# RECENT DEVELOPMENTS IN BIOTECHNOLOGY

Dr. Sunita Rao
Upendra Sharma

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# CONTENTS

# CHAPTER 1

# A STUDY ON CENTRIFUGATION TECHNIQUES AND ITS APPLICATION IN PHARMACEUTICAL INDUSTRIES

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***ABSTRACT:*** ***Centrifugation is the separation of the components of solutions using centrifugal force. The usefulness of centrifuged samples in pharmaceutical businesses has received little attention. Centrifugation is one of the most important and widely used research processes in molecular, biochemistry, or cellular biology, as well as the evaluation of suspensions but rather emulsions in pharmacology but not medicine. This review covers the fundamentals but also principles of centrifugation, but also different types of centrifuges, different types of centrifuge separation, different types of density gradient media, characterization of industrial centrifuges, or centrifugation implementations in the pharmaceutical industry. Centrifugation is important in the pharmaceutical industry for the production of bulk medications, biological products, identifying the molecular weights of colloids, and assessing suspensions and emulsions. In this paper, the author talks about the centrifugation techniques and their implication in pharmaceutical Industries or their benefits. In the future this paper help to understand centrifuges and centrifugation techniques and their use n Pharmaceutical Industry.***

***KEYWORDS:*** ***Centrifugation, Molecular,Pharmaceutical, organelles***,

## 1. INTRODUCTION

Centrifugation is a separation process that uses centrifugal force to separate things. The particles in a solution are sorted based on their size, density, medium viscosity, shape, or rotor speed. In biochemistry, molecular or cellular biology, and the assessment of suspensions, emulsification in pharmacy but also medicine, centrifugation is among the most essential and commonly used research procedures. Isolation of cells, subcellular organelles, and macromolecules, typically in large numbers, are used in current research and therapeutic applications. On a batch or continuous flow basis, a centrifuge employs centrifugal force (g-force) to separate particles from the surrounding liquid. Gravity causes many particles and cells in a liquid solution to settle to the bottom of a container over time [1]–[3]. The amount of time necessary for such separations, however, is unfeasible. Other, very minute particles will not separate in the solution until they are exposed to significant centrifugal force. Centrifugal force causes the particle to migrate radially away from the axis of rotation whenever a suspension is spun at a specific speed or revolutions per minute (RPM). Relative Centrifugal Force is the force acting on the particles in comparison to gravity (RCF).

A rotator is a device that utilizes a rotor to separate particles from an answer. In physics, particles are usually cells, subcellular organelles, and massive atoms, all of which are referred to as particles here. There are two types of axis systems: preparative and logical. The former is used to detach explicit particles, while the latter is used to estimate the real attributes of the sedimenting particles. A radiating power is provided to every molecule in the examples while a rotor revolves; the molecule will then silt at a pace that is proportional to the diffusive power given to it. The sedimentation rate of each molecule is also influenced by the thickness of both the examples arrangement and the physical qualities of the particles [4], [5].

The sedimentation rate of a molecule is proportional to its size (sub-atomic weight) and the difference between the molecule's thickness as well as the arrangement thickness at a reasonable radiating power or fluid consistency. In a solution, particles with a greater density than the solvent sink (sediment), whereas particles with a lower density float to the top (solvent). They travel quicker as the density difference between them grows, as shown in Figure 1 [6]–[9]. The particles remain constant if there is no variation in density (isopycnic circumstances).

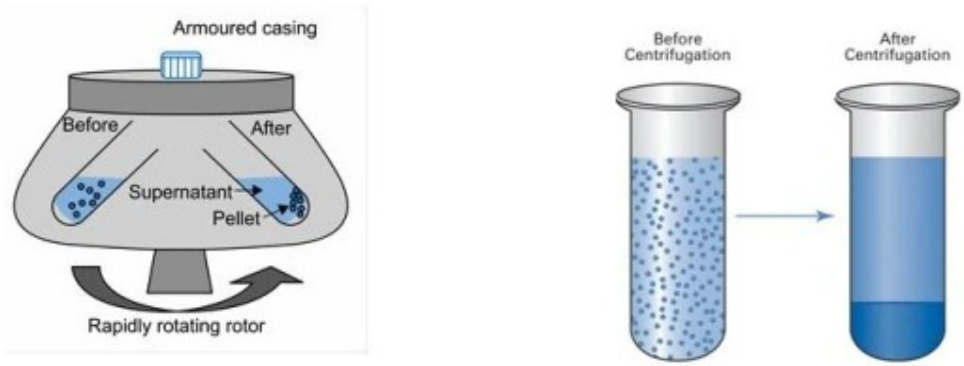


**Figure 1: The more substantial "diffusive power" provided by an axis might be used to leverage even minute variations in thickness to isolate unambiguous particles in an answer, replacing gravity.**

### *1.1. Principle of Centrifugation:*

- In a solution, particles with a greater density than the solvent sink (sediment), whereas particles with a lower density float to the surface. They travel quicker as the density difference between them grows. The particles remain constant if there is no variation in density (isopycnic circumstances).
- Gravity may be replaced by the far more strong "centrifugal force" generated by a centrifuge to take advantage of even minute changes in density to separate distinct particles in a solution.
- A centrifuge is a piece of machinery that spins an item in a circle around a fixed axis while delivering a potentially powerful force perpendicular to the spin axis (outward).
- The centrifuge operates on the sedimentation principle, which states that as the centrifuge accelerates, denser substances and particles migrate outward in a radial direction.
- At the same time, less dense things are displaced and travel to the center. In a laboratory centrifuge with sample tubes, radial acceleration causes heavier particles to settle at the bottom of the tube, while low-density substances rise to the top.

### *1.2. Types of Centrifuges:*

A rapid spin creates an increased gravitational force that separates the particles in centrifuges. This might either replace gravity in suspension sedimentation or act as a driving force for filtration via a filter material. The most common use is the separation of solids from highly concentrated liquids (Figure 2). It enables for dewatering with the formation of more or less uniform sediment depending on the kind of sewage sludge to be treated, as well as the thickening of low concentration sludge at a quicker rate when employed for sewage sludge treatment [10]–[13].

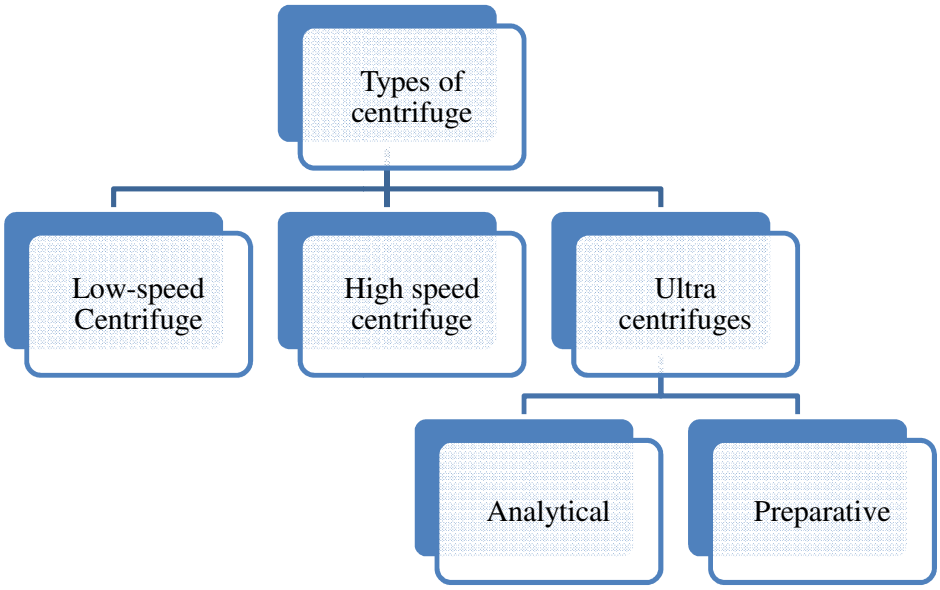


**Figure 2: illustrate the types of Centrifuges. Such as Dest top, High-speed centrifuge, and ultra-centrifuge.**

*1.3. Centrifuge with a Low Speed:*

- Most laboratories employ a low-speed centrifuge to sediment heavy particles regularly.
- The maximal speed of the low-speed centrifugation is 4000-5000 revolutions per minute.
- These devices are normally used at room temperature and have no temperature control.
- It makes use of two distinct types of rotors,
    - Fixed angle
    - Swinging bucket.

It's used to settle red blood cells until they're densely packed into a pellet or the supernatant may be collected by decantation.

*1.4. High-Speed Centrifuges:*

- Higher speeds or temperature control of rotor chamber are required in the more complicated biochemical application, hence high-speed centrifuges are utilized.
- The high-speed centrifuge spins at between 15,000 and 20,000 RPM.
- This instrument's operator can precisely adjust speed or temperature, which is essential for delicate biological samples.

For high-speed centrifugation, three kinds of rotors are available:

- The angle is fixed.
- Bucket those swings.
- Rotor that rotates vertically

*1.5. Ultracentrifuges:*

Fractionation, the method of extracting out all elements of a live cell1, includes centrifugation. Only with the introduction of the preparative ultracentrifuge machine was it possible to fractionate such cellular components. Ultracentrifuges are being used to separate very minute particles, including organelles, but much smaller molecules, including such DNA, proteins, or RNA, by spinning at very high speeds. Today's ultracentrifuges may spin at rates of up to 80,000 revolutions per minute. Large rotational speeds are required to generate high centrifugal forces, which are required to separate smaller particles. Equilibrium sedimentation or velocity sedimentation are two forms of ultracentrifugation. The particles split and travel across the media in equilibrium sedimentation until they come to a point where their density equals that of the medium. The particle sediment out at different rates depending on their size inside the velocity sedimentation type.

- It is the most advanced instrument available.
- The greatest speed of an ultracentrifuge is 65,000 RPM (100,000 x g).
- Because high speed generates a lot of heat, the spinning chambers need to be chilled and maintained at a high vacuum.
- It may be used for both preparatory and analytical tasks.

*1.6. Density Gradient Centrifugation:*

Thickness angle centrifugation is a centrifugation method used in natural exploration to separate particles based on density differences. This is a method that has been around since the 1950s. It's been used to isolate and segregate plant diseases, to fractionate cell organelles, but in a variety of other atomic science applications. Cesium salts or even sucrose may be used as the setup for this type of centrifugation. For example, to focus on cauliflower mosaic infection, centrifugation with a sucrose arrangement was used [14]–[17].

- A sucrose thickness inclination is formed by lovingly stacking smaller convergences of sucrose over higher fixations in rotator tubes.
- This kind of centrifugation is mostly used to decontaminate infections, ribosomes, films, and so on.
- In ultracentrifuges, the particle of interest is placed on top of the inclination and axis.
- The particles proceed across the angle until they reach a point where their thickness is equal to the surrounding sucrose thickness.
- A part of the dish is removed and inspected.

*1.7. Rate-Zonal Density-Gradient Centrifugation:*

- It is based on the notion of sedimentation coefficients but is also known as the band as well as gradient centrifugation (i.e., movement of the sediment through the liquid medium).
- A density gradient is formed in a test tube with sucrose at the top and high density at the bottom using this approach.
- The protein sample is put on top of a gradient or centrifuged.
- During centrifugation, faster-sedimenting particles in the samples move ahead of slower-sedimenting particles, resulting in the sample being divided into zones in the gradient.
- The fractions are collected by drilling a hole in the bottom of the tube but also allowing the proteins to settle as per their sedimentation coefficient.

*1.8. Isopycnic Centrifugation:*

- The inclination shaping arrangement is packed into the cylinder with the example (on top of or underneath pre-framed slope, or blended in with self-framing angle).

- In an axis tube, the natural example of cesium salt is consistently disseminated and spun in an ultracentrifuge.
- The cesium salts rearrange as a result of diverging power, forming a thickness slope all the way through.
- Particles migrate to the point where their light thickness reaches that inclination or structure groups. That is, the examples particles migrate to the district where their thickness equals the inclination thickness.
- It is a "legitimate" balancing approach since it is based on buoyant densities rather than speeds.

### *1.9. Types of Centrifugation Separations:*

Differential centrifugation or density gradients centrifugation is two kinds of centrifugal procedures for particle separation. Rate-zonal or isopycnic centrifugation are two types of density gradient centrifugation.

#### *1.9.1. Differential Centrifugation:*

Differential centrifugation is the process of separating particles based on their size differences. Through a series of repetitive turn cycles, regions within cells may be investigated and separated in this interaction. The heavier sections and organelles break out at lower speeds, whereas the more modest organelles separate at greater speeds. This is a method that has been used extensively in the study of exosomes in research. While speed sedimentation (an ultracentrifugation technique) focuses on measuring the pace at which particles separate, differential centrifugation is primarily concerned with where the particles end up after the interaction [18].

Differential centrifugation, also known as differential pelleting, is the easiest form of centrifugation division. Particles of different densities, as well as sizes, will silt at different rates in a suspension, with the larger or denser particles sedimenting faster. Divergent power may be used to increase these sedimentation rates. A suspension of cells subjected to a series of increasing radiating power cycles will result in a series of pellets comprising cells with a decreasing sedimentation rate. Particles of differing densities or sizes will settle at different speeds, with the largest and thickest particles sedimenting first, followed by the thinnest and smallest particles.

#### *1.9.2. Density Gradient Centrifugation:*

To refine subcellular organelles or macromolecules, thickness slope centrifugation is the preferred method. Thickness slopes may be made by layering a large number of layers of angle media, such as sucrose, in a cylinder, with the heaviest layer at the bottom as well as the lightest layer at the top, in either an intermittent or continuous mode. The separated cell division is placed on top of the layer but also centrifuged. Rate - zonal (size) detachment or isopycnic (thickness) division are the two types of thickness angle partition.

- *Rate-Zonal Centrifugation:*

By layering the example as a confined zone on top of the thickness angle in rate-zonal centrifugation, the problem of cross-tainting with particles of different sedimentation rates may well be avoided. As a result, unlike differential centrifugation, the faster regimenting particles aren't debated by the slower regimenting particles. Despite this, the thin burden zone

limits the number of tests that may be imposed on the thickness slope (usually 10%). The angle creates a manner of growing thickness and uniformity by balancing the groupings.

- *Selection of Suitable Density Gradient Medium:*

The primary function of thickness angle centrifugation is to separate particles depending on their lightness thickness and sedimentation rate. The inclination for rate-zonal divisions can offer a thickness slope that further improves the molecular objective while balancing out all the segments from convection fluxes. The fact that the inclination media have a greater thickness than the particles is a crucial factor in isopycnic partitions.

*1.10. Applications of Centrifugation:*

- Separation of two miscible compounds
- Mammalian cell filtration
- Fractionation of subcellular organelles (counting films/layer sections)
- Hydrodynamic characteristics of macromolecules Layer vesicles fractionation
- Separating the chalk powder from the water.
- Isolating particles from a wind current using cyclonic division.
- Wine explanation and modification.
- Partitioning of urine parts or blood parts in legal and research laboratories
- Assists in the partitioning of proteins via sanitization processes such as salting out and precipitation of ammonium sulfate.

*1.10. Centrifuge application in Pharmaceutical Industries*

*Production of bulk drugs:*

Centrifugation is used to remove the medicines from mother liquor after crystallization. Centrifugation, for example, is used to extract residues of liquid solution from aspirin crystals.

*Production of biological products:*

The majority of the biological product are proteinaceous or macromolecular. They stay in colloidal dispersion in water throughout the production process. It is difficult to remove colloid particles using conventional filtering procedures. Centrifugal procedures are applied in these circumstances. Insulin is separated from other proteins precipitated by centrifugation. The centrifugal technique is used to separate blood cells from plasma. The bacterial enzymes are separated from the bacterial growth medium by centrifuging the bacterial cells. Olive oil or fish liver oils are isolated from dirt and water.

*Evaluation of suspensions and emulsions*:

Sedimentation is an issue with suspensions, but creaming is a problem with emulsions. This issue may not arise right away once a suspension and emulsion have been prepared. The suspension as well as the emulsion is placed in a centrifuge or rotated at 200 to 3000 rpm to speed up the sedimentation and creaming process. If the difficulties persist, the suspension and emulsion may be considered a stable formulation.

*Determination of molecular weight of colloids*:

Colloidal dispersions are often formed by polymers, proteins, or other macromolecules. Ultracentrifugation may be used to estimate the molecular weights of such molecules. The heavier molecules will be at the core, while the bigger molecules will be on the perimeter.

## LITERATURE REVIEW

Jane M. Morrell et al. investigated Colloid Centrifugation of Semen: Applications in Assisted Reproduction. Colloid centrifugation may be used to separate heterogeneous populations of cells, such as spermatozoa at various stages of development, dead and passing on spermatozoa, and non-sperm cells that are seen in normal semen tests. Colloids might be utilized to increase the population of appropriate and functional spermatozoa, which are the spermatozoa that will be employed in Assisted Reproduction. Colloid centrifugation may lead you to believe that sperm tests are only for motile, morphologically normal spermatozoa with perfect chromatin. Since research has shown that colloid centrifugation may favor spermatozoa with certain characteristics, such as metabolic activity, the approach does not just progress for living spermatozoa by preventing dead or kicking the bucket spermatozoa from passing through the colloid. Other regenerating cells, such as spermatids or begetter cells, may also be cleaned using colloid centrifugation. This article looks at the history of colloid centrifugation for sperm selection, compares colloid centrifugation to other tactics, and looks at some of the processes' applications in the animal husbandry field. On most stud homesteads or semen assortment stations where there is a swing-out axis, and in IVF research centers, a comparable procedure could be used for different kinds of regenerative cells, like spermatids or spermatogonia for germplasm banking, or could be used to choose non-conceptive cells; a comparable procedure could be used for different kinds of regenerative cells, like spermatids or spermatogonia for germplasm banking,[19].

Concerning Putting a different spin on it: Gradient Centrifugation for Analytical or Preparative Applications, Helmut Colfen et al. discovered. In science, materials science, science or design, inclination centrifugation is a critical approach. It has immense potential beyond the well-known centrifugation for atoms as well as particle division. There are several prospects for remarkable particle inspection or division, as well as preparative applications such as the development of angle materials or regulated polymerizations. Centrifugation is used to generate an inclination of physical and material qualities in all models, which is then used for future applications. We give selected examples of slope centrifugation in this concept article to demonstrate important advancements in the area and to explore their uses, potential, and restrictions. Finally, we look at future slope centrifugation patterns that are crucial for academic and contemporary usage. Structures with tendencies of physical and material qualities are realistic in a regulated way when the developed cycles are upscaled to the current size. For electronics, optics, sensors, catalysis, or pharmaceuticals, such materials are exceedingly promising [20].

RICHARD M. BASEL ET AL. DISCOVERED THAT DENSITY CENTRIFUGATION MAY BE USED TO MONITOR MICROBIAL NUMBERS IN FOOD. BECAUSE OF SMALL FOOD PARTICLES THAT BLOCK THE COUNTING OF PROVINCES, A FEW FOOD KINDS CONTAIN TINY NUMBERS OF CREATURES THAT MAY BE DIFFICULT TO DETECT USING THE PLATE COUNT APPROACH. TO CREATE A NONTOXIC THICKNESS MATERIAL, LUDOX COLLOIDAL SILICON WAS COATED WITH DECREASING EXPERTISE. FOOD HOMOGENATES WERE ADDED TO A LAYERED 10% OR 80% CHANGED LUDOX MIXTURE AS WELL AS CENTRIFUGED AT THE LOWER SPEED. TRADITIONAL POUR PLATE PROCEDURES WERE USED TO EXAMINE THE LUDOX-CONTAINING PORTION, WHICH WAS LOCATED AT

THE TOP AND BOTTOM OF THE CYLINDER. THE PLATES COUNT OF LUDOX MIX AND THE MEAL HOMOGENATE ALONE WERE IDENTICAL. AT 4°COVER A 24-HOUR HATCHING PERIOD, ALTERED LUDOX WAS TESTED FOR ITS EFFECT ON MICROORGANISMS. THERE WAS NO IMPEDIMENT FOUND. THIS METHOD APPLIES TO FOODS SUCH AS DOUGHNUTS, TASTES, TOMATO PRODUCTS, AND MEAT, WHERE LITTLE FOOD PARTICLES COMMONLY SLOW DOWN ROUTINE PLATES COUNT AS WELL AS LOW WEAKENING MAY STIFLE SETTLEMENT GROWTH. FLAVORS MAY BE STRIPPED OF INHIBITORY CHEMICALS, RESULTING IN LARGER NUMBERS. LUDOX IS MORE PRACTICAL THAN SIMILAR PRODUCTS SUCH AS PERCOLL. THE INCREASE OF STANDARD RESEARCH FACILITY REAGENTS MAKES IT EASY TO DISTRIBUTE ALTERED LUDOX SAFELY. IN ADDITION, THE COMBINATION WORKS WITH MICROBIOLOGICAL MEDIA **[21]**.

THE SIZE-SEPARATION FOR SILVER NANOPARTICLES UTILIZING SUCROSE GRADIENT CENTRIFUGATION WAS DISCOVERED BY DALE A PELLETIER ET AL. NANOPARTICLE SIZE AND SHAPE CIRCULATIONS MAY DRAMATICALLY ALTER THEIR OVERALL QUALITIES, CHANGING THEIR INTERACTIONS WITH DIFFERENT CHEMOTHERAPEUTIC PARTICLES, NATURAL ORGANISMS, AND PERHAPS MATERIALS AND CELL TYPES. AS A RESULT, TO BENEFIT FROM THE PROPER USE OF NANOPARTICLES FOR VARIOUS BIOMEDICAL BUT ALSO BIOSENSOR APPLICATIONS, IT IS NECESSARY TO GET A LARGE NUMBER OF ISOLATED MONODISPERSED NANOPARTICLES. REGARDLESS, DEALING WITH THE MORPHOLOGICAL CHARACTERISTICS OF NANOPARTICLES DURING THEIR UNION IS A CHALLENGING TASK. AS A RESULT, POST-SYNTHESIS NANOPARTICLE PARTITIONING IS CRITICAL. IN THIS STUDY, WE SHOW HOW TO USE SUCROSE THICKNESS SLOPE SEDIMENTATION TO REACH MONO VARIATIONS VIA A ONE-POT POST-BLEND DETACHMENT OF ANISOTROPIC SILVER NANOPARTICLES. OPTICAL CONFIRMATION, AS WELL AS SPECTROPHOTOMETRIC OR TRANSMISSION ELECTRON MICROSCOPY ESTIMATES, WERE USED TO SHOW THE NANOPARTICLES' DETACHMENT. OUR FINDINGS DEMONSTRATE THE EASY DIVISION OF ANISOTROPIC SILVER NANOPARTICLES USING SUCROSE THICKNESS SLOPE SEDIMENTATION, PAVING THE WAY FOR NANOPARTICLES TO BE USED FOR VARIOUS MARKING, IDENTIFICATION, AND BIOLOGICAL APPLICATIONS. THE ADOPTED STRATEGY'S SIMPLICITY AND EASE OF USE PROVIDE AMAZING ADVENTURES FOR REASONABLE PARTITIONING, AND IT MAY BE USED FOR A VARIETY OF METALLIC NANOPARTICLES **[22]**.

## DISCUSSION

Centrifugation is a useful way for separating particles using a rotator, but it is a strategy with many applications in the life sciences, particularly atomic physics. Axes come in a variety of shapes or sizes, and they may be used for a variety of purposes, including studying organelles or natural particles. In molecular genetics lab, centrifugation is among the most useful but also used methods. Cells are collected, DNA is precipitated, viruses' particles are purified, and tiny changes in the conformations of the molecules are distinguished using centrifugation. Centrifugation is a cycle that uses divergent power to separate two fluids in a mixture. The denser section of the mix travels away from the pivot and towards the hub during this interaction. The sedimentation guideline is used by rotators: fluids separate as their thickness is influenced by gravitational force (g-power). ultrafiltration, Isopycnic, slopes of mass, stages division, or pelleting are examples of several forms of partition.

There are two types of radial techniques for splitting particles: differential centrifugation and thickness angle centrifugation. Separating thickness inclination centrifugation into rate-zonal or isopycnic centrifugation is also possible. In any event, centrifugation is undoubtedly more effective than defiltration and particle trading at removing LMW atoms, and centrifugation tubes should be filtered and cleaned as soon as possible. As a result, centrifugation's primary disadvantage is the rotator's limited example limit. Centrifugation offers several benefits, including being a very easy approach for separating chemicals and is superior to layer filtering procedures in some situations when film filtration frameworks are used. Ultracentrifuges can separate small particles, making centrifugation particularly useful in subatomic scientific research. While fast axes have been used to focus on a variety of biological particles and components, including extracellular vesicles, there are certain drawbacks, such as the possibility that particles would be injured due to the actual machine's high twisting speed. Centrifugation may also cause alterations in the existence of the vesicles or cause them to clump together on occasion. Another disadvantage of centrifugation is the high cost of the equipment.

## CONCLUSION

Centrifugation is a well-known and useful process used by scientists nowadays. It entails separating distinct particles using a centrifuge1, a machine that spins at a high rate to separate particles in an answer based on differences in factors such as molecule size and thickness. Contrasts in medium thickness and turn speed are crucial variables since larger particles split and frame a pellet at lower speeds, whereas smaller particles need much greater velocities. Centrifugation's applicability and importance are mostly in the biological sciences, with numerous processes and rotators being used in natural exploration. Surface moisture of fine coal and mineral particles were eliminated using an innovative outward filtering method at varied G-Force, air/vacuum pressure, spine time, cake thickness, or compound dosages. A 3.5-inch width bin was connected to an air/vacuum pressure source and turned at varying speeds to generate massive diverging powers on the channel cake's outer layer. This audit has provided insight into the importance of centrifugation in the pharmaceutical industry. Centrifugation is used to make large quantities of pharmaceuticals and natural products. During centrifugation, the purging of insulin, as well as the partition of platelets, are extracted. Centrifugation speeds up the sedimentation of suspensions or emulsion creaming.

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# CHAPTER 2

# SACCHAROMYCES CEREVISIAE SUSTAINABLE ENERGY ALTERNATIVE: BIOFUEL PRODUCTION

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***ABSTRACT:*** ***Microorganisms are critical to human survival. They are found in ordinary settings including water, soil, air, as well as odd sites like hydrothermal or soda lakes, have all been studied. Limestone lakes although Saccharomyces cerevisiae has been the most oftenUtilizing cerevisiae for industrial production of bioethanol, ethanol can be produced by several of yeast strains, bacteria or fungi. This study examines the present and non-molecular developments in S. cerevisiae-based ethanol production. Molasses, starch-based substrates, sugar cane extracts, lignocellulose, as well as other wastes have all been used to make ethanol. Adopt alternative signal transduction mechanisms to defeat these yeast cells. This review focuses oncarbon feedstock's that are both ubiquitous and underutilized but can be easily converted into bioethanol. These many genes, protectants, and processes that may be manipulated to design yeast- strains are addressed.The current study provided methods for using this profitable option for long-term bioethanol production.Transforming or over-expression of the gene linked with certain features (example cellulose) in S. - cerevisiae may be extremely significant in today's molecular era to tackle issues such as the inability to use polysaccharides and ribose. As a result, a thoroughlyEconomic or process research are it is necessary to design an industrial applications efficientproduction approach which would steadily increase production of ethanol to help the energy crisis. Mitigation of such concerns is undoubtedly conceivable with novel ways including the use of microorganisms to make better biofuels. Much more research will be required to give these solutions on an industrial scale, and they're becoming more accessible as time goes on.***

***KEYWORDS:*** ***Biofuels, Environmental Biotechnology, Microbial Ecology, Microbes***, ***Saccharomyces Cerevisiae.***

## 1. INTRODUCTION

Microorganisms are critical to human survival. They are found everywhere, including typical settings like soil, water, and air, as well as unusual locations like hydro-thermal –vents deep-sea, or soda- lime-lakes. They are widespread in natural habitats and are connected with a variety of activities such as biomass or fuel generation, nutrient recycling, energy recovery, mineral as well as among others. The concern about microbial research have been highlighted as an important area in which background knowledge is required for thenatural component recovery for our purpose.Applications for microbes, the research of environmental balance, and their linkage are all beneficial to live organisms. Microbes are utilized in environment protection and bio mediation processes that are necessary to direct to more sustainable life and greater resource use.

Microbes are everywhere in the biosphere; therefore, their very existence always has an impact on the ecosystem.The influence of microbes on the environment can be either helpful or detrimental. Because a large portion of this material is devoted to discussing the positive actions of microbes as these rely on human welfare. Microorganisms are extremely varied, and their roles in the environment are extremely particular. Recognizing the myriad important activities of microorganisms, this chapter of the book examines the environment and the function of microbes in a wide range of applications including such biomass- degradation, energy generation, minerals recovery, as well as nutrient- recycling [1].

Environmental biotechnology is concernedwith the microflora present in the soil, air, water, or air, around us; additionally, the digestive system, or even the "external world within live

animals," is sometimes seen as a component of environmental biotechnology (Figure 1). Microbial communities in the open environments are complex and continually changing.These communities might be thought of as either haphazard ensembles or meta-genomic functional assemblage's entities. One critical point is that they are helpful options with relation to genes and functionality. However,much more can be done than simply listing DGGE/T-RFLP/ clone libraries [2]. This efficient leadership of these microbiological resources will be critical in the next decades. Human resource management has become more important at all levels of our society, and several top-tier business schools are developing this; the focus of environmental biotechnology in the coming decades is on microbial-resource- management (MRM).

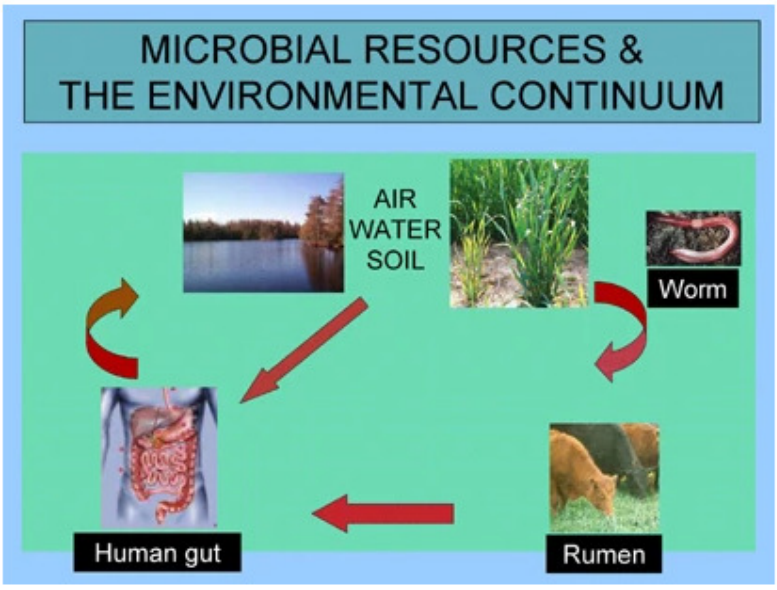


**Figure 1 : Depicts the wide range of disciplines addressed by environmental - biotechnology that are depicted schematically** [2]**.**

Based on an environment- biotechnology, and also microbial- ecology, must come to terms and build procedural rules for transparent as well as successful administration. This will allow humanity to grasp the myriad of services that microorganisms contribute to the overall quality of our planet, and specifically to our 'environmental continuum.' Researchersmust not be afraid of new challenges and the formation of new complicated connections between different types of microbial life. However, researchers must work hard to understand the complexities so the resources that represent are efficiently managed, utilized, and maintained. The challenges of global- warming and the depletion of fossil fuels have undoubtedly revived interest in alternative commercial fuel sources. The hunt for renewable fuels has sparked interest in research into biodegradation of lignocellulose biomass material to create biofuel including bioethanol, biodiesel, and bio-hydrogen. To create an enhanced the type strains for biofuel must be able to ingest a large amount of substrates, transport sugar via quick and uncontrolled pathways, endure inhibitory compounds or end products, and improve metabolic fluxes.[3].

Biofuel is the great alternative to traditional energy sources such as coal, gasoline, and diesel since it is generated from the plant's lignocelluloses.The fundamental structural element of plant cell walls is cellulose. It consists of a lengthy strand of molecule of glucose joined together by glycosidic- linkages. Lignocellulose,the second most common lignocellulose

component is a polysaccharides family composed of mono-saccharide unit. Hemicellulose,besides cellulose this is the most effective biomass component for the synthesis of bioethanol. Lignin is phenyl-propanoid-based 3-D polymer. Plant cell wall carbohydrate fractions may be transformed into microbial fermentation monomeric sugars by acidic as well as enzymatic (hemicellulose/cellulase) processes, which have been used to make biofuels such as ethanol, as well as butanol throughmethods of microbial fermentation Therefore, microorganisms convert simple carbohydrates into energy (Cyanobacteria, Yeast, Bacteria,) [4].

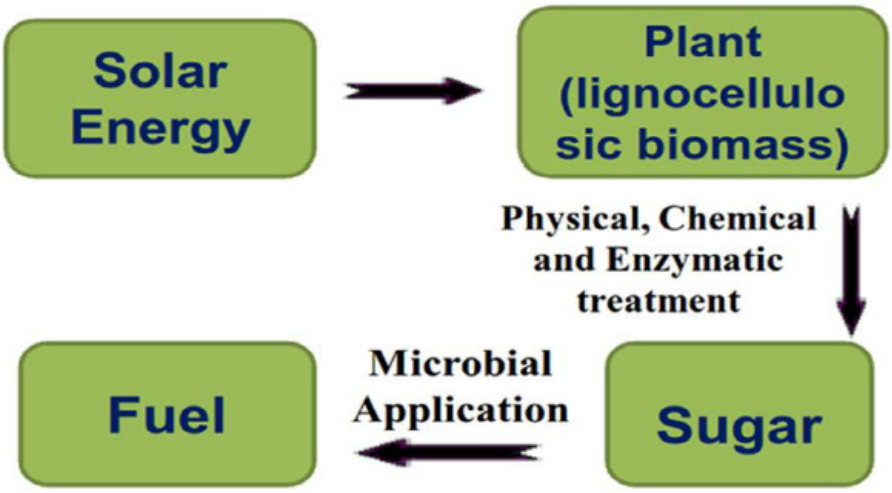


**Figure 2: Shows solar energy conversion by microbes into fuels** [5]**.**

The primary kinds of biomass known so far for ethanol generation include monoculture crops grown upon fertile lands (including such sucrose, corn, soya-bean, oilseed, grasses, willow, or hybrid- poplar); trash- biomass (also including wheat, corn, as well as waste wood); also municipal solid waste. Wood- cellulose, that includes *E. crassipes, L. camara, Prosopis juliflora, S. spontaneum, Typha latifolia, Crofton, Chromolaena odorat*a, as well as others, is another kind of biomass that is a possible and less expensive feedstock for fuel ethanol manufacturing. Photosynthesis is the process through which plants store solar energy, which may then be turned into fuel by microorganisms shown in Figure 2.

## 2. LITERATURE REVIEW

ZiheLiu et.al,conducted in this studythe growing concern about environmental repercussions and global warming necessitate an urgent shift away from the usage of fossil fuels and toward renewable alternatives. Biofuels are becoming increasingly popular as viable alternatives to fossil fuels. It is now feasible to construct microbial cell manufacturers for effective biofuels more accurately and efficiently using synthetic biology. In this section, we will look at recent advancements in biofuel derived from yeast generation.Regarding an overall view of an effects of artificial biology on biofuel production, we assess recent advancements within test, design, build, as well as explore steps of brewing process biofuel production, but also reach the conclusion with a conversation of the difficulties involved with by using biological methods to develop new biofuel production processes.Biofuels are important alternatives to fossil fuels for lowering net CO2 emissions. S. cerevisiae is a commonly used chassis with a plethora of synthetic biology techniques at its disposal. Synthetic biology improves biofuel production by being more accurate and efficient. Continuous innovation is still required to simplify biofuel manufacturing [6].

Mobolaji Felicia Adegboyeet.al, stated in this studymicroorganism's play a critical part in the synthesis of bio-fuel. Though, natural strains' production the products is inadequate, necessitating its development and enhancement by using biotransformation as well as genetic- engineering. Current study has focused on the use of metabolic- engineering for construct models strains in order to attain maximum efficient resource value at a lower production cost. Database mining has the potential to uncover additional unique pathways for biofuel synthesis soon.As a result, using these routes in fermenters hosts could help to overcome any limitations related to the utilization of lignocellulose biomass energy fermentation feedstock. Metabolic engineers must make use of advanced innovations such as technologies and the CRISPER/Cas9 scheme to design as well as related to the implementation stressors of microbes to enhance capacityto generate biodiesel from a variety of feedstock's by integrating critical alleles into the genome, as well as elimination of obstructive ones [7].

Nicolaas A. Buijs et.al, conducted in this study replacement of traditional fuel sources with biofuels will necessitate the development of molecules capable ofincluding the whole fuel spectrum, including gasoline to kerosene because they outperform alcohol,improved bio-fuels were considered to play an increasingly part in the replacement of fossil- fuels. Some of these biofuels may have had the energy density necessary to be used in vehicles, ships, or planes. Furthermore,improved bio-fuels might be used as a direct replacement fuel in today's combustion engines. *S- cerevisiae*, a yeast production plant, might be converted into the a producer of alcohols (1-butanol and isobutanol), sesquiterpenes (farnesene or bisabolene), and fatty acid ethyl esters (biodiesel), and so this understanding progress in metabolic engineering of S. cerevisiae for such production of these innovative bio-fuels. [8].

Shannon M.Hoffmanet.al, mentioned in this study Future ethanol generated from corn productionraises concerns about the food industry's sustainability and competitiveness. As a result, cellulosic biofuels made by there is a need for agriculture waste and energy crops. Slow and partial hydrolysis of cellulose, but also high enzyme costs, having impeded cellulose biofuels' economic feasibility to far, and while efforts including such hydrolysis as well as fermentation (SSF), which includes the application of thermo-tolerant bacteria, may enhance output, more progress is required [9].

## 2. DISCUSSION

Bio-fuels are produced by living creatures or even the wastes that they produce. Plants are being used to produce ethanol, among the most common bio-fuels. A plant material used is plant's food element, including such sugar- cane in Brazil, corn kernel within the United States, and fructose sugar beet in France, since it is readily broken down into sugar. This sugar can then be processed (broken down) by bacteria like S. cerevisiae to produce ethanol. Currently, sugar crops are utilized to make bioethanol and biodiesel. The food against fuel conundrum, on the other hand, jeopardizes its long-term existence. Biofuel may also be obtained from non-food plants (including such switch-grass, poplar, or willow), algae, as well as genetically engineered organisms.

Scientists are looking at usingcellulose for the production of ethanol.Alcohol derived from cellulose is like ethanol derived by edible plant- components as a bio-fuel. Ligno-cellulose is used to make cellulose ethanol,a lignin, hemicellulose, or cellulose blend. These plant- cell walls this three-part structure is comprised of these three parts. The glue that keeps the cellulose fibers together and provides the plant its stiffness is lignin. This plant component ligno-cellulose seems to be the only one that is not digested by humans or most animals i.e. wood -chip, stalks, as well as sawdust,there is an enormous amount of non-edible bio-waste which must be recycled. Our environment provides us with non-living and abiotic resources

including such, soil, minerals, temperature, air, water or solar energy. Plants, animals, and microbes were instances of 'biotic' and living natural components. Plants and animals thrive by relying on abiotic and microbial components. Microbes play an important part in the balance of biotic and abiotic components in the environment.

*3.1Biofuel Production by Microbes:*

Biofuel is a great alternative to traditional energy sources such as coal, gasoline, and diesel since it is generated from the plant's lignocelluloses. Lignocellulose is composed mostly of cellulose, hemicellulose, and lignin. The fundamental structural element plant cell walls are cellulose. It consists of a lengthy a glucose molecule chain joined togethervia glycosidic connections Hemicellulose is the second most common component of ligno-cellulosic biomass,is a polysaccharide family composed of monosaccharide molecules. Hemicellulose,besides cellulose, it is the most efficient biomass component forsynthesis of bioethanol. Lignin is phenylpropanoid-based 3-D -polymer. A wide range of creatures, including free-living organisms, have developed to make use of this source, comprising biomass breakdown, fuel generation, mineral recovery, and nutrient recycling in biomass-rich settings, organisms, or symbiotic animals–microbe consortia are invariably present.

Despite the growing usage of biofuels like biodiesel as well asethanol derived from sugar or starch, data shows the lignocellulosic biomass created transportation fuels arethe most versatile alternative fuel source is currently available. Plant materials containing ligno-cellulosic biomass provide a renewable, widely distributed, as well as low-GHGsugar source that can be turned to ethanol as well as other liquid fuels. Estimated economic costs of ligno-cellulosic feedstock, partlyaccording to their potential to thrive on marginal agricultural terrain, indicatethat they might very certainly have a substantial impact on transportation demands while not considerably lowering the land required for food agricultural production [5].Many unique enzymes or enzyme complexes theseorganisms that are tough to cultivate evolved to utilise cellulosic cellulose bacteria.The existence of a varied collection of natural lignocellulose-degrading enzyme sources enhances the likelihood of enzyme optimization success in industrial applications The development of a saccharification method is critical since the expense of cellulases continues to be a substantial obstacle to the expense synthesis of bio-fuelsA more diverse set of candidate enzymes identified by a mix of traditional cultured microbial research as well as environmental prospecting approaches increases the possibility of obtaining enzymes with the stability and activity required for a wide variety of commercial applications.This last phase'scellulosic ethanol production would need many of the same technology as sugar- or starch-based production of bioethanol; however, adjustments will be required to leverage the range of sugars released by biomass breakdown. Whereas starch-based biomass conversion produces mostly hexoses as well asD-xylose and L-arabinose are pentose sugars. Pentose sugars, unlike hexose sugars, fermentation was possible in wildtype *S. cerevisiae*.

*3.2 Metal and mineral recovery by microorganisms:*

It's exciting to discover certain bacteria capable of doing it properly. This potential of microorganisms has just lately been understood, and attempts are being undertaken to harness them to improve the extraction of mineral resources through natural deposits. Bioleaching or microbial mining is the technique of extracting metals or concentrates using microorganisms. Metals are commercially extracted in low-grade ore by utilising the metabolic activity of *Thiobacilli bacteria, specifically, T. thiooxidans, Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans as well as T. ferro-oxidans* They thrive in situations with pH is low (pH 1–2) or absorb eithercarbon or nitrogen derived from the atmosphere.It assists in

the dissolving of copper or other metals inside rocks and is critical to the bio-geochemical cycling of nutrients and metals in the environment.

The leaching procedure can be used to remove lead that is insoluble sulfate (PbSO4) by those additional metals included in the ore. Microbial activity can also improve the extraction of uranium, and nuclear fuel, which should aid in the resolution of the global energy issue. Low-grade ores include insoluble tetravalent uranium oxide (VO2). *T. ferro-oxidants* may indirectly convert VO2 to the leachable hexavalent form $VO_2SO_4$ and release S2- in the existence of some kind of growing medium S2- is oxidized and transformed to sulphur (S), which is required with in metabolism of *T. ferro-oxidants* and so completes the bioleaching cycle Figure 3.

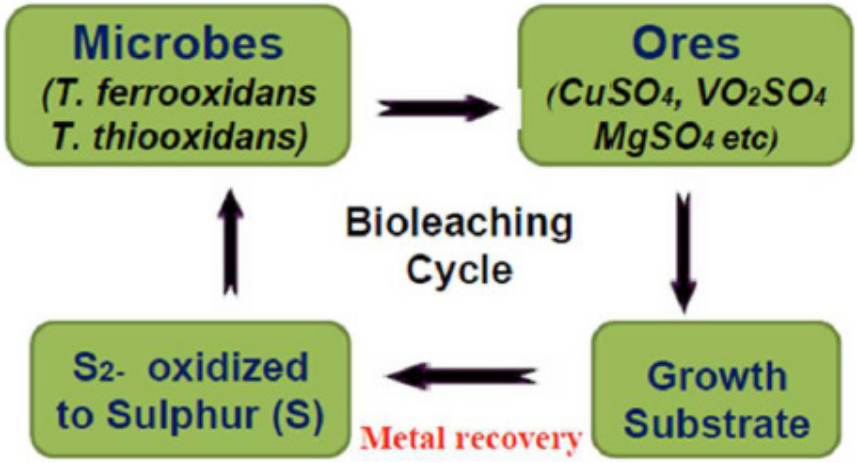


**Figure 3: shows the Cycle of bioleaching** [5]**.**

*3.3 Microbes and Nutrients in the Environment:*

Nutrients are required for the growth of all living organisms. Nutrients are also the chemical substances that plants and animals absorb in the greatest quantities. Nutrients are classified into two type's nutrients from nature: Proteins, vitamins, carbohydrates, or fats are all examples of macronutrients are examples of organic nutrients and inorganic chemical substances including, Oxygen, Nitrogen, Phosphorus, Carbon, Hydrogen sulphur, water, and oxygen are all examples of nutrients. The elements considered basic for cell development are carbon, hydrogen, oxygen nitrogen, phosphorus, as well as sulphur.They are utilized to construct and repair tissues, to control bodily functions, and to convert to and use as energy. Animals consume mealsmaterials are digested with such an interiorwhile most plants obtain nutrients in the soil through their roots either from their surroundings, others have a digestive system. Bacteria are the most prevalent microorganisms) plays a significant function in the ecosystem as mineralizers on organic waste and recyclers of key nutrients.

*3.4 New methods for manufacturing biofuels:*

Scientists are looking at bacteriato discover whether any can truly convert ligno-cellulose, cellulose, or even hemi - cellulose into ethanol. These residuals lignin besides can be burnt to generate electricity. Researchers had looked in the most unusual areas, such as Termite guts and dirt nearthey discovered a variety of bacteria that all had one thing in common they all make a set of enzymes known as cellulose.

*3.4.1An archaeon* called *Sulfolobus- solfataricus:*

In Italy, lives in volcanoCellulase are produced by streams nearby Mount Vesuvius. Scientists are looking at ways to genetically edit this microbe to boost its activities and produce additional cellulase. S. solfataricus might be utilized for making bio-fuel in the future.

*3.4.2 Trichoderma reesei*:

Another common wood digester is Trichoderma reesei. This can be present in almost all soils as well as secretes huge volumes of cellulase. During World War II, US soldiers discovered the fungus for the first time. This was in responsible of trying to break down the cellulose in the soldiers' tents and clothes, leading them to become slightly holey. It was called "jungle rot."

They utilize solar energy to turn they convert $CO_2$ to sugar, that they are subsequently metabolized to become lipids. Algal lipids can be converted into bio-diesel, whereas algae carbohydrates can be converted into bio-ethanol. It works well in experimental small-scale- bioreactors. Scientists are investigating if it is feasible to produce microalgae on a large scale in order to determine whether such algae could be the bio-fuel suppliers of the future. The optimum microbe for ethanol production is still regarded to be yeast, *S. cerevisiae*. It can produce bio-ethanol from a variety of feedstock, which is discussed further below.

*3.5 Mechanism of Fermentation Stress Tolerance*

*3.5.1 Fermentation of Ethanol*

S. the yeast cerevisiae is frequently used in ethanol production because of its efficacy in the fermentation sector sugar to ethanol conversion Figure 4. However, it is subjected to a variety of stresses during fermentation conditions. Stress circumstances and adaptive response overcoming mechanisms are referred to as a group 'Fermentation Stress Tolerance' (FST).

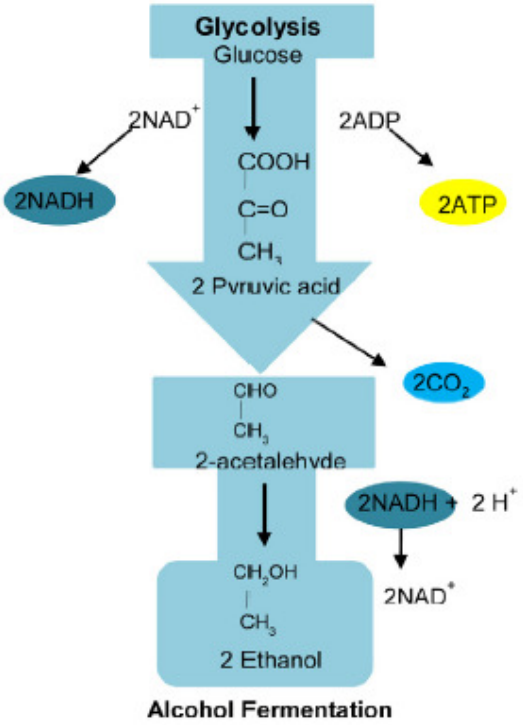


**Figure 4:represents the Fermentation of ethanol** [10]**.**

*3.5.3 Ethanol Tolerance*

S. Cerevisiae yeast fermentation sugar, starch, or ligno-cellulose to ethanol, however when the ethanol level surpasses a particular threshold, it restricts growth, induces mitochondrial loss, and eventually kills the yeast cells. Ethanol improves membrane stability, destroys protein, and damages cell membranes. Several investigations have revealed the fundamental mechanisms of ethanol stress tolerance as well as associated genes.

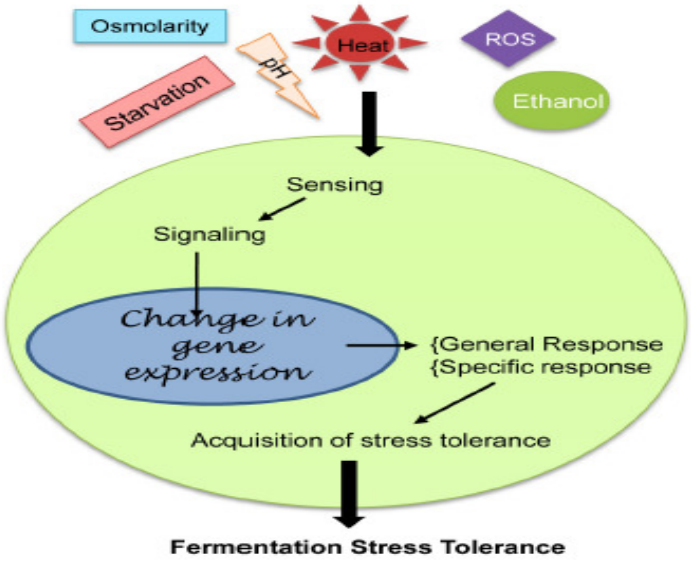


**Figure 5: represents the Mechanism of Fermentation Stress Tolerance** [10]**.**

In addition to damaging the plasma -membrane, ethanol denatured proteins with functional properties and proteins found within the membrane of cell. Cells had developed application has been designed tolerance mechanisms to endure varied environmental disturbances and preserve internal steady-state homeostasis. To induce a stress response, these biological responses cause changes affect gene expression and need signalling networks to transport information from sensors upon that cell surface or cytoplasm to transcription factor in the nucleus (Figure 5).

*3.6 Growth Factors Influencing Ethanol Fermentation:*

Oxygen ($O_2$), organic - acids, Temperature, pH, dissolved -solids, initial –sugar-concentrations and yeast immobilization are all critical elements in affecting the paceof yeast development and biofuels production. Medium conditions control yeast viability, and also the particular rate of fermentation or nutrient absorption [10].

*3.6.1 Temperature:*

The temperature has a significant impact on yeast cell Commercial bioethanol production requires enzymatic activity as well as membrane turgidity, as well as yeasts that really are active or tolerant at high temperatures. At 30°C and pH 3.5, Saccharomyces it has been established that yeast cultivated using sugar cane molasses produces more ethanol. [11]. Another study found that 30 to 40°C the ideal temperature of S. cerevisiae BY4742 was; warmer temperatures impeded the development phase of the yeast cell. Though temperatures of While temperatures ranging from 40 to 50°C were best for saccharomyces strain fermenting, S. cerevisiae JZ1C inulinases functioned well at temperatures ranging from 30 to 35°C [14]. As just a consequence, the yeast has to be active or temperature resistant in order to produce ethanol from inulin alone. Another study discovered that when the temperature

was increased to 30°C when alkali-treated-palm fruit-bench-fiber was used was used was used in a fed-batch SSF, ethanol yields decreased conditions with higher temperatures producing uneconomical ethanol.

*3.6.2 pH:*

The ideal pH of S. cerevisiae BY4742 had been in the 4.0–5.0 range. When the pH falls below 4.0, the incubation duration is extended however the ethanol concentration doesn't really fall much, and when the pH goes below 4.0, the incubation time is reduced level is greater over 5.0, its ethanol concentration decreases drastically. The acetic acid formation was favored so when pH was less than 4.0, but the when the pH was greater than 5.0, butyric acid production was preferred.**[12]**.

*3.6.3 Sugar Concentration at the Start:*

The impact of initially lowering the sugar content inthe effect of the effects of the effects of sweet- sorghum stalk juice on S. cerevisiae CICC 1308 immobilized by alginate were studied. As a result, increasing the initial sugar content considerably decreased mean specific growth rate as well as mean- biomass- yield while boosting, mean specific ethanol productivity, mean ethanol as well as average specific substrate uptake yield (sugar concentration within aAt 30°C, a ranging of 85–156 g/L was investigated) **[13]**.

*3.6.4External Nitrogen Sources and Growth Factors Supplementation:*

Saccharomyces, malt extract, peptone, or (NH4)2SO4 are examples of exogenous nitrogen sources boosted ethanol production in S. cerevisiae under natural growth conditions. Sugar intake can be improved by supplements, which may explain why enhanced substrates yield more ethanol. The availability of essential cofactors such as biotin and riboflavin was one explanation for increased ethanol generation with yeast extract supplementation **[14]**.

*3.6.5 Size of the Inoculum:*

Lowering the size of the inoculum decreases the production costs in the production in terms of ethanol a s example, 5% (v/v) as well as 12 hr. old inoculant sizes yielded approximately the same results as 10% employing Saccharomyces cerevisiae Y5 by non-detoxified steam-exploded maize-Stover enzymatic hydro lysate enhanced with CSL.**[15]**. When the ethanol yield of baker yeast reduced when the yeast concentration was raised to in coffee- husk-based substrates, 3 - 4 or 5 g/L [25]. Therefore, rather than using a source of nitrogen, 10% (v/v) S. cerevisiae TISTR 5596 was used to create the most ethanol using cassava starch waste **[16]**.

## 4. CONCLUSION

The world's energy consumption is rising in sequential with the world's population. Biofuels appear to be a cost-effective and long-term energy source. However, biofuel commercialization remains in its infancy. Some of the hurdles for commercialization are the initial investment cost, the paucity of arable land, as well as the seasonality nature of crops. For a long time, ethanol has been made from molasses and starch; nevertheless, ethanol production through starch leads food sources in terms of area and price. To address these issues, ligo-cellulosic agricultural leftovers might be utilized to produce ethanol. Co-culturing S. cerevisiae with some other bacteria boosts its output in a variety of ways.In this study, Saccharomyces cerevisiae is thought to be the greatest alternative for improving bioethanol production techniques. Biomass was a good resource for bioethanol synthesis. The investigation concentrated mostly on three types of biomass wastes. Starches and lignocellulose biomass were used to create bioethanol. Currently, the study is being conducted to enhance bioethanol manufacturing technology. Biomass was a good resource

for bioethanol synthesis. The investigation centered mostly on the utilization of biomass wastes. The immobilizing the yeasts improves efficiency and ethanol generation. This study focuses on yeast strains, fermentation processes, and variables that impact bioethanol production, particularly yeast immobilization for improved bioethanol production. Immobilizing yeast cells improves efficiency and ethanol generation.

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# CHAPTER 3

# THREE-DIMENSIONAL BIO PRINTING FOR TISSUE ENGINEERING PROGRAMS

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***ABSTRACT:Three dimensional (3D) bioprinting technology in conjunction using tissue engineering principles, has already been designed to allow the fabricationgenerating of new cells that matches their anatomic, morphological, and functional requirements, as well as functional characteristics of original various organs.A diverse spectrum of biomaterials has been stimulated its important impact in 3D printing (3DP),that serve as synthetic frames in the form of scaffolding, structures, as well as matrix.This is the main difficulty, or even the most difficult step, is to take the sector to new heights so that it is accessible for people. Present 3D bioprinting efforts are centered on the creation of bio-inks which offer not only physical stability but also provides offers stem - cell microenvironmental stimulation. This paper provides an overview of 3D bio-printing advancements in tissue regeneration.Components, as well as procedures in bioprinting, are also discussed, as well as potential clinical uses, problems, or field prospects. This research focuses on different three-dimensional printing techniques using biomaterials for tissue engineering (TE) applications also this study examines the various types of three-dimensional printing (3DP) developments in terms of their prospective uses, as well as a future.***

***KEYWORDS: Biomaterials, Cells, Extracellular matrix (ECM), Scaffolds, Tissue-Engineering (TE), Three-dimensional Printing (3-D).***

## 1. INTRODUCTION

Tissues were living structures made up of many types of cells, and Extracellular Matrix or a wide range of signaling chemicals.The Extra - cellular matrix is an important element of the cellular structure micro-environment, producing a complicated 3-dimensional structure.ECM is a three-dimensional network composed mostly of collagen and elastin fibers, as well as proteoglycans, multi-adhesive -proteins (such as laminin, fibronectin), and glycosaminoglycan's, with varying architectural shapes and components in different tissues (e.g., hyaluronan) [1]. 3-D printing (additive- manufacturing) is a helpful technology for fabricating biomimetic scaffolding of desired characteristics and as well spatial chemistry and architecture by layering materials to produce the final shape. 3-D printing entails creating a model with 3D software, importing the model into slicing software, or printing the model with a three-dimensional printer [2].

3D Bio-printing is a cutting-edge technology used in tissue engineering and cell transplantation to create complex tissues architectures that closely resemble natural organs or tissues.Tissue-engineered is one of several relatively young human endeavors. It blends biological, medical, material engineering, or mechanical aspects. Tissue engineering's main goal is to find ways for regenerating injured organs and tissues, particularly those that had previously been thought to just be non-regenerative. Conventional medical practice provides an example of these kinds of tissue and organ damage. These would be frequently serious bone, skin, or nerve tissue abnormalities [3].Various forms of trauma are the most prevalent source of such abnormalities, followed by those coming immediately from tumor activity, and also those ensuing from tumor excision locations. Traditionally,this partially healed tissue (cell growth) is grown in-vitro (within a bioreactor) before becoming transplanted in - situ somewhere at injury site. Tissue-Engineering scaffolds, which seem to be adsorbents and

provide a suitable environment for cultivated organisms while also allowing an open supply of nutrients or drainage of cell metabolic products, are utilized to guarantee an equal distribution of cells within the defective space.Another significant role of tissue scaffolds would be to replace the injured tissue's biomechanical properties (organ). As a result, they should have a rigidity that is adequate[4].

A healthy macro-tissue necessitates a precise micro-architecture that offers structural components support, enough nutrient supply, types of cells necessary, and also the ability to proactively rearrange whenever transplant. 3D printing makes the use biomaterials, printing methods, as well as cell transportation systems an effective way to construct all of these essential components shown in Figure 1. Earlier solutions included printing complicated scaffolds and then seeding cells, but contemporary efforts attempt to reduce processes and provide structure and cells concurrently via scaffold-based or scaffold-less designs.

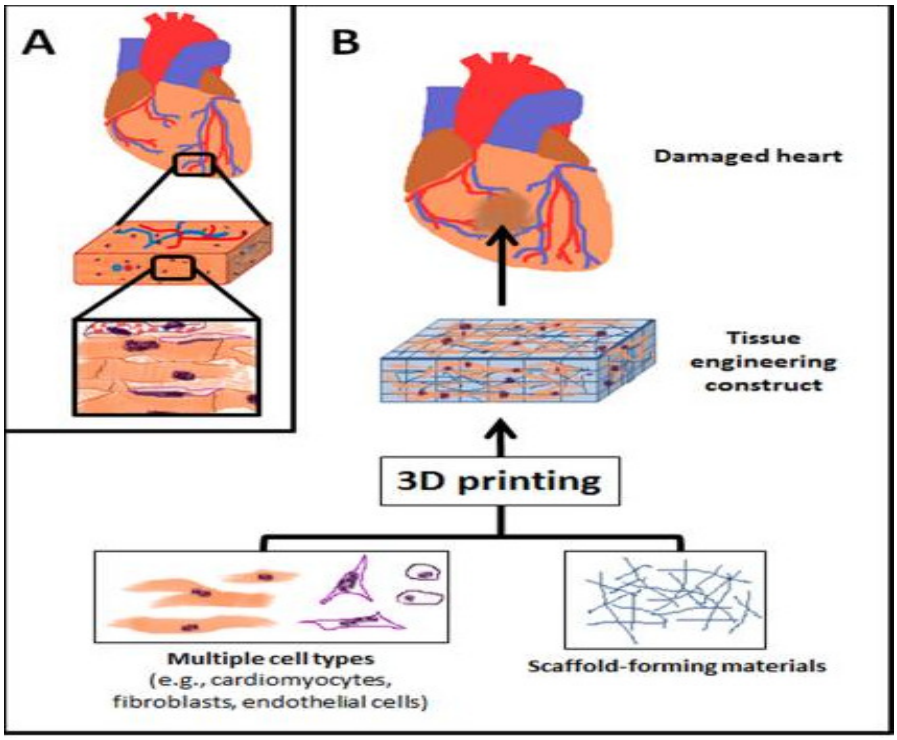


**Figure 1:The Schematic of Tissue Engineering Technologies, including Heart Tissue Engineering, are 3D printed. (A)Tissue samples are made up of various cell types which are arranged in a hierarchy structure. (B) Functional Tissue may be created via 3D printing from cells as well as scaffolding material** [5]**.**

The a development framework wherein the cells can migrate as well as proliferate to form a functional organ would have been the perfect 3D printed construct for tissue engineering. Though genetics may influence cell destiny, research throughout this sector had proven to be time-consuming as well as difficult.Epigenetics has proven that covalent or noncovalent alterations (– for example, DNA methylation) towards the DNA or The arrangement of histone proteins in chromatin serves as a connection between inherited genes also the environment as well as the phenotype.Though 3D printed tissue-engineered constructions have been built with the core features of biodegradability, biocompatibility, or fast prototyping in mind, tissue integration requires more consideration [6].

Fabrication of TE scaffolds can be done in a variety of ways. Among these are a few traditional technologies, likeSolvent -casting, phase -separation, as well as electrospinning

are three techniques that provide for minimal command over scaffolding construction. They are also characterized by a lack of consistency. These limits listed herewould not applicable to Additive- Manufacturing procedures, which are commonly referred to as 3D printing. The limitations and fundamental concepts among the most commonly employed additive manufacturing processes, notably extrusion-based and jetting techniques, are summarized in this paper. As a result, current printing processes, as well as printable materials, would be explored. In addition, the study discusses sophisticated scaffold construction techniques for tissue-engineered.

## 2. LITERATURE REVIEW

Udayabhanu Jammalamadaka et.al, stated in this study that Three-dimensional printing offers a lot of possibilities as a method of construction for scaffolds for tissue engineering. This diversity of biomaterials that may have been employed in 3d bioprinting limits its uses within the areatissue engineering as well as regenerative medicine. There were many various benefits to creating scaffolding via 3D printing, along with the capacity to generate complicated geometries, high porosity,and co-culture of various cells with growth factors.This research examines recent advanced biomaterials for diverse tissues. 3D printing using biomaterials include ceramics, composites also polymers. Generally ceramic were combined using polymers to increase printability because to the nature of screen-printing processes.Polymer-based bio-materials, that are usually three - dimensional printed via extrusion, offer a wider range of applications in tissue regeneration. This objective is regeneration of tissue is to create functioning as well as viable organs, which necessitates the investigation of several biomaterials as well as manufacturing techniques [7].

Gia Saini et.al, conducted this study on regenerative medicine is a new area that focuses just on the restoration or regeneration of damaged tissue's functioning components. Tissue engineering is a regenerative medicine technique that aims to build fully functioning tissue components or organs. Biomaterials, as well as live cells, could be used to generate natural tissue mimics employing 3D printing technology. To improve upon standard tissue engineering approaches, regenerative medicine had recently started to use 3D bio-printing technologies to build highly specific tissue models. They also described the technique of 3D bio-printing as well as its benefits over the other tissue engineering technologies in this article, which reviews the usage of 3D bio-printing within the progress of tissue engineering. Bio-printing materials and procedures are also discussed, as well as prospective clinical uses, problems, and field directions [8].

Dilara Goksu Tamay et.al, illustrated in this study, Three-dimensional (3D) as well as 4 (4D) printing having developed as another generation of construction techniques in domains such as architecture, chemistry, biological, computer programming, as well as materials-science.Active materials that alter shape or color in response to an external stimulus, generate an electrical flow, becoming active, or perform a specific function open the path for the development of dynamic three - dimensional structures, commonly known as 4D printing. This study conducted a complete review of 3D or 4D bio-printing techniques as well as the benefits of the use in tissue- regeneration
on one another scaffold producing advances[9].

Bon Kang Gu et.al, conducted this study on the 3-D printing techniques, also called Additive Manufacturing or rapid prototyping that have recently become very popular for their usage in art, architecture modelling, ultralight technology, and sometimes even tissue engineering Among such options, tissue - engineered via 3d printers have piqued the curiosity of a

number of researchers..Because of its quick fabrication, high- accuracy, as well as customizable output, 3D bio-printing proposeda benefit in the design of a scaffolding for tissue regeneration. Authors also stated in this study that they introduce the applications as well as the present conditions of the three-dimensional printing methods. Mainly concentrating on some of the current applications for biomedical as well as tissue engineering sectors employing printable 3D scaffolding [10].

## 3. DISCUSSION

There are several techniques for tissue engineering scaffolds fabrication. Among these are a few traditional processes,solvent- casting, phase- separation, and electrospinning, for example, give limited power and control over scaffolding form.Furthermore, 3D printing technologies facilitate the useapproaches for computer-assisted TE scaffolding development. This is currently an excess of 3D printing processes being used for TE applications. There have been recorded efforts to modify commercial printers such that theymight be used to make scaffolding for tissue - engineered. Currently, 3D printing technology allows for the production of TE structures utilized for the regeneration of several tissue types, including cartilage [6], skin [7], complete organs [8], as well as vascular networks [9].

### *3.1 Tissue Engineering Scaffolds:*

Tissue engineering could provide manynovel scaffold fabrication technologies, wherein the tissue compatible problemmight be readily remedied .The concept, as well as the goal, is to create a functionally adequate organ utilizing the individual's cells.Furthermore, because there are numerous elements connected to the physiology of the organism, like growing different cell types, a procedure could be a highly difficult undertaking.TE scaffolds serve as a substrate for cell migration/differentiation and the regeneration of new tissue. Therefore, materials qualities, particularly physical and chemical properties, and also design or morphogenesis, are critical for cell proliferation and viability. Furthermore, efficient treatment of abnormalities frequently necessitates regeneration ofcombination of tissues such as, muscles, arteries, ligaments, nerves, cartilage, bones, and glands.Among the most crucial components of printing technologyscaffolds is also the manufacturing process as well as the biomaterial used. Biomaterials connect within biological systems and thus are categorized according to numerous characteristics including biodegradability, physiochemicalconstitution, or so the use of particular changes. Polymers (both natural as well as synthetic) composites, or ceramics, are the primary categories of these materials. Regarding orthodontics applications, ceramics scaffolding was preferred over scaffolds for oral tissue engineering, as well as polymers for soft tissue engineering.

#### *3.1.1 3-D Printing Approaches vs. Conventional Tissue –Engineering Scaffold-Fabrication Methods:*

Several scaffold creation processes allow them to fulfill the needs of the various specialized applications.Furthermore, numerous biomaterials are continually being developed to be successful in tissue engineering.Electro-spinning, phase separation, additive –manufacturing, foaming, solution casting, self-assembly also extrusion are the most common scaffold construction processes.To mitigate several of the methodologies' drawbacks, they are frequently combined, which can produce some extremely fascinating or promising results.Figure 2 depicts numerous methods for fabricating three-dimensional scaffolds, with a few of them explained in further detail.

An electrospinning technique is among the most used scaffolding methods.The spinneret is linked to a high electrostatic field (many to hundreds of kV) under low current and filled with

electro-conductive polymer, which is generally a solution. Mostly on the journey between such a spinneret as well as a collector, the polymer is spinning into fibers even as the solvent evaporates.The collector is a fixed or spinning object that is electronically neutralized or has a low counter potential.

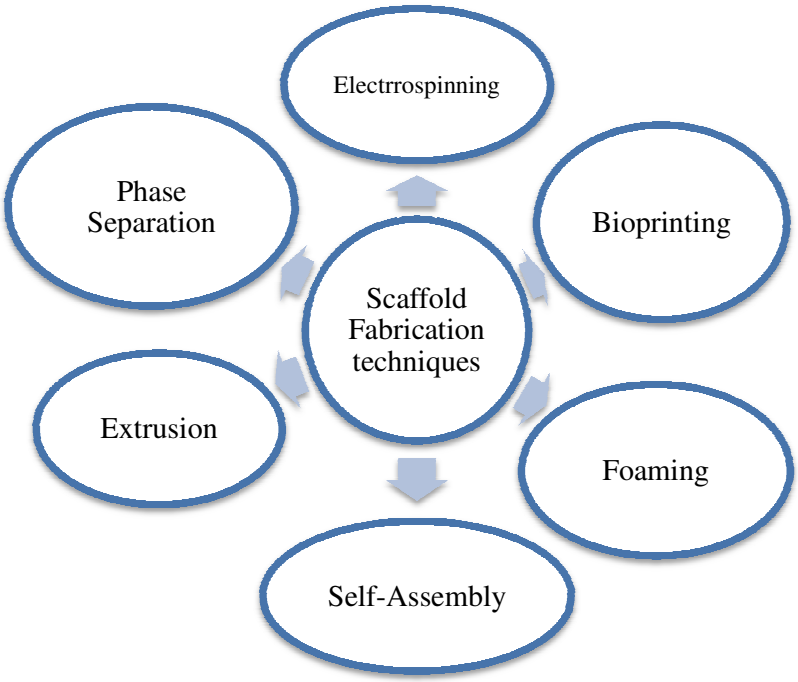


**Figure 2: It shows the representation of Techniques for making scaffolds** [3]**.**

This resulting scaffold features a submicron, nano-fibrous architecture also micron that isdepending on the collecting type or mode, the accumulation might be either random as well as regular. This kind of scaffolding manufacturing enables the production of elastic materials utilizing a form and structure which perfectly approximate the porous structure of an extracellular matrix, that would be essential for cell survival.As a result of the electromagnetic force between both the two liquids, a cone-shaped solute pours outas well as the collector. Whenever the electric field surpasses the surface energy of polymer liquid, the Taylor cone emits a polymer jet, causing multiple instabilities, including bending Jet instability caused by the repulsion of electrostatic force on the jet's surface the shape and design of the scaffolding might be drastically altered depending on the intended use besides modifying a few the spinneret-collector distance, collector rotational speed, solution flow rate, polymer concentration, voltage , weight, humidity, temperature also polymer- molecular are all electrospinning process, material, or exterior condition characteristics.

Another common approach for producing complicated or high-porosity 3-D scaffolds is called phase separation. There are many variations of this technology that are all depends on 2 processes: liquid-solid phase separation as well as liquid-liquid phase separation.Thermally or non-solvent-induced techniques are used to apply them technically. Separation is achieved in the first scenario by lowering a shift in solution temperature promotes polymer precipitate, resulting in polymeric soluble [11].This second instance involves submerging a liquid within a non-solvent (for the polymer) solutions in an attempt to draining off the polymer-solvent (wet phase -inversion -method).Also, there are ways for generating porosities that use phase

extraction processes which resulting in highly compressed gas, such as supercritical. Also, because phase-separated scaffolds' morphology is extremely susceptible to processing variables, required three-dimensional scaffold characteristics could be attained by modifying different methods or material parameters

The solvent casting/particulate leaching process involves combining an inorganic solution polymeric, adding porogen granule as well as spherical, subsequently cast the mixture into the mould, following by porosity frameworks separation. Porogen size, content, or distribution can all be used to influence ultimate pore size. When the solvent evaporates, as well as the porogen dissolves, producing a porous structure behind it. Due to the obvious homogeneous dispersion of endothelial cells, solvent scaffolds could be employed in cardiac tissue- engineered applications.These approaches allows for the development of structures having uniform porosity but it's a very restricted thickness [12].Table 1 provides an overview of these strategies.

**Table 1: Approaches for forming selected scaffold's main uses as well as disadvantages/advantages** [3]**.**

| Procedures | Implementations | Advantage | Dis-advantages | Reference |
|---|---|---|---|---|
| Electro-spinning | TE or bone, skin, cardiac, nerve | High-surface ratio, pore-volume, and simple processing | Polymer selection is limited. | [13] |
| Phase - Separation | drug release, or Protein delivery applications | Bio-active substances may include in this framework, and the construction has a high-porosity. | Pore size limitations, as well as residual solvent issues | [14] |
| Solvent Casting | Applications of Vascular Tissue-Engineering | Adjustable porosity, simple approach | Weak mechanical strength, thinness, or pore size | [15] |

*3.2 Examples of Advanced 3D-Printed TE Frameworks:*

The newest achievements in three-dimensional print of tissue –engineering scaffolding are described in this chapter, with an emphasis on the novel possibilities for complex tissue recapitulation architectures provided by contemporary 3-D printing methods.

*3.2.1 Nerve- Tissue:*

The peripheral –nervous- system also central- nervous- system have proven to be the most challenging to mend tissue. This 3-D printing brain model in vitro is developed by creating micro-channels utilizing collagen or utilizing needles as well as a 3-D printed structure.Brain cells of Mouse are cultivated in collagen micro-channels, resulting in brain microvasculature regrowth. These experiments demonstrated also that the brain-blood barrier model may be utilized in both physiological and pathological investigations, as well as a range of applications including medication administration, tissue repair, or tissue engineering [16].

### *3.2.2 Ocular -Tissues:*

Although curiosity in the use of 3D printing techniques in ophthalmology is increasing, the bulk of 3D printing does not integrate genetic engineering approaches. There were a few illustrations of efforts that use 3D printing to regenerate ocular tissue.This retina-like structure was 3D printed and contained mature rat retinal ganglion cells as well as glia. It has been demonstrated that those same different kinds of retinal cells could be effectively transplanted produced deprived of losing vitality nor specific phenotypic characteristics. Some other examples of three – dimensional printing within The study on the development of a tissue- engineering retinal framework composed of collagen-based bio-ink that incorporates encapsulating corneal keratocytes would have been considered ocular tissue engineering [17].

### *3.2.3 Ear:*

This bionic human ear was created using computer-aided design. For printing, a hydrogel matrix comprising cellsthe form of a human ear was created using a conducting polymer that incorporates nano particle.Controlling these signals from cochlea-shaped electrodes was possible as a result of the research.This analysis revealed that 3-D bio-printing utilizing the patient's lipid tissue or auricular cartilage may make the manufactured ear. Adipocytes or chondrocytes were differentiated by adipose-derived stromal cells or implanted there in lipid as well as cartilage tissue [18].

### *3.2.4 Kidney:*

PEGDA (Poly (ethylene glycol) acrylate scaffolds with sodium alginate or calcium sulfate additions were evaluated.It was demonstrated that the indicated composite materials have qualities that promote cell growth and vitality. Human kidney organoids were created using extrusion-based three-dimensional bio-printing (an organ is a reduced formin vitro creation of a live organ). This manufacturing approach employed permits exact adjustment of the organoid, cell quantity, conformation, and size. This kidney organoids in -vitro model produced might be utilized for drug screening and disease simulation [19].

### *3.2.5 Skin:*

A three-dimensional-printed skin was created using a laser-assisted process. Matriderm (for matrix stability) or collagen type I was combined and incubated by using fibroblasts as well as keratinocytes.The process of bio-fabrication producing skin equivalents (SE) bio-printed utilizing an open-market bio-printer or composed of fibroblasts as well as keratinocytes floating in a gelatin-based hydrogel were addressed. Skin - Equivalents build layers were used immediatelyover-extruded onto such a multi-well disk. This evolved structure is made up of three layers: dermis, laminin/enacting bottom layer, or epidermis. This generated SE might be used to model skin diseases in vitro [20].

### *3.2.6 Cancer Models:*

Rapid innovations in bio-printing allow the production ofnumerous three-dimensional in vitro models of many forms of cancerous tissue. These structures allow for these development of specific-pateint’s medicines and the study of carcinogenesis-related mechanisms including tumor extravasation.3-D bio-printing cancer models often are made up of numerous layered comprising various cell types such as tumour cells (mainly obtained from patients), growth factors, vasculature also ECM. 3d bio-printing tumor models may accurately represent tumor heterogeneity. These allow for the screening of anti-cancer therapies and also the examination of cell or cell orconnections between cells or cell-matrix. 3d bio-printing cancerous prototypes provide significant benefits beyond two-dimensional invitro structures that can replicate Cancer' structural complexity [21].

### *3.2.7 Cartilage TE or Bone:*

One of the most popular regeneration treatments is the restoration of bone and cartilage abnormalities. The primary goal of cartilage and bone tissue engineering would be to replace a broken bone. As a result, three-dimensional printing techniques attempt to produce an artificial bone structure that needed features including acceptable mechanical properties, form, as well as size. Trauma, congenital malformations, and cancer-related tissue excision are the most frequent cause of cartilage and bone deformities. Autogenous transplantation, for example, is distinguished because of many drawbacks, like insufficient donor site morbidity as well as donor tissue availability [22].

## 4. CONCLUSION

This 3D bio-printing technique had proven itself with a promising invention in the field of tissue repair, and it does have potential uses outside of tissue formation. It entails creating three-dimensional functional tissue that aids in tissue regeneration, repairing, as well as regeneration as well asa human bodily organ.Cells or biomolecules are mixed with scaffolds to accomplish this. Tissue- engineered 3-dimensional nature enables tumor architecture to be investigated in a more precise context. Tissue- engineering also often allows for the testing of prospective novel medications for all these disorders.Different techniques for manufacturing scaffolds for use in tissue regeneration applications were making tremendous progress. One of the most significant aims in the creation of 3D-printed scaffolds is to properly emulate the intricacy of a true living tissue. This structure would have adequate mechanical qualities,distribution of pore-size, also known as pore arrangement (allow to migrate the cells as well as diffusion).Although various tissues have been successfully cultivated as a working prototype, the construction of a completely functional and complicated human-sized organ remains a work in progress.Currently, the majority of TE product implantation attempts are carried out in collaboration with hospitals and research organizations. Typically, such collaboration was improvised and won't extend further than the scope of the research endeavor.

The absence of an ancient constitutional plan or verification methods for tissue-engineered goods is just a serious challenge for researchers, surgeons, or engineers. Current legal constraints, this is well known, restrict thescalingmoving from laboratories to a bigger scale. From the standpoint of present standards, evidencing of patient-specific TE devices also is troublesome.However, the real challenge as well as the most difficult step includes taking the industry to new heights it is available to patients, given a chance to enhance the equality of their lives. Recently, the patient's cells were used in the 3D-printed-assisted growth of TE constructions. Upcoming activities mustinvolve material testing for medical record 3D printing processes, as well as the development of novel printers capable of providing exceptional precision of TE scaffolds., developing strong and united scaffold standards,enhancing market oversight to optimize transplant for medical use, as well as developing a 3D printing platform to increase communication across several hospitals, businesses, also research institutes. Such developments could aid within the evolution of 3D-printed tissue- engineered technologies.

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# CHAPTER 4

# PROCESSING'S EFFECT ON THE CHEMISTRY ANDFUNCTIONING OF DIETARY PROTEINS

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***ABSTRACT:*** ***Proteins are a common class of macromolecules that are used as ingredients in the food business to impart nutritional, functional, or sensory qualities to food compositions. Proteins' capacity to behave in different capacities is based on their distinct physicochemical qualities, which are in turn reliant on protein structure at various organizational levels (i.e., primary, secondary, tertiary, and quaternary). This study addresses the chemistry and molecular foundation of protein complexes as well as functional interrelationships, as well as how they are influenced by various food processes.This study explores and shows how traditional approaches, such as those used in chemistry and biochemistry, have developed to intertwine with new functional genomic methods of creating progress in our knowledge of food problems through an exhaustive review of the abundant literature dedicated to the applications of omics sciences in food analysis. This study concluded that present supply volumes are sufficient to support the nutritional needs of a growing global population, it is anticipated that this could change in the future as a consequence of a combination of driving or opposing factors, as well as a tendency of policymakers to enforce austerity meals on individuals.***

***KEYWORDS:*** ***Food- Science, Food-Additive, Flavoring Agents, Monosodium Glutamate (MSG), Macro-nutrients, Vitamins***.

## 1. INTRODUCTION

Most of the food products consumed, other than the water content, are made up of macronutrients, substances that require energy for humans in large amounts. The food is available plenty much of which is available in processed form,although its use of added chemicals is widespread. All of those concerns have come under the domain of food science, which is defined as science related to the physical, chemical, as well as biological properties of food concerning durability, affordability, quality, preparation, safety, nutritional value, hygiene standards, and benefits.Food science is a multidisciplinary field. It involves primarily the areas of bacteriology, engineering chemistry, also biology.An important section of food science, food chemistry, is concerned with the composition and characteristics of food, as well as the alterations in chemical properties which occur during storage, management, as well as preparation. Molecular biology, Chemistry, biochemistry, physiological chemistry, botany, also zoology are all intertwined disciplines.

Food consumed by humans contains hundreds, sometimes thousands of different molecules. However, the majority of nutrients present in the food are in the form of huge macromolecules that need to be converted to much simpler and smaller forms so that they are absorbed into the blood. For example, sucrose, commonly known as table sugar, cannot be absorbed into the bloodstream before being disintegrated into glucose and fructose, enzymatically. The branch of food chemistry comprises the study of various changes when a food product is subjected to any kind of processing and evaluating the ways for intensifying the desirable ones, simultaneously preventing the undesirable ones. For example, maintaining proper storage conditions and the addition of lactose-to-lactic acid converting microorganism’s conversion during fermentation of milk to produce curd helps in intensifying the sensory attributes of the final product. Alternatively, the prevention ofusing lemon juice or other acidulated water to avoid browning on the surface of newly cut red apples is evidence of preventing an unwanted alteration.

Food Chemistry and biochemistry are interrelated, as the latter alsoit is concerned with food components such as carbohydrates, lipids, proteins, water, vitamins, as well as mineral elements.Furthermore,it comprises the discovery and development of additives that may be used to safeguard food quality or to change the color, taste, or scent of food. Food chemistry is concerned with the changes that take place in food throughout its manufacturing, processing, storage, and cooking, as well as the composition of raw materials and completed goods or end-products.Food's extremely complicated structure creates a variety of positive and unfavorable reactions that are determined by several parameters. The aspects of food chemistry have been known since the 18th and 19th centuries, during which many scientists have discovered various chemicals present in food. Based on the gathered information, in 1813, "Elements of Agricultural Chemistry," written by Sir Humphry Davy, was the first book dedicated to food science [1],in a series of talks for the Board of Agriculture that went on to form the global basis of this profession.About 60 years later, this Society of Public Analysts was founded, to bring the notion of food science to the general public by using analytical procedures to comprehend various foods, beginning with bread, wine, as well as milk. It also handled complaints received, the most prevalent of which was food poisoning caused by adulteration or improper processing.

Famous discoveries were made by several scientists during the phase of 1780-1850, many of which were related eitherdirectly as well as indirectly related to food chemistry. Carl Wilhelm Scheele (1742–1786) [2], a Swedish- physician, will go down in history as one of the world's finest chemists. Besides his well-known discoveries of chlorine, glycerol, or fruits for the existence of citric, malic, and tartaric acids (17 Malic acids from apples (1785) and also tested twenty commons) (1785). Antonie Laurent Lavoisier (1743–1794), a French scientist, proved instrumental in disproving the phlogiston concept and laying the groundwork for organic medicine[2].

Theodore de Saussure, a French scientist, contributed much to defining and explaining Lavoisier's concepts of food and agriculture chemistry. Sir Humphrey Davy, an English chemist (1778-1829),In 1807 and 1808, the elements K, Na, Ba, Ca, or Mg were separated. The writings of Swedish chemist Jons Jacob Berzelius (1779-1848) or Scottish chemist Thomas Thomson [6]. (1773-1852) [3],developed in the early stages of organic formulations. It is worth noting that the latest trend coincided with the onset of major or widespread food adulteration.In the early 1800s, there was a boom in public concern over food supply quality.Therefore, between 1820 to 1850, chemistry or food chemistry gained prominence, particularly in Europe. Some vital nutritional elements were found during the first part of the twentieth century, while compounds that help in the development, production, and marketing of foods were discovered in the nineteenth century. In comparison to what existed in the 1800s, the present food supply appears to be nearly ideal.

### *1.1: Introduction to Food Chemistry:*

This science of biochemical activities and interaction among biological and non-biological food components is known as food chemistry.This is focused on food ingredients like carbohydrates, lipids, proteins, water, minerals as well as vitamins.Since prehistoric times, humans have been altering the status of food to lengthen its longevity or improve its flavour. Humans used fire to build tools to prepare or preserve meat as early as 300,000 years ago and later realized that salt could be used to prevent spoilage without cooking. The wine was widely mixed with honey, herbs, spices, and even seawater, chalk, and lead as a sweetener and preservative in ancient Rome and Greece. [4].

Food Chemistry in terms of food safety is critical to emphasize because safety is the primary requirement of any food, which implies that a percentage of food must be free. This is any

dangerous chemical or microbiological contamination that is present at the moment of intake. This concept takes on a more operational version for practical purposes. In the canning business, for example, "commercial" sterility is attained whenever the food product has indeed been proven to be free of live Clostridium botulinum spores [5].A customized combination ofthis may be accomplished by adjusting the heating conditions for a certain product in specific packaging. Specific time-temperature combinations that aid in the retention of quality features can be determined by examining the heating needs. Therefore, with a product like peanut butter, operational safety is essentially defined by the absence of aflatoxins carcinogenic substances produced by certain species of moulds [6].

*1.2: Bio-molecules of Food:*

To regulate various life processes efficiently, all living things need to obtain fuel in the form of food, whether they produce on their own through the process of photosynthesis (plants), or by ingesting food and breaking it into smaller components (animals/humans). The food consumed by humans contains some kind of components that are certain organic substances or minerals that are referred to as nutrients. The primary purpose of nutrition is to supply energy to the body that is needed for the growth, repair, and maintenance of damaged tissues. Hence it is important to understand the exact role of each nutrientwithin food or identify the numerous dietary providers for these nutrients. Not all foods contain the same proportion of these nutrients. The biomolecules present in the food, which are responsible for various life processes are:

*1.2.1: Carbohydrates:*

Carbohydrates are a wonderful source of energy, but they are also helpful for energy storage and delivery, as well as structural support in some circumstances. Carbohydrates' primary function is to provide energy to the organism, namely the brain or neurologic system. Amylase is an enzyme that catalyzes the breakdown of carbs producing glucose (blood-sugar), which also provides energy to the body. Carbohydrates have a variety of tasks in living creatures, including energy, transportation, and structural components in plants and arthropods (prawns, fish, silkworms, etc). Carbohydrates are molecular molecules composed of three elements: carbon, hydrogen, and oxygen. Figure 1, depicts the simple carbohydrates, which comprise single sugars (monosaccharides) and polymers, oligosaccharides, and polysaccharides.

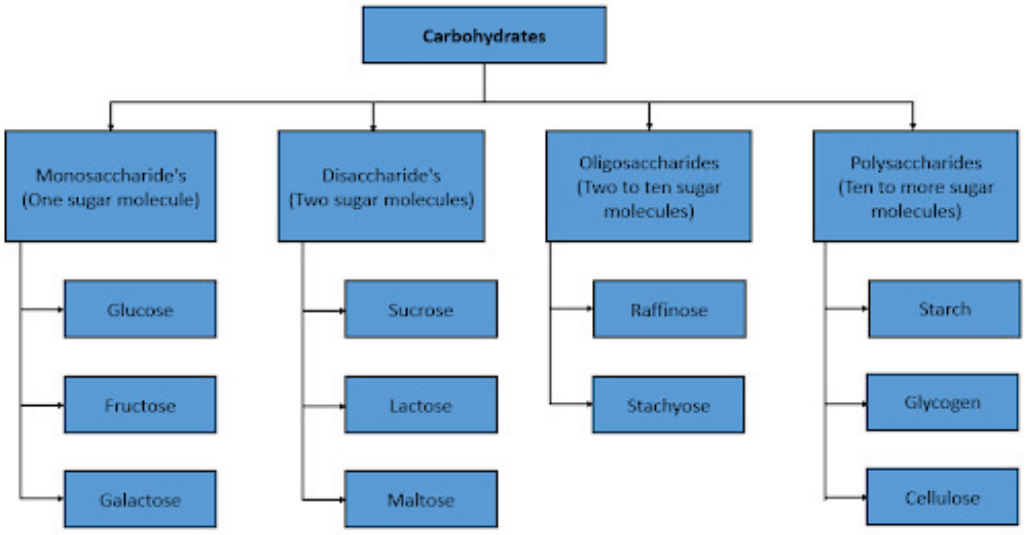


**Figure 1: Displays the Carbohydrate Classification (Types of Carbohydrates)**

- *The function of Carbohydrates within the Human -Body*:

Carbohydrates' principal function is to provide energy to the brain as well as the body. A sufficient intake of Function of Carbohydrates in the Human Body (carbohydrates also spare proteins aids in fat metabolism This simple carbohydrate sugar is used for energy by all bodily cells, however, the brain requires glucose in particular. As a result, one key function of carbs is to provide energy to the brain. Carbohydrates play a major role in promoting health and ensuring overall fitness.

*Deficiency Disease: Ketosis:*

A carbohydrate deficit indicates that perhaps the body would be unable to produce adequate energy. This might lead to exhaustion and weakness.Some cells, like brain cells, require glucose as fuel. Long-term carbohydrates deprivation increases the production of complex compounds referred to as ketone bodies (a state known as ketosis), which gives the breath a distinctly pleasant odour. Ketosis as well as other harmful effects of a low-carbohydrate diet might be prevented by taking 50-100 grams of carbs every day.

When there are insufficient carbs to create energy from glucose, the body begins to burn fat for energy alternatively. Ketones are acids found within the blood which are produced when fat is burned for energy. The buildup of acidic ketones leads the body to lose minerals necessary for regular health activities such as fluid balance, neurological dysfunction, or muscle contraction over time.In diabetics, dangerously high amounts of ketones in the circulation raise the risk of electrolyte imbalance, dehydration, tiredness, and digestive disruption. This deficit could result in ketoacidosis, a potentially fatal illness.

Ketoacidosis is not the same as the typical benign nutritional ketosis associated with ketogenic diets and fasting conditions in the body. Benign dietary ketosis would be a controlled, insulin-regulated process that causes the release of fatty acids as well as the creation of ketone bodies.

Ketoacidosis is a condition in which excessive levels of ketones are produced in an unregulated metabolic process as a result of a low carbohydrate diet and increased fat consumption. To enter ketoacidosis, the body must produce inadequate insulin to regulate the passage of fatty acids and the creation of ketones.

*Recommended Daily Allowance (RDA) and Sources:*

The Recommended Daily Allowance of carbohydrates is 130 grams for adult men and women. Most of the carbohydrate in our diet is provided by sugars. Consuming too many added sugars can harm health. Soft drinks, pastries, candies, and other sweets are high in added sugar. Common added sugars include fructose, dextrose, corn- syrup, brown- sugar, or white- sugar. The major sources of carbohydrates are cereals, pulses, and potatoes. Fruits and vegetables also contain carbohydrates and even milk.

### *1.2.2. Fats or Lipids:*

Fat is an essential component for the optimum physiological role. Fats, which have been composed of many chemicals are soluble in organic solvents but insoluble in water. Fats are chemically recognized as triesters of fatty acids and glycerol[7]. Fats are a kind of lipid found in the body that has the overall property of becoming hydrophobic (they are insoluble in water). The fats seen in Figure 2, are also known as triglycerides, which are molecules created by mixing one molecule of glycerol with fatty acid chains.

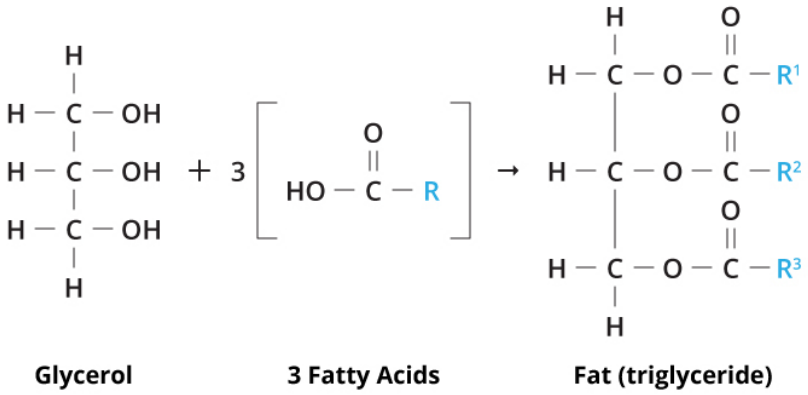


**Figure 2:In a fat molecule the R's within three fatty acids represent a long C-C-C chain. The R's in a triglyceride may or may not be the same.**

- *Function:*

Lipids and fats' primary function within the body is to provide energy. Proteins were protected from being used for energy by fats, beginning to focus on their more important duty of tissue formation or repair.Whenever fats are oxidized, they create about double the amount of energy that carbs do. Whereas carbohydrates are our bodies' primary source of energy, fat is employedwhen carbohydrates are scarce as a second energy source.

- *Deficiency Disease:*

Lipids, or fats, give energy and protect against crucial fatty acid shortages. Fat may also absorb fat-soluble vitamins A K D or E, preventing their deficiency in the body.Dryness, hair loss, scaly skin, loss of menstruation, cold sensitivity, increased susceptibility to infection, bruising, poor development, poor wound healing, and low body weight are all symptoms of a lack of fat in the human body. Because many vitamins and impact needs are fat-soluble, a lack of fat impacts the level and action of vitamins as well as the entire body.

- *Recommended Daily Allowance (RDA) and Sources:*

The Recommended Daily allowance of fats is 65 grams/day for adult men and women.

*1.2.3: Proteins:*

Proteins were massive structures composed of amino acids which are required for the cells in the human body to operate properly.It carries out a multitude of critical functions in the body. These would be necessaryfor the formation, functioning, or control of the internal tissues, and then do most of their activity in cells. Proteins consist of hundreds and thousands of smaller parts called amino acids which are connected within long sequences.

- *Deficiency Disease: Marasmus and Kwashiorkor:*

Young children or newborns are especially sensitive to the repercussions of protein deficiency. Marasmus is induced by a substantial nutritional deficit. Chronic diarrhea, dizziness, exhaustion, and fast weight loss are all signs of marasmus.Kwashiorkor is caused by a shortage of proteins from carbohydrate sources such as rice, yams, and bananas. It is a severe form of malnutrition that typically affects older children.A bloated stomach owing to fluid retention is one of the illness's symptoms. This also features marasmus-like symptoms like irritability, diarrhea, exhaustion, stunted growth, cognitive development, and mental health problems.

- *Recommended Daily Allowance (RDA) and Sources:*

Every day, a certain quantity of each essential amino acid is necessary. However, according to the RDA for total protein,women need 46 grams each day, while males need 56 grams. Complete proteins are meat-based meals that contain all of the essential amino acids in a single serving. Except for soy and quinoa, which are high in plant proteins, plant-based meals are incomplete proteins because they lack at least one necessary amino acid.Different forms of incomplete proteins fill in each other's lacking amino acids, resulting in,consuming a variety of grains, legumes, and veggies throughout the day nets complete. Although rice and pulses (dal) are both good sources of protein, it must be remembered that the protein in both is incomplete. While rice is deficient in the amino acid lysine, dal is deficient in the amino acid methionine. Therefore, to reap the benefits of the protein in rice or dal, rice and pulses are consumed in combination which would provide all the essential amino acids.

*1.2.4. Vitamins or Minerals:*

Minerals and vitamins are important nutrients also they serve hundreds of various functions within the body. Micronutrients are vitamins and minerals that are often consumed since the body requires just trace amounts of them. Nonetheless, failure to obtain even those little amounts almost ensures sickness [8].

*1.2.5. Dietary Fiber:*

Dietary fiber, commonly known as "roughage,"is a class of compounds found in plants that are incapable of being decomposed by human digestive enzymes.Waxes, lignin, including polysaccharides like cellulose or pectin are examples of all these. Certain fibers in the large intestine may be fermented by gut bacteria, producing gases or short-chain fatty acids (carbon dioxide, methane, also hydrogen,).These fatty acids enter the blood and supply a small amount of energy. This amount of gas generated is influenced by the type of fiber consumed as well as the gut microbes present. This may help prevent heart disease, insulin, obesity, and certain forms of cancer, as well as enhance digestive health. Although roughage is not absorbed by the human body, this is essential for other meals to be digested.

*1.2.6. Water:*

Water is the only material found in abundance in all 3 physical states on Earth (ice, water, and vapour).This is the most commonly available pure solid, and also the only common liquid. This is required for life as a reaction medium also reactant, plasticizer or lubricant, a likely facilitator of macromolecule dynamic behaviour, a stabilizer of biopolymer conformation, including catalytic (enzymatic) properties, waste products, as a solvent, a carrier of nutrients, or in other ways.

*1.2.7.'Recommended Daily Allowance:*

The nutrients listed above are ingested following their 'Recommended Daily Allowance (RDA).' RDAs were developed during WWII to explore nutritional concerns that may affect national defense. The RDA is the estimated quantity of a nutrient (or calories) per day recommended by the National Research Council/National Academy of Sciences Food and Nutrition Board for the preservation of good health [13]. The RDA is periodically updated to include fresh information.

## 2. LITERATURE REVIEW

Nivia M. M. Coelho et.al, stated in this study that,focus on foods, their manufacturing techniques, and quality assurance. In an increasingly crowded world,this research establishment has worked tirelessly to enhance analytical methods for effectively regulating

the safety and quality of human meals. This, which would be a major concern within industrialized nations, requires special attention in underdeveloped regions to expand food security to all stages of the food chain manufacturing as well as all eaten goods worldwide. As a result, the focus of this special issue is on food science and technology: food quality and novel analytical techniques.This special issue attempts to provide the most recent achievements in food science and technology as a result of previous improvements in quality food standards and novel research methodologies. This issue's topics have been carefully chosen to address recent research difficulties in the quality of food as well as a safety control, general f&b analysis, food forensics, biologically active components, or recent improvements in food sample preparation procedures including food chemical speciation [9].

Mrs. Munteanu Irina-Georgiana et.al, concluded in this study, that the scientific community is very interested in the research ofantioxidants as well as their uses in a wide range of fields, including food engineering pharmacy to medicine, are examples.This present study is a comprehensive review of one of the most essential assays for determining antioxidant potential, as well as the detection method, applicability, advantages, and downsides of such procedures.These tests were defined as the transfer of a hydrogen molecule and included the Hydroxyl Radical Antioxidant Capacity (HORAC), the Oxygen- Radical- Absorption - Capacity (ORAC), and Total Oxyradical Scavenging Capacity (TOSC), Total Peroxyl Radical Trapping Antioxidant Parameter (TRAP). The Ferric Reducing Antioxidant Power (FRACRAC), and the Folin–Ciocalteu tests all are based on the single-electron transfer also the Cupric Reducing Antioxidant Power (CUPRAC). The 2, 2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) test also the [2, 2-di (4-test-octyl phenyl)-1-picrylhydrazyl] (DPPH), are both mixed tests that include the exchange ofboth a hydrogen atom and an electron.These assays have been used effectively in antioxidant analysis or determining the antioxidant capacity of complicated substances. Approaches based on electrochemical (bio) sensors, which require accuracy assessment stages, could be employed as a complement in these kinds of research. The use of such chemical methods in combination with electrochemical techniques might lead to a better knowledge of the operational mechanisms or kinetics of processes that involve multiple antioxidants [10].

Payal Singh et.al, discussed in this study, Food omics, which includes diet and nutrition, omics tools, as well as bioinformatics, is meant to be a worldwide subject. It also involves the use of molecular technologies in the creation and monitoring of novel transgenic foods. Using capillary electrophoresis and mass spectrometry, these technologies have already been utilized to conduct a proteomics investigation on transgenic soy. The food omics movement opens up a plethora of possibilities, including the ability to design particularly customized food items that improve the health and very well of groups of individuals identified based on their unique genomes.Food omics still have several significant gaps and constraints to solve before they can exhibit their full analytical potential. Despite these facts, it is possible to infer that food omics represents the future of methodical technique in food products [11].

## 3. DISCUSSION

Food Science is concerned with the biological, physical, also chemical, aspects of foods concerning their durability, quality, production, safety, nutritional value, wholesomeness, accessibility, as well as cost. Food Science is a multidisciplinary field that includes bacteriology, engineering, chemistry, also biology. Food chemistry is a branch of food science that is concerned with the composition and quality of food, as well as the chemical changes that occur during handling, preparation, as well as preservation.

*3.1 Food- Additives:*

Additives are compounds that are added to food either directly or indirectly intentionally to intensify its sensory attributes (colour, flavour, odor, etc) or to enhance its storage life.Various food additives have multiple applications. The following example demonstrates or promotes the usage of additives to improve performance:

*Food's Nutritious Value*:

To boost the nutritional value of food, additives such as vitamins, minerals, amino acids, and derivatives are used. A specialized diet could also demand the use of thickeners, emulsifiers, sweeteners, or other additions.

*Sensory Value of Food:*

Pigment, smell, flavour, as well as firmness or texture, all of which contribute to food's sensory value, may deteriorate throughout preparation or preservation. Additives such as pigments, scent compounds, or taste enhancers can be used to compensate for or modify such declines. Antioxidants can prevent the development of off-flavors, such as those caused by fat or oil oxidation. The inclusion of minerals or polysaccharides, for example, can help to control food texture.

*Food Storage Life*:

The modern methods of food production have raised the need for products with a longer shelf life. Moreover, this world's food supply crisis necessitates conservation by preventing degradation to the greatest extent feasible.Protecting prevent microbial deterioration, such instance, by employing antimicrobial additives or active agents that inhibit and prevent unwanted physical and chemical changes in food, is one method of extending shelf life. This is accomplished by pH stability with buffering additions or texture stabilization using thickening and grilling agents that are resistant to biological polysaccharides.

*Value in Practice*:

The widespread trend for easy-to-prepare foods (convenient foods) may demand an increase in the usage of chemicals.

*3.2: Some common types of food additives used in the food industry are:*

- *Sweeteners:* Substitute the sweetness normally given through sugars in meals without considerably increasing the energy available.
- *Preservatives:* Food spoilage can be avoided if the deterioration of food by microbes is slowed or prevented.
- *Flavors enhancers:* Improve a food's existing flavor and/or odor.
- *Colourings:* Meals are given new or restored color. This icing mixture, for example, is colored to make it attractive on cakes.
- *Antioxidants:* Food oxidative degradation can be slowed or prevented. When fats and oils, for example, are exposed to air, rancid odors can develop. This is prevented by antioxidants.
- *Acidity regulators / Alkalis:* It aids in the maintenance of a steady acid level in the diet. This is important for flavor and also for determining how the other elements in

the dish operate. An acidified diet, for example, can inhibit the development of some microorganisms.

- *Anti-caking- agents:* Individual food particles are less likely to attach as a result, and flow properties are improved. Anti-caking seasoning, for example, flows freely and therefore does not clump together.

*3.3: Properties of the Food- Additives:* The food- an additive used in the food should have the following properties:

- It should not produce toxic elements in the food by reacting with the components of the food and the packing products.
- It should not alter thearoma and taste of the item to which it is introduced.
- It should not cause any type of physical, chemical, or biological changes in the food.
- It should not produce any kind of exothermic or endothermic reactions.

The above-listed food additives have been explained below briefly:

*3.3.1.* Preservatives*:*

The main rationale for employing preservation is to make meals safer by reducing the impact of biotic factors.The most serious concern to consumers would be that food would decay or become poisonous due to the presence of microorganisms (such as bacteria, yeast, mold) in it. Most of these species can emit toxins, which are harmful to human health and can also be lethal.

*3.3.2. Anti-oxidants:*

Antioxidants, which are found in some foods, may help to decrease a fewby removing free radicals, we can reduce the amount of harm they do. This included antioxidant substances such as vitamins A, E, or C, as well as minerals such as zinc and copper, which have stronger antioxidant properties than vitamins and nutrients. Non-nutrient antioxidant includes compounds found in tomatoes, including lycopene, and anthocyanin present in cranberries.Even though the usage of anti-oxidants stretches while flavourings have been used to store food for decades, contemporary antioxidant technologies are just around 60 years old. Even though free radicals were proven to be the cause of fat oxidation, thousands of organic and inorganic chemicals that have been implicated were investigated for their capacity to serve as scavenging free radicals or have various inhibitory activities.

The most active dietary antioxidants are phenolic and polyphenolic substances. This structural variety of phenolic antioxidants does have a direct influence as a result of changes in physical qualities, leading to variances in antioxidant properties BHT or BHA, are 2 examples of phenols with alkyl groups within the aromatic ring (hindered -phenols), which are incredibly potent antioxidants Butylated Hydroxy-anisole (BHA) is a white, sticky material that comes in the form of flakes or tablets. This is a fat-soluble mono-phenolic antioxidant that is commonly found in bulk oils or oil-in-water emulsions. It works well in animal fats and not so well in vegetable oils. Because of its great efficacy in blocking the oxidation of short-chain fatty acids, BHA is commonly used to keep palm oil and palm kernel oil fresh in cereals and desserts.

*3.3.3.Substances for Flavoring:*

Natural flavouring compounds are those thatare derived from plant or animal sources by physicochemical, microbial, or enzymatic methods. Humans might eat these natural flavourings in either their natural or processed form. These must not, however, include any naturally or artificially flavouring components.Monosodium glutamate (MSG), is a well-known, commonly used, and sometimes contentious taste enhancer MSG. This MSG, which is the sodium salt of the naturally occurring amino acid glutamic acid, is largely regarded as safe. MSG injections into young mice, on the other hand, have previously been shown to cause brain harm.However, whenever exposed to excessive doses of MSG in food, some people have symptoms akin to a heart attack. This subject is presently being discussed. Over the years, the FDA has received reports of people experiencing symptoms including headaches and nausea after eating MSG-containing foods. Consequently, the FDA has never been able to determine that the MSG was responsible for the alleged adverse effects.

*3.3.4. Coloring Agents:*

Colour is an important determinant of food selection.Colourants have an important function in boosting the visual attractiveness of food.A color enhancer, as prescribed by law, is any dye, pigment, or even other material that could give color to an amount of food intended for human consumption. Color enhancers are important ingredients of many goods because they make them more appealing, appealing, delicious, or instructive to customers.The entire annual color output in the globe is expected to be 8 million tons. Both have had a huge quantity of natural or synthetic dyes removedbecause of their mutagenic or carcinogenic properties, food colors are prohibited on national and international levels.The majority of mutagenic chemicals appear to be carcinogenic as well. Active substances (E129) include amaranth (E123), sunset- yellow (E110), carmoisine (E122), Allura red, also, tartrazine (E102), [17]. A result of customer Issues over artificial food colorings has led to the commercialization of a growing range of organic food colorings about synthetic food colorings, and a rising variety of natural food colorings are being commercially developed. Here are a few examples from Table 1.

**Table 1 : Displays Several Examples of Food Colors** [12] **.**

| **Colorant** | **Color** |
|---|---|
| Anthocyanin | Blue-reddish shades |
| Annatto | Orange shades |
| Beta-carotene | Yellow-orange |
| Canthoxanthin | Orange red-red |
| Paprika | Orange-red |
| Saffron | Yellow |
| Crocin | Yellow |
| Lucin | Yellow |
| Beet powder | Bluish Red |

Coloring agents are classified as either certified or non-certified. Non-certified are those that are natural or commercially produced. Some of the permitted natural colors are ß-Carotene, Chlorophyll, Caramel, Annatto, Saffron, turmeric, etc. Commercially produced colors include, curcumin - turmeric, paprika - red chili, Caramel-Sugar, etc, whereas certified are

those whose limits are restricted for their use within the food, they are synthetic colors. Some examples of permitted synthetic colors are shown in Table 2.

**Table 2: Shows the Permitted Synthetic Colors Used in Food Additive.**

| Color | Common Name |
|---|---|
| **Red** | Ponceau 4R<br>Carmoisine |
| **Yellow** | Tartrazine<br>Sunset Yellow |
| **Blue** | Brilliant Blue |
| **Green** | Fast Green FCF |

*3.3.5. Artificial Sweeteners*:

A sugar replacement, sometimes known as an artificial sweetener, is a food additive that acts as a substitute for the taste effect of corn syrup or sugar and useless food energy. By giving low-calorie or sugar-free alternatives, artificial sweeteners replace the sweetness traditionally delivered by sugar.

*3.3.6. Sweeteners used in Food Industry:*

*Sucrose Alcohols*:

Hydrogenated, sorbitol, mannitol, xylitol, isomalt, and starch hydrolysates are major sugar alcohols present in foods. Sugar alcohols are made from plant materials including fruits and berries.Although some fruits contain trace levels of sorbitol, the commercial source of sorbitol is dextrose (glucose) made from cornstarch. Sorbitol is created by hydrogenating sugars (adding hydrogen to the recovered dextrose). Although glucitol (similar to glucose) is another name for sorbitol, sorbitol is the word used throughout the food sector. Sorbitol is around 60% as pleasant as sucrose and contains 2.6 calories per gram.

*Saccharin:*

Saccharin was discovered more than a century ago, making it the oldest artificial sweetener.This sweetness varies depending on how it is used, however, it is 300-500 times sweeter than table sugar. Saccharin could be present in many low- and no-calorie foods and drinks, and also in Sugar-free foods and products, such as tabletop sweeteners, baked products, jams, chewing gum, canned fruit, confectionary, various dessert toppings, as well as condiments. Extensive personal population research shows that saccharin is safe for all age groups, especially youngsters and pregnant or nursing mothers.This is authorized forusage in food and drinks in over a hundred countries worldwide. Because saccharin is not digested by the human body, it provides no caloric to the diet. It is removed in the same quantity as it was ingested. Saccharin might aid in weight maintenance, blood sugar regulation, as well as the reduction of dental cavities [13].

*Sucralose:*

Sucralose is the most recent non-nutritive sweetener to enter the marketplace.This is most well-known for proclaiming to be made from sugar. It is 600 times sweeter than sugar and it is used alone and in Splenda (Splenda is the commercial name or registered brand of a sucralose-based artificial sweetener made from sugar owned by the British firm Tate & Lyle and the American corporation Johnson & Johnson) (table sugar). Whenever consumed individually, it provides essentially little caloric and is not entirely digested.

### *3.4. Flavor in Food:*

Understanding of taste chemistry is often regarded as a relatively new advance in food chemistry, resulting from the introduction of gas chromatography as well as other current extraction technologies.Whenever we eat food, the mix of taste, aroma, and texture feeling creates an overall impression best described by the word "flavour." Aromatic compounds are volatile molecules that are sensed by odor receptor sites in the odor organ, namely the nasal cavity's sensory tissue. Fruits and veggies could contain a variety of chemicals.Most recognized volatile chemicals are categorized according to their food or compound class. The scent is dependent on only a few volatile molecules.The desired objective of taste surveys has long been the measurement of chemical flavor characteristics to offer definitive information on the flavor intensity and quality of meals.Although many advancements have been made in the use of methodologies for correlating subjective sensory data with objective taste chemistry data, the regular assessment of flavor quality using solely analytical methods remains restricted. This feeling created by a substance consumed in the mouth but mostly experienced through the senses is referred to as "flavor."This flavor research encompasses the structure of food molecules with taste or fragrance, as well as their interactions with taste and aroma receptor cells.

### *3.5. Importance of Flavor in Food:*

Natural flavor is characterized by any, essence, essential -oil, or oleoresin as well as extractive, distillate, protein hydrolysate, or any product of enzymolysis, roasting, and heating containing flavor enhancer constituents obtained from a bud, root, leaf, edible yeast, herb, bark, also fruit- juice, fish, poultry, eggs, dairy products, vegetable or vegetable juice, spice, fruit, or comparable plant material, meat or fermentation products thereof, whose main role in food. Food flavor is the most important element of its flavor as well as plays a significant impact on its intake or acceptability. Furthermore, the flavor, taste, and scent of food promote salivary flow, which aids digestion or metabolism. Flavorings are necessary elements in the creation of food that today's customers expect. This nutritionally complete diet is better accomplished by eating a range of foods that do not sacrifice flavor.

## 4. CONCLUSION

The development of Food Chemistry began when malic acid was isolated from apples by the famous Swedish Chemist Carl Wilhelm Scheele. Thereafter, it opened many doors for Food Chemistry to continue. There has been development in methods of conserving compounds for preservation or restoration of flavors, extending the keeping quality of food with effective processing methods. Food Chemistry describes the role of chemicals and compounds present in food and their relative need and function in various life processes. Without amino acids, the human body would not be able to function properly.Carbohydrates, Lipids, and Proteins are critical components in food chemistry. Carbohydrates are made up of large organic compounds that are part of 80% of our everyday intake.Lipids are insoluble in water and are composed of fatty acids. Moreover, proteins make for further than half of a normal living cell's dry weight.They play for amusement. These play an important part in the basic structure and functions of the human body's biological processes. Overall Food chemistry has benefited the food industry by allowing the discoveries and new ways to the retention of nutritive as well as sensory attributes in food and hence, improving the overall acceptance of the food products.

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# CHAPTER 5

# A MOLECULAR DOCKING STUDY ON THE IMPACT OF APIGENIN ON DENGUE FEVER

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***ABSTRACT:Dengue fever, caused by Mosquito-Borne Dengue Virus (DENV) serotypes 1-4, is the most common arboviral infection affecting individuals in tropical and also subtropical areas of the world. DENV remains the most frequent viral disease spread by mosquito vectors. Transmission with DENV produces mild dengue fever, acute dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Dengue virus type 2 (DEN-2) protein NS3 is the virus's second-biggest nonstructural protein and also is recognized to have numerous enzymatic activities, along with a serine proteinase in the N-terminal region or an NTPase-helicase in the rest 70percent of the protein. These compounds were docked to three dengue targets, Glycoprotein E, NS2B-NS3, and NS1, and seven ligands were selected as possible high-affinity ligands. Current research has been conducted to examine the impact of Apigenin against DENV by molecular docking i.e. Apigenin could reduce the dengue disease by interacting with viral translation or preventing viral proteins from reaching the cell surface. The receptor was identified as a possible target in the late infection stage by molecular docking. This research suggests that Apigenin is regarded safe, even at large dosages, with no reports of harm. However, in large dosages, it might cause muscular relaxation or sleepiness.***

***KEYWORDS: Apigenin, Dengue Virus (DENV), Docking, Hydrogen Bond, Non-Structural Protein (NS3).***

## 1. INTRODUCTION

Dengue fever is caused by a mosquito-borne sickness that still infects approximately 100 nations, including the Western Pacific, the Americas, and South-East Asia. It is anticipated that 390 million dengue cases would happen each year, with 3.9 billion people within 128 countries affected by dengue fever infection [1].This condition does have the potential to create a serious flu-like sickness. Dengue disease can lead to deadly consequences if not treated properly, based on the kind of infections (primary or secondary) and strain of the dengue virus.However, there is no specialized antiviral medicine for dengue, as none of the prospective dengue antiviral alternatives examined so far have been clinically successful. Dengue is among the most common water-borne or mosquito-borne illnesses in the world today. As a result, dengue sickness viral hepatitis infection causes viral illness and the onset of dengue disease.

Dengue fever is estimated to affect 50 to 100 million citizens globally each year.Dengue development and severity are linked to immunological dysregulation produced by virus infection. Increases in particular pro-inflammatory cytokine and chemokine levels, for example, have been observed in the blood of patients diagnosed at various stages of the disease and clinical signs [2].As a result, looking for new anti-dengue candidates exist that can control immunological dysregulation caused by the dengue virus. Combating the disease's development would be among the more effective ways.

### *1.1.Epidemiology:*

Between 1960 and 2010, the prevalence of dengue fever increased by a factor of 30. This rise is thought to be the result of a combination of reasons, including fast urbanization, population expansion, increased international travel beyond endemic areas, as well as, finally, global

warming. This geographical distribution is centered on the equatorial, mostly impacting Asia - pacific.

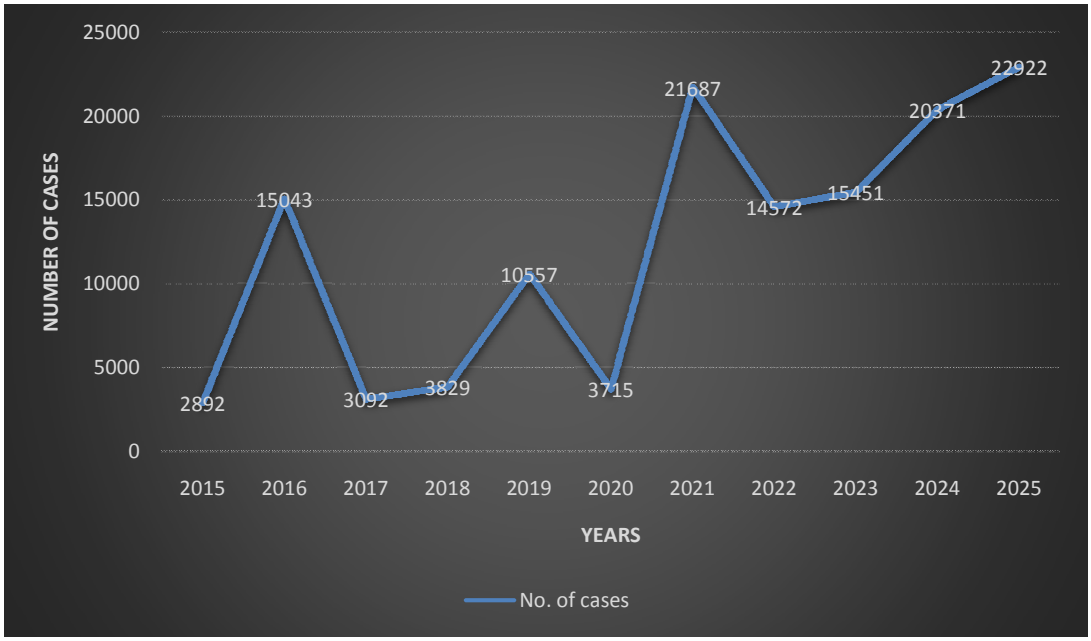


**Figure 1: Graph Depicting the Number of Dengue Cases in Uttar Pradesh from 2015.**

The next Dengue/Dengue Hemorrhagic Fever epidemic was recorded in Uttar Pradesh in 2016, as stated in Table 1. The data shows the previous 10 years have revealed that in 2016, the highest number of Dengue/DHF cases were recorded, (15,043), with the following rise occurring in 2024 (20371), as seen in Figure 1.

*1.2.Virology:*

Dengue (called Den-gee) is a virus-borne illness induced by any of the 5 strongly linked dengue (DEN1, DEN2, DEN3, and DEN4) viruses.The anopheles mosquito, Aedes -Aegypti, transmits these viruses to people, whereas the Aedes-Albopictus mosquito transmits them to animals and is also capable of transmitting them mosquito is responsible for the 2001 epidemic in Hawaii. Asian DEN-2 and DEN-3 genotypes are usually linked with a serious infection.

Dengue is an RNA virus that refers to the Flaviviridae family, which is commonly known as arboviruses. There are 11,000 nucleotide bones within the genome of the dengue virus.They are made up of three different protein complexes that constitute viral genes (C, prM, and E), as well as seven additional biological proteins present within the infectious host cells as well as so therefore essential for replication of the virus (NSI, NS2b, NS2a, NS4a, NS4b, NS3 or NS5), all these types of viral, are transcribed as a single protein complex as well as cleaved at specific sites by viral and host cellular proteases. To highlighting a function of NS proteins is:

- NS1:discovers NS1 the nonstructural protein of the dengue -virus. NS1 protein is excreted within the blood while dengue infection occurs. NS1 tests are allowed to use in serum. NS1 promotes viral replication to inhibit the host's immune response found within the cytoplasm or nucleus of the host- cell.
- NS2a and NS2b: NS2a is a viral RNA synthesis or assembly of the virion. The NS2 regions encode the 2 proteins which are NS2A and NS2B. In which NS2a is a non-

structural protein i.e., 2a is the 22 kilo-Dalton hydrophobic transmembrane protein that is recognized to connect to the endoplasmic reticulum membrane. Additionally, NS2a is part of the viral replication compound and the function within virion assembly is to counteract the host immune response.

- NS3: NS3, is an essential non-structural protein because it constructs the non-covalent compounds along with the NS2B cofactor, therefore, the formation of the functional viral proteases as well as it also includes a C-terminal ATPase/helicase sphere which is essential for the replication of the RNA.
- NS4a and NS4b: NS4a is a protein or NS4B from the dengue virus (DENV). There are extremely high hydrophobic transmembrane proteins and it is accountable for the arrangements of the membrane which is lead to the making of viral replication compound, and it is also important for the viral life cycle.
- NS5: The non-structural protein 5 comprises a methyltransferase for capping the RNA as well as the polymerase for the synthesis of the viral RNA. This NS5 non-structural protein plays several functions within the cytoplasm of contaminated cells, and it is also responsible for enabling the replication of RNA and antagonizing the host antiviral responses.

Viruses are classified into four strains, for example, DEN1, DEN2, DEN3, and DEN4. All four serotypes have the potential to cause the dengue disease. Although the infection including one serotype is thought to give permanent protection to the serotype,in the future, this might be contaminated with additional serotypes.

*1.3.Mechanism:*

Whenever a DENV-carrying mosquito stings a human, these viruses can enter the skin including the insect's mucus. It adheres to or penetrates the white blood cells, where it replicates within the cells as they travel all across the human body. The White blood cells (WBCs)react to release a range of signaling molecules.Interferon, for example, is involved in several symptoms such as fever, common cold symptoms, or acute pains. Virus generation inside the body is substantially higher in serious illnesses. Fluid pressure in more tissues (like the liver or bone -marrow) could be compromised, or fluid leaks fromthe flow of blood through the walls of tiny blood arteries flood into body cavities.As a result, blood circulation through the blood arteries is reduced, and blood pressurefalls to the point where it cannot give enough blood to essential organs. Furthermore, bone marrow failure leads to a decrease in the number of thrombocytes, which are also essential for proper blood coagulation;this raises the risk of bleeding, which is the second most serious complication of dengue fever. In a serious illness, it is unclear why subsequent infection with a specific variety of DENV increasesthe possibility of developing dengue shock conditions as well as hemorrhagic fever. The *Antibody-dependent -Enhancement* hypothesis is the most widely accepted (ADE) [3].

*1.4.Clinical Aspects of Dengue Fever:*

Dengue fever is distinguished by the following symptoms: a fast onset of temperature, a rash, headaches (typically behind the eyes), muscle or body pains, as well as a redness; another term for dengue. Dengue fever is often known as break-bone fever because it causes problems with the connecting bones or muscles. This infection's progression has three stages: febrile, severe, as well as recovery.

*1.5.Associated Issues:*

Dengue fever can infrequently damage other bodily systems. This can occur alone or in conjunction with the characteristic dengue symptoms. In 0.5–6 percent of severe instances, there is a loss of consciousness.This could be due to the viral disease in the brain and, more indirect means, to the disorderofessential organs like the liver, or neurophysiological illnesses such as *Transverse -myelitis* or *Guillain-Barre Syndrome*, have been observed in the setting of dengue. Acute liver failure is one of the more uncommon dengue complications.

*1.6.Plants Used in Traditional Dengue Treatment:*

- *Carica- papaya-(Papaya- Leaf-Extract):*

The *Carica- papaya* plant grows throughout the tropics and subtropicsknown for using all of its nutrients. This plant keeps growing over the winter as a tropical species,even though growth slows as well as the production of fruit ceases during the winter season. As a result of papaya overgrowth infection, which might damage the plant's future, papaya output has fallen, coupled with the rise of new obstacles to its front [4].Papaya leaf extract is frequently used in Asia to cure high fevertriggered by viral illnesses such as dengue fever, malaria, or chikungunya.The usage of Carica papaya leaves in the therapy of thrombocytopenia, a condition wherein the white blood cell count is less than 150,000 per Liter of blood, is presently being investigated for dengue therapy. These key ingredients in papaya induce arachidonate synthesis. The Platelet-activating factor receptor (PTAFR) gene, results in enhanced megakaryocyte synthesis as well as platelet transformation [5].

**Table 1: Displays the phytochemical compounds discovered in papaya leaves**.

| Classes | Compound | Pharmacological -Effect |
|---|---|---|
| **Flavonoids** | Deoxyquercetin, protocatechuic acid, galic acid, Apigenin, catechin deoxykaempferol, and kaempferol, are all antioxidants. | Antibacterial, Antidengue, Antioxidant [6]. |
| **Flavonoid-glycosides** | Uercetin 3-(2-rhamnosylrutinoside), kaempferol, myricetin 3-rhamnoside, 3-(2-rhamnosylrutinoside), Quercetin 3-rutinoside | Anti-oxidant [7]. |
| **Cyanogenic glycosides** | 2S-sambunigrin, R-prunasin | Anti-cancer [8]. |
| **Coumarins** | p-coumaric acid and alcohol, 5,7-dimethoxycoumarin, o-coumaric acid | Anti-oxidant [9]. |
| **Quinones** | Anthraquinone | Anti-diabetes [10]. |
| **Cinnamic acid** | chlorogenic acid, acrylic acid | |
| **Phenol** | 2-6-dimethoxyphenol | Anti-oxidant [11]. |
| **Alkaloid** | carpaines, pseudocarpaines, dehydrocarpaines I and II, carposides, emetines | Anti-malarial [12]. |

Table 1, highlights or categorizes the natural chemicals discovered in papaya leaves into several classes. There are lists of the chemicals found within the papaya pulp and also within the seeds. Among the various therapeutic plants having Anti-DENV potential,many clinical

studies have been conducted on *Carica papaya*. It was shown that giving patients 25 ml of papaya leaves extract reduced the severity of dengue fever by increasing platelets, white blood cells, or neutrophils levels in the blood. Recent research found that C. papaya therapy lowered interleukin-6 levels, and also the plasma tumor necrosis factor-interferon the dreadful dengue NS1 (non-structural protein). These outcomes verified the plant's immune-suppressive effects observed in the animal study [13].As compared with the control group, administered orally to the 228 patients having dengue fever, as well as dengue hemorrhagic fever, 30 ml of papaya leaf extract for 3 or more days, raised white blood cell count [14].As a result, it also can reduce thrombocytopenia problems or shorten patients' hospital stays [15]. Recent research [16], found that C. papaya therapy loweredthe TNF-, interferon-, and also the interleukin-6 levels in plasma.

- *Giloy (Tinospora cordifolia) :*

Tinospora cordifolia (giloy), a medicinal plant commonly utilized in Indian medicine, is believed to alleviate a variety of illnesses due to its therapeutic properties.Guduchi is another name for Tinospora cordifolia. This plant is used in Ayurveda Rasayanas to enhance the immune system and resistance to infections in the body. Tinospora cordifolia is used to treat a variety of ailments. There are numerous medicinal plants around the globe, but Guduchi is said to have the most medical benefits. This plant's pharmacological importance stems mostly from the presence of different bioactive chemicals like glucosides or alkaloids, notable berberine. Tinospora cordifolia appears to be safe as taken in small doses. Long-term usage (greater than 8 weeks) is just not known to be safe. Regarding seasonal allergies, a particular Tinospora cordifolia aqueous stem extraction (Tinofend, Verdure Sciences) was provided 3 times daily for 8 weeks.

Guduchi is another name for Tinospora cordifolia. The herb is used in Ayurveda Rasayanas to boost the immune system as well as resistance to illnesses. Tinospora cordifolia is being used to treat a variety of ailments. There are several Guduchi is thought to have the highest medical value of any herbal plant in the world. The existence of several bioactive chemicals, like glucosides or alkaloids, particularly berberine, contributes to the plant's pharmacological relevance.Tinospora cordifolia has been shown to contain a wide range of chemical elements, including alkaloids, diterpenoid lactones, steroids, glycosides, aliphatic chemicals, as well as polysaccharides. T. Cordifolia's leaves include calcium and phosphorus. It was also mentioned that the stems contain Clerodane- furono -diterpene glucoside (amritoside A, B, C, or D), alkaloids, glycosides, steroids, phenolics, aliphatic compounds, polysaccharides, or protein (11.2 percent), also the structure has already been discovered using several spectrophotometric techniques [17].

## 2. LITERATURE REVIEW

There is the various study that has been done to understand the impact of potential plants used in herbal medicine to treat dengue fever has been reviewed in such studies. Ram Pentewar et.al, described the Carica papaya as a nutritional and therapeutic plant with several pharmacological actives. The entire plant has therapeutic properties. Carica papaya is a nutritional and therapeutic plant with several pharmaceutical actives. This entire plant offers therapeutic properties. This same current review covers all of Carica papaya's notable pharmacological activity, home cures, and negative effects. Without a question, papaya extracts provide a low-cost and potentially effective therapy for dengue. In acute febrile sickness sufferers having thrombocytopenia, as an alternative or supplement, papaya extract of leaves could be used as a supplemental medicine; it accelerates platelet count growth as well as shortens hospitalization duration, lowering healthcare costs dramatically [18].

In another study, Jenny G. H. Low et.al, stated in this study, Dengue fever is a major worldwide health issue. Even though a dengue vaccine is now available,thislong-term effectiveness against any of the virus's other four dengue virus variants has yet to be established. As a consequence, drugs that attack and target the virus or essential host processes and can be used effectively as preventive or therapeutic to effectively reduce illness or avoid disease length or fatalities also arerequired to lower down burden of dengue.This study examines the brief outline of the present level of research and development in dengue therapies. Although no antiviral drug has yet been shown to be successful in proof-of-concept studies against acute dengue,various chemicals or biologics are still in the medicinal study pipeline and would be tested therapeutically shortly. At this point,the benefits and costs of developing an antiviral drug that can survive vaccinations are uncertain. Despite the availability of a safe vaccination, the reappearance of yellow fever outbreaks must serve as a warning as well as motivation to capitalize on the present momentum in viral studies targeting DENV or similar flaviviruses, like Zika virus. The latest advancements of potent directly acting antiviral medications against Flaviviridae family member hepatitis C virus implies that discovering a dengue cure in the next generation is quite likely [19].Pooja Chawla et.al, concluded in this study, that Dengue fever is a common viral illness that is often lethal, affecting mostly the tropical. The Aedes mosquito is the disease's carrier, therefore dengue epidemics can cause massive death. This study focused to provide an understanding of the causes, etiology, signs, spread, diagnosing, principal organs impacted, and prevention, or possible treatments for this condition, with a focus on medications naturally derived. This disease can spread like an epidemic, often taking multiple lives, necessitating collaborative efforts to develop improved treatment alternatives. Conventional medicine provides an alternate remedy and may be investigated as a healthier therapeutic choice. Their authors' goal was to provide an introduction to dengue, a frequent pathogenic illness. A thorough, cumulative, and in-depth literature study was utilized to describe the specifics of theetiology, transfer, or therapeutic interventions, with a particular emphasis on environmental drugs. This pathophysiology alterations in various main bodily organs have been thoroughly discussed [20].

## 3. METHODOLOGY

### *3.1 Design:*

The crystalline structure of dengue virus *NS3 protease –helicase (2VBC)* was obtained through *RCSB* (Research Collaboratory for Structural Bioinformatics) and converted into PDB (Protein Data Bank) format or three-dimensional structure of Apigenin recapture from Pubchem.In this RCSB, the format of the file is in .pdb, however, files obtained from PubChem are necessary to be converted to .pdb format. This is possible with the Open Babel tool. Both the files are utilized for docking after being converted.PDB format. AutoDock, a docking utility provided by MGL tools, is used for docking. After obtaining the .dlg file (docking log file), Docking findings for one of the chemicals in the NCI Diversity library are included. This part displays the energy, as well as structural coordinates from the very first genetic algorithm, run being shown, this complex is created in the Autodock and picturized within the BOVIA drug discovery studio. All the steps followed to obtain the docking are represented in Figure 2.

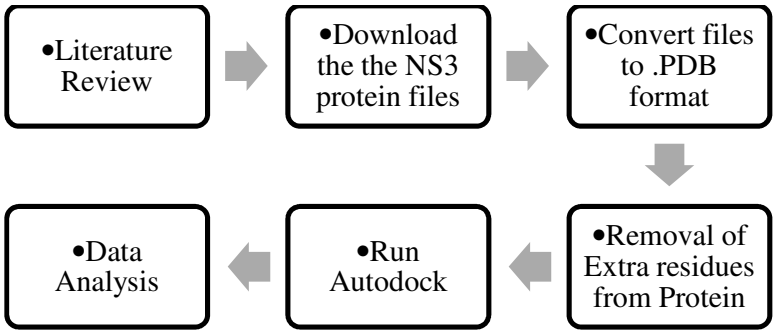


**Figure 2: Shows the Steps to Obtain the Docking Results.**

*3.2: Instrumentation:*

The three-dimensional structure of NS-3 protein is downloaded in *PDB* format from *RCSB.*RCSB helps in scientific research or education globally by providing access to the information about the 3D structures of macromolecules (proteins, nucleic acids) also linked with the small molecules (like cofactors, drugs) within the PDB. This RCSB webpage sets implements for searching, visualizing as well as PDB databank, like easily explore for the chemical interactions which stabilize the macromolecules and play an essential role in the interaction and the functions of proteins and compounds.

The three-dimensional structure of the Apigenin (2VBC) compound is downloaded from *Pubchem.*The *National Institutes of Health (NIH*), *Pubchem* database is a searchable chemical resource. Easily accessible to Pubchem means that we can upload the scientific data to Pubchem as well as other users could also utilize it. PubChem aids drug development in a variety of ways, including lead optimization, lead compound–target profiling, polypharmacology investigations, and also the explanation of unknown chemical identities. PubChem has also proven to be an invaluable resource for the creation of secondary databases, informatics tools, and online applications.Compound–target profiling, polypharmacology studies, and elucidation of uncertain chemical identification are all examples of research. P PubChem also has proved to be a great tool for the development of secondary databases, bioinformatics tools, or web-based applications.

Furthermore, the structure of Apigenin is used in molecular docking by using the tool Autodock4. Autodock4 employsa grid-based approach for evaluating the efficiency of trial morphologies to investigate the vast conformational available space to a ligand around a protein.The target protein is embedded in a grid in this approach. This Autodock4 program is used to predict how tiny compounds, such as substrate or medicines, would attach to a given three-dimensional receptor. After the docking was completed by using the Drug Discovery Studio tool we can visualize the interactions between protein and compounds.BIOVIA Discovery Studio makes use of the toolbox for the Catalyst Pharma-cophore Design and Prediction to aid in the assessment of specific molecular therapeutics both with and without target-structured information. It provides de novo drug discovery, and multi-target drug design, including activity profiling to advance small molecule R&D.

*3.3: Sample:*

The Quaternary structure of NS3 Protein is displayed in Figure 3. In the structure of NS3 Protein havean amino-terminal region of NS3 containing sequencing or structural patterns caused by bacterial or cellular trypsin-like proteases. In its C-terminal region, NS3 also contains an ATPase/helicase also an RNA triphosphatase domain, which is required for

replicating the viral RNA and also in capping. Its capacity to split into the various portions of the polyprotein precursor also plays a role in viral replication. This NS3 protein is an essential target for showing therapeutic candidates or evaluating their effectiveness.

The structure of the Apigenin compound is displayed in Figure 4. Apigenin is the class of Flavonoids found in plants. This Apigenin has antioxidant, anti-dengue, or antibacterial pharmacological effects. The empirical formula of Apigenin is C15H10O5 and also the CAS (Chemical Abstract Service) number is 520-36-5. Apigenin is in short supply in fruits and vegetables like grapefruit, as well as beverages. It's also found in basil and parsley, among other herbs.

**Figure 3: Quaternary Structure of NS3 (Non-structural -3) Protein.**

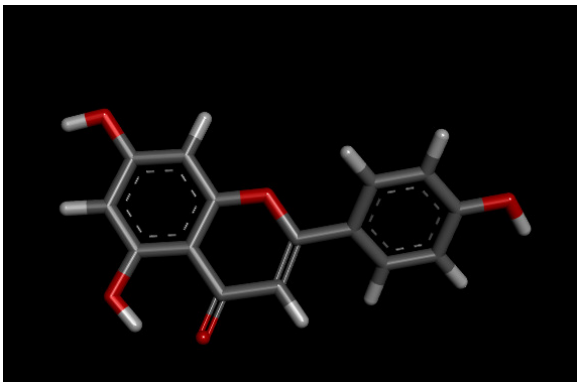

**Figure 4: Displays the structure of the Apigenin compound.**

*3.4. Data Collection:*

The docking file is extracted in the format of the DLG file i.e, a docking log file that contains docking results. After viewing this file, the interaction between the NS3 Protein (Non-structural protein) and the Apigenin compound formed the three-dimensional complex structure and visualize with the help of BIOVIA Drug Discovery Studio, shown in Figure 5. Figure 6, shows the interaction between protein and the compounds to see the precise amino acids involved in the bond formation, water and hydrogen atoms were eliminated.

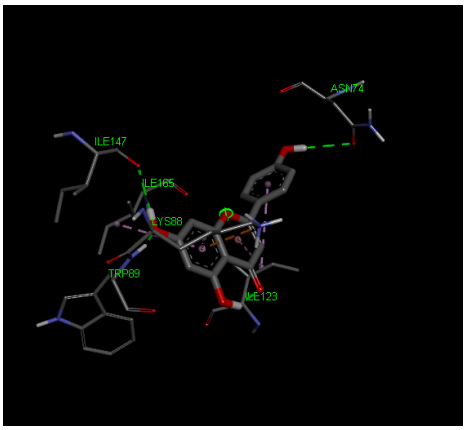


**Figure 5: Displays the 3-Dimensional structure of NS3 Protein and the Apigenin compound.**

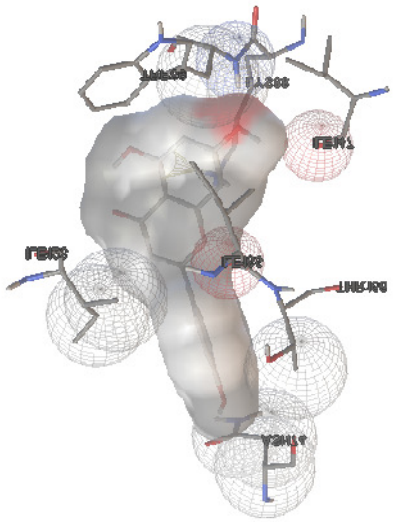

**Figure 6: Displays the interaction of NS3 Protein and Apigenin compound.**

*3.5. Data Analysis:*

A more detailed explanation of the distance between formed bonds and the type of bonds formed within the interaction between the non-structural protein (NS3) and the compound Apigenin is shown in Table 2. In this, we observed that are 3 Hydrogen (H) bonds formed between the ligand and the amino acids i.e TRP89, ASN74,ILE147, whereas 2 Electrostatic bonds were formed between the ligands and the amino acids LYS88. Four Hydrophobic bonds were formed with the amino acid sequence which were ILE123,LYS88,ILE165, and ILE123.In Figure 7, the amino acids ASN:74, TROA:89, and ILE A:147 are linked with conventional hydrogen bonds whereas LYSA:88 or ILEA:123 or ILEA:165 are attached with Pi cation and Pi Alkyl respectively.

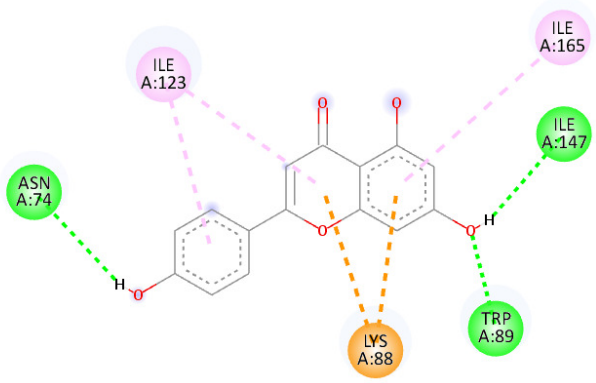


**Figure 7: Displays the 2D structure of Types of bonds generated as a result of Protein-Compound interaction.**

**Table2: Displays the types of bonds formed between the interaction of Protein and the compounds.**

| Amino acids | Bond Length | Type of Bond |
|---|---|---|
| LIG1:O - TRP89:HN | 1.73087 | H -Bond |
| LIG1:H - A: ASN74:OD1 | 2.619 | H- Bond |
| LIG1: H - A: ILE147: O | 2.28912 | H- Bond |
| LIG1- A: LYS88:NZ | 4.18824 | Electrostatic Bond |
| LIG1- A: LYS88:NZ | 3.75807 | Electrostatic Bond |
| LIG1 A: ILE123 | 4.77918 | Hydro-phobic-Bond |
| LIG1 A: LYS88 | 5.06804 | Hydro-phobic-Bond |
| LIG1 A: ILE165 | 5.47081 | Hydro-phobic-Bond |
| LIG1 A: ILE123 | 4.56175 | Hydrophobic -Bond |

## 4. RESULTS AND DISCUSSION

The energy needed to detach particles or to scatter all of the particles in the system is referred to as binding energy.Binding- Energy is equal to the sum of Total Intermolecular -Energy, Final Total- Internal -Energy, or Torsional -Free -Energy, and this issubtracted from the energy of the Unbound -System. Following docking, this data would provide a listing of ligands together with their binding energy or affinity. A common rule of thumb is that the greater negative the energy for binding energy, the better the ligand. It was observed out of 10 runs that the binding energy was conformational cluster found -5.04/cal/mol, which shows the complex formed between the docked compound of Apigenin and the viral nonstructural protein NS3 (2VBC) protease – helicase from Dengue virus was stable as shown in Figure 8.

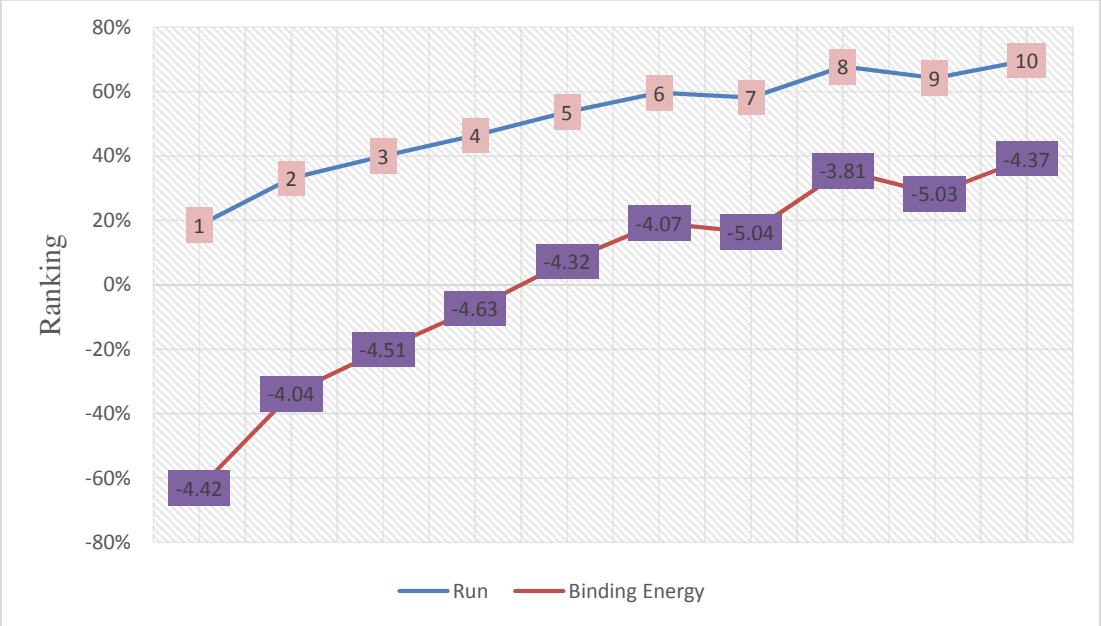


**Figure 8: Represents the Number of multi-member conformational clusters.**

## 5. CONCLUSION

Dengue fever is quickly one of the most recurrent viral diseases transmitted by mosquitos. This dengue- viruses were spread to transmit through the bite of affected wildlife. Various tactics have been offered, but some of them have shown to be successful, and there are still certain diseases for which there is no effective therapy, emphasizing the need of developing and discovering possible anti-viral medications.This study highlights the key pathogenic functions of NS3 in dengue pathogenesis as a whole. NS3 is the virus's second-biggest nonstructural protein, and this is known to have a variety of enzymatic functions. Therapeutic techniques, as well as vaccine development that target NS3, may give new avenues for combating dengue sickness.

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# CHAPTER 6

# CURRENT DEVELOPMENTS AND PROBLEMS IN NANOPARTICLE APPLICATION IN CANCER THERAPY OR TREATMENT

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***ABSTRACT:Cancer is a very deadly illness that is the leading reason ofmortality as well as poor lifestyle around the globe. Although various treatments are now being developed to minimize these fatalities, and also chronic pain, as well as to enhance the quality of life, there is a break within the sufficiency of these chemotherapeutic drugs. The accurate diagnosis of cancer cells and also the medication administration with higher sensitivity to prevent toxicity are two critical stages towards assuring the optimum treatment of cancer. Because the traditional cancer screening and the therapeutic technologies have increased their systemic toxicity with resistance, new strategies, including nanotechnology, have been used to enhance the detection or prevent disease severity.This study examines the most widely utilized nano-materials in the diagnosis of cancer as well as for the treatment of cancer. Focusing on their physicochemical and biological features, they have emphasized the applicability of these nanomaterials for cancer treatment. It also discussed the difficulties connected with different nano-materials, including restricting their usage and hindering the potentialtranslationofsome cancer types into a medical environment.***

***KEYWORDS: Biomarker-Mapping, Computed Tomography (CT), Cancer cells, Magnetic Resonance Imaging (MRI), Nanoparticles, Positron - Emission –Tomography (PET).***

## 1. INTRODUCTION

Cancer is a prominent cause of death and has a huge impact on world health. This is predicted that through 2018, there would be 18.1 million fatalities every year, with 9.6 million of them being cancer-related [1]. Cancer is a fatal illness due to excessive multiplication of cells which extends from an initial focus area to various sections of the body. For such factors, it is important to ensure that malignancies are detected and treated as early as possible to prevent infection that spreads and mortality. Nanotechnology is one of the most extensively employed approaches in cancer research today. Nanotechnology has produced some promising discoveries to monitor and diagnose cancer and also involves medication delivery,[2]the “gene therapy”, “diagnosis or detection”, “drug transportation”, “biomarker – mapping”, the “targeted- therapy “, and “molecular imaging” are all examples of cellular imaging. Nanotechnology has been used in the creation of nanoparticles [3], like quantum dots, and also the gold nanoparticlesthat also are utilized for diagnostic procedures at the molecular scale. Nanotechnology is dependent on molecular diagnostics, such as the detection and differentiation that could identify the tumors correctly and promptly [4]. Nanotechnology therapies, also including Nano scale medication delivery, could assure accurate malignant tissue trying to target with minimal adverse effects [5]. Nanomaterials could easily overcome the cell barriers due to their biological origin [6].Because differences in properties are caused by the quantum-mechanical framework of mechanics in the realm of the very tiny, the term quantum is associated with all three types of nanotechnology. Materials could be nanostructured to achieve a new characteristic and performance. This sector is concrete the way for new opportunities in technology and science.

The discovery and development of Nanoscale materials, procedures, as well as occurrences, and also the establishment of innovative theoretically and experimentally research tools, open up new avenues for the introduction of unique Nanosystems and nanostructures [7].The

characteristics of nanoscale materials may differ significantly from those of the larger size. When a material's dimension is reduced from a big size, the qualities stay the same at first, later minor changes happen, and eventually, when the size falls below 100 nm, significant changes in properties may happen. Because of its active and passive targeting, Nanomaterials have been applied in the treatment of cancers for many years. Although numerous medications could be used to treat cancer, the responsiveness of the treatments often results in insufficient outcomes and could have a variety of adverse effects, including harm to healthy cells. As a result, multiple research has examined various kinds of nanoparticles, including liposomes, polymers, molecules, or antibodies, concluding that a combining of such Nanomaterials in cancer medication development could strike a balance between enhancing effectiveness and minimizing drug toxicity [8].

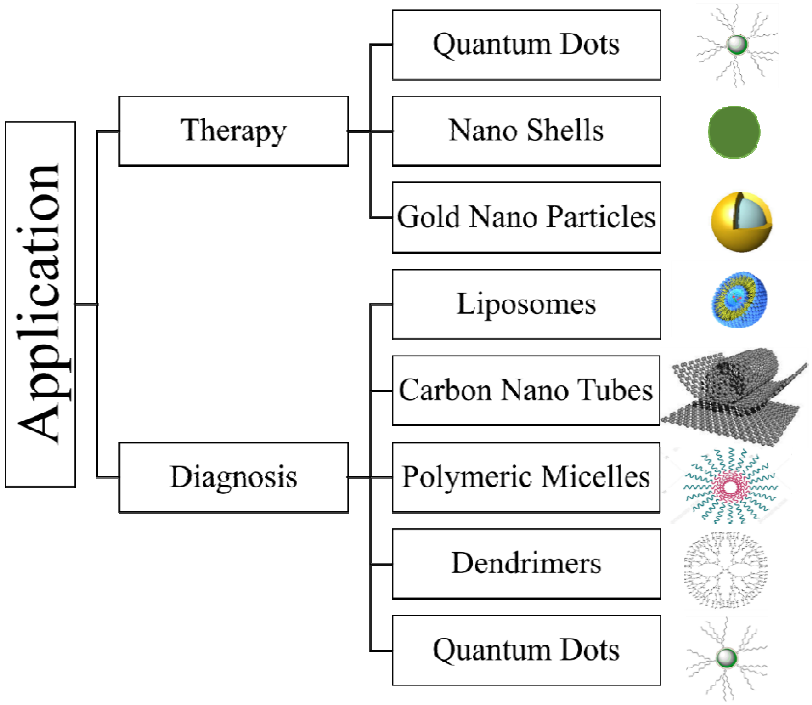


**Figure 1: Represents the Use of Nanomaterials in Cancer Detection and Treatment.**

Furthermore, the given possible instability of nanoparticles, there is a consideration of research going on them until they could be used within the clinic to treat cancer [9]. With the fast application of nanotechnology, this research will explore the use of cancer detection and therapy, and consider the benefits and limits of their usage shown in Figure 1. Nanotechnology is a branch of research that works with objects ranging in size depending on the application, it might sort from several nanometers (nm) to hundreds of nanometers (nm)[10]. Throughout the last decade, there has been a lot more interest in creating specific controlled drug delivery due to the multiple advantages it provides in overcoming the constraints of traditional formulations [11].

## 2. LITERATURE REVIEW

Several studies have been conducted to examine the current advances and issues within the utilization of nanoparticles in the therapy of cancer which has been analyzed in such research.Kumar Bishwajit Sutradhar et.al, discussed in this study,Nanoparticles are quickly developing and trying to solve different constraints of current medication delivery systems

and areas developing as unique cancer treatments. Traditional chemotherapeutic drugs have some significant side effects, such as harm to the immune response as well as other organs from growth and division cellsinduced by nonspecific targeting location, a lack of solubility, as well as a difficulty to access the core of tumors, resulting in a delayed diagnosis by reducing the dosage or decreasing the probability of surviving.Nanotechnology has made it possible to gain immediate access to malignant cells while increasing medication localization and cellular absorption. Nanoparticles may be engineered to recognize malignant cells as well as enter the bloodstream selectively and precisely while coming into contact with healthy tissue.Their study was focused on the potential of nanomaterials to recognize cells using diverse methodologies that have unique identification qualities that set them apart from past anticancer medicines. They concluded that precise medication distribution by nanomaterials into cells displays several positive results, and also nanoparticles reduce the adverse effects of traditional chemotherapy drugs [12].

Burcu Aslan et al. stated in a study that cancer disease is among the leading causes of illness and death around the globe, therefore improved therapies are critically required. The discovery of new nanomaterials or nano emulsions has enabled a tremendous push to enhance cancer medication delivery. The primary goal of most nanocarrier uses was to preserve the medication from fast degradation following systemic distribution and also to permit it to access tumor locations at therapeutic levels and limiting the delivery of drug to regular areas as often as possible to limit adverse reactions. The goal of their study was to advance Nanomedicine to provide new chances to strengthen the oncology arsenal. Targeted or non-targeted nanomaterials are now in preclinical studies, demonstrating the influence of delivery methods on the sector. They concluded that ongoing research in Nanomedicine would broaden the therapeutic effects of medications while drastically reducing side effects, resulting in better outcomes of care [13].

P. N. Navya et al. concluded in a study, that advanced Nano-materials-based formulations, as well as drugs, have shot to the forefront of the latest technology, appearing as promising cancer therapeutic or therapy qualities. The requirement for creating and producing a collection of nano-materials, as well as efficient flexibility on their physical and chemical properties or surface treatment accessibility to improve accuracy, is certainly dedicated to the achievement of tumor therapeutic uses.According to the published studies, nanotechnology would give upcoming technologies for diagnosis and management as well as anticancer therapies. As a result, in this comprehensive examination, they highlight a variety of nanoparticles presently used in anticancer therapy that explore the essential importance of their physicochemical features in treating cancer. They go through the most recent advances in nanoparticle technology for cancer treatment, as well as current medication targeting as well as releasing tactics for effective cancer delivery. They also talk about nontoxicity, which is an often-overlooked aspect of nanotechnology. Furthermore, they highlight the present issues in nanomedicines and give a prognosis for the field's future [14].

Zhannat Ashikbayeva, et al. stated that cancer is a major health concern that is becoming more prevalent across the globe. Despite the current traditional treatment of cancer procedures,every year, the frequency of cases of cancer or fatalities rises. As a result, innovative methods of cancer therapy are necessary to advance established cancer treatment methods. Therefore, the application of nanotechnology, which employs nanoparticles or Nanocomposites, has enormous potential and opens up new avenues for process improvement of treatment for cancer when combined with therapeutic applications. Because of their mechanical features, nanomaterials could create or precisely boost heat transfer at the tumor site. The above-mentioned special features of nanomaterials enable the induction of

temperature as well as the destruction of malignant cells.Their study gave an overview of the use of nanoparticles as well as nanomaterials in the heat therapy of tumors, proving their benefits over previous heating methods including magnetostrictive iron oxide nanoparticles, nanowires, nanostructures, nanostructured materials, carbon nanotubes, as well as other nanoparticles [15].

Ancuta Jurj et al., discussed in this study, Nanostructures have sparked tremendous attention in recent years as a means of safely delivering medicinal medicines. Numerous improved access to healthcare, also including molecular diagnostics, illness monitoring, nanoscale immunology, or anticancer drug delivery, were documented but might be incorporated into clinical applications. The purpose of their work was to emphasize the information that proves the usage of nanoparticles in conjunction with certain medicines in therapeutic strategies or medical research, with a focus on hematopoietic cells.Its functional assessment of non-viral delivery techniques, such as metal nanoparticles, nanostructured lipid carriers, and dendrimers, is a specific emphasis. In addition, the study discusses important published findings on microRNA or RNA interference delivery employing nanoparticles in cancer treatment [16].

## 3. DISCUSSION

Cancer nanotechnology has been investigated and utilized in the treatment of cancer, which represents a high improvement in the detection of the disease, diagnostics, or therapeutic interventions. Numerous research is being done in an attempt to develop better precise nanotechnology-based cancer treatments while limiting the adverse the treatment's side effects. The nanoparticles were presently becoming developed to help medicinal medicines cross biological boundaries, mediate molecular interactions, and detect molecular events. Dendrimers, nanospheres, nanocapsules, liposomes, polymeric micelles, and nanotubes were instances of the latest nanotechnology-based medicine delivery techniques for cancer therapy that are presently accessible or have been investigated or evaluated [17].

### *3.1.Cancer Diagnosis using Nanotechnology:*

Genetic mutations could alter the production of specific biomolecules, resulting in unregulated cell growth or, eventually, malignant tumors.Cancer was classed as malignant or benign. Malignant cells regularly release cells that enter surrounding or internal organs, whereas benign tumors were isolated to the site of cancer growth. Cancer screening and therapeutic procedures were aimed at detecting as well as inhibiting malignant cell development and spreading as early as possible. The utilization of “Magnetic Resonance Imaging (MRI)”, “Computed Tomography (CT)”, “Ultrasonography”, and also “Positron Emission Tomography (PET)”, as early detection tools for cancer, is notable [18].These scanning technologies, therefore, were constrained by a lack of valuable diagnostic information about various cancer kinds or stages. As a result, it is hard to acquire a comprehensive assessment of the illness condition against which to build optimal therapy [19].

### *3.2.Tumor Imaging is Aided by Nanotechnology:*

The use of nanotechnology in tumor detection and management has sparked considerable attention, having various nanoparticle kinds now being employed for cellular diagnostics. They have acquired significance in current cancer research or detection because of their benefits such as tiny size, strong biocompatibility, or greater atomic number. Nanoparticles utilized in the treatment of cancer, including such semiconductor, quantum dots, as well as

iron oxide crystals, have visual, electromagnetic, and physical features not found in the other materials [20].Anti-tumor medications or macromolecules, such as peptides, antibodies, as well as other compounds, could be combined using nanoparticles to mark extremely specialized tumors, which could be utilized for early identification as well as detection of cancerous cells [21].Imaging tumor tissue with nanoparticles had allowed for the detection of cancer through its initial stages in cancer diagnosis. Metastases in lung cancer can be discovered by creating immunological as "SPIONs' goal," superparamagnetic iron oxide nanoparticles might be employed in Magnetic resonance with tumor cell lines " [22].Recent research has demonstrated that SPIONs have a high selectivitywith no known negative consequences, these model elements for aerosol in lung cancer magnetic resonance [23].

*3.3.Cancer Diagnosis Using Nanotechnology Tools:*

Nanotechnology, according to existing research, can verify cancer screening at the tissues, cells, or molecular scale [24].This is performed by using nanotechnologies to investigate the tumor's surroundings. A pH-response to fluorescence nano-sensor, for example, might aid in identifying activating fibroblasts proteins mostly on cell membranes of tumor tissue [25]. Numerous spatially and temporally nanotechnology-based methodologies for monitoring the fluctuating cellular activity in malignancies and live cells are explored.

*3.3.1.Quantum Dots in the Near Infrared (NIR) Spectrum:*

The inability of visible and infrared scanning to penetrate things restricts its application. To overcome this issue, the development ofQDs produce light in the near-infrared spectrum (700-nanometers), These are better appropriate for tracking colorectal cancer, liver issues, stomach cancer, as well as leukemia [25].A second near-infrared (NIR) window (NIR-ii, 900-1700 nm) with increased tissue film thickness and better high - the resolution has been introduced to improve cancer screening. Further, using a sulphur source to make silver-rich Ag2Te Qd's has been demonstratedto result in better geographic image enhancement across a broad infrared range.

*3.3.2. "Nano-shells":*

The usage of nanoshells is also another frequent nanotechnology application. Nanoshells were dielectric centers ranging in size from 10 to 300 nanometers, often constructed of silicon or encased within a delicate metal casing (typically gold).These nanospheres generate illumination by exchanging plasma-mediated energy, and they can be actively changed using UV-infrared emission/absorption arrays. So although its applications are restricted by their huge size,nanoshells were intriguing due their scanning is free of heavy metal ions.

*3.3.3.Nanoparticles of Colloidal Gold:*

Because of their small dimensions, biocompatibility, or higher electron number, gold nanoparticles (AuNPs) are a better contrast agent. According to one analysis, AuNPs attack cells in both active and passive modes. Due to the obvious permeability of EPR (Enhanced Permeability Retention) in Tumor Sites, this controlled delivery method is directed by a collection of gold nanoparticles to increase visibility (Stress Impact). On either side, active targeting is performed by combining AuNPs with tumor-specific targeted medicines, like epidermal growth factor receptor (EGFR), targeted treatments shown in Figure 2 . If the frequency approaches within 80keV, gold's mass attenuation rate rises quicker than those of other elements like iodine, showing a bigger promise for gold nanoparticles combined AuNPs with liver cancer cells as well as the discovery that the groupingsthe effects of gold

nanocomposite groups on liver cancer cells are significantly more powerful than those on liver cancer cells just utilizing X-ray imaging. These discoveries offer substantial ramifications for timely identification, as the technology may identify cancers as tiny as little as a few millimeters in size throughout the organs [26].

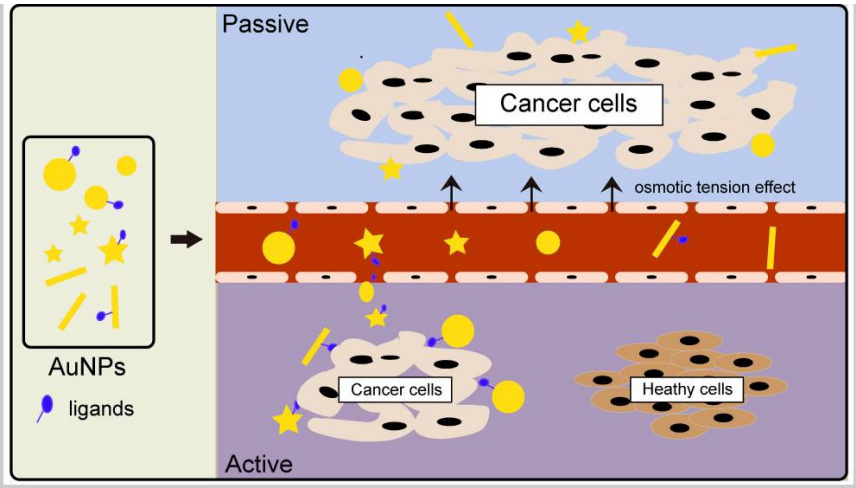


**Figure 2: Shows theDifferent Forms Of Gold Nanoparticles (All Sizes, Shapes, As Well As Ligands) Aggregate In Cancerous Tissues By The Osmosis Tension Impact (known as Passive- Targeting) and connect using Ligand-Receptor Interaction to select malignant cells (Referred as Active- Targeting).**

*3.4. "Nanotechnology Tools for treatment of Cancer":*

The utilization of small molecular structures and nanoparticles as pharmaceutical procedures and techniques is driving the expansion of nanotechnology. "*Liposomes, micelles, dendritic macro-molecules, quantum- dots"*, or tubes are a few types of nano-carriers used in cancer therapy.

- *Liposomes:* Liposomes, which are tiny spherical made fromNatural or manufactured lipid molecules with a water phase nucleus membrane, are one of the most researched nanostructures.Liposomes develop naturally due to the currently contributes of phospholipids, permitting hydrophilic medications to predominantly remain in the monolayer lipid membrane whereas hydrophobic drugs develop before the multilayered lipid membrane.Liposome-bound antibodies target tumor-specific antigens and afterward transport medications to the tumor to ensure active targeting. Certain liposomal medicines have been licensed for therapeutic use due to their numerous pharmacokinetic advantages. Liposomal versions of adriamycin, for example, have been used to treat malignant ovarian cancer and also have shown significant therapeutic advantages [27].

- *Carbon nanotubes*:Carbon nanotubes (CNTs) were classified into two types based on their structure as well as diameter: "Single-Walled Carbon Nanotubes (SWNTs)", and also the "Multi-Walled Cnts (Mwnts)". Nanotubes were just a viable option for large-scale biological applications because of their chemical characteristics, which include contact area, mechanical properties, metal properties, as well as electrical and thermal conductivity.Carbon nanotubes also absorb the energy around the near-

infrared (NIR) range, allowing them to warm up and thus targeting the tumor cells. Carbon nanotubes in their natural condition promote noninvasive biofilm penetrating and are thus considered extremely efficient carriersto transfer a variety of medicinal chemicals into living cells. Because of the evident suitability of nanotubes in cancer treatment, pharmaceuticals have been developed and tested in vivo [28].

- *Micelles Made of Polymers:* Polymeric nanoparticles (PNPs) were developments involving a homogenous micelle withdiameters ranging between 10 and 1000 nm. PNPs, also called nanoparticle-based polymers, nanostructures, microcapsules, or polymeric microcapsules, were the first polymers to be described for use in medicinal applications. PNPs made the self-assembly of amphiphilic polymers with a lipophilic and hydrophilic block possible. quickly with a covalent link or connection through a hydrophobic region, permitting PNPs to absorb hydrophobic drugs in an aqueous phase due to the obvious hydrophobic interactions. Hydrophilic charged molecules, including protein, polysaccharides, and nucleotides, are swapped to enable core linkages while neutralizing ions [29].

- *Dendrimers:*

Dendrimers were nanocarriers having a cylindrical polymeric center and regularly spaced branches. This inclination to lean toward that spherical structure grows as the diameter of the dendritic macromolecule rises. There are typically two main methods for synthesizing dendrimers: a divergence technique wherein the dendrimers could expand outwardly from the central area and a converging method for which dendrimers expand inwards towards the margins as well as eventually wind up in the central area.Numerous studies also revealed that cancer cells having high folate receptor expression may generate foils using dendritic molecules coupled to folate.Dendrimers may also As evidenced by the DNA-polyamides clustering DNA-poly (amidoamine), they are particularly efficient at triggering death in cells carrying folate detectors.

- *QuantumDots (QDs):*

Quantum dots (QDs) are tiny semiconductor materials nanoparticles or nanostructures with diameters between tens of nanometers. Furthermore, QD immunoreactivity is more precise than standard immunochemical approacheswhen protein levels are low and the context is poor.QD immunostaining is a viable approach in diagnostic processes for identifying tumor biomarkers, like proteins or even other features of tumor sample heterogeneityQuantum dots may cluster in certain areas of the body and transport medications there. QDs' capacity to accumulatethey now have a reasonable alternative to untargeted medication delivery in a specific internal organ, or also a potential way of avoiding chemotherapeutic side effects. Recent advances in the surface functionalization of QDs, that could be used to target tumors or combine with macromolecules In vivo, peptides, and antibodies, for example, might be utilized to target tumors, allowing them to be used in medical diagnostics and therapies.Many studies have employed quantum dots in the combination of prostate-specific antigens to treat cancer, while some have used quantum dots to createmarkers that could speed up the procedure, such as immunologic biomarkers with a more constant intensity of light than standard fluorescence immunologic indications.

## 4. CONCLUSION

Nanotechnology is shown significant potential for the treatment of cancer. Nanomaterials' enhanced pharmacokinetic and pharmacodynamic characteristics had helped to better cancer

detection and therapy. Nanotechnology, due to its unique features, provides targeted medicine administration in damaged tissues with minimal neurotoxicity. Due to the constraints of nanotechnology, further work must be made to improve pharmaceutical delivery, increase efficacy, and minimize adverse effects. By strengthening the links between the physical andthe chemical characteristics of the nanomaterials used,better and more efficient chemicals for cancer detection and treatment may be produced. This study highlights the potential of nanoparticles to recognize cells using diverse methodologies that have unique identification qualities that set them apart from past anticancer medicines. It also addresses precise medication delivery by nanoparticles into cells, displaying several beneficial studies, and also how nanotechnology minimizes the negative effects of traditional cancer treatments.This study focuses on the potential of nanoparticles to recognize cells using diverse methodologies that have unique identifying qualities which set them apart from past anticancer medicines. It also addresses precise medication administration by nanotechnology into cells, displaying several positive studies, and how nanoparticles reduce the adverse effects of traditional cancer treatments. Furthermore, Nanotechnology's therapeutic benefits, as well as future advancements, may make it a therapeutic option for usage in a variety of medical circumstances. Ischemic stroke and rheumatoid were 2 illnesses that would demand the provision of suitable pharmacological treatment to the affected area.

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# CHAPTER 7

# LITERATURE SURVEY ON COMMERCIAL AND INDUSTRIAL APPLICATIONS OF BIOTECHNOLOGY

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***ABSTRACT:*** ***Biotechnology is assisting in the healing of the globe by utilizing nature's toolbox and utilizing our individual genetic composition to rehabilitate as well as direct directions for research. Patients now have access to more than 250 biotech items for their healthcare and vaccinations, many of which are for disorders that were previously resistant to treatment. Biotechnology is the large-scale production of biopharmaceuticals and biologicals using genetically altered microbes, fungus, vegetation, vertebrates, etc. Biotechnology applications include the detection of genetic modification for agriculture, packaged foods, bioremediation, waste management, or power production.The purpose of this study is to assess biotechnology in terms of its applicability. Environment, medical, agriculture, food processing, and industry are some of the major application areas described in the literature. With advances in science, it is predicted that the domains and extent of use of biotechnology would expand. It was found that as the breadth of biotechnology use expands, research efforts should concentrate on the highlighted hazards and obstacles, particularly in agricultural applications.***

***KEYWORDS:*** ***Biomaterials, Bioprocessing Techniques, Biotechnology, Genetic Engineering, microorganisms.***

## 1. INTRODUCTION

Biotechnology is a broad term that includes a variety of technologies. It is, nevertheless, a type of technology which have two (similar) characteristics: it works with living beings or their molecules, as well as it includes a wide selection of functional applications that help us make a good life. Biotechnology is defined broadly as "the use of microorganisms or their products for economic purposes. As a result, (conventional) biotechnology has been used since the beginning of time. The early days of biotechnology use included baking bread, manufacturing alcoholic drinks, including growing food crops or domestic animals. In contrast, the advancement of molecular biology has given biotechnology new meaning, relevance, and promise. The attention of the public has become focused on contemporary biotechnology. Biotechnologies seem to have the capability to affect the world economy and society [1].

Biotechnology is a term that refers to the use of genetic modification to create goods or procedures for humans in a limited sense. Genetic engineering, which is a method of altering the chemistry of genetic material, is used to achieve genetic alteration (DNA or RNA).Another biotechnology approach is the use of sterile ambiance conditions in chemical engineering processes. The development of the required microorganism in great amounts for the production of biotechnological goods such as antibiotics, vaccinations, and enzymes [2]. There are several domains of biotechnology that is relevant: important ones are in health care (medical), agriculture, and the production of the crop (industrial) utilized crop and also other products (like biofuels, vegetable oils as well as biodegradable- plastics) and also applied in the environmental application shown in Figure 1.

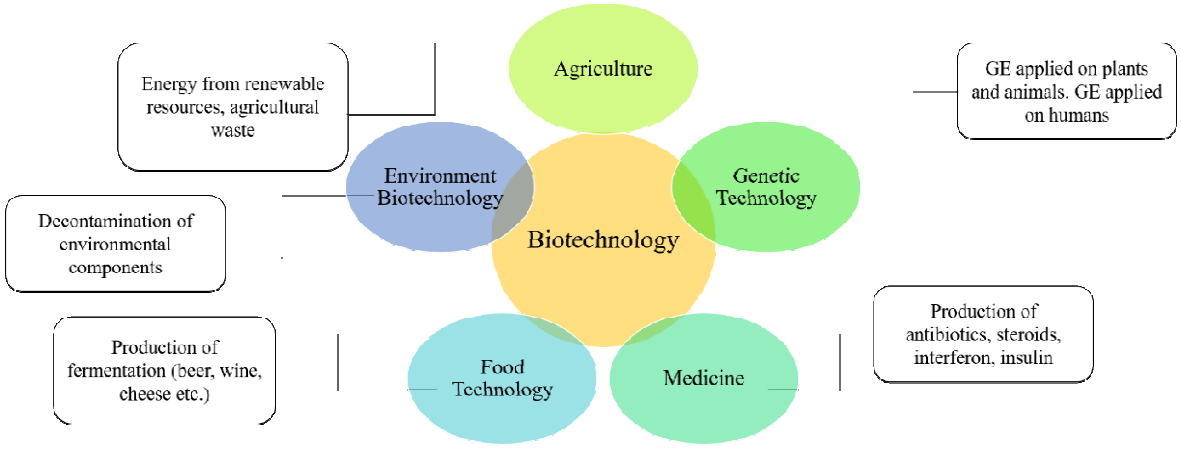


**Figure 1:Biotechnology's Application in Anthropogenic Activities (Industry, Agriculture, Medicine, Health, and Environment).**

Biotechnology is also an option for promoting human lives. While producing the technologies using the desired organism for the benefit of human beings is named as a "Biotechnology". Biotechnology has affected everyday life through various innovations [3]. Biotechnology has opened up a new world of scientific progress. In agriculture, biotechnology has a wide range of applications. To eliminate the need for pesticides, Bacillus thuringiensis (Bt) crops have been introduced.The goal of biotechnology in watershed crop production is to produce small yields and grains.The development of a shelf-life tomatoes' productivity or stability has been improved by enhancing genes. In the market, there are also BT brinjals.The number of livestock produced has grown dramatically. Biotechnology also resulted in the12s rRNA sequencing being utilized to identify bovine meat.

For disease identification and cure, biotechnology has had a significant impact on human life. The development of new drugs such as insulin or generation 3 antibiotics has simplified the treatment and management of human diseases.Vaccines are the most recent major medical innovation. Mullis and his colleagues' invention of PCR is a defining moment in biotechnology history [4]. In today's world, biotechnology's application in the defense industry is a fast-growing domain. Bioweapons and other technologies based on organisms are far more precise and precise. The method of employing cells to make industrially useful goods is known as industrial biotechnology (also known as white biotechnology). It has been used in a range of industrial processes in various ways, most notably in the employment of biocatalysts in manufacturing [5]. It employs living cells and enzymes derived from yeast, moulds, bacteria, and plants to develop readily degradable goods, consumes less energy, and generates less waste during manufacture.

One of the earliest aims of white biotechnology was the development of biodegradable polymers. These studies have mostly focused on polyesters of 3-hydroxy acids (PHAs) that are naturally generated by a diverse spectrum of bacteria as an energy store and carbon source. These compounds have characteristics comparable to manmade thermoplastics and elastomers ranging from propylene to rubber, yet microorganisms in soil or water fully and swiftly destroy them. Poly (3-hydroxy-butyrate) (PHB) is the most prevalent PHA, which bacteria produce from acetyl-CoA. "*Ralstonia Eutropha",* a bacterium that grows on glucose, may accumulate it contains up to 85 % of its weight basis in PHB, creating it a mini-bio-plastic factory[6].For a long time, biotechnology has played a role in industrial operations. Bacterial enzymes have long been employed in food processing and as active

ingredients in laundry detergents to avoid the need for artificial surfactants. In large-scale fermentation tanks, transgenic *Escherichia. Coli* is utilized to manufacture human insulin.Natural processes and products have numerous advantages, including the fact that they do not rely on fossil fuels, are more energy-efficient, and have biologically degradable substrates and waste, all of which help to reduce their environmental effect [7].

In this review article, we discussed the most recent developmentsin biotechnology to enlighten researchers about its significance in all sectors of science and technology. This article takes into account the whole biotechnology sector as well as its related applications.Some of the key application areas addressed within the study include the environment, medical, agricultural, food processing, and industrial. Biotechnology's fields and scope of application were expected to increase as science progressed. As the use of biotechnology broadens, it was determined that research efforts should focus on the identified risks and limitations, notably in agricultural applications.

## 2. LITERATURE REVIEW

Rehana conducted a study to assess biotechnology in terms of its application. Environment, medical, agriculture, food processing, and industry are some of the major application areas described in the study. Goal of Rehana's study was to cover all elements of biotechnology in the field. In most developing nations, biotechnology has been used to improve food processing by using microbial inoculants to enhance food and dairy product attributes such as taste, fragrance, storage stability, uniformity, or nutritive quality Biotechnological techniques are utilized in the milk, beef, seafood, as well as beverages processing sectors to improve food, functional, and sensory characteristics of food. Author concluded that whenever utilized wisely, biotechnological solutions can help decrease the incidence and number of harmful ingredients in meals while also eliminating allergic compounds [8].

Abdul Hannan et al. proposed in a study that human population growth has offered a particular challenge to science in terms of combating hunger and increasing health. With the advent of biotechnology technologies, a whole new world of research has emerged to address these concerns. Biotechnology is the use of biological systems for the development of helpful technologies for human benefit. Biotechnology is even being employed in the military to improve poultry meat, feeding, or milk production. Whenever it relates to GM crops, biotech crops were unbeatable in terms of productivity performance. The major aim this study was to enlighten current advances in biotechnology and also to educate research professionals about its significance in all sectors of science [9].

Adeogun B.K. conducted a study in which they evaluated biotechnology in terms of its applicability. Biotechnology may be used in the environment, medicine, agriculture, food processing, or industry, according to the study. As science progresses, it was noticed that the fields and extent of application would continue to expand. It was determined that biotechnology research efforts should be focused on the highlighted dangers and obstacles, particularly in agricultural applications. The purpose of their study was to assess biotechnology in terms of its applicability. Environment, medical, agriculture, food processing, and industry are some of the major application areas described in the literature. With advances in science, it was predicted that the domains and extent of use of biotechnology would expand. It was decided that, as the breadth of biotechnology use expands, research efforts should concentrate on the highlighted hazards and obstacles, particularly in agricultural applications [10].

Jasia Nissar et al. proposed in a study today, that the advantages of biotechnology overshadow the disadvantages. Because it is critical to boosting food production to fulfill the needs of a growing population. Before authorizing genetically modified (GM) crops to be cultivated and sold, the United States Department of Agriculture (USDA), the Food and Drug Administration (FDA), as well as the Environmental Protection Agency (EPA) investigate their safety. As research and breakthroughs in the department of food biotechnology continue, researchers might discover a speedier method of identifying hazardous viruses and bacteria in food. This may help to lessen the chance of foodborne illnesses or keep food safe to consume. Biotechnology is also being used to generate crops that can flourish in difficult climatic circumstances such as excessive heat or drought. This may result in crop cultivation in the land that was previously inappropriate for agriculture. In the expectation of one-day permitting patients with food intolerances to comfortably consume previously problematic foods, researchers have started to target particular allergy-causing proteins in meals. Food-biotechnology has the potential to lead to more nutritious meals for humans and animals [11].

## 3. DISCUSSION

Biotechnology is an essential approach that has achieved significant progress in two main areas: molecular biology as well as the manufacture of industrially essential bio-chemicals. Scientists are now directing their efforts toward biotechnological firms, resulting in the growth of several biotechnological industries.More than 225 firms have been formed and are effectively operating in the United States alone, including Biogen, Cetus, Geneatech, Hybritech, and others. The globe, the United States, Japan, and several European nations are leaders in biotechnology research and are supported by manufacturers [12].These firms are concerned with human welfare and have chosen the following topics for research and innovation:

- Bio-screening for medicinal compounds that is automated.
- Alkenes are bioprocesses into useful oxides and glycols.
- Immobilized cell or enzyme systems are being developed for the chemical industries.
- Microorganisms' genetic enhancement for medicinal product manufacture.
- To extract the crude mineral oils, xanthan gum is produced in oil fields.
- Transgenic plant and animal production.
- Production or development of a bacillus’s vaccine
- Monoclonal antibodies for organ transplant tissue typing are being developed.

### *3.1. Biotechnology's Industrial Applications:*

The industrial use of molecular biotechnology is sometimes categorized into red, green, grey, and white biotechnology. This distinction pertains to the application of technology in the fields of medicine (including human and animal medicine), agriculture, the environment, or industry.

#### *3.1.1. Biomaterials' Applications within Biotechnology:*

Biomaterials biotechnology is utilized for metals as well as chemicals as a biomaterial and bio-soft materials. It contains (Zirconia, Alumina, and Hydroxyapatite), all of which are utilized in dental implants. The manufacture of Hydroxyapatite from the gardening snail shell (Helix Aspersa) is another wonderful biotechnological technique[13].Titanium is a mechanism that increases osteoblastic bone marrow stem cell development before this. Silicone dispersions were employed in the fabrication of mouse ventricular expansions for the

Langendorff cardiac grounding [14]. A comparatively recent as well as potential use of "Membrane Transglutaminase (mTG)" is the production of so-called "Bioplastics," both of which are biodegradable as well as edible[14].

The development of methods namely the Response Surface Method (RSM) has created an opportunity to optimize lipase-mediated catalytic properties[15], It made the extraction of Anthocyanin from "Solanum melongena: much easier, and it also eased the production of mycoprotein from Fauna like Fusarium venenatum ATCC 20334 [16], Regardless of all of these uses, methodologies for avoiding microbial adhesion have been developed as if formation is not the only factor of biotechnology, as well as the protection of the created, and that protection is provided produced by the same bacterial community which generates microbial bio-surfactants

### *3.1.2. Biotechnology's Impact on the Environment:*

The technique of using microorganisms to enhance environmental quality is known as biotechnology in the environment. Environmental biotechnology is a word used to describe this process.Conversion of organic wastes, environmental bioremediation of hazardous pollutants, environmental protection, and monitoring are some of the applications [17].Organic wastes can be converted into usable bioresources utilizing biotechnological methods involving microorganisms. Plants, agricultural wastes, and municipal leftovers are among the organic waste sources under study. These plant-derived wastes are made up of lignin, cellulose, and hemicellulose.

Environmental biotechnology can help to safeguard the environment. Carbon dioxide mitigation, for example, has been documented in the literature as CO2 recovery from pollution gas emissions from businesses or on-site CO2 fixation by bioprocess utilizing cyanobacteria.To avoid global warming, CO2 must be removed from the atmosphere or mitigated as gaseous pollution, which can be accomplished through the use of biotechnology. However, the CO2 that has been extracted may be transformed into biodegradable polymers that can be used in place of petroleum-based plastics [18].The use of biosensors in biotechnology allows for environmental monitoring. Biosensors can detect heavy metals, herbicides, pesticides, or organic compounds are examples of pollution that may be measured.

### *3.2. Genetic Engineering:*

Genetic engineering is the activity of transferring individual genes across organisms or modifying an organism's genes to delete or add a desired feature or quality. However, the possibilities of genetic engineering will be covered in this paper. Genetic engineering is used to generate genetically modified crops or species. GMOs, or genetically modified crops, have been used in the production of biotechnology products. Consumers and consumer organizations appear to be especially interested in and concerned about this specific sort of modern biotechnology, genetic engineering [19].The current work investigated comparison Insilco analysis or Insilco gene sequencing of ascorbate peroxidase protein sequences from several species of plants as well as glycine betaine biosynthesis genes in Bacillus subtilis, accordingly. During the recent decade, several gene treatments have been identified, as well as genetic differences and epigenetic variations in autoimmune and behavioral disabilities such as ADHD and Endophenotypes.

Metabolite Profiling and Gene Expression is a biotechnology exploration approach that is Already more typically used to identify all (or the majority) of the biochemicals involved in

an organism's metabolism. Metabolism monitoring is related to methods for measuring changes in gene expression, like 2-D gel as well as mass spectrometric protein sequencing, sequential gene expression analysis, as well as cDNA microarray analysis.Both metabolic or gene expression techniques look at an organism's phenotypic flexibility, although from distinct angles. *Streptomyces tenjimariensis*, as well as many other species, were the subject of registered study[20].

### *3.2.1. Biotechnology's Medical Applications:*

In medicine, the purpose of biotechnology is to combat and treat illnesses. As a result, biotechnology may be used in the following fields of medicine: medication and therapy development, genetically modified organisms, gene analysis in hereditary illnesses, genetic defect rectification, and so on. The following are the important subjects in medical biotechnology, each of which requires a detailed explanation: Bioinformatics, genomics, proteomics, biopharmaceuticals, and biomedicines are all examples of gene therapy.Modern biotechnology has potential applications in medicine, such as pharmacogenomics, genetic testing, gene therapy, or medication development. The study of how a person's genetic composition affects his or her body's response to drugs is known as pharmacogenomics. Gene testing requires inspecting the complete DNA molecule.

The application of engineering and technology concepts to the area of living or biological systems is known as biomedical technology. Biomedical usually refers to a focus on issues of human health and illness. Biomedical engineering and biotechnology are commonly referred to as biomedical technology or bioengineering.Biotechnology is assisting in the fast advancement of medicine today. Molecular medicine will progressively replace traditional medicine. Soon, no sickness will be unknown, and no disease mechanism will be uncontrolled. Biotechnology is now being used by medical research to assess the disease's most extreme forms. Biotechnologists will locate genes in many features and abnormalities since the entire sequence of the human genome was discovered in 2001. Many genes involved in the development of illnesses, such as cancer, cardiovascular disease, respiratory disease, and mental illness, have been discovered so far. Individual genes and their generated proteins can be detected to deliver highly selective and effective treatments to deal with illness [21].

### *3.2.2. Bioprocessing Techniques Using Biotechnology:*

In bioprocesses such as biochemical processes, the down streaming process accounts for the majority of the experiment's cost (50 percent).Biotechnology or bioprocessing were 2 main technologies for improving economic and social well-being. The industrial, academic, or government sectors were bound to encounter technical hurdles whenever establishing competing biotechnological products as well as processes that use synthetic biology, heredity, or molecular genetics as replacements for chemical-based applications.Many approaches have been developed in the phenomena of time reduction and main recovery of the down streaming process.

Biotechnology offers a wide range of uses, involving food design, preparation, and nutrient consumption improvement:

- The development of monoclonal antibody purifying procedures for the diagnosis of various diseases, as well as the characterization of host proteins,
- Hematopoietic stem cell (HSC) generation for therapeutic applications,
- The creation of microbes capable of digesting and converting biomass into fuels,

- Raw material synthesis depends on fermenting methods, like ethanol and butanol,
- And some other bioprocesses compounds that were formerly sourced from chemical sources, like aliphatic, aromatic, as well as other macromolecules.

Several more significant bioprocesses also include a larger quantity of secondary metabolites applied to food, beauty products, pharmaceutical sewage treatment, as well as bioremediation businesses (all of which are high-value procedures) utilizing bacteria and cell organelles produced in vitro to preserve against endangerment or nonrenewable plants or even to obtain metabolic pathways as well as enzymes produced by fungal species, trying to leverage genetic engineering or molecular biology developments [22].For bulk and quality manufacturing, the proper and effective strain must be chosen.In some of the procedures, an indicator test like the Dehydrogenase experiment for able to monitor the Development of Streptomyces venezuelae in a Nutrient-Rich Medium has been used to evaluate to bigger or smaller production capability of a potential antibiotic known as Jadomycin (anti-carcinogenic cytotoxicity) was feasible.

*3.3. Agriculture Biotechnology Applications:*

As explained in Wieczorek [23], the use of biotechnology in agriculture has several advantages, including greater agricultural output, improved crop protection, improved food processing, improved nutritional content, and better flavor. Although the author mentioned possible hazards linked with the application, it is prudent to exercise caution.Agricultural biotechnology is an important part of theResearch instruments used by scientists to better understand and regulate the genetic makeup of agricultural species such as agriculture, cattle, forest, or aquaculture. In addition to genetic engineering, biotechnology includes genomic or databases, markers-assisted selection, micropropagation, cell cultures, replicating, fertility treatments, embryo transfer, and other techniques. Furthermore, in the domain of biotechnology, genetic engineering is used mostly in the agriculture industry and has the most direct impact on agriculture in underdeveloped nations, as well as the most pressing public concerns as well as policy difficulties.Animal diets frequently contain genetically modified crops, products generated from them, and enzymes made from genetically engineered microorganisms. Compound feeds are produced from a variety of basic materials, including maize and other grains, as well as oilseeds like soybeans and canola. They are mostly used for poultry, pigs, and dairy cows.

Biotechnology has been used by humans since the dawn of agriculture. Its applications ranged from the planting of seeds to the breeding of animals. The agricultural industry includes businesses that cultivate crops, raise animals, and gather fish and other creatures from farms, ranches, and natural ecosystems. Agriculture is the lifeblood of any country's economy. Agri goods add to the country's GDP and gross revenue. As the world's population grows, so does the number of hungry people, which has never been higher in human history. Biotechnology plays a critical part in feeding the world's growing population. Biotechnology has played an important role in the performance and development of agriculture, and it has been very turbulent in the past few decades. It is currently considered a safe transaction. Environmental changes, overpopulation, and human damaging actions are still causing problems in the agriculture industry. Water is a basic requirement of all living things; however, our planet's water resources are not evenly distributed.Floriculture is developing day by day as new types are introduced to the market. Insect-pest assaults and disease outbreaks were major hazards in previous decades, particularly in developing nations. Gene modification has also resulted in the introduction of pesticide and herbicide-resistant crops; such cultivars provide economic benefits even at the small-scale farming level. Transgenic

organisms with high-quality features have also been introduced into agriculture, animal husbandry, and fish production. Plant and animal illnesses are difficult to identify because the symptoms might be deceiving or even missing until the disease has caused significant harm.

These transgenic crops can thrive in a variety of environments and have higher nutritional value, as well as being used to produce vaccines and healthcare products for the human population. Basic tools for transgenic variations include tissue culture, micropropagation, and DNA marker aided techniques. These techniques are already being used to aid in the diagnosis, treatment, and prevention of plant and animal illnesses, as well as breeding and conservation efforts. Biotechnology is used to supply researchers with fresh information and to make their work more efficient and productive. Biotechnology also addresses environmental issues that influence agriculture, either directly or indirectly [24].

### *3.4. Biotechnology in the Food Processing Industry:*

Biotechnology is employed in food processing in the form of fermentation bioprocesses, food additives, and processing aids in food formulations. Enzymes, amino acids, vitamins, organic acids, specific carbohydrates, and flavoring compounds are examples of items made by genetically engineered microorganisms.The use of biotechnology in the food business is quite similar to the usage of biotechnology in agriculture as a whole. In this post, we'll look at biotechnology in the food sector and how it's changed through time. Biotechnology's application in the food business is mostly dependent on the usage of enzymes found in various microorganisms.

The food business is known for being cautious about how much it invests in research (usually 1.5 percent or less of sales) and how quickly it adopts new technologies. Biotechnology research is currently financed mostly by traditional power bases such as DuPont and Monsanto, as well as an expanding number of tiny research businesses known as boutiques. The majority of these small businesses are highly driven, responsive, and productive in their fields of specialization. They exemplify what we've come to understand as the entrepreneurial spirit.T The genetic engineering technique to boost food enzyme synthesis is definitely in place. Many key food industry enzymes have had their genes cloned, and gene transfer techniques that allow for their introduction and expression are generally regarded as safe (GRAS) organisms have been established. -amylase and chymosin are two new and essential uses of genetic engineering in enzyme synthesis.Biotechnology is used to make a variety of everyday food products that have been around for hundreds of years, such as wine, beer, vinegar, cheese, bread, or curd, since enzymes, as well as microbes, play a part in their production. Take a look at a handful of these foods and learn how biotechnology may help them become even better [25].

### *3.5. Biosensors and Bioelectronics have Biotechnological Relevance:*

Cadmium could induce bone demineralization in two ways: directly by bone destruction and indirectly through renal failure. In the manufacturing of milk and dairy products, Pb, Cd, Zn, Cu, Cr, and other trace metals are possible bioaccumulative toxins.Cadmium may enter the bloodstream by foods, beverages, and breath, and is absorbed through the skin, although among the non-occupationally exposed population, food, and smoking are the predominant sources of exposure.Because of their great sensitivity and quick reaction time, nano-scale materials have become more popular for electrochemical biosensors.Mn2+ treatment in TiO2 thin films depositing on conductive substrates via the dip-coating method, for example, is being utilized to construct a urea biosensor having ordered as well as self-organized nano-

array structures. In contrast to the sensor research, a parallel study named "Nano-biosensor" has been conducted, which integrates biomarkers using nanoparticles.

The stiffness of the ECM that can influence surviving, multiplication, development, as well as motility are all examples of anchorage-dependent cell activity. Adhesions influence biochemical signaling pathways and mediate force transfer from the cell to the flexible substrate, guiding a variety of processes.Amperometric sensors based on the enzymes from "Brassica napus" roots and leaves to detect ochratoxin, a colorless crystalline compound that belongs to a group of highly associated derivatives of iso-cumarin connected to L-phenylalanine therefore categorized as pent peptides.Every scientist understands that all bioactive molecules, as well as cell organelles, were governed by chemo-mechanical systems. This construction of microtubule (MT) molecules that are modeled to function as just a diode in electronics variables is a multidisciplinary art that allows them to be activated and work as an electrical device. Aside from the foregoing, building an immunological sensor is a job that is connected to (or dependent on) the immobilization of antibody molecules, which is a vital factor in the manufacture of the function of the immune detectors.

## 4. CONCLUSION

Biotechnology may be used in the environment, medicine, agriculture, food processing, or industry, according to the study. As science progresses, it was noticed that the fields and extent of application would continue to expand. It was determined that biotechnology research efforts should be focused on the highlighted dangers and obstacles, particularly in agricultural applications.In the current world, this is the most important field, which can change the future of the society.This study was focused on biotechnology's attention in crucial fields like biomaterials, biosensing, genetic modification, biometrics, bioinformatics, as well as bioprocesses. This study thoroughly reviewed every sub-head in the current review and attempted to assume that the procedures or occurrences explained in each sub-head comprise unique applications of biotechnology methodologies; the aforementioned industries and commercial products are extremely fast-growing but besides a few issues in genetic engineering

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# CHAPTER 8

# A STUDY ON DEATHS PER MILLION POPULATION FROM COVID-19 IN INDIA

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***ABSTRACT:Coronavirus disease2019 (COVID-19) has impacted every country in the globe, with India being amongst the worst affected. Coronaviruses are enclosed viruses, positive-sense coronavirus genomes are single-stranded or non-segmented which leads to difficulties in development of an effective drug against them. Seven distinct coronaviruses have been revealed to be capable of entering human hosts and causing respiratory illnesses. Lockdowns have been enacted in various countries, barring individuals from leaving their homes unless essential. It is excellent that the Indian government is taking proper actions to restrict COVID-19 transmission in the general population. COVID-19 may have less of an impact on India for several reasons, the topic of this study. This study includes the most current incidence of SARS-CoV-2 infection, diagnosis, and death in India. This study also focuses on environmental and biological elements that may aid in reducing the detrimental effects of COVID-19. A few research has been conducted on topics such as cross-immunity, the innate immune system, polymorphism in the Angiotensin Converting Enzyme (ACE) enzyme, and viral genetic changes.***

***KEYWORDS: Angiotensin-Converting Enzyme (ACE), Coronavirus disease2019 (COVID-19), the Middle East Respiratory Syndrome (MERS),Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2), World Health Organization (WHO).***

## 1. INTRODUCTION

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), produces COVID-19, which is considers as a very lethal viral disease worldwide. There are a wide variety of COVID-19 indications, including temperature, cough, and respiratory problems. Extreme symptoms of pneumonia or hypoxemia have a high death rate [1].SARS-CoV-2 is more often seen in adults and the elderly, although it can also affect children and babies. SARS-CoV2 infection poses a larger danger to those over the age of 65 who already have a significant medical condition. A pulmonary pandemic has grown since the first instances were discovered in Wuhan, China, in December 2019 [2]. COVID-19, also referred to as Novel Corona Virus is linked to a human respiratory ailment that has been labeled a global pandemic or epidemic declared by the WHO for the first quarter of 2020[3].According to the most recent statistics (6th April 2020) from John Hopkins University [4], as well as other monitoring databases,The Novel Corona Virus has infected more than 1.3 million individuals throughout the globe, and at least 75,000 people have died as a result. Coronaviruses infect their hosts and cause sickness [5].

United States; Spain; Italy; Germany; China; Iran; Turkey and the United Kingdom are among the top 10 countries affected by Ebola. The most deaths have been documented in Spain, Italy, the United Kingdom, the United States, and France.On the list of countries with the most people who have successfully recovered from the virus. There was a time when India had a secure place at the top of the ranking of infected nations; however, current revelations have propelled it up to the 27th position, which is the reason for fear.The rate of death is now under control at less than 3%, which is lower than the global mortality rate of 5.5 percent, however, the pattern of spread is progressively approaching an exponential trend, which might result in huge loss of life and equipment [6].Nations all around the globe now

look to India as a global leader in the fight against this epidemic, and even WHO has conceded as much. Over one-fifth of the population lives in India, which is also the world's second-largest nation. India is the second sign to global GDP and is one of the world's most renowned emerging countries, with relatively good economic expansion statistics [7]. Following the finding of the causal culprit,As soon as the outbreak in China became known, WHO officials notified the organization, and sequenced data was communicated to the rest of the globe. The WHO acted quickly by organizing diagnostics discovery, releasing recommendations on healthcare management, laboratory testing, and treatment, or disseminating up-to-date epidemic data. Several nations in the region, including the United States, we're keeping an eye on Wuhan visitors for signs of 2019-nCoV infection. Symptoms of 2019-CoV seem to be minor compared to SARS furthermore, the Middle Eastern Respiratory Syndrome (MERS), has been documented in China, Thailand, Korea, as well as Japan [8].

## 2. LITERATURE REVIEW

Muhammad Qasim et al. in study modeled the transmission of COVID19 using WHO data and daily government report from Japan, China, and Korea. They defined the two states data to obtain the mean ratio of current cases to total cases. This ratio indicates COVID-19's future.The predicted case ratio was 0.485, while the death ratio was 0.49. Despite precautions and therapies, the ratio of new cases to the previous day is comparable to daily fatalities to last-day deaths. The authors predicted a minimum of 8660 fatalities and a maximum of 117397 in the following 30 days. In Iran, a substantial rise in fatalities is projected in the next 30 days, from 1140 to 598478. Japan contained COVID 19 and minimized fatalities, they discovered.Countries with insufficient health resources suffer more from sickness and mortality. The author concluded that the approach should be regarded as the basis for future development by incorporating each country's health resource index [9].

M. Mahbub Hossain stated in a study that COVID-19 has become a worldwide health issue because of its pathogenicity and spreading.The existing studies' features were evaluated quantitatively, and representations of COVID-19 research's knowledge domains were developed utilizing statistical and text-mining methods and bibliometric tools, and R software.This study included 422 journal articles, editorials, emails, as well as other references. Maps of author, institution and country networks illustrate academic relationships. Genetic, epidemiological, zoonotic, or other biological subjects were found using keywords and textual information. The author concluded that socioeconomic factors and COVID-19 effects need additional investigation. Encourage worldwide research cooperation to pool resources. The worldwide knowledge base should be improved for evidence-based decision-making to avoid and treat the COVID-19 pandemic and its consequences [10].

Tarek Alsaied et al. stated in the study thatcoronavirus illness 2019 is caused by SARS-COV-2, a newly identified viral infection with devastating cardiovascular repercussions.COVID-19 infection has been linked to a higher risk of serious injury in patients with cardiovascular-related abnormalities, such as those with congenital heart disease (CHD), according to current research. Myocarditis, arrhythmias, and myocardial infarction are among the COVID-19 cardiovascular symptoms. Furthermore, the pandemic has strained healthcare infrastructures, and so many individuals on the care team are in danger of getting and perhaps transferring the disease, therefore making it more difficult to treat cardiovascular disease patients. With a focus on sufferers of congenital heart disease, the author examines theyoung people's response to the COVID-19 vaccine, and also the cardiovascular system's involvement in the research [11].

## 3. DISCUSSION

This viral genera belong to the family Coronaviridae, under the group Nidovirales, and may be split into three groups based on antigenic and genomic features [12]. Canine (CCoV) and Feline (FCoV) coronaviruses, together with pig transmissible gastroenteritis virus (TGEV), Porcine Respiratory Coronavirus (PRCoV), or Pork Epidemic Diarrhoea Virus (PEDV), are all members of coronavirus subgroup 1A in this study. Group 2 is further classified as 2a and 2b based on whether they are bovine-like coronaviruses and SARS-like coronaviruses. The latter includes the recent Canine Respiratory Coronavirus (CRCoV). The third coronavirus (CoV) category includes avian-derived CoVs such as the avian bronchitis virus [13].The genome sizes of coronaviruses range from 27 to 31 kilobytes, making them the biggest RNA viruses that have been discovered to date. They have an envelope that is generally spherical and is between 100 and 200 nanometers in diameter, and they have a fringe of petal-shaped spikes that measure around 20 nanometers in length and contain single-stranded positive-sense RNA seen in Figure 1, in addition, the genomes of the various CoV strains are structured in the same manner, sharing the same order for a group of five genes.

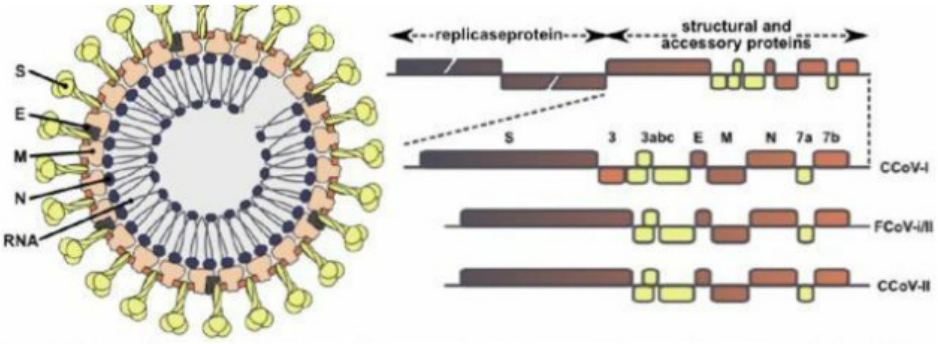


**Figure 1: Depicts the Coronavirus Structure as well as Comparison of the Ccov and Fcov Genomes** [14]**.**

*3.1.Types:*

The "Coronavirinae" subfamily is a part of the "Coronaviridae" family. Coronaviruses are members of this subfamily. The severity of the ensuing sickness and the extent to which it can spread varies across different strains of human coronaviruses. There are now 7 varieties of coronaviruses that may spread disease, according to doctors.

- *The common type of Coronavirus:*

Alpha coronaviruses 229E, NL63, OC43, and HKU1 are all examples of this kind of virus (beta coronavirus).

- *Transmission:*

There has been little investigation into how Human Coronavirus (HCoV) transmits from person to person. Furthermore, experts believe that viruses spread through fluids in the respiratory system, like mucus.

*3.2.Coronaviruses can be transmitted in the following manner:*

Droplets can be expelled into the air when a person sneezes or coughs without covering their mouth. Touching or holding hands with someone infected with the virus could spread the infection among people.Attempting to make touch with a surface or item that is contaminated with a virus, followed by making irritation to the eyes, nose, and mouth.Feces may spread certain animal coronaviruses including the feline coronavirus (F-CoV).It's also unclear whether this holds for human coronaviruses. Some people, according to the National

Institutes of Health (NIH), are more likely than others to have COVID-19-related issues in this list:

- Infants and adolescents.
- People above the age of 65.
- Women who are expecting.

Coronaviruses would affectpeople of all ages and backgrounds are affected at some time. Coronaviruses are very infectious due to their ability to evolve.If a person's condition worsens, they should remain at home and rest to avoid the spread of the disease. They must also refrain from making direct eye interactions with people. While coughing or sneezing,using a napkin or towel to cover one's mouth and nosein addition, coughing or sneezing might reduce the risk of infection.To maintain a clean and sanitary atmosphere, it is vital to properly dispose of any tissue that has been used. Home cleanliness is important.

*3.2.1. Typical symptoms include:*

- Cough
- Fever
- Breathing Problem
- After infection, this might take 2–14 days for an individual to detect indications.

*3.3.Corona Virus Life Cycle:*

- The virus's attachment and subsequent penetration into the system
- Expression Replicase for proteins
- Transcription and Replicating
- Assembling and Deploying

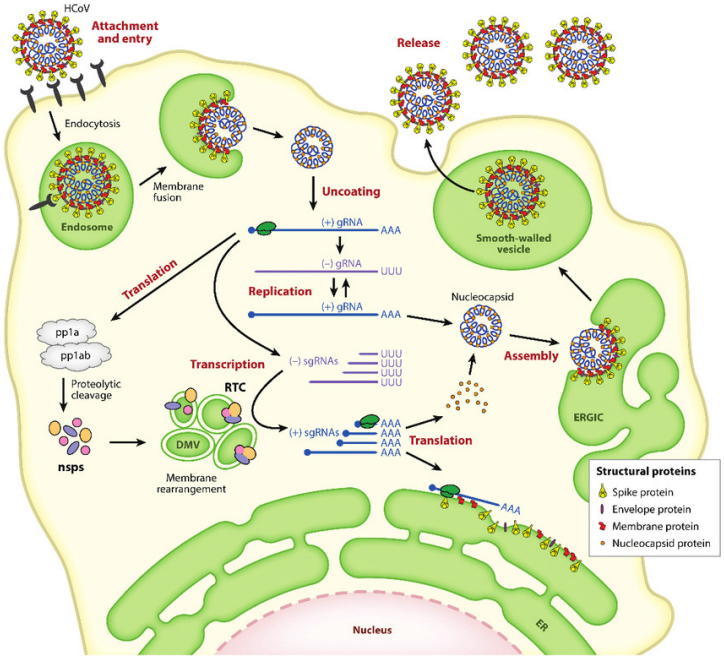


**Figure 2: Representing Entry and Release in the Life Cycle of Corona Virus** [15]**.**

Covid-19 infection begins with the attachment of the spike (S) protein to certain host cellular receptors. The host possesses a specific receptor that is a primary determinant of the virus's pathogenicity, tissue tropism, and host range shown in Figure 2. The S protein is made up of two domains: S1 and S2. When the S1 domain interacts with its corresponding receptor, the S protein undergoes a conformational shift, which promotes membrane fusion between the viral and cell membranes through the S2 domain.Some host cells utilize their substances to prevent viral invasion as well. Many groups have identified a family of Interferon-Inducible Transmembrane Proteins (IFITM) that may prevent worldwide circulating Human Coronavirus HCV-229E and HCV-NL63 S protein-mediated entrance, as well as the highly pathogenic SARS Coronavirus and MERS Coronavirus. While the route of action of IFITM remains unknown, cell-to-cell fusion experiments done by several research groups show that IFITM3 prevents enveloped virus entrance by blocking viral envelope fusion with the plasma membrane or endosomal membranes by altering host membrane fluidity.

Coronavirus replication begins with the translation of Open Reading Frame (ORF) 1a and 1b into polyproteins pp1a (containing 4382 amino acids) and pp1ab once the viral nucleocapsid has been released and uncoated into the cytoplasm (7073 amino acids). The ORF1b that follows it in the translation chain is translated by a ribosomal frameshifting process, which involves the translation ribosome moving one nucleotide in the 1 direction from the reading frame of ORF1a to that of ORF1b.Virion assembly is quickly followed by the accumulation of additional genomic RNA and structural components. The helical nucleocapsid holding the genomic RNA combines with other viral structural proteins (such as S, E, and M proteins) to create the formed virion during this stage of the infection life cycle. Coronavirus particles are formed by the helical nucleocapsid budding through membranes early in the secretory route from the endoplasmic reticulum to the Golgi intermediate compartment. The host's role in this stage of the infection process has received little attention.The M protein now orchestrates the whole assembly process by choosing and ordering the viral envelope components at the assembly sites and encouraging interactions with the nucleocapsid to enable virions to bud. The M protein interacts with other viral structural proteins, such as the E protein, to form a mature virus. This interaction creates the virion envelope scaffolding, stimulates the budding and release of the M protein-modified membrane, and collaborates with the S protein to assemble the spikes into the viral envelope. After assembly and budding, the virions are carried in vesicles before being exocytosis.

*3.4.Epidemiology:*

According to reports, Wuhan had an unusually high number of pneumonia cases in December of this year.It was on December 12, 2019, that the first instance of the COVID-19 epidemic was discovered, which would be an unusual case of pneumonia. As of the 31st of December, 2019, several 27 cases of viral pneumonia have been reported, 7 of which have been considered serious. Due to comparable virus backgrounds of these patients,research into the causes of disease in people who have sought to be patients at the hospital has been carried out, which has increased the likelihood that an ailment has beenanimal-to-human transmission. Group 2 of beta-coronaviruses that contains the Severe Acute Respiratory Syndrome Associated Coronavirus (SARS COV), was discovered on January 22, 2020 [16].

Even while SARS-COV and COVID-19 are both members of the same beta coronavirus subtype, only 70% of their genomes are identical,new group has been found to differentiate from SARS-COV in terms of genetics. Due to these factors, the highly contagious disease was able to spread rapidly, posing enormous challenges for pandemic treatment and prevention. As seen in Figure 3, the next Summer Festival of China will take place between the dates of January 10 and February 18 in the year 2020. Furthermore, between January 10

and 22, there was a dramatic spike in COVID-19 instances. Wuhan, the epicenter of the outbreak,10 million people populate the city, which serves as a vital transportation center during the springtime. India, like every other nation, has been fighting a tough battle against the coronavirus (COVID-19) epidemic. A large population, income disparity, and poor healthcare are some of the causes that have contributed to the situation being even more severe. The Indian state of Maharashtra was able to identify more than 1,900 instances of the disease in April 2020, after carrying out the highest number of diagnostic tests ever carried out in the nation. During the same period, the state of Sikkim conducted the fewest possible tests, and there was not a single instance of the disease found in Figure 3 [17].

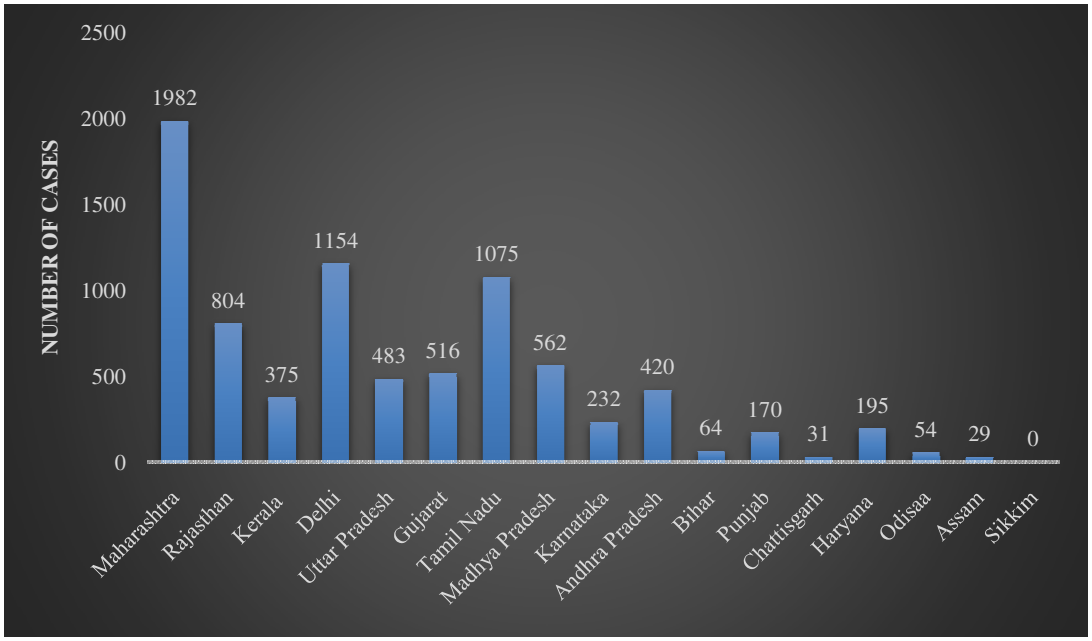


**Figure 3:As of April 12, 2020, the following is a breakdown, by state, in India, of the number of cases reported of COVID-19** [18]**.**

*3.5.Signs and indications:*

Coronavirus infections often cause mild cold or flu-like symptoms that manifest anywhere from two to four days following the first sickness. In addition, the symptoms might Virus strains may vary from person to person, and some strains are deadly.

*3.5.1. Among the symptoms are:*

- Runny –nose
- Diarrhea
- Sneezing and Cough
- Asthma
- Sore-throat
- Fever

In contrast to rhinoviruses, which are another potential source of the cold virus, human coronaviruses are extremely difficult to grow in a controlled environment. Because of this, evaluating the coronavirus's impact on the nation's economy and also the issues it faces in terms of public health is difficult. In the absence of a cure, treatment options are limited to self-care and over-the-counter medications. There are several options available to those who want to protect themselves against the effects of climate change:

- Getting enough water
- To use a fresh humidifier or vaporizer with a cold mist
- A clinician can identify the virus involved by collecting samples of pulmonary fluids, like nasal mucus or plasma.
- Common precautions to avoid infection transmission
- For pain and fever, take acetaminophen, ibuprofen, or naproxen.
- Rest and avoidance of overexertion

It includes hand washing regularly, covering one's nose and mouth while coughing or sneezing, or fully frying meat or eggs. Prevent direct contact with someone who has pulmonary sickness breathing difficulty or sneezing.

*3.6.Treatment, Diagnosis, and Preventive Measures:*

In the majority of cases of self-limiting sickness, coronavirus testing is unnecessary because the condition will resolve on its own. Therefore, identifying an etiological agent may be necessary for specific clinical and veterinary contexts, as well as in epidemiological investigations.Detection is especially critical in areas where a significant COV epidemic is happening,where MERS-COV is still spreading in the Middle East. Impact the formation of public health measures to control epidemics by reporting incidents [19].It is essential, not only for the management of these infections but also for the preservation of food sources, to identify people that suffer from severe COV-induced illnesses, such as PEDV and IBV, in animals. Multiplex real-time RT-PCR techniques that can diagnose all 4 respiratory HCOVs or would be further tailored to new COVs have been made to diagnose human COV, RT-PCR is the technique of choice. In cases when RNA is difficult or impossible to extract, and also in observational studies, serologic tests might be beneficial [20].

Because there are no effective interventions or vaccinations, the approach to controlling human coronaviruses is to have a strong public health monitoring system in place, along with swift diagnostic imaging or quarantine whenever appropriate. Coordination between political bodies, public health agencies, and health care professionals is crucial in worldwide epidemics. During easily transmitted veterinary epidemics, PEDV, more harsh measures, including the eradication of whole pig herds, might well be required to limit the spread of these lethal viruses [21].

## 4. CONCLUSION

There have been reports of a wide range of ailments in both people and animals. Because of their ability to recombinant, modify, or transmit multiple species and cell types, those viruses are expected to continue to emerge or mutate, creating veterinary or human epidemics. The upcoming coronavirus study will focus on various areas of viral replication or pathogenicity. Recognizing these viruses' proclivity to leap among species, develop infectionsthe host has been changed, or find substantialreservoirs for coronaviruses will considerably enhance our ability to predict the timing and location of prospective outbreaks.Therapeutic targets for the treatment of infections should significantly grow as a result of these investigations. ADP-ribose1-phosphatase, one of the specific enzymes generated by coronaviruses, is also observed at higher eukaryotes, finding coronavirus research essential for molecular formula or bioinformatics research more generally. Understanding the peculiar RNA replication or transcriptional methods adopted by these viruses will be outlined in this study by giving a full explanation of the Replication-Transcription Complex (RTC). The host's immune-pathological response to coronaviruses can be examined which might considerably enhance our ability to develop vaccinations or decrease the strain of illnesses.

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# CHAPTER 9

# ANALYZE THE POSSIBILITIES IN FORENSIC SCIENCE OF DNA FINGERPRINTING

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***ABSTRACT:****The study of human genetics and scientific technology hasrecently made significant progress. The finding of deoxyribonucleic acid (DNA) was a watershed point in scientific history. DNA fingerprinting has changed criminal investigation, person or connection analyses, hereditary illnesses, as well as many other applications for examination of migration patterns.The genome's uniqueness has been discovered to be due to repeated sequences. The forensic application of DNA profiling is a significant commitment to an invention that can assist not just the criminal but also the innocent.This research delves into the principles of DNA fingerprinting as well as the various technologiesoften used in the laboratory, like, AFLP, touch DNA RFLP, RAPD also STR. The applications of such information are not yet completely realized, the area has endless potential. This study tries to discuss the fundamentals of DNA fingerprinting as well as its applicability in forensic science.This study also discusses an attempt that has been made to expand on the shifting scenario of the technology in recent years, as well as to present some genuine circumstances in which several variants of the DNA fingerprinting technology were effectively connected in comprehending criminal proceedings in the research center.*

***KEYWORDS:*** *Chromosome, DNA (deoxyribonucleic acid), DNA Fingerprinting, Forensic Science, Genetic Fingerprinting,Short Tandem Repeats (STR).*

## 1. INTRODUCTION

Human genetics has advanced tremendously in recent times, so scientists were capable of distinguishing between individuals using DNA owing to this field of science and its accompanying approaches.Following the discovery of Dr. Alec Jeffery, the geneticist from British has created a new sophisticated forensic analysis instrument[1].It is more precisely described as the discovery of a sequence of binding to a genetically designated stretch of DNA Strands inside a chromosomal segment or the possibility of being related to those other members of the family.Because uni-ovular (identical)the sequencing of twins will be the same; however,the probabilities of two distinct individuals who possess the same sequences are one in a million billion, while siblings have a one in 10,000 million probability. Regardless of the type of blood, the quantitative accuracy of samples of DNA beyond the entire population of humans is trustworthy and specific to every individual[2].

The ringmaster of life, deoxyribonucleic acid (DNA), is an essential component of the biological system. It is a molecule containing all of the necessary instructions for the biotic community's survival, development, as well as reproduction.DNA mutation patterns in genes are linked to hereditary disorders[3]. They have decoded the tri-nucleotide code of amino acids [4] to investigategenetic engineering or DNA fingerprinting techniques [5], and it is being widely employed in genome editing approaches to create a strong microbiological variant and also animals and plants varieties [6]. Every cell's blueprint is made up of sequences that carry directions for the creation of key proteins or molecules in the form of nitrogen bases such as “Cytosine (C), Thymine (T), Adenine (A), Guanine (G)”. Forensic science is a subject that focuses on the identification and examination of evidence obtained from crime scenes. It entails collecting and analyzing fingerprints, footprints, calligraphy,

tire markings, gunshots or armor-piercing, bite marks, scars, and biological material like blood, hairs, bones, and so on [7].

Since the development of fingerprints, a couple of centuries ago, DNA has been used to identify people, analysis has been regarded as one of the most significant technological achievements in criminal investigation.Biochemical DNA identification studies are likely to reject a cleared citizen while linking the offender.When a DNA test is carried out correctly, the findings are extremely replicable as well as definitive enough to confirm the accused defendant's guilt or connect the accused to the crime.When the DNA test is completed correctly, the results are highly reproducible and conclusivesufficient to establish the suspect's defendant's client is guilty.Genetics is the study of inheritance.The human body is made up of approximately ten trillion cells, and each has a nucleus (save for red cells) with microscopic fiber structures known as chromosomes. The chromosome contains some genes that influence many aspects of a person's appearance, like the color of skin, posture, facial structure, hair texture, etc. Every cell of the system has created 46 chromosomes (44 autosomes and 2 sex chromosomes) which are found in 23 pairs in typical healthy people. Because once fertilization happens, the chromosomes, containing the genetics of the ovulation (from the mother's ovaries) and sperm (from the father's testicles), are likewise transferred to the infant [8].

The chromosome is made up of nucleic acids (DNA) and proteins. The DNA molecule contains the information that permits the body to function and also provides everyone with a unique appearance. The discovery of the DNA structure, the basis for people, in 1953 by James Watson and Francis Crick, revolutionized molecular biology or genetics, and they were given the Nobel Prize in 1962 [9].The DNA molecule consists of two strands. Every strand comprises a sugar-phosphate framework with the nucleotides Adenine, Cytosine, Guanine, or Thymine attached to it and directed toward the double-stranded molecule's core.DNA is composed of 2 strands of 4 chemical bases (A, T, C, and G) and acts as a recipe book for cellular proteins. The precise arrangement of the four chemical bases in a DNA strand determines whether protein is generated. A fragment of DNA opening with ATTCGC generates a much more protein isolate than one beginning with CCGTAT. This is made by reordering the characters of a word. Although the letters are the same, the interpretation is different.

The 23 pairs of individual chromosomes contain around 25,000 - 30,000 functional genes that control numerous physiological activities. A gene is a little bit of DNA that encodes for a specific bodily function.A locus is a location or place on a chromosomal that requires a unique sequential gene. Sir Alex Jeffery, a British biologist, created the phrase "DNA fingerprinting." Since then, DNA fingerprinting has grown in importance as a tool in law enforcement. Only 10% of entire genomic DNA encodes for specific functional properties, whereas the other 90percent of the total isnon-coding DNA, often known as junk DNA. The non-coding DNA includes the most variety of polymorphisms as well as forms the foundation for forensic DNA fingerprints[10].

According to the Human Genome Project (HGP), the world's biggest collaborative research initiative for sequencing the human- genome, only 1.5 percent of the human genome's genes are encoded, with the remaining DNA considered "junk DNA."Variable number tandem repeats (VNTRs) or minisatellites were particular repeating sequences that make up a major component of junk DNA. DNA fingerprinting is among the most revolutionary discoveries of the twentieth century [11], and it may be used by people considered the same species and from a little number of biological samples.It has primarily been used in forensic science to identify suspected criminals utilizing biological samples and to recognize damaged dead

bodies, as well as for medicinal reasons such as order to detect genetic conditions, finding appropriate tissue donors for organ transplants, trying to link close connections as well as blood relatives, etc. This study also discusses or explains the many kinds of DNA fingerprinting methods used in forensic science applications in this article.

## 2. LITERATURE REVIEW

The objective of the study conducted by aLutz Roewer was to investigate to analyze the possibilities of DNA fingerprinting in forensic science, the DNA fingerprinting is among the most important innovations of the twentieth century, so it revolutionized the forensic investigation.This overview outlines 30 years of progress in forensic DNA analysis, which assists in the convictions of criminals, the investigation and prosecution of falsely accused persons, and also the identifying the victims of crime, natural disasters, or conflicts. Current conventional approaches focused on small tandem repeats (STRs) and also lineage indicators (Y chromosome, mitochondrial DNA) were addressed, with casework examples illustrating applicability. The advantages and disadvantages of increasing forensic DNA libraries were examined, as well as researchers speculate on the development of forensic DNA fingerprinting [11].

Fatima Farrukh et al. stated in a study according to study, DNA evidence can assist us to hold the genetic system in living beings. Every person in the cosmos has their identity embedded in every cell of their body. Using the paternity test, you may identify the link between a person's DNA and alter it. However, DNA fingerprinting in Forensic science significantly contributes nowadays in that it makes it easier to identify a person involved at the crime scene using samples gathered by the team, which aids the police in finding the guilty. Criminology is a science that works with a wide range of alternatives to arrive at an appropriate conclusion. Several types of studies are continuously being conducted to improve the precision of DNA fingerprinting [12].

Laura K. Palmer discussed in a study this employs personalitiesusing a "hook" from the Harry Potter films to stimulate students' curiosity in commencing forensics. Students conducted "RFLP (restriction fragment length polymorphism)" evaluation was carried out utilizing low-cost devices and was intended to analyze the information from a modeled criminal investigation. However, the course provides for consideration of the constraints of using DNA fingerprinting for forensic purposes, and also an assessment of a common misconception that the advanced scientific involved in solving crimes is perfect [13].

Jónathan Heras et al. proposed in a study that DNA- fingerprinting also known as genetic fingerprinting technology enables the comparing of DNA patterns as well as the measurement of genomic relatedness across samples.This method may be applied in a variety of areas (food- industry, agriculture, medical diagnosis, forensic science, parentage testing, also many others). This comparability of pulsed-field gel-electrophoresis (PFGE) sequences is a significant challenge in infectious disease epidemiology.This is used to identify the genetic variation of microorganisms in the aftermath of epidemics or to monitor certain copies of particular interests. The pictures generated by DNA profiling can be difficult to interpret at times, as well as several tools have been created to help with this process. They give a survey of techniques for analyzing DNA fingerprints in this paper.Researchers analyze 33 tools in particular to use a set of predetermined parameters. Hands-on experiences or inspection of tool documentation were used to compare the tools wherever feasible. Because no system is preferable in all potential cases, we designed a worksheet that investigators may use to find the optimal system for their purposes [14].

## 3. DISCUSSION

Finding fingerprinting a century ago, DNA analysis has been regarded as one of the most significant technological achievements in criminal investigations. DNA profiling studies performed on genetic evidence were likely to reject an innocent civilian orconnect the criminal.Whenever DNA profiling is implemented right, the findings are extremely repeatable and conclusive enough to affirm a criminal's defendant guilty and link the guilty to the crimes.When DNA testing is performed appropriately, the findings are highly replicable or definitive enough to show the accused defendant's case beyond a reasonable doubt.

### *3.1. DNA and Its Structure:*

The genetic substance of living creatures, DNA, is located largely in the cell nucleus. Chromosomes are long threadlike structures made up of DNA. Chromosomes have related proteins, primarily histone modifications, which arrange the DNA into its natural form. Many human cells contain 46 chromosomes, one pair of each kind. Exception of male and female sex cells (gametes), which are haploid and have just one of each kind of chromosome, the majority of cells contain pairs of chromosomes and thus are termed diploid (Figure 1). The zygote, or fertilized egg, has 46 chromosomes, 23 of which are maternally derived or 23 of which are paternally derived [15].

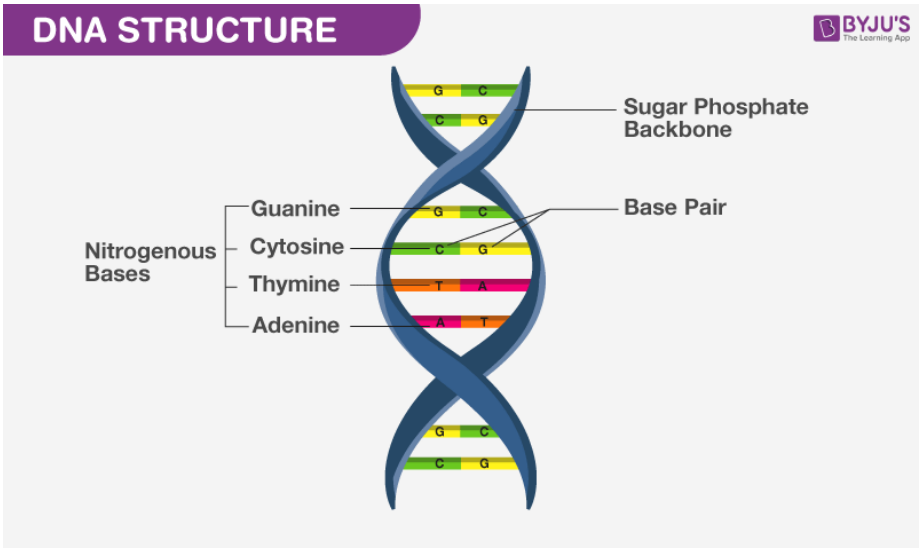


**Figure 1: Represents the structure of DNA and its nature** [16]**.**

The unique biological characteristics that exist between humans and other living species originate from the variable arrangement of the nucleotide "*Adenine (A), guanine (G), cytosine (C), or thymine (T)"*. Humans' genomic DNA has roughly 3 billion nucleotide sites shown in Figure 1[17]. Genes are located on the 46 chromosomes in distinct areas known as genetic loci. Furthermore, several genes occur in various versions known as alleles. Genetic polymorphism refers to the presence of several alleles at the same genetic locus. Multiple alleles at a particular locus may exist in a population, and so this genetic variation is the biological basis of DNA Evidence fingerprinting [18].

### *3.2. Methods for Fingerprinting DNA:*

In the area of forensic science, DNA fingerprinting has been the most recognized sort of forensic evidence. Since its inception, enormous progress has been achieved in DNA fingerprinting procedures. In general, four procedures are completely conducted in DNA Analysis:

- Evidence collection: a sample is collected andreferred to a forensics lab for examination.
- DNA isolation: To acquire pure DNA, DNA is extracted from biological samples using various techniques such as extraction procedure, ion exchange, solid-phase extraction, and so on.
- PCR amplification: certain repeating sections of DNA are replicated to increase the volume of DNA available for future analysis.

DNA fingerprinting: a variety of methods have been used to obtain the distinctive profile of the DNA, including "short tandem repeats/simple sequence repeats (STR/SSR), amplified fragment length polymorphism (AFLP), mitochondrial DNA (mt DNA), touch DNA as well as restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD)" analysis to confirm the sample's relationship with the suspect. The specifics of these various DNA fingerprinting techniques have been described here.

*3.2.1. "Restriction Fragment Length Polymorphism (RFLP)":*

RFLP is a procedure that involves treating genomic DNA with one or even more restriction endonucleases which reduced the DNA whenever a specific sequence of nucleotides takes place (so every restriction enzyme means cutting in a varying restriction enzyme). As an outcome, a variety of DNA fragments of various lengths is generated. Several kinds of restriction endonuclease enzymes are found in bacterial cells. As part of the bacterial body's immune response, these enzymes aid in the battle against invading phages as well as other external genetic material [19].The enzymes identify certain nucleic acid patterns that are referred to as restriction sites. This restriction endonuclease recognizes a distinct restriction site.Once the restriction endonuclease enzyme recognizes the specific sequence, it activates its nuclease activity as well as destroys the phosphodiester link (a covalent connection in DNA or RNA which connects polynucleotide chains).

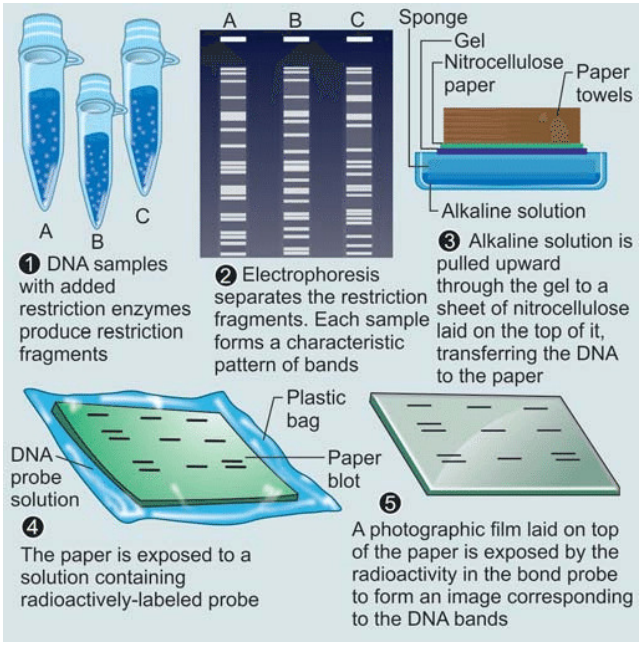


**Figure 2: Depicts the Procedure of Using RFLP Probes In Genome Mapping And Variation Analysis** [20]**.**

The utilization of restriction fragment length polymorphisms is the foundation of DNA profiling. Because restriction enzymes have a three-dimensional structure, they may connectto the double-strand DNA molecule or move down a helix until they come across a precise series of sequential pairs that tells the enzymes to cease. When a restriction site exists at more than one position on a DNA molecule, a restriction enzyme would break at each of those spots, producing several fragments [21]**.**Following that, electrophoresis isolates DNA fragments based on their relative size. Smaller fragments would move farther than bigger bits over time. Fragments of the same size recombine and move in single DNA "band." An RFLP probe is a labeled Segment of DNA that hybridizes with one or more fragments of processing DNA throughout gel electrophoresis, resulting in a particular blotting pattern diagnostic of specific genotypes at a single location. Figure 2 depicts how RFLP probes are frequently used in genome mapping or variation analysis (genotyping, forensics, paternity testing, hereditary illness diagnoses, and so on).

*3.2.2. "Random Amplification of Polymorphic DNA (RAPD)":*

Polymorphism is the type of genetic variation that occurs at a specific location in the DNA. RAPD is a technology that uses PCR to identify polymorphisms, as well as the resulting product, and is submitted to band pattern detection. RAPD, like PCR, employs random markers in its reaction. To replicate the sequences in RAPD, primers of random nucleotide sequences are utilized [22].A primer of 10-15 nucleotides designed for RAPD fingerprinting of any random sequence, for example, would link to the genomic DNA molecule's complementary regions.The transitional sequences between both the two primers will not be replicated during PCR [23], if the 3' ends of the two primers are far apart nevertheless if the 3' ends of the two primers are near together, the sequence will be amplified.

*3.2.3. "Short-Tandem-Repeats (STR)/Simple Sequence Repeats (SSR)":*

One type of DNA polymorphism is short tandem repeats (STR) or microsatellites. These repetitive sequences may be found all across the genomes of eukaryotes and prokaryotes including humans.

STRs are typically composed of a 1-6 bp short repeated sequence of DNA,producing a sequence that emerges as a repeated, tandemly stitched sequence of nucleotides that may be up to 100 nucleotides long each repeat STRs were most typically found in non-coding areas, while around 8% were detected in coding regions [24].The probability of STR in the genetic code is one every 2000 bases on average (Lander et al., 2001). The STR region is denoted as D19S1234, where D remains for DNA, 19 is the chromosomal number on which the STR locus is found, S represents STR, as well as 1234 is the unique number for that specific STR.

STRs can be categorized based on the length of the repeating units or the repeat structure's foundation. STRs are categorized as mono- or single-stranded, di-, tri-, tetra-, and poly-stranded hexanucleotide or pentanucleotide repetitions STRs discovered in the human genome were dinucleotides in nature. STRs are based on repeated patterns. Sequence stretch repetitions can be characterized as basic or perfect repeats consisting of only one type of repeating unit, and complex or imperfect repetitions, i.e. sequences made up of a varied mix of repeats[25].

*3.3.DNA Fingerprinting Applications:*

All of the following instances have made use of DNA fingerprinting:

- Because of DNA typing, accused and convicted offenders may be released.
- Identifying human connection

- Investigating ancient people's kinship
- Families' DNA testing
- Evaluating the efficacy of bone marrow transplantation
- Demonstrating the kinship of immigrants.
- Finding biological parents (paternity testing)

The first disclosure in the research of a case involving the use of DNA fingerprinting in a 'paternity' situation to determine to identify the unidentified decomposing human bones. Although standard procedures are unable to confirm the victim's positive identity, DNA typing approaches utilizing blood from the victim as well as the victim's presumed parents were utilized to establish the victim's identity [26]. The mitochondrial DNA derived from Enamel is kept for a period of 3 months to twenty years. Donor blood derived from teeth or their maternal relations was used as a based-on comparison. They also discovered that teeth were a richform of DNA which might be useful in prolonging the degradation time. Human remains might be genetically identified [27]. When dealing with potential DNA evidence, two significant problems are degradation and contamination.Degradation is the breakdown of DNA into smaller fragments caused by physical or chemical causes, while the infection is the introduction of foreign material into the sample. Extended exposure to temperature or moisture, and potential causes of degradation include conditions that might leadto the development of bacteria, mold, and mildew on a specimen. The contamination may happen when two DNA samples are intermingled, or if a deposition is accidentally left in the evidence. Both degradation and infection may lead to inaccurate or irrational consequences [28].

*3.4.DNA Finger printing's Possible Applications:*

The uniqueness or progress of DNA fingerprinting technology has been a benefit in terms of producing significant enthusiasm in the field of criminal justice. These tools are no longer restricted to legal analysis; they are also being used to solve mysteries at the molecular scale.

*3.4.1. Identification of Criminal:*

DNA fingerprinting techniques have been generally acknowledged by courts across the world as sensitive and reliable proof of the case. Blood, hair, saliva, or sperm specimens are gathered from crime scenes and used as conclusive evidence in a case. Because the DNA in each cell of the body is unique, the DNA may be used to identify a person. It is feasible to identify a guilty person from a list of targets using only a small amount of DNA.DNA fingerprinting technology has been used to solve several cases throughout the world [29].

*3.4.2.Diagnosis of Inherited or Genetic Diseases:*

Because the kid inherits both parents' DNA, there is always the risk that if one parent has a hereditary condition, the trait will be passed down to the child. Evaluating flaws in DNA sequences from persons with or without a condition might help forecast the emergence of hereditary illnesses.DNA fingerprinting is a quick process that helps scientists to discover genetic illnesses such as hemophilia, thalassemia, sickle cell disease, or cystic fibrosis using the parents' samples, which could be passed on to offspring. It might be valuable for illness detection, treatment, as well as therapy [30].

## 4. CONCLUSION

DNA fingerprinting is a reliable as well as an important tool in forensic science for linking criminals with biochemical tests in incidents. Newly developed approaches have

outperformed traditional techniques such as RFLP, RAPD, STR, and now determining.Over the last decade, technologies for collecting and processing specimens have improved, increasing the likelihood of effective DNA profiling. By employing mt-DNA analysis, it is feasible to identify the individual even in collections that have been degraded, damaged, and festered. Furthermore, a variety of automated or robotic devicesthere have developed robotcapabilities for a wide range of tasks, from quick extracting robots to lab automation environments.Indian Biosciences offers DNA ancestry services DNA testing, paternity testing, and also genetic testing. Expertise or DNA genealogy in India offers unequivocal answers to emotive inquiries professionally or discreetly.This study presents that DNA analysis techniques are fairly specialist, and several future simplifications may increase the simplicity of use. Better methods for preserving DNA samples in safe locations will presumably lead to bigger advancements in the futureTrying to cure genetically susceptible disorders will assist many people in need and people of the future. These DNA fingerprinting applications were virtually infinite. Profiling has proven to be among the most important technological resources in history and also helps forensic science.Because of advancements over the last 3 decades, Forensic evidence is today considered one of the most credible proofs in the courts. This is beneficial not just in criminal cases, but also in predicting hereditary ailments, analyzing population dynamics, selecting the appropriate match for organ donors, paternity testing, recognizing victims in catastrophes and conflicts, and building lineages. In the future, the introduction of nanotechnology technologies, as well as a microfluidics platform and a universal DNA database, will make studies easier, faster, and more efficient for subsequent processing.

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# CHAPTER 10

# ANALYSIS OF EFFECT OF EXERCISE AND PHYSICAL ACTIVITY IN THE PREVENTION OF CHRONIC DISEASES

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***ABSTRACT:*** ***Chronic diseases are long duration, non-communicable, and sometimes permanent conditions that change over time. The global healthcare burden is tremendously increasing as treatment costs and management of these diseases are becoming difficult day by day. According to recent reports, non-communicable diseases (NCDs) are accounting for 41 million death each year globally including cardiovascular diseases (CVD), cancers, respiratory disease, and diabetes on the top of the list. Physical activity and exercise are now being considered the most important interventions for the management as well as prevention of major chronic diseases. The focus of this study is to provide evidence that supports the effectiveness of physical activity as cost-effective therapy for non-communicable diseases. This review also provides a deep insight into the direct relationship between sedentary behavior and chronic diseases. The present study evaluates physical activity as a capable measure to prevent a wide range of chronic diseases. Initiatives from both the public sector and government are required to ensure the active participation of individuals in physical activity to deter the increasing burden of chronic diseases.***

***KEYWORDS:*** ***Cardiovascular Disease, Chronic diseases, Coronary Artery Disease, Non-Communicable diseases, Physical Activity.***

## 1. INTRODUCTION

Chronic disease is a condition that is not communicable, progresses slowly, usually last 1 year or more, and is a result of one or more factors like genetics, environment, or poor lifestyle. Chronic diseases are now requiring immediate attention for their prevention and effective management. One of the reasons for the rise in chronic disease prevalence is associated with the adoption of physical inactivity. The increasing trend of chronic diseases is more frequently seen in the population of low-income nations [1]. However, the significant impact of these diseases has also been noted in developed nations. Heart disease, cancer, and diabetes are some of the leading chronic diseases as per "The Centers For Disease Control and Prevention (CDC)" and the latest reports [2]. They are also fueling the demand for healthcare systems and ultimately increasing the health care cost of the nation [3]. Poor nutrition, tobacco, excessive use of alcohol, and physical inactivity are the modifiable risk factors that can increase or decrease the susceptibility of an individual to chronic conditions [4]. Many chronic conditions benefit significantly from minimum physical activity and structured exercises. These advantages include improved quality of life, fitness, and muscular strength, in addition to the prevention of the condition. This is especially essential for older adults since regular physical activity can help them maintain their independence. Physical activity and exercise have been shown to lower the occurrence of chronic diseases and increase disease-free life expectancy, making it a preventative strategy for the health industry.

### *1.1.Understanding concepts behind physical activity and exercise:*

Many people get confused with the terms "physical activity" with "exercise". However, they both have a significant difference which is needed to effectively prevent and manage the prevalence of chronic diseases. Any bodily movement produced by the muscle contractions with energy requirement is known as physical activity whereas the exercise is organized, planned, and executed to improve and maintain standards of physical fitness and thus defined

as a subset of physical activity [5]. There are significant researches that show all types of physical activity help in improving the health and well-being of an individual irrespective of his/her conditions. On the other hand, exercise contributes more to physical fitness. Performing one of them or both of them can significantly improve the quality of life by preventing chronic conditions.

*1.1.1. Types of Physical Activity and Exercises:*

Physical activity like walking, wheeling, cycling, participating in sports, or engaging in active recreation gives major health advantages for the majority of chronic illnesses. Coming to cardiovascular conditions physical activity benefits in different ways: directly by regulating risk factors such as obesity and elevated BP or by enhancing endothelial function as well as by having a positive influence on the coagulation mechanism [6]. Exercises are classified into four categories endurance, strength, flexibility, and balance with each having one or more benefits for patients with chronic diseases. Endurance exercise also referred to as cardiovascular exercise has proven to be beneficial for the performance of the heart, lungs as well as whole cardiovascular system ultimately reducing the risk of associated diseases.

*1.2. Common Chronic Diseases and their relation to Physical Inactivity*

A mix of physiological, genetic, environmental, and behavioral factors contributes to the cause of chronic illnesses which are usually of long duration [7]. They are also referred to as non-communicable diseases as they don't possess the characteristics of communicable diseases like infections. NCDs impact people of all ages, from one country to another. Although these chronic diseases are typically associated with older age groups, evidence suggests that a large portion of NCD-related mortality occurs between the ages of 30 and 69 [8]. The risk factors that lead to NCDs affect all including children, adults, and the elderly. Physical inactivity is one of the important factors to be stressed on. Figure 1 shows more deaths associated with non-communicable diseases while communicable diseases in the row with 29% of deaths.

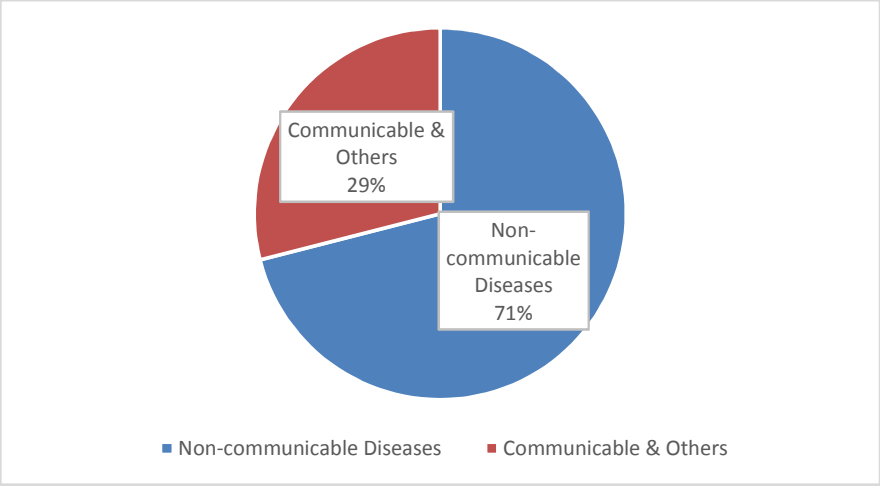


**Figure 1: Illustrating the Global Death Distribution from Communicable and Non-Communicable Diseases Accounting for 29% and 71% Respectively.**

Physical activities and continual exercise habits have been demonstrated to be effective in several studies concerning the management of chronic conditions. Physical activities are helpful to the health of both young children as well as for elder people. The progression of various chronic conditions such as diabetes, cancer, heart diseases, respiratory diseases, and others including obesity are coupled with a lack or complete absence of physical activity and

a bad diet [9]. According to current studies, an increase in chronic conditions such as obesity, cancer, respiratory diseases, diabetes, cardiovascular disease (CVD), and stroke would reduce life expectancy for future generations. Figure 2 shows the separate contribution of non-communicable diseases to the deaths occurring globally. Research suggests that including daily physical activity (PA) and exercise in lifestyle significantly reduces the risk of chronic conditions and related deaths while also serving as a major disease prevention strategy.

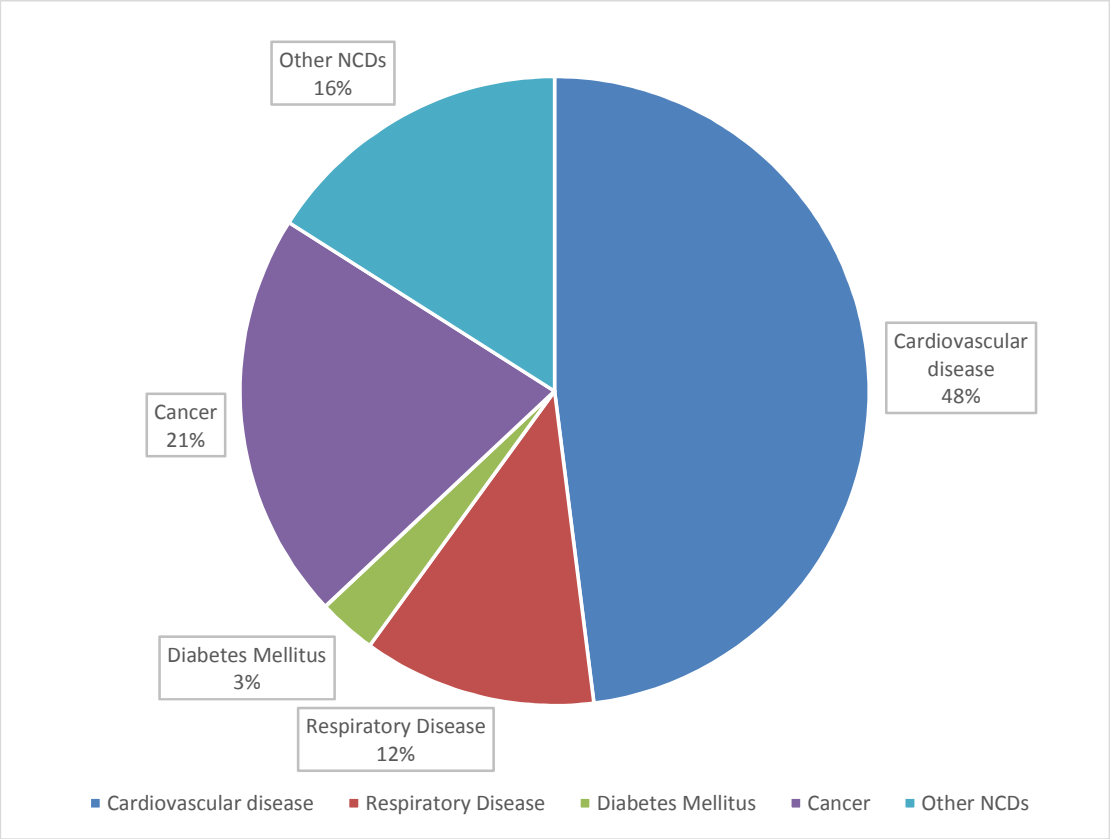


**Figure 2: The above chart illustrates death distribution from different non-communicable diseases in which cardiovascular diseases account for 48%, the respiratory disease accounts for 12%, cancer accounts for 21%, diabetes mellitus accounts for 3%, and other NCDs account for 16%.**

Some of the chronic diseases that are greatly associated with physical inactivity and a sedentary lifestyle are highlighted as follows:

### *1.2.1. Cardiovascular Diseases:*

Cardiovascular disease (CVD) is one of the prevalent chronic illnesses affecting the majority of the population practicing a sedentary lifestyle. CVD is all in one term for the conditions associated with a heart as well as the other accessory components of the cardiovascular system. Commonly its association has been noted with an accumulation of fatty deposits into blood vessels called arteries as well as the increased risk of developing blood clots. Type of CVD including coronary artery disease (CAD), strokes, arterial disease, aortic disease, and many others can be largely prevented by leading a healthy lifestyle.

When it comes to heart conditions, physical inactivity is considered one of the important modifiable risk variables after age.In US, CAD is the major cause of mortality. Healthcare services for CAD are expected to cost more than $200 billion each year in the United States

[10]. Exercise and physical activities have been shown to lessen the risk of coronary artery disease and its development. Physical activity and exercise do this by:

- Increasing the diameter of the artery
- Reducing myocardial oxygen demand
- The dilating capacity of coronary arteries
- Slowing the development process of coronary artery atherosclerosis
- Increasing collateral artery development.

Furthermore, increased activity is linked to reduced blood pressure, higher levels of high-density lipoproteins which are healthy lipoproteins, and lower levels of low-density lipoproteins which contribute to the development of CAD and improved insulin sensitivity and glucose tolerance [11][12].

*1.2.2. Diabetes:*

Diabetes happens when enough insulin is not generated by the pancreas or when the body system of an organism is unable to make effective use of produced insulin. As per the raw estimates released in the CDC report, 34. 2 million population of all age groups has diabetes which is making this chronic illness to have immediate attention [13]. People are becoming less physically active which can be seen globally. Non-communicable diseases (NCDs) are on the rise as physical activity declines. Diabetes is one of the four major NCDs, accounting for the majority of disease burden and early death together with CAD and other diseases [14][15]. Current research has been supporting the link between sedentary habits and the progression of diabetes. Physical inactivity is a modifiable risk factor that can initiate as well as fuel up the progression and pathogenesis of diabetes from normal glucose metabolism. However, regular exercise and physical activity can reverse the pathogenesis from different stages of diabetes with the potential to completely reverse the process.

Physical activity has been regarded as an effective therapy for Type 2 diabetes. Apart from other factors such as selecting energy-rich foods and population aging, physical inactivity is well-known as one of the key causes of the increasing epidemic of type 2 diabetes mellitus.Exercise of any intensity has been shown to be a positive element in reversing the most of known type 2 diabetes mellitus factors to a healthy condition. Exercise has been shown to provide therapeutic advantages such as enhanced insulin sensitivity and higher peak oxygen consumption (VO2peak), all of which show preventative effects on Type 2 diabetes [16]. Another study showed that in comparison to sedentary rats, diabetic rats with exercise training have lower TG levels, lower blood glucose levels, and lower body weight [17]

*1.2.3. Obesity:*

Obesity is defined as a chronic condition described as abnormal or excessive fat deposition that presents a health concern. However, obesity is frequently misinterpreted and portrayed as a lifestyle problem that can be remedied by encouraging individuals to 'eat less'. However, the level of physical activity is a factor that also contributes to the condition of excessive fat accumulation.Physical activity and exercise increase total energy expenditure, which can aid in maintaining energy balance or even weight loss. Weight lifting, push-ups, muscle-strengthening exercises, and other workouts build muscle mass. This increases the number of calories burnt during the day and simplifies weight control even if the body is at rest.

*1.2.4. Cancer:*

The word "cancer" covers a range of conditions having an effect on any body part [18]. It is the biggest reason for mortality globally, with 10,000,000 deaths in 2020. Breast, rectum, prostate colon, and lung cancers are the most frequent malignancies that have been reported

[19][20]. The advantages of exercise training and physical activity for patients having cancer are also becoming more apparent. Physical activity has been demonstrated to lower tumor development and cancer incidence with various molecular mechanisms that are now being investigated intensively [21].

As per the latest reports of the WHO, 1800000 deaths due to lung cancer, 916000 deaths due to colon including rectum cancer, 830000 deaths due to liver cancers, 769000 deaths due to stomach cancer, and 685000 deaths due to breast cancers were reported in 2020 which is mentioned in Figure 3 [20].

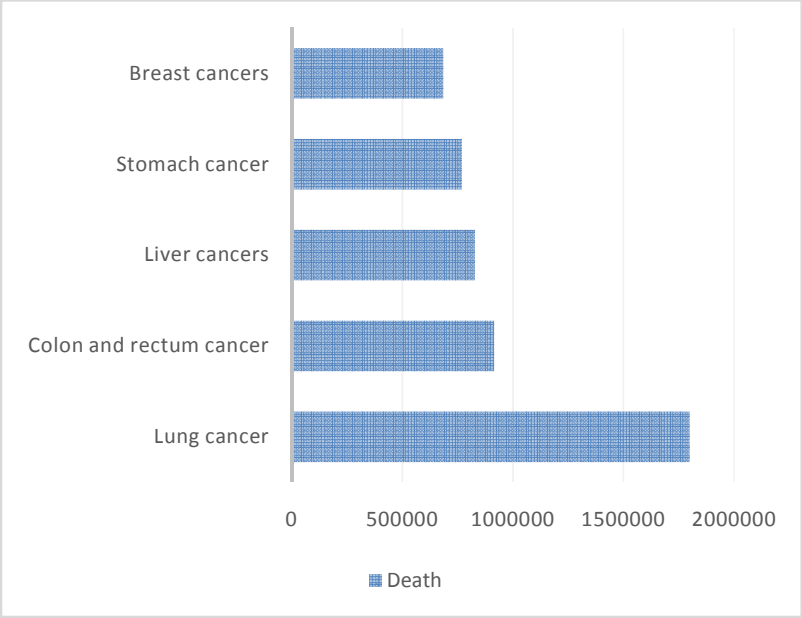


**Figure 3: Graph Represents an Increasing Number of Deaths from different kinds of Cancers Including lung Cancer Accounting for Major Deaths. Data of Death Distribution from different kinds of Cancer.**

Physical activity and exercise have been found linked with a lower risk of a variety of cancers, including lung, breast, colon, and endometrial cancer. Physical exercise lowers all-cause and cancer-related mortality in patients suffering from colon and breast cancer as well as enhances their quality of life and functional status throughout cancer treatment. Physical activity demonstrated a reduced cancer risk through several physiologically plausible processes. A sedentary lifestyle needs to be minimized to reduce the risk of colon, endometrial, and lung cancers.Furthermore, physical activity and exercise after and before a cancer diagnosis is considered to be beneficial for individuals with breast and colon cancer to have a better chance of survival.

### *1.2.5. Chronic Respiratory diseases (CRDs)*

CRDs including “pulmonary hypertension”, “pulmonary sarcoidosis”, “chronic obstructive pulmonary disease (COPD)”, “asthma”, and “occupational lung diseases” impose a health burden making CRD a major public health issue worldwide. The research has revealed an exponential increase of 39.5% in the total number of CRD cases, a 49.8% increase in the cases of COPD, increase of 81.1% has also been reported for the cases of pneumoconiosis from 1990 to 2017. The covid-19 disease has also affected the quality of life of a significant population from every country [22]–[24]. Research reveals that older adults who are involved

in physical activity and exercise had improve lung function throughout life. Conversely, it has also been noted that patients with COPD are less active in their course of life [25].

### *1.3. Other Benefits of Physical Activity and exercise*

Physical activity (PA) is considered a strong gene modulator that produces functional and morphological alterations in the brain leading to considerable advantages in cognition and well-being. PA may also help to prevent neurodegeneration. According to current data, many types of studies have been undertaken to investigate the influence of exercise and physical activity on neurological disorders such as Alzheimer's disease. Elevated cerebral blood flow, transport of oxygen to cerebral tissue, decrease in muscular tension, and increased serum concentrations are all benefits of exercise and physical activity demonstrated by several research studies. Neurotransmitters have also been noted to rise with physical activity and exercise. Exercise and physical activities have been shown to reduce depression in several studies. It has also been shown that those who exercise regularly are less likely to be suffering from anxiety. As a result, physical activity can be used as a no-cost treatment for many chronic illnesses. Cross-sectional and longitudinal studies have also suggested a significant relationship between lower levels of stress with increasing physical activity. Conversely, it has also been found that individuals practicing a sedentary lifestyle (physical inactivity) are more susceptible to psychological distress.

### *1.4. Physical Activity in Quality of Life (QOL)*

The concept of QOL has experienced a major shift. WHO defines the quality of life as a multidimensional concept associated with "individuals" perception of a complete state of psychological, social, and physical well-being. It does not entail only the lack of any disease condition. Figure 4 illustrates the three components; social well-being, psychological well-being, and physical health which determine the quality of life of an individual.

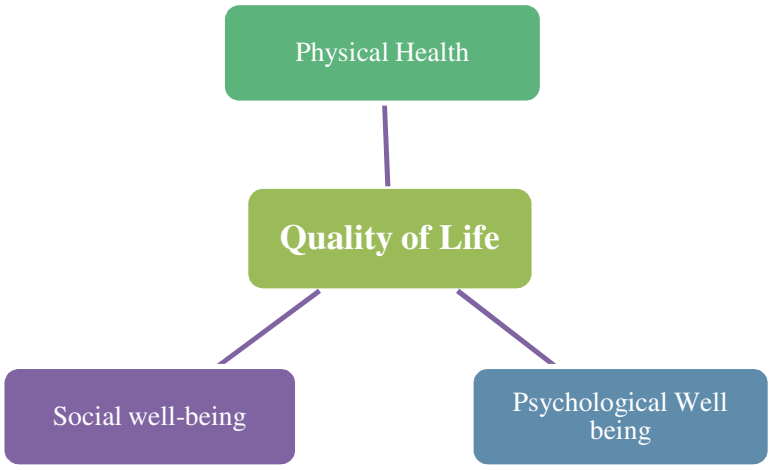


**Figure 4: Quality of Life: A component Determined by Physical Health, Social well-being, and Psychological Well-being.**

While it is critical to have a deep understanding of the relationship between quality of life, psychological well-being, and physical health, it is even more critical to understand what element or variables may concurrently enhance all three and it can be found that one of the most essential aspects of increasing quality of life, both psychologically and in terms of physical health-related well-being, is frequent physical activity, as seen above. This study provides a thorough overview of the increasing healthcare burden of chronic diseases. Physical activity in the management of chronic diseases was also reviewed in a set of

research studies. The author has evaluated and discussed different levels of exercise and physical activity as interventions to improve the symptoms of patients having different chronic diseases such as CAD, obesity, respiratory diseases, diabetes, and cancer which can help researchers and scientists in their study to confirm the effectiveness of researched interventions in the management of chronic diseases.

## 2. LITERATURE REVIEW

Research by Chan et al. assessed the effectiveness of Tai Chi exercise on cardiovascular disease in comparison to brisk walking. A randomized controlled trial was performed with 246 adults having a cardiovascular disease with one-three modifiable risks. Tia Chi and brisk walking were assigned to two different groups with a moderate intensity which revealed that They found that Tia chi is superior to brisk walking for the management of cardiovascular diseases [26]. A survey study by Brugnara et al. assessed the association among physical inactivity and the development of diabetes with the help of a cross-sectional, population-based, and randomly sampled study. A total of 4991 individuals were studied with the help of physical activity questionnaire. As a result, they found that physical inactivity in 32.3% of men as well as 39% of women. Low physical activity was also found to be linked to 44% of known diabetic individuals, 43% of prediabetic diabetic individuals, and 38% of individuals with normal glucose regulation [27].

Way et al. evaluated and reported the positive influence of low-volume high-intensity interval training (HIIT) on patients with Type 2 Diabetes. A comparison of low-volume HIIT was carried out with a sham placebo control on hemodynamic responses and arterial stiffness in obese and Type 2 diabetes patients. Participants were allocated randomly to HIIT or PLA. Groups were exercised three times per week for 84 days. They found out that 12 minutes of low volume HIIT significantly improves heart health and arterial stiffness in physically inactive individuals with Type 2 diabetes and obesity [28]. Lee et al. assessed 160 patients with COPD, out of which 103 patients were found to be lacking in physical activity which was demonstrated by a univariate analysis [29]. Apart from COPD, asthma patients also benefited from the different intensity of exercise. Hansen et al. assessed the effect of aerobic exercise on asthma patients with a meta-analysis where they included 11 studies with 543 adults with asthma with heterogeneity in their age and body composition and reported improved asthma control with interventions such as walking, jogging, spinning, and treadmill running [30].

Galvao et al. evaluated the influence of exercise in preserving physical functionality in patients that were suffering from bone metastases and prostate cancer. In their study, they studied 57 patients with bone and randomized them to different exercise programs thrice a week. They found the significant differences in the groups with significant improvements that were assessed in terms of self-reported improvements in physical functions as well as no reported complications and bone pain [31]. Using a randomized clinical trial, Minnella et al. investigated the impact of exercise on functional capacity in patients with esophagogastric cancer. The primary analysis included 51 of the 68 patients. After comparing with the control group they found that exercise improved the functional capacity of patients with esophagogastric surgery [32].

The above research studies help to determine the therapeutic and preventive effects of exercise on specific sets of diseased conditions using randomized trials, cross-sectional studies, population-based studies, and meta-analyses. But the focus of this paper is to provide an assessment of physical activity and exercises as a measure for a wide range of chronic illnesses. This review also provides a large body of supportive studies for the reverse association among a sedentary lifestyle and the risk of chronic disease development.

## 3. DISCUSSION

Chronic diseases are becoming a major public health concern across the world, costing billions of dollars in yearly healthcare costs and more than millions of deaths each year. Physical inactivity and other lifestyle variables are strongly linked with the development of many chronic diseases. New primary and secondary disease preventive strategies are critically needed to help mitigate the harmful consequences of these illnesses. Physical activity and exercise are increasingly considered preventative strategies for chronic illnesses. Investigations and research studies conducted over the last century strengthen the link between physical inactivity and the development of chronic diseases. Even though physical inactivity is not the core factor in the development of chronic disease, it has drawn a lot of attention in recent years. Chronic diseases are the silent killers because they pose health until the condition worsens to an advanced stage that too without showing any symptoms. Patients with these conditions, or those who are at risk of developing them, require treatment that is tailored, proactive, and long-term. Not only chronic diseases but physical inactivity is also associated with a range of other diseases thus making physical activity an important part to be included in the life of an individual. People of all ages ranging from children to older adults have been found to benefit from a short time of physical activity such as five times walking for 30 minutes a week can retard the disease conditions. Although there are many benefits of being physically active, most adults, as well as many children, practice a sedentary lifestyle which is defined as not engaging in leisure time activities such as sports, exercises, and hobbies that involves the movement of body parts and muscle contractions in a period of two weeks.

This review clearly states the need to encourage the population to be more physically active. However, making people more physically active is now a challenge and further requires an awareness program as well as the commitment to do so. The need of the hour is to do the collaboration between private and government sectors for encouraging individuals to incorporate physical activity into daily life. Regular physical activity is essential for maintaining good health. Therefore, aiming for at least one hour of exercise every day for teenagers is important. Regular physical exercise benefits most organ systems, and thus aids in the prevention of a wide range of health problems and associated diseases. Physical activity provides significant health advantages and lowers the chance of acquiring or dying from several of the most common chronic illnesses. Primary healthcare may plan and implement healthcare program to manage NCDs in each community and diagnose illnesses early. As a result, they can effectively address the issues associated with high healthcare costs.

## 4. CONCLUSION

A sedentary lifestyle shows a proportional link with the development of chronic illnesses. Physical activity and exercise have demonstrated their effectiveness in two ways: i) by serving as a preventative measure, and ii) effective in treating the diseases. Wise selection and incorporation of physical activity in the daily routine of an individual can help decrease the risk of developing chronic disease and can also manage the symptoms of one's chronic diseases. Physical activity has far more complex effects than can be taught from the outside. Much more has to be learned about the mechanisms that cause these effects to occur. Nonetheless, the benefits that may be obtained should lead to research and motivate future studies to achieve the aim of a physically active, and hence healthier, global population while saving significant amounts of public money. Physical activity has far more complex effects than what can be taught from the outside. Much more needs to be learned about the mechanisms that lead to the emergence of these complex effects. Nonetheless, the available

benefits should drive research and inspire future studies to achieve the aim of a physically active, and hence healthier, global population while saving significant public resources in the management of chronic diseases.

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# CHAPTER 11

# ROLE OF MICROORGANISMS IN CLIMATE CHANGE AND THEIR ASSOCIATION WITH BIOGEOCHEMICAL CYCLES

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***ABSTRACT:*** ***Climate change is a widely discussed topic, and global warming is a serious issue, so it is a huge concern. As everyone knows, microbes are part of many processes, such as the nitrogen or carbon cycle, or the demand for and production of greenhouse gases such as carbon dioxide and methane. Microorganisms can react positively or negatively to temperature, making them an important part of climate change models. The level of greenhouse gases is increasing day by day due to both human and natural systems. Examples are plant degradation and the burning of biomass, and the burning of oil, coal, or other fossil fuels. Global warming, often referred to as climate change, is the most pressing concern in the world. A wide range of biological components is damaged or destroyed by it. This affects the form and function of their microbial populations as well as their energy metabolism. Microorganisms hold a lot of promise, especially when it comes to nutrient recycling and greenhouse gas treatment. The main objective of this paper is to learn more about the role of microorganisms in climate change. The prospect of reducing greenhouse gas emissions by regulating terrestrial microbial activities is an exciting future option for tackling climate change.***

***KEYWORDS:*** ***Biogeochemical, Climate Change, Greenhouse Gases, Microorganisms, Mitigation***.

## 1. INTRODUCTION

Microbes have a role in the nutrient cycle, biodegradation/bio deterioration, infection prevention, food spoilage, climate change, control, and biotechnology, among other things. Microbes can be employed for several purposes, including the development of life-saving pharmaceuticals, biofuels, environmental remediation, as well as the manufacturing of food and beverages [1]. For billions of years, microbes have influenced global temperature, but humans have only just begun to alter the composition of the atmosphere and the planet's energy balance. Microbes have a significant role in the generation and ingesting of greenhouse gases, and both natural or manmade $CO_2$, nitrous oxide fluxes, or methane are dominated by microbiology [2], [3]. Microorganisms, on the other hand, exist in complex relationships with other species as well as with ecology, making it impossible to predict their impact.

It is believed that human actions have facilitated the production of additional greenhouse gases by microbes. Major concerns of the twenty-first century include health, climate change, energy supply, disease, and a sustainable environment. Politicians, corporate executives, environmentalists, society, and the media are all discussing climate change at the moment. Microorganisms and biogeochemical cycles are two sides of the same coin. It can be found in both confined and open conditions, including oceans and dirt. Both make it easier to produce and consume greenhouse gases. Microbes react to climatic changes as well as global warming by sending short and long encouraging or discouraging signals. Because microorganisms can recycle and modify the essential elements that make up cells, including nitrogen or carbon they play an important function in the ecosystem as either producers or consumers of these gases. In terms of nutrient recycling, biological methods for reducing greenhouse gas emissions are essential. Microbial diversity in several environments plays a significant role in climate change prevention and response since their metabolism is extremely adaptive or they may develop in a broad variety of climatic circumstances. Microorganisms may readily absorb, store, or release gases [4], [5].

### *1.1. Causes of Climate Change:*

The human or natural forces that alter the Earth's atmosphere are referred to as climate change causes. Several natural and human elements that impact global climate change have been recognized by scientists as shown in Figure 1.

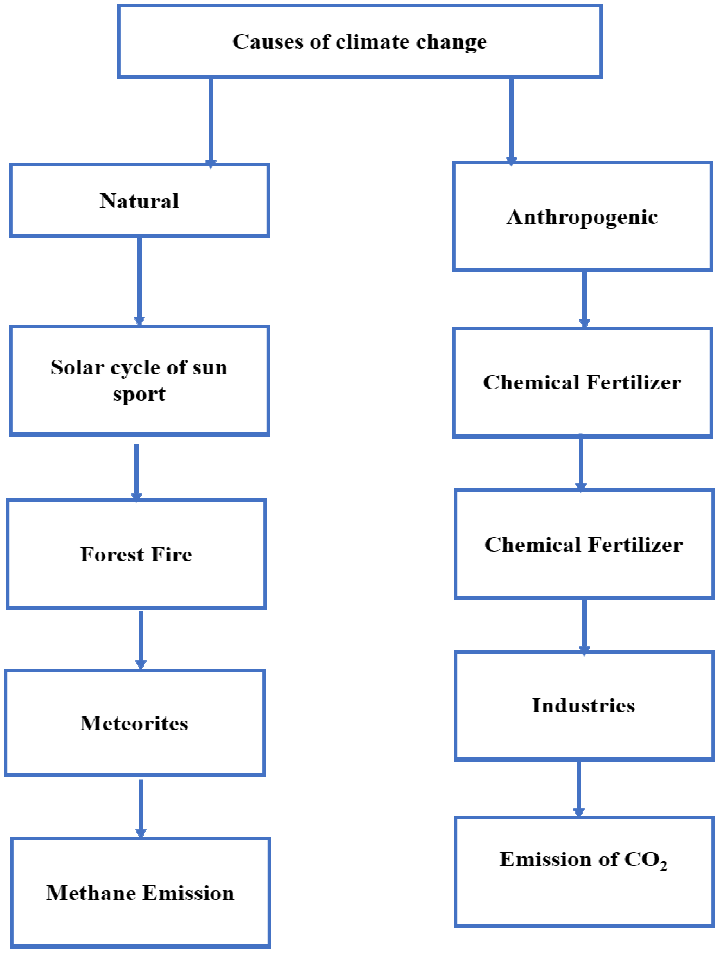


**Figure 1: Illustrate the Major Factors that Causes of Climate Changes.**

### *1.2. Change in the Earth's Orbit:*

The seasonal dispersion of sunlight reaching the earth's surface is affected by the earth's orbit. A little movement in the Earth's orbit can create changes in the planet's distribution. The number of sunshine changes relatively little daily. However, it has a significant influence on regional and seasonal distribution. Changes in the earth's eccentricity, the tilt angle of the Earth's axis, or precession of the Earth's axis are the three forms of orbital variations. The Milankovitch cycle, which has a large influence on climate and is well for its relation to glacial and interglacial eras, might be caused by a combination of these factors. According to the International Panel on Climate Change, the Milankovitch cycles changed the behavior of ice production [6], [7].

#### *1.2.1. Plate Tectonics:*

The mantle plumes or convection currents drove the Earth's Plates to adapt when the temperatures in the center of the Earth changed, causing the plates to rearrange. This has the potential to affect domestic and global climate and atmospheric patterns. The location of the continents determines the shape of the seas. As a result, the pattern of the ocean is influenced

by the position of the continents. The sea's position is also important in managing the passage of moisture in the air around the world, as well as determining the global climate. The formation of the Isthmus of Panama, which prevented the direct mixing of the Atlantic and Pacific oceans about 5 million years ago, is a striking example of the geological influence on ocean circulation.

### *1.2.2. Volcanic Activity:*

Whenever a volcano erupts, gases or dust particles are released, partially blocking sunlight. This may cause the weather to chill down. Although volcanic eruptions usually last a few days, the gases or ashes emitted by them can persist for months, impacting climatic patterns. Volcanic sulfur oxide can mix with water to generate small drops of Sulphuric acid. Because these droplets are so minute, many of them can linger in the air for years. One of the primary climate systems is the ocean current. The movement of something like the water against the sea surface is caused by horizontal winds. The climate of the region is influenced by the temperature fluctuations in the sea.

### *1.2.3. Anthropogenic Elements:*

Climate change induced by human activity has been researched by scientists since the beginning of the twentieth century. Climate change is characterized by a long-term enhancement in the global overall temperature of Earth's climate systems. The increase in average surface temperature is primarily due to human activity. The following are the manmade elements that are creating climate change.

### *1.2.4. Greenhouse Gases:*

The greenhouse gases absorb the sun's heat radiation and the output of greenhouse gases has grown rapidly since the start of the Industrial Revolution. This has resulted in higher heat absorption and retention in the atmosphere. As a result, the global temperature has risen and the greenhouse gases trap most of the infrared released by the Earth's surface rather than solar energy, and the most common greenhouse gases include:

- Vaporized water (the bulk of GHG in the atmosphere, although with a smaller impact)
- Carbon dioxide emitted as a result of natural and manmade processes spends longer in the atmosphere, increasing its influence. Since the beginning of the industrial revolution, the concentration of $CO_2$ has increased by 30%. Deforestation, in addition to the industrial revolution, adds to the increase of $CO_2$.
- Because of their damaging effects on the ozone layer, chlorofluorocarbons, which are used in industry for refrigerants or air conditioning, are a man-made material that is regulated under the Montreal Protocol.
- When organic matter decomposes, methane is created. Because it can absorb more heat than $CO_2$, it is more potent.
- The agricultural industry produces nitrous oxide, particularly in the manufacture and application of organic fertilizers, as well as when fossil fuels are used.

The number of halocarbon gases in the atmosphere is increasing as a result of both human and natural activities. Chlorofluorocarbons (CFC-12 or CFC-11) were widely employed as refrigeration specialists and in other contemporary activities until it was discovered that their amounts in the air caused stratospheric ozone depletion. Because of global rules aimed at protecting the ozone layer, the number of CFCs outflows is currently decreasing. Ozone is a greenhouse gas that is constantly created and destroyed in the atmosphere as a result of chemical reactions.

Anthropogenic activities in the troposphere increase ozone levels by producing chemicals like hydrocarbons, nitrogen oxides, or carbon monoxide, which combine to produce ozone. Human-made halocarbons degrade ozone in the stratosphere, resulting in the Antarctic ozone hole, as previously stated. The most prevalent and significant greenhouse impact is water vapor. On the other hand, human activities have only a minor direct impact on the amount of water vapor present in the atmosphere. Indirectly, the environment's capacity has a significant impact on water vapor. In a warmer climate, for example, there is more water vapor. Since $CH_4$ undergoes chemical decomposition in the stratosphere, resulting in a little amount of water vapor, human activities have an impact on water vapor [8], [9].

### *1.3. Climate Change's Impact on Microorganisms:*

Climate change affects the composition or structure of terrestrial bacterial communities both directly and indirectly. Some of the consequences of climate change on microbes include: Death or disruption are both impacted, enzymatic activity is influenced both directly or indirectly, biomass stimulation/reduction, variety or composition leads to extinction/shift, all of which have varying effects on physiology as well as greenhouse emissions. Whenever the temperature rises, the bacterial community's structures alter, and processes such as fermentation, respiration, or methanogens speed up. The impact of climate change on abiotic or biotic components includes the danger of damage, disease, and mortality from concomitant heat waves, wildfires, violent storms, distinction, extreme heat, floods, natural catastrophes, and drought, poor air quality, as well as the spread of emerging illnesses. Bacteria, fungus, algae, and archaic have the effect of speeding up global warming by degrading organic materials and so, therefore, enhancing $CO_2$ flow in the environment. The microbial decomposition of soil carbon is a major contributor to rising temperatures. Microbial biomass or catalysts are fantastic tools for combating global warming since they consume carbon-based natural resources while also releasing hazardous combinations into the atmosphere and preventing environmental change. The temperature has a direct impact on protein transport and microbial physiological characteristics. Their carbon reactivity to the environment is determined by the efficiency with which soil microbes consume carbon.

#### *1.3.1. The Carbon Cycle and Microbial Communities:*

Microbial communities are crucial aspects of the global carbon cycle because they fix atmospheric carbon, encourage plant development, or break down as well as replace organic materials in the ecosystem. Permafrost, grasslands, tropical forests, as well as other environments at high latitudes, are currently storing huge quantities of organic carbon. Organisms, on the other hand, are in charge of carbon's lifespan and soundness, as well as whether it is released into the environment as an ozone-depleting material, meaning that they are in charge of the carbon cycle's activity. Microorganisms contribute to the reduction of climate change, which also influences crucial ecological processes like the nitrogen cycle, which is dependent on microbial activity. Microorganisms play an important role in the breakdown of decomposing natural materials and their transformation into structures that can be used by various living forms. Consequently, the linked microbial enzymatic framework is considered a fundamental 'motor' for Earth's biogeochemical cycles. The balance between respiration and photosynthesis affects the terrestrial carbon cycle. Carbon-fixing autotrophic microorganisms, such as photosynthetic organisms or photo as well as chemoautotrophic bacteria, transform atmosphere light energy into chemical energy-matter or transport it to the soil. Since they absorb a large amount of carbon dioxide from the environment, organisms require carbon as just a metabolic substrate [10], [11].

Primary activities are mediated by soil bacteria in the terrestrial carbon cycle. To attain their primary goal of survival through reproducing, soil microorganisms must move carbon across

environmental matrices. Microbes obtain fermentable carbohydrates from a variety of chemical carbon sources. The balance between photosynthesis and respiration affects the terrestrial carbon cycle. Carbon can also be found in the Earth's hull, although in the form of limestone or lamp oil. Unlike phototrophic living forms, chemoautotrophic organic organisms obtain their nutrition through the oxidation of non-natural substances. Carbon is found in the air in two forms: carbon dioxide and methane. Carbon dioxide is directly absorbed into the water bodies as a result of climate change. Raindrops also disintegrate in the rain as they descend from the sky. When carbon dioxide comes in contact with water, it reacts with the water molecules to form carbon dioxide, therefore contributing to ocean acidification. Microbes are a component of a larger carbon cycle that takes place on a global level. Microorganisms aid in the removal of carbon from the non-living resources or the availability of carbon to living organisms, with carbon dioxide accounting for a significant portion of the carbon cycle.

This type of carbon is released into the environment but must be converted into a useful natural structure before it can be consumed by living things. Carbon obsession is the unusual interaction in which carbon dioxide is "fixed" from the atmosphere or in natural mixtures. Photosynthesis, which involves using energy from sunlight to make natural atoms, is one of the best-known examples of carbon addiction. In this case, major microorganisms are photosynthetic algae or chemoautotrophs. Carbon dioxide may be converted by bacteria or archaea into a cube of sugar that could be used to make phone calls. The majority of natural carbon is discharged into the air as $CO_2$. The natural carbon that the pecking order's surviving pieces may cycle from one animal to the next. Microorganisms decompose dead organisms and release ozone-depleting chemicals into the atmosphere or soil. Green growth, plants, or microbes convert $CO_2$ to natural carbon when it is broken up in the water. Carbon may be transported from creation to use between species. Bacteria degrade their tissues over time, releasing $CO_2$ into the water or atmosphere. A variety of bacteria and fungi cycle carbon in watery settings. In large-scale oxygen-free settings, such as the deep silt of ponds, lakes, and other bodies of water, anaerobic carbon conversion may occur. In the presence and absence of oxygen, both forms of conversion occur. The process of participating in algae needs the utilization of oxygen. Fermentation is a process through which microorganisms cycle carbon molecules to create energy in anaerobic environments. Carbon cycling may also include other microbes.

### *1.3.2. The Methane Cycle or Microbial Communities:*

Carbon dioxide, or carbon cycling between organic compounds, is seen as vital in terms of the environment. Both eukaryotes ("plants or algae") and autotrophic bacteria (particularly cyanobacteria) are important in the fixation of carbon dioxide in organic compounds. Organic molecules are used by consumers, releasing $CO_2$. Methane ($CH_4$) is a greenhouse gas released into the environment mostly through microbial processes. Microbes that consume methane are vital to the planet's survival. Bacteria use methane as a fuel source in their metabolism. Methanotrophic bacteria get all of their energy from methane, which they convert to ambient $CO_2$ during digestion. These microorganisms can absorb a lot of methane, which helps industries and landfills minimize methane emissions. Microbes eat a lot of $CH_4$ molecules, which are everywhere around us. In anaerobic settings, such as soils heavily compacted by carbon dioxide, methanogen bacteria are easily converted to methane. This procedure necessitates hydrogen and results in the production of water or energy for methanogens.

### *1.3.3. The Nitrogen Cycle or Microbial Communities:*

Nitrogen is the most prevalent element in the atmosphere, accounting for around 78.00 percent of all carbon dioxide. Different nitrogen gaseous components can also be found in the environment, such as NO, $NH_3$, or $N_2O$. Nitrogen is a very stable chemical ($N_2$) that animals or plants cannot utilize until it is fixed. The conversion of gaseous nitrogen into chemical compositions that may be utilized by living organisms is known as nitrogen fixation. Biological fixation allows N2 to reach the biosphere. In intensive agriculture, biological nitrogen-fixing will never completely replace industrialized nitrogen fixation. Mycorrhizal fungi bacteria cause nodules to grow on the roots of legumes such as soybeans and alfalfa. The microorganisms are plant-specific; for example, germs that infect soybeans will not infect alfalfa. When bacteria attach to a plant's root hair, the plant responds by producing a hollow thread that extends into the root. Bacteria multiply along the infected thread until the root becomes a nodule. Bacteria can account for up to 30.00 percent of a nodule's weight [12], [13]. Microorganisms get nutrients and energy from the plant, while microbes, as well as organisms, fix nitrogen from the air for the plant to consume. This is an example of a group of people that are obsessed with nitrogen. Several tiny organisms ("Rhizobium Trifolium") contain nitrogenized chemicals that might be used to fix nitrogen from the atmosphere into a synthetically desirable structure ("ammonium particle") for higher species. The plant converts the 'fixed' ammonium particles to nitrogen oxides as well as amino acids as part of the harmonic interaction, delivering proteins and other compounds such as alkaloids. The nitrogen cycle revolves around the movement of nitrogen from one stage to the next. Microbes frequently pressurize the system to collect energy or nitrogen in a form that is beneficial to their development or growth. There are numerous frequent phases in the $N_2$ cycle:

#### *1.3.3.1. Nitrogen Fixations:*

The initial step is the conversion of the nitrogen to a form that plants can utilize. Microorganisms that break down nitrogen into ammonia and nitrogen-fixing bacteria come in two varieties. Free-living bacteria (non-symbiotic) include cyanobacteria, algae, Anabaena, Azotobacter, blue-green, Beijerinckia, Nostoc, or Clostridium. Inside the soil of leguminous plants, mutualistic (symbiotic) bacteria, particularly Rhizobium, are discovered. Both free-living and symbiotic bacteria are capable of nitrogen fixation.

#### *1.3.3.2. Nitrification:*

Living organisms transform ammonium into nitrates, which are then absorbed by the microorganisms. Soil-dwelling microscopic organisms and other nitrification microorganisms complete the alkali to nitrate conversion. The oxidation of ammonium by bacteria such as the Nitrosamines species, which converts smelling salts to nitrites, is the most important phase in nitrification ($NO_2$). The conversion of nitrites to nitrates is carried out by Nitrobacteria and other bacterial species ($NO_3$). Because ammonia gas is harmful to plants, it is converted to nitrites or nitrates.

#### *1.3.3.3. Assimilation:*

This stage shows how plants obtain nitrogen through their mechanisms. Plants use their root hairs to absorb nitrates from the soil. It is eventually employed in the creation of biological components such as nucleic acids, amino acids, or chlorophyll. In crops having a cooperative partnership with rhizobia, some nitrogen is retained directly from the knobs as ammonium particles. Nitrogen is desired by other living creatures in the pecking order.

#### *1.3.3.4. Ammonification:*

The decomposition stage is when decomposers such as fungi and bacteria convert nitrogen into ammonium when living things die. The process of ammonification, also known as

mineralization, produces one kind of ammonia. The only enzymes involved are Gln synthesis ("plastid or cytosolic"), Glu 2-oxoglutarate aminotransferase ("ferredoxin or NADH dependent"), and Glu dehydrogenase. In the soil, it takes the form of the positively charged ammonium ion ($NH_4$+). This charge binds nitrogen to clay soil minerals, preventing nitrogen loss by leaching and runoff.

*1.3.3.5. Denitrification:*

After the cycle, more nitrogen atoms in the soil are released into the atmosphere. The process of turning nitrates back into practically harmless nitrogen gas is known as de-nitrification ($N_2$). Two bacteria that specialize in this task are Pseudomonas and Clostridium. Rather than using oxygen as an electron acceptor, they utilize nitrate. Denitrifying bacteria in the soil transport or create an incredible number of nitrates or nitrogen gas, which is genuinely latent and unavailable to plants.

- Some of the climate change mitigation strategies
- Less chemical use on farms due to a reduction in the need to spray crops.
- Reduce the use of artificial chemical fertilizers in agriculture or replace them with plant-promoting microbes that operate as biofertilizers via bio-inoculation. Furthermore, GHS emissions can be readily stopped.
- Bio-based items may be produced in a variety of businesses by replacing enzymes and microorganisms for fossil raw materials or fuel (wood).
- Utilizing biofuels or putting bio-based strategy and goals in place take, for example, bioethanol.
- Live organisms or their waste are used to make biofuels.
- Ethanol, which is made from plants, is one of the most well-known biofuels. As a result, biofuels made from food, such as sugar sticks, are unlikely to provide a long-term replacement for non-renewable energy sources.
- Bio-based chemicals and polymers have the potential to replace fossil-based counterparts, resulting in large and quantifiable greenhouse gas emissions reductions.
- Introducing new species to the environment is critical.
- Improving the drought tolerance of biotic organisms.
- Reducing and preventing agricultural water loss
- It is thus a straightforward undertaking to implement a worldwide afforestation program and carbon sequestration.

An increase in greenhouse gases in the atmosphere causes positive climatic forcing or warming. The cumulative warming effect of greenhouse gases emitted by humans into Earth's atmosphere increased by 45% in 2019. This paper is divided into various sections, such as the introduction, literature review, discussion, and conclusion. In this paper, the authors discuss climate change, greenhouse gases and the role of microbes in climate change, how microorganisms are beneficial to climatic conditions. In the literature review section, the author discusses a previous study on climate change.

## 2. LITERATURE REVIEW

James M. Tiedje et al. studied the involvement of microbes in climate change. Microorganisms create and consume the three primary greenhouse gases methane, carbon dioxide, and nitrous oxide, and certain microbes cause illnesses in humans, animals, and plants, which can be aggravated by climate change. As a result, microbial study is required to aid in the mitigation of the warming trend and cascade impacts caused by heat, drought, or severe storms. The author gives a quick overview of what they know about microbial reactions to climate change in three key ecosystems: oceanic, terrestrial, and urban. The

author also suggested new research areas for reducing microbial greenhouse gas emissions and mitigating microbe pathogenic effects. These include conducting more controlled studies on the impact of climate change on microbial activities, system interdependencies, as well as human intervention responses, utilizing microbes but also their carbon or nitrogen alterations for useful stable products, working to improve microbial process data for climate models, and studying microbes as well as climate change using the one health approach [14].

Brajesh K. Singh et al. studied terrestrial feedbacks, microbes, and climate change, according to the author, microorganisms play a crucial role in global biogenic greenhouse gas fluxes. It's unclear if microbial changes have a net beneficial or negative impact on greenhouse gas emissions. Determining the methods by which bacteria control the flow of terrestrial greenhouse gases is essential for improving climatic model prediction. This necessitates taking into consideration bacteria's complex interactions with diverse biotic and abiotic factors [15].

Athanasia Kavadia et al. studied the significance of microbial inoculants in changing climate agriculture. Climate change has acquired prominence as a result of its devastating effects on many facets of life. Climate change, drought, or greenhouse gas emissions have direct and indirect effects on natural and agricultural ecosystem production, but also human health. Because of their potential to operate as biofertilizers and stimulate plant development, functional microbial guilds can be used as an alternative or even an addition to standard agricultural techniques. When compared to chemical inputs, the use of microbial inoculation has a substantially reduced environmental effect, while the agriculture industry benefits financially and consumers gain access to high-quality goods. Microbial inoculants have the potential to help farmers manage stress and mitigate the harmful effects of climate change. The study's author underlines the importance of bacteria in assisting agricultural operations in adapting to changing climate conditions. The multi-functionality of microbial inoculants is questioned, and the author emphasizes future demands and concerns [16].

C. Mangodo et al. examined the difficulties of climate change that emerge from greater concentrations of the greenhouse gases in the environment, which cause the world to warm. Biosphere reserves are locations that are intentionally and purposefully preserved across the world to encourage a green economy or, most significantly, to reduce the effects of global warming or climate change. Although bacteria have been associated with greater exposure to ambient methane gases during digestion, such as methanogens in ruminants' rumens, the importance they play in transforming greenhouse gases into useful forms in the water and soil, hence decreasing global warming, cannot be understated. Overall, the goal of this study emphasizes the relevance of the biosphere reserves in protecting soil or crops bacteria in response to climatic changes [17].

## 3. DISCUSSION

The importance of microorganisms in global warming cannot be overstated, since they are both producers and consumers of greenhouse gases. Microbiology dominates both healthy and natural methane, carbon dioxide, or nitrous oxide fluxes. Microbes are linked to climate change and play an important role in maintaining a sustainable ecosystem on Earth. Assessing the effects of climate change on diverse microbial communities is a current issue in this regard. These include a worldwide assessment of microbial functional traits, the inclusion of bacterial processes and activities in ecosystem modeling, and innovative tactics to overcome restrictions in laboratory microorganism growth methods. These initiatives should help policymakers or the general public understand the importance of micro biota in the many earth ecosystems. Microorganisms have a dual function in GHG generation and consumption through the nutrient cycle. They use GHG to meet their energy needs, reducing climate

change and global warming. Microorganisms have a significant role in carbon sequestration. Microbes also have a significant role in greenhouse gas emissions by participating in methanogens ($CH_4$), heterotrophic respiration ($CO_2$), or Denitrification ($N_2O$). The importance of microorganisms in influencing the number of greenhouse gases in the atmosphere cannot be overstated; nonetheless, further study in this area is still needed. Microorganisms might be a valuable natural resource for combating climate change if they are properly harnessed. However, if a thorough investigation is not carried out, it might operate as a mechanism that hastens the effects of global warming.

## 4. CONCLUSION

Microorganisms normally work by breaking down organic matter as part of the nutrient cycle, emitting greenhouse gases, and hastening global climate change. But on the other hand, converting different gases into useable natural shapes for themselves because of others, decreases or compromises emissions as well as slows or stops climate change. In natural cycles, organisms play a significant role in the change, usage. Natural cycles control the exchange of carbon and nitrogen between land, water, and climate. Microbiological biological systems are important for adjusting the atmosphere or setting barometric conditions, both of which are essential for estimating the Earth's carbon cycle. Methylotrophs can utilize ozone-depleting chemicals as a substrate to meet their energy and carbon needs. Exhalation (breathing), decay, or combustion all release greenhouse gases into the environment (burning). Within the biogeochemical nutrient cycle, nature also performs an excellent job of balancing carbon and nitrogen. This paper's major goal is to understand more about the function of microorganisms in climate change. Regulating terrestrial microbial activity to reduce future greenhouse gas emissions is an interesting potential approach to combating climate change.

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# CHAPTER 12

# IMPACT OF ECOLOGICAL CHALLENGES ON FUTURE PERSPECTIVES OF PLANT BIOTECHNOLOGY

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***ABSTRACT:*** ***The term "genetic engineering" refers to a set of approaches that allow organisms to create a product that was previously either possible or impossible for their species to produce. The technical ability and innovative potential of Biotechnology have useful applications in plant breeding practices to develop new crop varieties with high yields, better quality, disease, and stress resistance ability. A wider range of future perspectives for plant biotechnology breakthroughs may help to the huge impediment to feed the population with lots of nutritious food. By the use of gene manipulation techniques, certain environmental sacrifices are avoided. While analyzing the necessity to direct agriculture toward sustainability, ecological challenges cannot be ignored. Based on the study, technology alone would not be enough to solve this problem. Three fundamental factors influence our world system today, e.g., Science, Economics, and Society. Researchers need a new social compact to keep these factors in control. The use of emerging technology must be guided by ethical and moral considerations. As a result of this study, it is possible to shed light on how scientific advancements have shaped contemporary society.***

***KEYWORDS:*** ***Bacillus thuringiensis (Bt), Deoxyribonucleic acid (DNA), Genetic engineering, Plant Biotechnology, Ecology, Sustainability.***

## 1. INTRODUCTION

Plant biotechnology is predicted to contribute for quality food supply enhancement. Researches of plant physiological processes, Phyto-chemistry, biosynthetic pathways, molecular biology, genomics, proteomics, as well as metabolic control have all advanced. Plants are now being changed for enhanced photosynthesis rate, adaptation to harsh conditions like cold, droughts, saltiness, as well as alkalinity, resistance to disease-causing microorganisms or insect pest damage, as well as increased response to agrochemicals for improved agriculture production. A desire to enhance agricultural plants for higher yields, and higher quality, and to meet changing human tastes has existed since the beginning of agriculture. The growing population of the developing countries has increased the severity of this problem [1].

The scientifically established totipotency of plant cells is the foundation of plant biotechnology, as well as the transport, stable integration, or activation of transgenes in plant cells, regenerating of transgenic plants, or Mendelian inheritance of transgenic organisms to offspring. Around 10000 years ago, man domesticated wild plants and animals. Over the years, suitable consideration has resulted in crop genotypes fit for personal consumption [1].Controlled breeding has changed agriculture and increased agricultural productivity since Mendel discovered the principles of heredity in 1865. In the 1960s, recombinant DNA technological tools brought in a new age of biosciences that would change every aspect of human existence safely and sustainably during the following century [2]. Plant biotechnology is one of the numerous technologies competing to treat a given agricultural issue; nevertheless, a specific pest problem might also be addressed by traditional breeding, a transgenic strategy, Integrated Crop Management (ICM) technique [3].

Synthetic Biology provides novel ways for developing new biological systems and modifying current systems for practical uses shown in Figure 1. It has been described as a breakthrough

technology that sits at the core of the bio-economy and can provide novel solutions to issues about the healthcare system, agricultural production, industrial production, and environmental issues. There is a general perception that synthetic biology has not yet reached its full potential, although it has been successful in the synthesis of several high-value molecules and medications [4].Researchers now have more access to genetic information and, as a result, more powerful genetic engineering tools than ever before because of the significant decrease in the barrier to DNA sequencing and synthesis. Additionally, synthetic biology is increasingly becoming an answer to most of our existing and future needs in healthcare, energy, food production, cleaning, production, and national security [5].

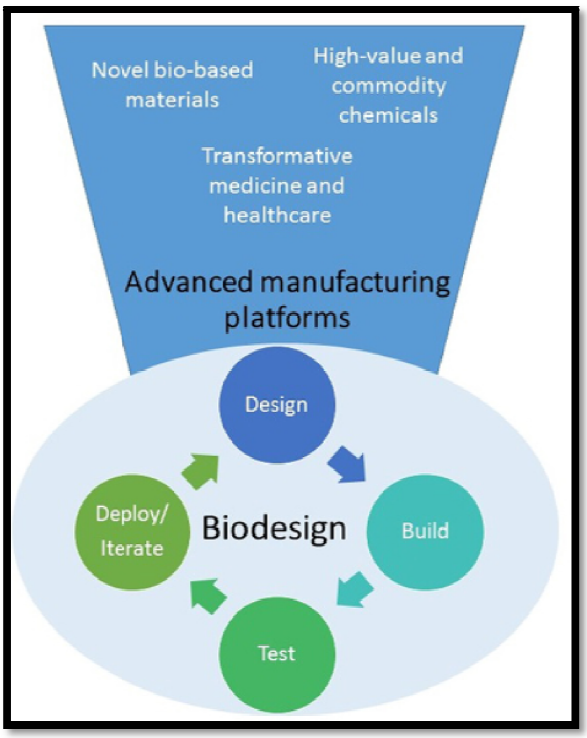


**Figure 1**: **Shows the Innovative ways to develop new or existing biological systems** [6].

## 2. LITERATURE REVIEW

Changshuang Jing et al. stated in a study that the Pollen-specific regulators of maize Profilin 1 or Profilin 3 (pZmPRO1 and pZmPRO3) were used to induce Cas9 expression, while Opaque2 (O2) was used as the target sequence. Control was pZmUbi-driven CRISPR/Cas9 (UC). The author created the transgenic plants for pollen-specific CRISPR/Cas9 (PSC) or UC and studied three events for each. The pZmPRO1 PSC system produced no target gene alterations in T0 but 0–90% in T1. T2 might inherit 31 of 33 T1 mutations. 89%–97% of T2 mutations came from T1. The UC system created T0 mutations, and 0%, 50%, and 92.9% of T1 mutations came from T0. The findings show that the PSC system produced persistent, heritable mutations in the following generation. This strategy might be used in other crops employing germinal cell-specific CRISPR/Cas9 systems to ease plant breeding [7].

Javier A. Fernandez et al. conducted a study that biotechnology has aided in the development of maize hybrids with enhanced nitrogen (N) consumption as well as the increased expression of the zmm28 transcription factor (Event DP202216) boosting the yield of maize crops. In this study, the author compares the maize absorption and use in DP202216 transgenic hybrids to wild-type (WT) controls. Isotope 15N labeling shows that DP202216 hybrids have better N

absorption during late-vegetative stages (causing N storage in lower canopy leaves) and N uptake efficiency compared to WT. Transgenic plants improved N use efficiency by increasing N harvest index and reproductive N remobilization (yield to N uptake ratio). This study suggests that DP202216 might improve maize's N absorption and use.

Saleem Ur Rahman et al. discussed in a study that among legumes, soybeans are among the most significant crops. Soybean has several uses throughout the food, feed, and energy industries due to its highly nutritious seed (proteins, carbohydrates, oil, fatty acids, and amino acids).New plant breeding technologies (NPBTs) have recently evolved, allowing for more precise genetic manipulation of soybean, such as zinc-finger nucleases, transcription activator-like effector nucleases, and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9). There is hope that these NPBTs may be used to enhance soybean via the use of gene functional characterization and precision genome engineering for trait enhancement.This study examined the GE and NPBT soybean improvements. Different GE limitations have enhanced important features. NPBT-improved characteristics, possible NPBT-improved soybean targets, and ethical and public acceptability solutions are also discussed.

## 3. DISCUSSION

For the production of novel plant characteristics and varieties, plant biotechnology is a helpful and effective tool. To be commercially successful and meet farmer demand, new cultivars must be mass-produced on a massive scale. Seed propagation was traditionally used to create new kinds. To safeguard ecological balance, environmental safety, and natural resources, several agricultural inputs, as well as techniques which have been proven to be hazardous during the last few decades, as well as techniques that have been proven to be hazardous during the last few decades must be taken out [8]. In this field, biotechnology and plant genetic engineering will be critical. Plantlets generated by vegetative propagation are presently a suitable option for many plant species. Plant biotechnology has evolved into a new era of science and technology in which secondary metabolite synthesis, beneficial plant genetics advancements, germplasm conservation, and also the creation of massive numbers of disease-free and novel varieties were prioritized. This study examined plant biotechnology's progress over the last few decades and speculates on its possibilities in the new century.

### *3.1. Genetic Engineering of Plant:*

The insertion of DNA into the genome of an organism is the technological basis of genetic modification (GM). To create a genetically modified plant, new DNA must be introduced into plant cells. In most cases, the cells are subsequently developed into plants via a process called tissue culture, where they continue to develop. The modified DNA would be passed down through these plants' offspring, which are the seeds. Today, introducing genomes into crops to develop new economically viable types might appear to be a worthwhile endeavor.A major obstacle to a new agricultural era that may have resulted from discovering and using restriction enzymes was removed in the early 1980s when that enzyme was discovered and put to use, accompanied by the rapid development of bacterium genetic engineering for industrial and pharmaceutical purposes.Gene delivery systems for important crops have increased in productivity since the early 1980s, which was a big accomplishment for the tiny biotechnology enterprises that were driving the field ahead at the time[9].

To learn more about the architecture, physiology, and disease of plants, researchers have turned to gene delivery. There has been so much study done utilizing this method that I could not even begin to list its many successes here. Molecular pharming (remember from Red Meets Green Biotech) involves the development of plants for the delivery of genes that

encode pharmaceuticals; other explanations also include the advancement of crops with increased nutrient content (Golden Rice); the cultivation of plants that are resistant to diseases and herbicides; as well as the advancement of plants which can make their insecticides (Bt Corn). It didn't take long after the soil bacteria "*Agrobacterium tumefaciens"* was proven to transfer DNA from a native plasmid into the plant genome to create the very first model genetic variation.Early plant genetic engineers saw the technology's promise for boosting yields or solving serious social challenges, such as food scarcity. While technology has improved at a breakneck pace, the beneficial influence it may have on the globe has been neglected [10].

### *3.1.1. Biotic stress resistance:*

Biologic stress occurs when organisms such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and artificial or natural plants cause damage to one another. There is a distinction between biotic stress and abiotic stress, which refers to the harmful effects of non-living variables including temperature, sunshine, wind, salt, floods, and drought on living things. The environmental conditions in which an organism must survive and the species' inherent resilience determine the nature of the biotic stressors it must endure. Some obstacles exist for researchers studying biotic stress, including the fact that it is more difficult to manage biotic pressures in an experimental setting than abiotic stresses. A wide variety of organisms, including parasites, viruses, and pests, are to blame for the infection of plants and the resulting biotic stress. Both necrotrophic (which means they destroy the host cell by secreting toxins) and biotrophic lifestyles are possible for fungal parasites (feed on living host cells). They have the potential to cause plants to suffer from vascular wilt, leaf spots, and cankers. Many living species like bacteria, fungus, nematodes, protozoans, pests, viruses, or viral vectors produce biotic stressors on plants.It's only a handful of the many instances of historical biotic stresses, such as the famine in Bengal during World War II and also the coffee rot that decimated Brazil's coffee harvest[11].

Pathogens are responsible for around 15% of worldwide food losses, therefore creating resistant crops is a big issue. Pesticides have been used extensively and, in many cases, indiscriminately to regulate this stress throughout the last four decades, resulting in negative impacts on human health as well as environmental degradation and vulnerable ecosystems. As a result, there is a pressing need to minimize pesticide use by using biotechnology techniques and making crops naturally resistant to insects and pathogens.

### *3.1.2. Insect Pest Resistance:*

On a global basis, insect pests are estimated to be responsible for 14% of crop yield loss [12].Cry proteins are entomocidal endotoxins from Bacillus thuringiensis that are expressed in GM corn (maize), potato (potatoes), or cotton (cotton plants); were initially marketed in the mid-1990s [13]. The integration of genes expressing Bacillus thuringiensis insecticidal proteins into agricultural plants resulted in durable resistance.Consolidated efforts resulted in insect-resistant cotton, maize, and potatoes being produced in the 1990s. *B. thuringiensis* (Bt) was discovered in Japan in 1902 at a silkworm-raising facility. Berliner in Thüringen isolated it again in a flour moth population in 1911 as well as described it in Germany [14]. Many Bt strains generate several crystalline proteins (Cry proteins), including one with a very limited host range. Multiple insecticidal protein genes will be carried by the next generation of transgenic crops that will be created in the upcoming decades.

### *3.1.3. Viral Resistance:*

Crop yield is severely harmed by viral infections. Maize, cassava, sweet potato, banana, papaya, common bean, and rice, are one of the many subsistence crops infected by DNA and

RNA viruses that are resistant to insecticides.Crop growth is often hampered by a lack of resistance sources or by the genetic complexities or difficulties of transferring a resistant gene to cultivars. As a result, biotechnology creation and transmission of tolerance to crops is an appealing alternative strategy [15].As a solution to this problem, a potato cv. Russet Burbank was produced that had genetically engineered virus resistance to Potato Virus Y (PVY) and Potato Virus X (PVX), currently the second crop plant (after tobacco) to have this capability[16].The most efficient and cost-effective strategy to reduce plant virus losses is to utilize viral-resistant plants. The inevitability of resistance disintegration due to the emergence of a new viral disease and species is one restriction of resistant crops. Pesticides, on the other hand, are expensive and have negative environmental repercussions when used to control insect vectors. In the approach, it will be critical to employ tactics that give long-lasting and broad-spectrum tolerance.

*3.1.4. Fungal Resistance:*

Many significant infections in agricultural plants are caused by fungi. For many years, the only successful technique for their control was to use insecticides. The discovery and sequencing of genetic variants in plant defensive responses have progressed significantly in recent years. A vast number of antifungal peptides and proteins have been identified and tested using plant molecular biology or biotechnology in vitro bioassays [17].As a result, a significant trend is the advancement of technology that allows for the creation of cassettes that includes various features. This is already possible to some extent, as evidenced by gene stacks including three NLRs that detect "*P. infestans"* [18].Cyp51 genes, which seem to be critical to the azole fungicide sterol demethylase in Fusarium species, have been silenced utilizing host-induced gene silencing. Plant genetically modified for disease resistance, either through the production of novel proteins from alien species or the overexpression of a component of their protective arsenal, has become a possibility [19].

*3.2. Abiotic Stress Resistant:*

Abiotic stressors have been found to have a significant impact on plant development and agricultural production over the last 50 years, whereas agricultural production has stalled or fallen in economically vital crops when large inputs guarantee high yields. Drought causes calcium ion levels to fluctuate, causing Calcium-Dependent Protein Kinases (CDPKs) to activate through the calmodulin-like domain [20].Crop resistance to these environmental dangers is anticipated to become even more important in the future as the variety of environments in the area used to grow crops grows, and so does the frequency of severe thunderstorm occurrences.

*3.3. Increasing the level of quality:*

Plant biotechnology has enormous potential for improving the quality of food for animals and human health and quite well in perspective of protein, amino acids, vitamins, oil, or carbs. There are 2 main techniques for improving theprotein content and nutritional value of plant proteins:

- Amino acid content changes in plant protein.
- Improving the nutritional content of proteins through the use of gene transfer.

The Amaranthus crop protection protein (2s) gene was shown to be transferable to agricultural plants because of its well-balanced amino acid composition. An auxin-inducible promoter was used to regulate thetransgenic potatoes producing p-casein, a protein seen in human milk.These results provide the opportunity for human milk protein to be reconstituted in plant meals. Plant foods comprising nutrients, minerals, or phytochemicals are essential for

human nutritional support and well-being [21].Saturated fatty acid biosynthesis may be replaced by genetically engineered oil crops that produce high quantities of stearic acid in their seeds. Seed-specific transcription of the altered enzyme, along with site-directed mutagenesis of the "Acyl Carrier Protein (ACP)" thioesterase gene from "Garcinia mangostana," produced transgenic plants which accumulated 55-68 % more stearic than plants produce the wild-type enzyme.

### *3.4. Phytoremediation:*

Phytoremediation refers to the use of plants to restore a contaminated environment. Mining, industry, and urban operations have all led to widespread water and soil pollution during the last decade. Transgenic Arabidopsis encoding the bacterial gene encoding organ mercurial lyase (MerB) has been produced to degrade highly harmful organ mercurial pollutants (for example, methyl mercury) [22].Overexpression of enzymes engaged in the making of PCs or their precursor GSH greatly improved arsenic tolerance however did not improve arsenic deposition.In Arabidopsis, co-expression of both ECS and PCS had a larger effect on arsenic sensitivity or deposition than over-expression either of gene separately. Transgenic plants having high arsenic tolerance and increased arsenic accumulation in branches were created by co-expressing two strains' genes [23].

### *3.5. Molecular Breeding:*

These most basic plant organizations need to modify a huge group of genes, the rearrangement of significant genotypes, and also the identification of their chromosomal placement. Molecular approaches that use genetically related molecular markers to monitor important alleles in genetic variability are now accessible.Tomato fruit had anthocyanin levels equivalent to blackberries and blueberries in 2008 owing to the expression of two transcriptional regulators from snapdragons. Transgenic strawberries producing dog interferon- were produced and sold as an oral medication in Japan beginning in March 2014. This is the first instance of powdered transformants being used as a medication [24].Because it is difficult to locate closely connected genetic markers, gene tagging for crucial agronomic parameters is not widely used.

### *3.6. Genetics of Plants:*

The innovation of decoding the entire plant genome of select plant species marks a watershed moment in plant biology research. Genome sequencing would broaden the scope of plant biology research, particularly the identification of morphological, physiological, or growing and changing.Investigating gene expression on a more comprehensive scale was the first application of plant genomics. RNA profiling, which would be accomplished by hybridizing transcripts to a collection of DNA molecules that are affixed to a solid matrix, has contributed significantly to generating profiles of overexpression.The technique, which is more popularly known as DNA chip technology, has successfully bridged the gap between sequence information and genetic testing.It was made possible by automation in DNA chip technology to simultaneously gather massive amounts of data for thousands or millions of individuals' DNA sequences at a faster pace, followed by data processing using computer hardware. Arrays have the benefit of providing hundreds or even thousands of individual genes at the same time. As a result, 2 categories of DNA chips or microarrays have emerged: Microarrays based on DNA fragments and oligonucleotides [25]. For plant populations, DNA microarrays could also be used to look for 'expression fingerprints' of polymorphisms. They could then be connected to a complex process such as drought resistance to evaluate genetic variations and gene clusters in the metabolism of that plant.

## 4. CONCLUSION

Application of biotechnological tools and techniques for genetic improvement in crop plants plays a pivotal role in agriculture. It will be in practice in upcoming decades. Plant biotechnology relied on a few applications just a few decades ago, like cultured cells, recombinant DNA technology, or monoclonal antibodies.Currently, transformations, marker-aided selection and micro-propagation are only a few types of biotechnological applications produced in response to the need for crop management program. A plethora of scientific tools are available for the screening and genetic alteration of crops for better quality and yield. Techniques of plant biotechnology is also helpful for the conservation of medicinal and valuable plant and plant products. Nanotechnology interventions might be considerably improving the competence of tools and techniques. The use of nanomaterials as vehicles for gene or protein delivery and application of nanocrystals for high-resolution imaging of plant cells and organs, might be another avenue for researchers to improve the uniqueness of crop production. In the realm of transgenic biology, the primary issue in judging the efficacy of added genes is gene silencing. The existence of numerous clones or hyper-methylation of the inserted gene and homologous sequences in several plant genome segments, might be all leads to transgene silence.The promise of genomic information must be realized as soon as possible. Regulation of bioethics and biosafety rules should be in practice and strictly followed. IPR rule and Patent laws can be the passible safeguard indicators. Indigenous efforts or discoveries must be established as well as simplified and make available worldwide.

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# CHAPTER 13

# CHIKUNGUNYA VIRAL DISEASE: EPIDEMIOLOGY, CLINICAL FEATURES, VIRAL CO-CIRCULATION COMPLICATIONS AND ITS MANAGEMENT

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***ABSTRACT:Chikungunya diseases have been a serious issue in recent decades due to their re-emerging nature. CHIKV or Chikungunya Virus is an alphavirus that is spread by mosquitos, an acute febrile sickness with body rash, muscle aches, or polyarthralgia that could last for years. The most common chronic symptom of post-viral Chikungunya is polyarthralgia, which requires multi-drug treatment.This study looked at current CHIKV -related topics including epidemiological and propagation. Many signs of infection (acute, chronic, and atypical) were presented, with a relation to clinical management of co-circulating Zika virus (ZIKV) and Dengue Virus (DENV). Organic and inorganic chemicals are being studied for the therapy of chikungunya sickness, as well as medications already approved for other uses. Furthermore, the previous and present vaccination techniques, and also CHIKV transmission control through integrated management, were thoroughly discussed. Therefore, the present study paves the path for future researchers to have a snapshot of epidemiological data, clinical manifestations, associated complications, and its management.***

***KEYWORDS: Chikungunya (CHIKV), Dengue Virus (DENV), Envelope Protein (E1), Non-Structural Protein, Togaviridae.***

## 1. INTRODUCTION

Chikungunya is an arthropod-borne infection carried most often by the “Aedes” species, the chikungunya virus (CHIKV) [1]-[2]. Chikungunya virus (CHIKV) is an alpha-virus that produces a severe illness with severe infection, skin irritation, and polyarthralgia [3]. The significance of studying viral immunopathology, contributory factors, and epidemic control measures there is currently no particular therapy or vaccination possible to stop the condition, but numerous options are being developed.

As some of the additional symptoms of Chikungunya virus infection, joint swelling, muscle discomfort, and headache have been observed. CHIKV has also been reported in epidemics in Europe, Asia, Africa, America, the Caribbean, the Indian Ocean, and also the Pacific Ocean. Infected travelers can spread the disease to untouched areas [4].Despite the growing burden of diseases and danger, no vaccine or medication seeks to prevent or cure chikungunya viral replication. Mosquito bite avoidance is the only effective method known for protection. When visiting countries where the chikungunya virus is common, wear insect repellent, long-sleeved shirts, and trousers, or remain in places having air conditioning or screened windows and doors [5].The sickness is usually not deadly, and also the acute phase lasts three to five days, with the arthralgia state lasting longer. CHIKV is an alpha-virus of the “Togaviridae” family including an 11.8 kb linear, positive-sense, single-stranded RNA genome. People are harmed by the biting of CHIKV-infected mosquitos, specifically Aedes aegypti[6].

*1.1. CHIKV Taxonomy:*

CHIKV is classified as an old-world disease because of its ability to induce acute arthralgia, yet it has been linked to unusual occurrences of neurological problems or bleeding.CHIKV, along with other alphaviruses, is an antigenically related species of the Semliki Forest virus family. Despite CHIKV being antigenically or clinically most similar to the virus (which causes severe arthralgia), the genetic diversity between both two strains is substantial [7].Several artificial antiviral medications have been produced as a consequence of enhanced scientific study over the last several decades because they are efficient against a wide spectrum of virus contagious disorders.However, these synthetic medications have been linked to a host of adverse effects. In some cases, they could become inefficient for emerging viral-resistant organisms. Further, the underdeveloped world's population cannot afford these expensive synthetic medications for viral illness therapy. Given the widespread prevalence of virus infection and also the high cost of antivirals, new approaches for developing efficient and affordable antiviral medicines are considered necessary [8].

Chikungunya is an illness that must be reported. Therefore, based on the distribution of nations in the area, it may become essential for primary healthcare centers, private clinics, or hospitals inthe public and private sectors to inform the authorities of suspicious incidents. A laboratory would provide a positive test for chikungunya, where in case the laboratory must notify the local authorities. The presence of a cluster of instances matching the characteristics of symptoms suggestive would suggest the possibility of an epidemic that should be reported directly to the local state health department.Despite the significance of CHIKV disease to global health,there seems to be no vaccination or possible antimicrobial medicine available for the treatment and prevention of Chikungunya Fever. In way of comparison to other arboviruses, like Dengue Virus (DENV) and Zika Virus (ZIKV), where substantiated and trustworthy experimental studies are widely available and also used, experimental studies for having studied CHIKV infectious disease were also diversified as well as frequently replicate only a portion of the pathophysiology noted in humans, creating new challenges for the vaccine as well as drug discovery.

## 2. LITERATURE REVIEW

Mst. Sharmin Sultana Shimu et al. stated in a study that the global spread of the Dengue virus and various outbreaks of distinct serotypes have caused a huge number of fatalities and a medical emergency since no effective treatments have been developed.In this study, 27 molecules were docked with the Dengue protein target NS2B/NS3. Phellodendroside, quercimeritrin, and quercetin-7-O-rutinoside were chosen depending on their binding free energy in MM-GBSA. The studied chemicals interacted with Lys74, Asn152, and Gln167 residues in NS2B/active NS3's areas, which inhibits the protein. RMSD, SASA, Rg, RMSF, and hydrogen bond characterize the stable mode of docked complexes. ComputationalAbsorption, Distribution, Metabolism, Elimination, Toxicity (ADMET) characteristics simulations indicated no toxicity in the compounds' pharmacological properties. This computational study may help discover Dengue virus inhibitors.

Hussain et al. used a functional and molecular docked concept technique to identify & select flavonoids using medicinal plants from Pakistan and India against the four NSPs of CHIKV. After assessing the compounds' pharmacological characteristics, they performed a docking investigation against 4 CHIKV NSPs. An 8.5 kcal/mole criterion is used for screening and decreasingthe variety of flavonoids to be studied in the upcoming. The sensitivity of flavonoids that have previously been screened was examined using Dry film thickness (DFT). They discovered found Tamarixetin, Cirsimaritin, 5, 7, 3′, 4′-tetrahydroxyflavone, as well as Apigenin from "Andrographis paniculate", used to have a strong affinity for NSP-1, whereas

or rhamnetin, tamarixetin, and medioresinol had a strong affinity for NSP2, whereas only apigenin had a substantial affinity for NSP4. As a result of their study, the following eight screened compounds have promising antimicrobial activities that may be investigated further in future studies utilizing in vitro and in vivo models experiments [9].

Chaudhary and Sehgal discussed in a study that natural substances with antiviral characteristics were identified in silico as possible inhibitors of the NSP3. Apigenin, Rutaecarpine, Luteoloside, Baicalin, Amentoflavone, and Baloxavir were 6 plant-based natural antiviral medicines that have been found as effective CHIKV NSP3 inhibitors. According to ADMET predictions as well as target analyses, the chemicals picked showed drug likely hood. Simulations demonstrated that the NSP3 and also the antiviral medicines of choice form energetically advantageous complexes. Furthermore, employing the principles of each trajectories interactions experiments were carried out. The data suggest that these compounds are likely to impede NSP3 and could be investigated for pharmaceutical research and development for Chikungunya fever [10].

Keramagi and Skariyachan used information from 43 herbal sources to analyze lead chemicals, their potency, or pharmacokinetic properties. They also carried out docking studies to investigate the binding interactions of specified plant-based ligands against the specified targets, which included non-structural proteins or envelope proteins. Chymopain and Kaempferol, natural flavonols discovered in "Carica papaya," or Gossypetin, flavonoids discovered in "Hibiscus sabdariffa," displayed potential binding interactions to the putative pharmacological target of DENV or CHIKV, according to their research. The selected compounds demonstrated optimum drug likeliness and also the Absorption, Distribution, Metabolism, Elimination, and Toxicity (ADMET) qualities required for pharmaceutical research, indicating the phytoligands' possibility for scale-up.The current work focuses on the interactions of a new triterpene called "Arjungenin," which is discovered in Combretum punctatum, Rudgea burnsides, as well as other species, with the NSP3 macro domain of CHIKV [11].

## 3. DISCUSSION

CHIKV nonstructural proteins (NSPs) were synthesized as one or two polyproteins' intermediates from viral genomic RNA. To produce developed NSPs, these polyproteins were properly processed. The precursors or developed NSPs are required for CHIKV replication. CHIKV NSPs, like all other alphaviruses, were essential not only for viral RNA duplication but also for infected individuals or toxicity. Second, NSPs have a variety of positions inantiviral evasion responses in host-virus interaction These actions are far more viral (and/or cell-type) specific) particular, necessitating additional attention and care in the development of a powerful drug that could employ NSP protein as a possible target [12].

They are categorized into 3 membrane proteins which comprise viral genes (E, C, or M,), and also 7 new biological proteins observed within the contagious host cells and thus important for virus replication (NSI, NS2b, NS2a, NS4a, NS4b, NS3 or NS5). Each of these viral types is translated as a single protein complex or cleaved at specific locations by viral and host cell membrane proteases. It is important to emphasize the role of NS proteins:

- NS1: NS1, the nonstructural protein of the dengue disease, is discovered. NS1 protein is secreted in the blood after dengue infection. The use of NS1 assays in serum is permitted. NS1 increases viral replication in the host cell's cytoplasm or nucleus to suppress the host's immune system response.
- NS2a and NS2b: NS2a is a viral RNA production or virion assembly protein. The NS2 domains encode proteins, NS2A and NS2B. Although NS2a is a non-structural protein,

2a is a 22-kilo-Dalton hydrophilic transmembrane glycoprotein that would be known to link to the endosomal membrane. Furthermore, NS2ais part of the viral assembly complexes, and its purpose during virion assembly is to suppress the specific immune system.

- NS3: NS3 is an important non-structural protein because that creates non-covalent molecules with the NS2B cofactor, resulting in the creation of functioning viral proteolytic enzymes. This also comprises a C-terminal ATPase/helicase sphere, which would be required for RNA replication.
- NS4a and NS4b: NS4a is a protein, while NS4B is a protein from the dengue virus (DENV). There are a lot of hydrophobic membrane proteins, and they are responsible for the membrane arrangements that contribute to the emergence of viral replication compounds. They are also vital for the viral replication cycle.
- NS5: The non-structural protein 5 contains a methyltransferase for encapsulating the RNA and also a polymerase for viral RNA production. This NS5 non-structural protein performs various activities inside the cytoplasm of infected cells, including RNA replication or antagonizing host antiviral defenses.

*3.1. The Vector, The Virus, and The Communicable Disease:*

CHIKV is a Togaviridae family encapsulated RNA alphavirus. The approximately 12kb single-stranded positive sense (+ve) genomic RNA functions as mRNA to generate nonstructural specific proteins (nsP1–nsP4) [13].The genetic architecture of CHIKV is comparable to those of other alphaviruses: 5' capnsP1-nsP2-nsP3-nsP4-nsP5-nsP6-nsP7-nsP8 (junction -region) -C-E3-E2-poly (A) 3' shown in Figure 1. A specific change in the E1 envelope protein was discovered in multiple tropical variants of the Indian Ocean pandemic CHIKV. The specific alteration proved critical in the epidemiology of mosquito-borne viral illness. Firstly, no mutations have been identified, but eventually, more than 90% of alterations were found in strains of the virus.

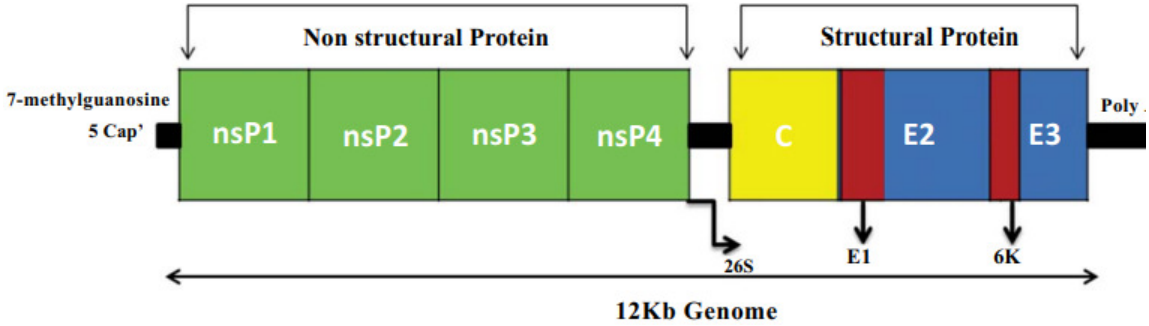


**Figure 1: Chikungunya virus genome RNA structure (S27 strain), containing structural and non - structural and structural poly protein-coding sequences, as well as the 26S and sub chromosomal regulator** [14]**.**

The spikes components of the E1 and E2 envelope proteins extend the viral edges in a membrane-anchored way.Viruses can connect to targeted cell surfaces or infiltrate the mosquito's intestinal epithelium owing to these spike proteins. The envelope-1 glycoprotein is a glycoprotein of type II which facilitates membrane fusion caused by low pH during viral illness [15].On the other hand, the envelope protein is a type I membrane protein necessary for certain ligands to connect following alphavirus adherence to the host organism[16].The amount of cholesterol in the cell membranes determines the efficacy of alphavirus entry. The E1 mutation at position 226 improves A. albopictus infectious illness and enables effective

reproduction into midgut cells, and is among the key inflammatory sites for CHIKV strains. Other changes in E2 proteins, which govern CHIKV adaptability within the mosquito, had been found. The E1 or E2 proteins are important for the human host's serological reaction to chikungunya infection [17].

### *3.2. CHIKV Infection Symptoms:*

#### *3.2.1. Acute:*

The clinical disease affects between 50–97% of CHIKV-infected patients including temperature & arthralgia. CHIKV infection has been linked to the quick onset of febrile (>38.9 °C) in 92% of individuals, arthralgia in 87% of patient populations, back problems in 67% of patients, migraine in 62% of patient populations, as well as exhaustion in 12% of patient populations [18].One of the most prevalent symptoms of CHIKV Fever is polyarthralgia, which is often bilateral as well as affects mostly peripheral joints (ankles, wrists, or phalanges) as well as certain major joints (knees and elbows) [19].

Cutaneous symptoms have been recorded in around 50 percent of cases of acute. The transient maculopapular eruption or macular that might cause swelling or irritation characterizes the lesions, most frequently arising in the body extremities, palm, soles, chest, or cheeks.During the acute phase, gastrointestinal symptoms like diarrhea, vomit, nausea, or stomach pain occur in 15 to 47 percent of patients.CHIKV infection has been shown in many investigations to result in high viral loads ranging from 105 to 109 viral RNA transcripts per mL, which relates closely to the intensity and frequency of clinical signs [20].

#### *3.2.2. Chronic:*

Polyarthritis or Polyarthralgia were prevalent chronic chikungunya side symptoms, affecting both small and large joints such as phalanges or wrist (like shoulders, ankles, and knee). The disease is usually severe and causes people to remain immobilized. Polyarthralgia has been demonstrated to endure for a variety of periods, ranging from weeks or even months or, in certain situations, up to five years, based on the demography studied [21].There is currently no agreement on whether viral antigens have replication competency (possibly with modifications to enhance persistence) and are simply the product of prolonged removal of non-replicating viral proteins.

An investigation on CHIKV found macrophages carrying CHIKV genetic information or viral vectorsfor eighteen months in the synovium of an individual with a chronic infection. CHIKV has been found to survive in NHP lymphoid tissues, liver, joints, muscles, as well as macrophages[22].The pathophysiology of rheumatoid arthritis in CHIKV Fever remains unknown. Although some research has also shown that infectious disease may cause the onset of this inflammatory response illness, other investigations have found no inflammatory cytokines in affected people with chronic illness.

### *3.3. Clinical Advantage:*

Chikungunya virus infection during the acute phase induces arthralgia and arthritis, and in more than 50 % of cases, musculoskeletal signs could be protracted or, in certain circumstances, becomes chronic.Even though polyarthralgia has been the most common cause of chronic symptoms, polyarthritis, tenosynovitis, or enteropathy also are prevalent.Muscle discomfort, headache, exhaustion, and rash are other frequent indications of chikungunya sickness. The joint pain with this condition is frequently excruciating, yet it normally only lasts a few days or might last for weeks. As a result, the virus causes acute, subacute, and chronic illnesses.

Many chikungunya illness patients make a full recovery; however, joint discomfort can last for months and even years in certain situations. Isolated incidences of ocular, neurologic, or cardiac problems, and also stomach issues, have been observed.This should be noted, although, that some other arboviruses, like Zika, Dengue, as well as other alphaviruses, could have comparable clinical manifestations, like temperature or arthralgia.

*3.4. Diagnosis:*

Multiple techniques for diagnosing CHIKV illness were accessible, including viral RNA, IgM, or IgG antibodies against the pathogen detection of chikungunya or antiviral nanoparticles in cell line control cells that are now in vitro subjected to specimens of patients' blood. The major testing procedure used to identify infections in serum was taken 6 days following disease start is RNA.Anti-CHIKV IgM or IgG serological testing might be performed within several weeks or months of sickness, while false-negative findings are possible based on the stage of illness.Elevated concentrations of CHIKV IgM antibodies were detected in Indians suffering from post-CHIKV rheumatoid arthritis (RA).

*3.5. Advanced Imaging for Clinical Evaluation:*

The majority of the study concentrates on using conventional radiography to evaluatebones inCHIKV-infected individuals, with varying results.The 24 months after diagnosis, Magnetic resonance imaging (MRI) showed bone fissures in the hands of 5 of 6 patients, showing that MRI could be more provides productivity for early detection of joint illness.Joint examination in CHIKV-infected patients using conventional radiography is a common topic in the present literature, however, the findings are inconsistent. A subchondral bone of the hands and wrists, particularly the interphalangeal joints, may undergo major alterations, such as visible erosions, by 10-18 months post-infection (P.I), however many patients have entirely normal radiographs throughout this same period. Evidence of joint space narrowing and bone erosions was seen in individuals who had normal radiographs at 10 months post-infection suggesting that early alterations due to CHIKV-associated joint illness may not be apparent by radiography.

*3.6. Prognostication:*

Human CHIKV sickness is distinguished by severe knee pain, a fever, also rashes. Since the illness is self-limiting, serious complications normally go away for a week. However, polyarthralgia recurs in 30–40% of afflicted people and might endure for decades. CHIKV infections can cause severe joint pain, affecting even the most fundamental daily activities.In addition, neutralizing antibodies has been tested as a potential therapeutic strategy in mice models. If given before infection, it reduced viremia and virus levels in ankles to undetectable levels; if given after infection, it reduced viremia but had no impact on viral RNA levels in the inoculated foot. Though passive vaccination with monoclonal and polyclonal neutralizing antibodies has shown protective effects against CHIKV infection in vitro and mouse models, its preventive or therapeutic value in humans has yet to be determined. It has also been established that pre-infection with CHIKV IFN- therapy is effective in preventing the development of arthritis in a mouse model.

*3.7. Laboratory testing:*

Because the manifestations of, ZIKV, CHIKV, and DENV illnesses are so identical medicaldiagnostics in co-circulation regions are challenging, and lab investigation is neededto establish the viral etiology. Hematological results linked with CHIKV infection are often unspecific; nevertheless, lymphopenia, as well as hypocalcemia, have been the most prevalent symptoms, whereas acute thrombocytopenia is uncommon.Elevated levels of liver

enzymes, and also creatinine or phosphokinase, have also been documented. The different testing patterns identified throughout ZIKV, CHIKV, and DENV, infections, when combined with medical symptoms, could be used to confirm an actual diagnosis. They are more precise and faster than virus detection because they allow for highly precise RNA identification from all CHIKV lineages. CHIKV diagnosis by molecular detection technologies is often possible with serum samples obtained up to 7 days after symptom onset [23].

*3.8. Treatment:*

Since there are no approved particular antivirals for controlling CHIKV replication, therapy methods should be supporting or symptomatic, involving hydration intake.Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) like paracetamol are used in this situation to lower temperature and treat arthralgia suffering.Therefore,NSAIDs that interfere with platelet aggregation, as well as otherclotting agents (such as aspirin) should have been ignored. The use of NSAIDs in conjunction using systemic corticosteroids at modest dosages is currently recommended in the treatment of acute chikungunya patients with arthralgia to minimize discomfort or increase the quality of life. Previous fears that corticosteroid therapy might aggravate alpha viral arthritis appeared to be unfounded because serodiagnosis has revealed antiviral immunity.

## 4. CONCLUSION

There is a saying that "Prevention is better than Cure", this is particularly true in the case of the Chikungunya. Treatment is accessible even though the vaccination or specific medicine still has not been developed.The therapy pattern is similar to dengue; however, the discomfort is severe, unlike dengue. Because the mosquito is the major vector of this virus, avoiding mosquito bites is the key preventive approach for chikungunya fever.These viral diseases have a typical clinical spectrum and need prompt laboratory detection, which is very important for monitoring networks. Furthermore, CHIKV infections are a severe public health issue.The present spreading of previously undetected CHIKV indicates the globalization of infectious diseases.As a result, it is essential to comprehend the characteristics of CHIKV infections, autoimmune illness, or antigen type and quality employed in the development of diagnostic assays. Because CHIKV is a relatively new area of research, it is believed that breakthroughs in detection would help all individuals at risk, particularly those in impoverished nations. Given current breakthroughs in vaccination methods, the key difficulty in the establishment of equilibrium among immunogenicity or tolerability, especially thedecrease in negative effects like secondary arthralgia after infusion using attenuated infections, remained a priority in CHIKV vaccine development. Furthermore, given the effectiveness of vectors or arbovirus control strategies, we propose that CHIKV infection prevention should be planned on a worldwide and multidimensional scale. This multidisciplinary method, which is presently articulated within the One Health idea, should thereby incorporate all elements of human, animal, and environmental health care.The widespread use of bioinformatics or artificial intelligence technologies in monitoring infectious pathogen alterations and their implications for symptom severity or transmission efficiency could be used to research other viral illnesses such as CHIKV. This suggests that creating a bioinformatics network for an infective agent could be extremely beneficial in tracking virus spread and designing a treatment and preventive plan. In the search for CHIKV vaccinations, mRNA vaccine technology provides fresh promise.

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# CHAPTER 14

# SIGNIFICANCE OF PROBIOTICS FOR PREVENTION AND MANAGEMENT OF DIARRHOEA: A STUDY ON INDIAN POPULATION

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***ABSTRACT:*** ***Probiotics described as live microorganisms having significant health benefits, especially in the case of gut health are now being used and explored for the treatment of Diarrhoea. Probiotics foods and probiotics have received much interest and attention in recent years, both from researchers and the general population. The most common probiotics are Bifidobacterium and lactobacilli, and probiotic-rich foods include kefir and yogurt. Diarrhoea has been managed with probiotics due to the advent of antibiotic resistance. Bacteria, viruses, and protozoan parasite infections, as well as antibiotic therapy, are all causes of diarrhoea. In this research, a survey study was conducted to investigate the use of probiotics, their significance, health benefits as well as commonly encountered side effects during the management of diarrhoea, in children, youth, and adults. The results showed that a significant proportion of the population is not aware of the proper management of Diarrhoea and still prefers antibiotics for the treatment which sometimes make the condition even worse. However, the individuals who have used probiotics and probiotic foods have reported significant health benefits and reduced diarrhoeal episodes in a very less period. This study can provide a foundation for future research to explore the more precise combination of probiotics that can be used to increase the efficacy in diarrhoea management.***

***KEYWORDS:*** ***Antibiotics, Bifidobacterium, Diarrhoea, Lactobacillus, Probiotics.***

## 1. INTRODUCTION

Diarrhoea is among the most prevalent gastrointestinal (GI) diseases, with a significant economic burden. As per a World Health Organization (WHO) report, diarrhoea is considered the second largest cause of death in children that fall under the age of five, causing deaths of around 1.5 million per year. This condition is defined as having three or more loose stools per day [1]. It is a sign of gastrointestinal infections induced by several organisms, including bacteria, viruses, and parasites. It is usually self-limiting and does not need antibiotic treatments. Individually, acute diarrhoea impairs intestinal absorption of both vitamins and macronutrients, resulting in malnutrition and growth retardation [2]. Therefore, a major public health problem is preventing acute diarrhoea with widely available, simple, safe, and low-cost solutions to avoid acute diarrhoea. Hand washing has been shown to lower the occurrence of diarrhoea. Attempts to increase handwashing rates, however, are hampered by insufficient evidence of cost-effectiveness, a lack of evidence of effectiveness, and the inherent challenge of changing human behaviors. Improving hygienic facilities, drinking water, food storage, and handling procedures can all help to avoid acute diarrhoea. Regardless of the origin of diarrhoea, it is generally treated with fluid replacement treatment to correct nutritional deficiencies and shorten the length of the diarrhoeal episodes. Despite fluid replacement decreasing the risk of dehydration and mortality, it is ineffective in minimizing the duration of vomiting and diarrhoea. consider alternatives to antibiotics. However, the current management practices for diarrhoea revolve around probiotics that have been used to fight infection for over a decade.

Even though the majority of these deaths occur in developing countries, they are a common reason for consultations and hospitalizations in Western societies. When probiotics, also known as "good bacteria," are consumed, they can give health benefits in several ways. Because the preponderance of health effects attributed to probiotics are directly or indirectly connected to the gastrointestinal system, using them to prevent or cure gastrointestinal illnesses is an obvious method and arguably the most prevalent application of probiotics. Probiotics are characterized as live microbial supplements that, when consumed in appropriate amounts, benefit the host by improving its microbial balance and giving health benefits beyond those offered by basic general nutrition.

### *1.1.Human gastrointestinal system and distinct types of diarrhoea:*

The human gastrointestinal system is home to diverse populations of commensal flora that are constantly in touch with epithelial cells of the intestine. These microflorae develop after birth and, through a succession of colonization, obtain over 400 species of bacteria till adulthood. Most of these bacteria are present in the colon [3]. The variety of this microflora, as well as the intricate host–bacterial interplay, have lately been studied using molecular genetic techniques.

#### *1.1.1. Antibiotic-associated diarrhoea (AAD):*

AAD is most prevalent in individuals undergoing antibiotic therapy for a microbial balance in the intestine. Clostridium difficile is one of the most important pathogenic bacteria involved in the pathophysiology of AAD. When antibiotics affect the equilibrium of flora in the digestive tract, C. difficile can swiftly proliferate [4]. C. difficile bacterium produces toxins that attack the gut lining. Clindamycin, cephalosporins, fluoroquinolones, and beta-lactams are the antibiotics most often related to C. difficile infection, while practically any antibiotic can put people at risk [5]. Several research studies and review of existing literature suggest *L. Rhamnosus* and *S. boulardii* as potential probiotics for the management of AAD [6][7]. Recent research on probiotics to reduce the incidence of AAD also suggests that instead of using an individual species, multistrain probiotics can help reduce its occurrence [8].

#### *1.1.2. Travelers' Diarrhoea:*

Travelers' diarrhoea affects up to 20–70% of those who visit high-risk areas. Although antibiotics can be used to prevent infections, this method is not generally advised, due in part to the advent of resistant bacterial strains [9]. Probiotics for traveler's diarrhoea prevention offer an appealing strategy for this self-limiting condition. A recent review study combined multiple previous research and concluded that LGG is the most well-characterized probiotic to prevent traveler's diarrhoea using placebo-controlled clinical trials [10].

#### *1.1.3. Rotavirus Infection and Acute Infectious Diarrhoea:*

The main pathogenic microorganisms involved in diarrhoea-associated infections are rotavirus, *Escherichia coli, Campylobacter, Salmonella, Shigella, and clostridium.* However, rotavirus infection is the major cause of acute infectious diarrhoea [11]. Infectious diarrhoea is caused by two fundamental changes in normal intestinal physiology: excessive electrolyte and fluid secretion, primarily in the small bowel, and reduced electrolytes, fluid, and often nutritional absorption, which can impact both the large as well as the small intestine. Various systemic reviews and research based on double-blind, placebo-controlled trials have revealed various probiotic strains that can help manage acute infectious diarrhoea, out of which

Lactobacillus GG has shown the most consistent positive effect with shortening of diarrhoeal episodes [12]. A preclinical model is also used to investigate the probiotic properties of different strains of lactobacilli and bifidobacteria for the prevention of diarrhoea associated with rotavirus infection [13].

#### *1.1.4. Irritable Bowel Syndrome (IBS):*

IBS is a functional gastrointestinal condition affecting 10–15% of the populace and is characterized by diarrhoea or bloating abdominal pain, constipation and flatulence. The most commonly reported indication is altered bowel habits, with the syndrome usually linked with constipation (IBS-C), diarrhoea (IBS-D), or a combination of both diseases (IBS-M). Moreover, Patients with IBS frequently experience stomach pain, which can be triggered by eating or emotional stress and is generally alleviated by stool passing [14]. Recent research by radomanska et al. assessed the efficacy of synergy of preparation from lactobacillus and Bifidobacterium probiotic strains in patients with IBS with the help of a randomized double-blind, placebo-controlled study which suggested that the prepared multi-strain preparation demonstrates synergistic activity for the improvement in patients with diarrhoea-predominant IBS.

### *1.2. Potential Mechanism of probiotics for treatment and management of Diarrhoea:*

Elimination of pathogenic microorganisms by competing for accessible substrates and binding sites, decreasing luminal pH and generation of bacteriocins, and stimulation of mucus synthesis are all potential mechanisms through which probiotics combat infectious diarrhoea. The ability of pathogenic bacteria to make mucinase might explain some of the advantages of probiotic treatment in viral diarrhoea. Probiotics aid in pathogen elimination by increasing intestinal motility and upregulating genes involved with innate immunity. Lactobacilli and bifidobacteria create short-chain fatty acids, that govern cell development and thus have trophic effects on the epithelium of the intestine [15]. The aim of this research is to determine the prevalence of diarrhoea and its awareness among the Indian population to effectively manage and treat diarrhoeal episodes.

## 2. LITERATURE REVIEW

Research by Lai et al. investigated the effect of "*Lactobacillus casei*" on the management of clinical symptoms of Diarrhoea by changing the gut microbial community and inflammatory markers. A randomized trial was performed with children hospitalized for acute diarrhoea and provided with probiotics and no probiotics by oral administration for 7 days. Microbial composition was also determined by using methods such as next-generation sequencing (NGS) and the agar plate method. Different components such as specific antibodies and other immune components were assessed. Three-time points were used to evaluate the results from 81 individuals included in the study. It was found that the group that received probiotics had higher levels of Immunoglobulin A when compared to the group that did not receive the probiotic. Improvements have been observed after one week of probiotic Lc administration. Apart from that, it was also found that prevalence of Bifidobacterium and lactobacillus species was higher in the individuals receiving probiotics. Therefore, the study suggests Probiotic Lc as one of the potential candidates to be used for the management of childhood diarrhoea [16].

Another research by Kolodzeij and Szajewska assessed the effect of *Lactobacillus reuteri DSM17938* on cases of antibiotic-associated diarrhoea with the help of a randomized clinical

trial. Children hospitalized due to diarrhoea were randomized to receive a placebo or *Lactobacillus reuteri* at a specif concentration of strain two times a day. The study was performed after the cessation of antibiotics for one week. Analysis was performed with 247 out of 250 children that were randomized for the study. The results demonstrated that the occurrence in the group receiving the selected probiotic strain resulted in more occurrence of diarrhoea when compared to placebo. Therefore, their study demonstrated the ineffectiveness of using "*Lactobacillus reuteri DSM17938"* or the management of antibiotic-associated diarrhoea generally caused by *C. difficle*[17].

Yang et al. evaluated the positive effect of "*Bifidobacterium breve FHNFQ23M3" and "Bifidobacterium bifidum FSDJN7O5"* on E. coli-induced diarrhoeal condition. They utilized a mice model to study the effect of the selected probiotic strain on the diarrhoeal episode caused by *Enterotoxigenic Escherichia coli (ETEC)*. They found that the selected strains of probiotic bacterium significantly restored the villi structure in the intestine as well as lowered the water content in the stool. They also found that *B. bifidum FSDJB705* significantly enhanced the interleukin-10, on the other hand, it was also found that *B. breve FHNFQ23M3* can also aid in the restoration of body weight as well as reduce interferon- γ. Therefore, their research suggested the great potential of Bifidobacterium strains in the alleviation of Diarrhoea caused by ETEC [18].

Another study by Yue et al. evaluated the protective effect of *Lactobacillus plantarum* in relief from diarrhoea caused by ETEC. They performed a comparative study on the different strains of *L. Plantarum* as well as explored the potential for remission manner. Their finding revealed that strain Lactobacillus plantarum CCFM1143 is the most promising strain to possess the protective effect against diarrhoea caused by ETEC. Apart from those other strains namely CCFM1143, FCQNA30M6 and FGDLZ1M5 reduced interferon- γ, interleukin-6 as well as the, and tumor necrosis factor-alpha. Therefore their study suggested that the strains of the *Lactobacillus plantarum* can potentially reconstruct the unbalanced flora of the gastrointestinal tract [19].

Dall et al. carried out a randomized controlled trial to assess the effectiveness of the probiotic "*Lactobacillus Rhamnosus GG (LGG)"* strain in order to prevent the colonization of multi-drug resistant Enterobacteriaceae during Travelers' Diarrhoea. For their study, they made two groups from the Danish travels traveling to India for 10-28 days for receiving a selected probiotic strain or no probiotic.

Apart from those questionnaires as well as rectal swabs were collected from the participants immediately after return as well as after 6 months of return in which 30 travellers were randomized to the control and 31 travellers to the probiotic group. They found that 41 travellers out of 44 were found to be colonized from "extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E)" which were not colonized before travel. Apart from that 5 out of 50 were also found to be colonized with ESBL-E and 11 out of 36 were also found to be colonized after six months of return. Hence it was found that there was no significant effect of LGG strain on the risk of colonization from ESBL-E [10].

On the other hand, a study by Siedlecka et al. involving the analysis of cases that utilized "*Saccharomyces boulardii* CNCM I-745" for treatment of specific diseases suggested the superiority of non-bacterial probiotics in the effective management of Travelers' Diarrhoea with improved clinical symptoms during diarrhoeal episodes as well as other conditions associated with diarrhoea. Therefore, their study revealed the potential of "*Saccharomyces boulardii CNCM I-745"* as a safe probiotic strain to be used for the management of traveler's

diarrhoea [20]. The above studies assess different probiotics strain that can be further used to explore their potential in probiotic food to manage the different types of diarrhoea due to the increasing inefficacy of antibiotics in diarrhoea management.

## 3. METHODOLOGY

### *3.1. Design:*

The study is conducted using an online survey throughout different regions of India which involved individuals from different age groups, genders, ethnicity, and culture. In this research, all data collection was performed by asking questions to respondents including people, children as well as youth irrespective of whether they encountered an episode of diarrhoea in their lives. The primary data of the study is collected by asking questions to the respondents and taking personal interviews from selected areas to gain relevant information about their knowledge of diarrhoea and its management by probiotics. The secondary data for this research was collected from an online survey which was sent to the respondents by mail. In addition, other patient records were included in the research.

### *3.2. Sample:*

The collection of data is performed using a set of questionnaires. Survey questions are the easiest and most useful way to obtain data for research purposes. All kinds of possible biases in sample collection were eliminated. Several types of questions were designed to gather information about people's knowledge of diarrhoea and its management using probiotic strains. The survey was asked 100 people of different ages, ethnicity, gender, and culture.

### *3.3. Instrument:*

In this section, the researcher used survey questions to gather information and determine whether people know about the use of probiotics for the proper management of diarrhoea or not and if probiotics improved the condition in diarrhoea patients. The author evaluated the survey questionnaire from respondents and performed their analysis on specific parameters. Some of the survey questions which are used to frame this research study are listed below:

- Have you ever suffered from Diarrhoea?
- Have you taken probiotics for treating diarrhoea? If yes then what kind of probiotic? Specify the name such as yogurt.
- Did probiotics help to overcome Diarrhoea?
- If yes then how much time did probiotics take to overcome diarrhoea?
- What are the positive results encountered after taking probiotics?
- What do you prefer for treatment and management of diarrhoea? Probiotics or antibiotics
- Are there any side effects encountered after taking probiotics? If yes then specify.

### *3.4. Data Collection:*

The collected data is shown in different figures which are illustrated below. The collection of data is performed with the help of responses that are collected from an online survey sent to people including adults, children, and youth.

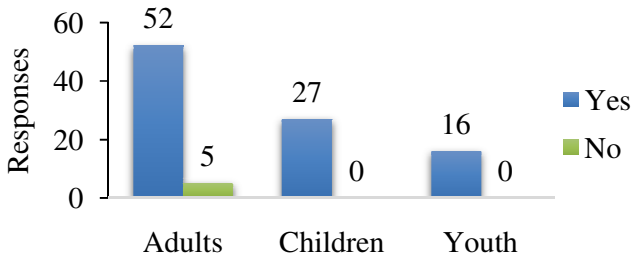


**Figure 1: Illustrates the different responses of the population to diarrhoea encounter with time duration of diarrhoeal episodes.**

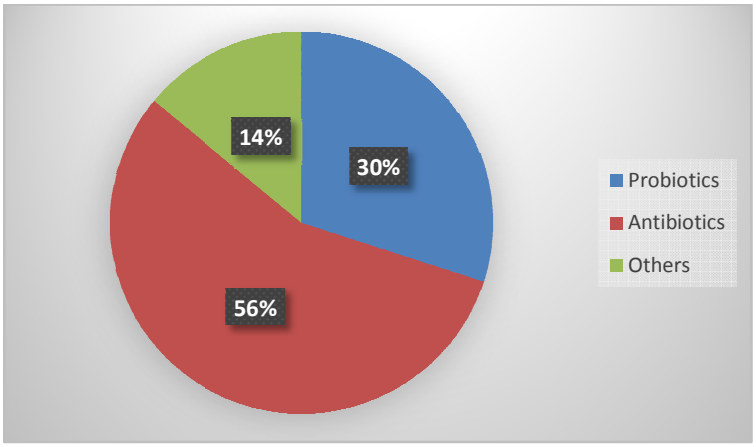


**Figure 2: Illustrates Different Interventions used by Respondents for Treatment and Management of Diarrhoeal Episodes.**

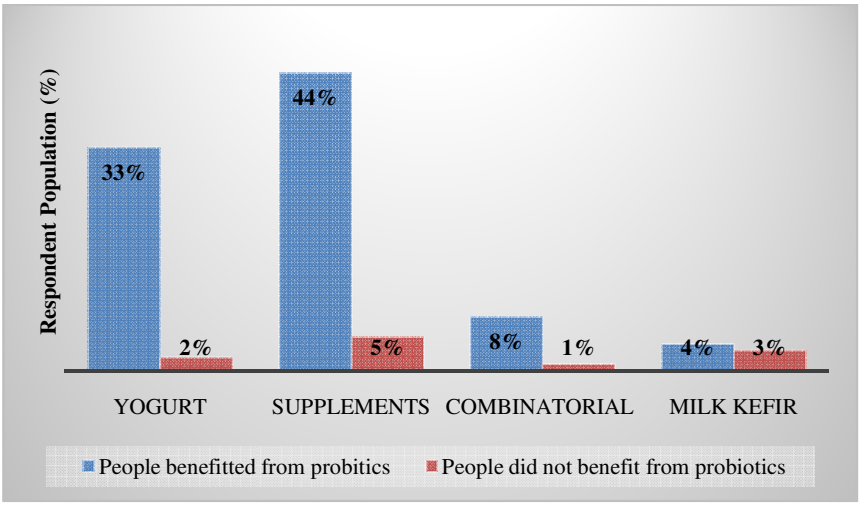


**Figure 3: Illustrates Different Types of Probiotics Used by the Respondent Population for Treatment and Management of Diarrhoea and their Effect.**

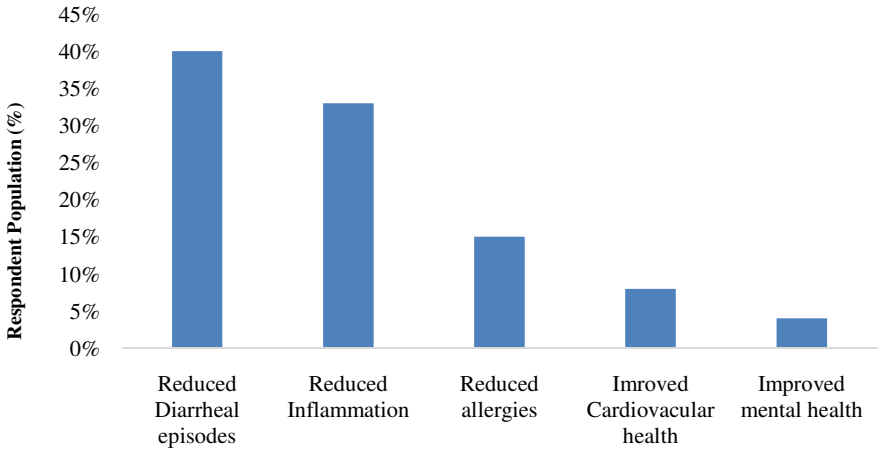


**Figure 4: Illustrates Benefits Experienced by Respondent Population After Consumption of Probiotics During Diarrhoea.**

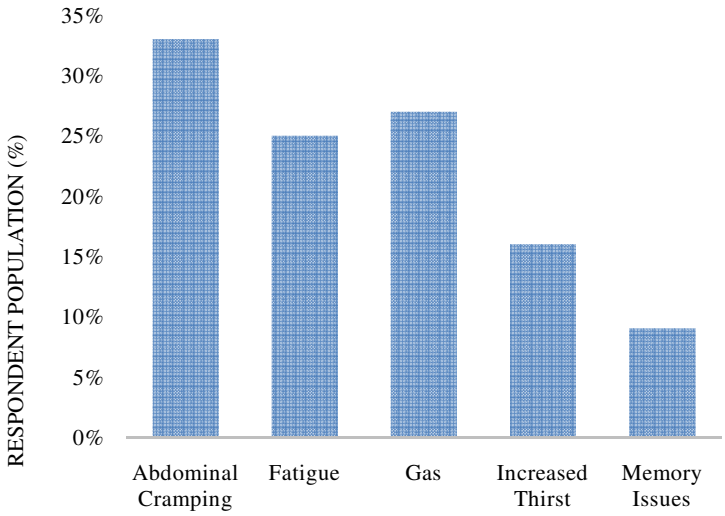


**Figure 5: Illustrates different types of side effects experienced by an individual after taking probiotics during diarrhoeal episodes.**

*3.5. Data Analysis*

This survey is used to analyze and evaluate the data as well as to define the problem of Diarrhoea as one of the major health burdens on the economy of developing nations. It is easy to identify that the probiotic can be used as a treatment and prevention measure for Diarrhoea in children as well as in adults. From Figure 1 it can be seen that the majority of people who have responded to the survey experienced Diarrhoea in their life accounting for 95%. Out of which 52% of individuals experience diarrhoeal episodes of 1-2 days, 27% of them experienced Diarrhoeal condition between 2-14 days, and 16% experience Diarrhoeal for more than 15 days making it a condition of lot more significance with the standpoint of global health burden. When asked about the interventions used for the treatment and management of Diarrhoea, antibiotics stand out to be on the top accounting for 56%, trailed by probiotics at 30% and then followed by other interventions accounting for 14% suggesting

the need to make people aware about the potential of probiotics as well as the ineffectiveness of antibiotics in the management of Diarrhoea. It has been seen that among individuals who used yogurt as a probiotic, 33% of gets benefitted from it and 2% of them did not experience any changes or improvement in their health. Apart from that, it was also found the majority of the population consider supplements for the management of disease, from which 44% of individuals get benefitted and 5% of them did not. Efficacy of the combination of both the supplements as well as yogurt showed amazing results that benefitted only 1% of non-benefitted individuals demonstrated in Figure 3. Different kind of health benefits has also been analyzed including a significant reduction in inflammation, diarrhoeal episodes, allergies, and improved mental health Figure 4. A very few side effects have been reported which as abdominal cramping, Fatigue, gas, increased thirst, and memory issues accounted for 33%, 25%, 27%, 16%, and 9% respectively demonstrated in Figure 5.

## 4. RESULTS AND DISCUSSION

Malnutrition, unhygienic conditions, as well as undeveloped nations, are all factors that revolve around the increasing burden of Diarrhoea in children as well as in adults. In the data analysis of the performed survey, it has been demonstrated that there is little knowledge on the use of probiotics for proper management of Diarrhoea. The majority of the population is experienced Diarrhoea encounters at least once in life accounting for 95%. And very less no. of individuals has used probiotics for treatment, however, it can be seen that the people who have used probiotics got benefitted from it. Moreover, it can be seen that when probiotic foods and supplements are used in combinatorial supporting therapy, it demonstrated their efficacy with a very less rate of failure accounting for 1%. With the help of a survey, it was found that 95% of people suffered from Diarrhoea, out of 30% of individuals taking probiotics, 56% took antibiotics, and the rest 14% took other interventions. The below table illustrates the responses of individuals who filled out the framed questionnaire of the existing study.

**Table 1: Shows the responses from the respondents who answers Yes and No according to the questions they were being asked.**

| **Questions** | **Yes** | **No** |
|---|---|---|
| Have you ever suffered from Diarrhoea? | 95% | 5% |
| Did probiotics help to overcome Diarrhoea? | 85% | 15% |
| Did you encounter side effects after taking probiotics? | 16% | 84% |

In above Table 1, the main questions that were asked by the researcher to the respondents have listed in which 95% of the respondent population responded Yes when asked whether they encountered any diarrhoea episode in their life. The population that received probiotics for alleviation and management of Diarrhoea found that they overcome diarrhoea when using probiotics during disease. It was also been reported that a very less population has encountered minor type of side effects when consuming probiotics accounting for only 16% given in Table 1.

## 5. CONCLUSION

With multiple modes of action, probiotics are quickly emerging as an alternative to conventional antibiotic treatment because they are relatively inexpensive and are less prone to developing resistance. Aside from their function in diarrhoea management, there is also evidence of improved mental health, reduced allergies, improved cardiovascular health as well as improved microbial balance. It has also been noted that the people who usually take probiotics in their day-to-day lives have not encountered diarrhoea or encountered it for a very less period suggesting the significance of probiotics and probiotics food for smart management of Diarrhoea in children, youth as well as in adults.

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# CHAPTER 15

# IMPORTANCE OF BIOTECHNOLOGY TECHNIQUES FOR CLIMATE CHANGE MITIGATION AND ADAPTATION

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***ABSTRACT:*** ***In its simplest form, adaptation is the act of reversing the effects of climate change on the present and the future. Mitigation is the process of reducing or reducing greenhouse gas (GHG) gases in the environment to reduce the severity of the consequences of climate change. To combat climate change, a variety of mitigation and adaptation strategies are available, but none of them are sufficient by themselves. Effective implementation requires policies and cooperation at all levels, but integrated solutions that combine mitigation or adaptation strategies with other social objectives can be helpful. The far-reaching effects of climate change on security, health care, and food security necessitate the creation of new climate adaptation strategies. Climate change is more visible, and while there is no reason to believe it will go away too soon, action must be taken now to allow quick adaptation and avoid unintended and undesirable effects. Finally, the study discusses the current constraints of biotechnology and the possibilities for climate change mitigation or adaptation. The main objective of this study is to understand the importance of biotechnological techniques for climate change adaptation or mitigation. This paper help to understand the role of biotechnology in future adaptation or climate change mitigation.***

***KEYWORDS:*** ***Adaptation, Biotechnology, Climate Change, Greenhouse Gas Emissions, Mitigation.***

## 1. INTRODUCTION

Increased temperatures, altered rainfall patterns, as well as the incidence of pests and diseases are all variables linked to climate change that have a detrimental influence on agricultural productivity, output, as well as quality. Production risk is considerably increased by climate change effects, particularly in areas where soil and water resources are consistently scarce. Climate change's impact on agriculture might well be influenced by not only the changing weather conditions but also by the capacity to adapt due to advancements in technology as well as the increase in global food demand. Modern biotechnology has a favorable impact on reducing the consequences of climate change. Utilizing genetically engineered, stress-tolerant, as well as high-yielding transgenic plants, modern biotech has the potential to considerably mitigate the harmful consequences of climate change.

### *1.1. Mitigation and Adaptation:*

The two options for dealing with climate change are mitigation and adaptation. A technique for mitigating climate change is to increase greenhouse gas sinks or cut greenhouse gas emissions. An alteration in natural or human systems about current or predicted climatic stimuli or their repercussions, which minimizes damage or optimizes benefits, is the definition of adaptation. Mitigation and adaptation are the two alternatives for responding to climate change. Mitigation is a technique for lowering greenhouse gas emissions while also boosting carbon sinks. An alteration in the natural or human system concerning current or future climatic stressors or their effects that minimize damage or optimize benefits, according to the definition of adaptation [1], [2].

*1.2. Distinctions Between Mitigation and Adaptation:*

There are several significant contrasts between change in climate mitigation or adaptation, particularly in terms of their goals. While climate change adaptation focuses on mitigating the phenomenon's effects, climate change mitigation focuses on its causes (building up of greenhouse gases). Both strategies are required. On the one hand, even with robust mitigation measures, the climate will continue to change in the next decades, necessitating adaptation [3], [4]. On the other side, mitigation will be required to prevent the climate system from altering too much because adaptation won't be able to completely remove all negative consequences. Figure 1 illustrates how mitigation or adaptation of climate change is related.

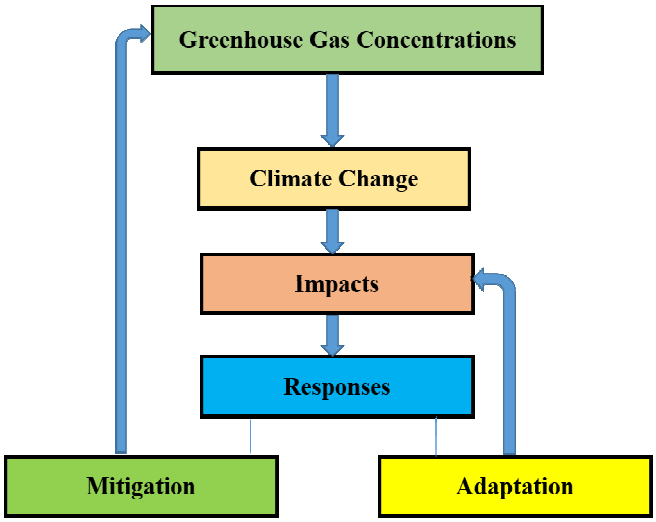


**Figure 1: Shows the Relationship Between Climate Change Adaptation and Mitigation.**

One of the most difficult problems that people are currently facing is climate change. It covers many areas of science, politics, economics, and culture, as well as ethical and moral challenges. This is a global issue with long-term implications for local scales. Since carbon dioxide, the high-temperature greenhouse gas responsible for current global warming can persist in the environment for millennia, the Earth takes a long time to adapt to the warming. As a result, even if humans cease creating greenhouse gases today, future generations would still be affected by global warming or climatic changes. As a result, humanity has agreed to some degree to climate change [5], [6].

The long-term average change or fluctuation of its characteristics is what the "Intergovernmental Panel on Climate Changes" (IPCC) refers to as climatic changes. Climate change is mostly caused by anthropogenic influences, including human-induced changes in land use, as well as natural factors, such as volcanic eruption, solar cycle fluctuations, and continental drift, according to the IPCC report. One of the most serious dangers to farming in the foreseeable future is climate change [7]. Temperature, precipitation from insect pests, diseases, soil, weeds, water quality, or soil would be influenced. Methane (48.00%) or nitrous oxide (52.00%) from the rice fields are mostly produced by agricultural activities, which account for 25.00% of greenhouse gas emissions. Natural and anthropogenic greenhouse gas emissions block radiation from being reflected into the atmosphere, resulting in a hotter environment. The most frequent gases generated by industry and other activities are methane ($CH_4$), sculpture hexaoxide ($SF_6$), nitrous oxide ($NO_x$), hydrofluorocarbons (HFCs), and carbon dioxide ($CO_2$). Various human activities have raised air concentrations throughout time, resulting in global climate change [8].

It is feasible to adapt to changes in climate by minimizing the sensitivity of natural and social systems. By lowering greenhouse gases through human impact on the source or the sink (or both), addressing climatic changes is a policy reaction to the phenomena that seeks to offset its negative effects. By employing trees and other $CO_2$ sinks to remove $CO_2$ from the atmosphere, but also by transitioning from biomass to renewable energy sources, climate change can be mitigated. As a result of drought, rising temperatures, more frequent and powerful precipitation events, and other sorts of poor weather, crop output and quality are declining, making it harder to feed a growing population. Increasing agricultural output is necessary to feed the world's expanding population [9], [10]. Agricultural biotechnology is the use of biological organisms or their cellular components in agriculture. Existing techniques include tissue culture, molecular marker-assisted breeding, conventional breeding, and genetic engineering. Biotechnology can lower greenhouse gas emissions, carbon sequestration, fertilizer use, and abiotic or biotic stress tolerance, all of which can help mitigate the detrimental effects of climate change.

In this context, the new study stresses the relevance of biotechnology in climate change adaptation for long-term agricultural productivity and food security [11], [12]. Increasing the number of beneficial alleles that contribute to stress tolerance is one way to breed increased stress tolerance. The introduction of one or more genes from organisms with which plants would not normally reproduce is not the only, or most important, biotechnological contribution to agricultural adaptation to climate change. A key feature of modern biotechnology is the ability to trace genetic material from other plant lines or organisms or transfer it to the crop of interest, the presence of plant phenotypic expression as a proxy for the presence or stress response solely on eliminating the need to trust. The process of evaluating how a plant line behaves in a given habitat is still important, but once a genetic region has been identified as conferring an evolutionary advantage, it can be removed much faster than previously thought. Adaptation refers to steps taken to mitigate the dangers of climate change consequences, whereas mitigation refers to activities taken to lower the emissions that cause climate change, as shown in Figure 2.

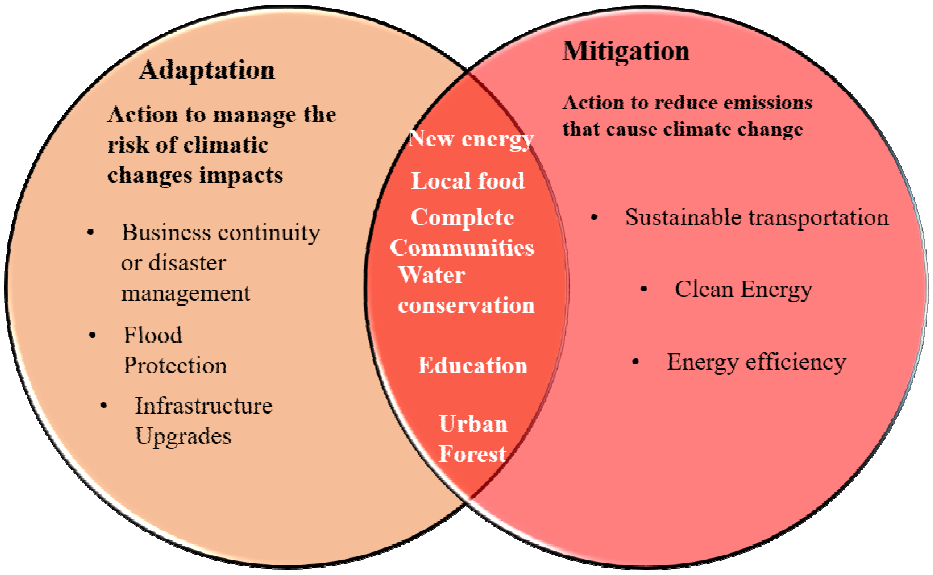


**Figure 2: Illustrate the Building Climate Resilience, Including Adaptation and Mitigation.**

### *1.3. Mitigation and Adaptation Futures and Challenges:*

New climate adaptation strategies are required due to the extensive impacts of climate change on food security, safety, and health. Climate change is becoming increasingly visible, and there is little reason to assume that it will be reversed very soon. As a result, immediate action is required to adapt quickly and avoid unintended or undesirable consequences. The present world's population of 7.00 billion people is expected to climb to 8 billion by 2025, with a high of over 9 billion in 2050. To feed their rising populations, emerging nations will need to plant 120 million extra hectares of crops. Crop yields should be raised as a consequence of employing contemporary farming technologies. Efforts should be undertaken, in compliance with national laws or regulatory frameworks, to integrate local or traditional biotechnologies with current biotechnology methods to strengthen the resistance of native crops to a variety of environmental stresses. Although contemporary biotechnology has yielded promising results, several biotechnology procedures still have a lot of space for improvement. Among several others, the primary problems were enumerated here.

- Uncertainty regarding the origins of climate change ("Natural or Human-made").
- Abiotic or biotic stress poses a danger to the food supply as the world's population continues to grow.
- Express concerns about environmental and health issues affecting public safety, such as the emergence of more dangerous pests or diseases, the escalation of the effects of current pests, disruption of biotic communities, harm done to non-target species, genetic diversity, or extinction of species or genetic diversity within the species. It brings up moral or social issues, such as the apprehension of an uncertain future and the abolition of conventional agriculture. Understanding polyamines' function in resistance to abiotic stress is still in its infancy. Understanding the molecular pathways behind the protective effect of SPM, SPD, or Put in abiotic stress tolerance would require further research.

Governments should establish sufficient biosafety or biotechnology legislation as well as legal frameworks before introducing modern biotechnology to solve the existing issues in its research and use. Anxiety about the bad consequences of GMOs must be founded on science, and each instance should be examined in detail with accurate data. To solve the problem, both traditional and contemporary biotechnology must be included. Polarized thinking should be based on science rather than self-interest or politics.

Climate change-related variables such as rising temperatures, changing rain patterns, and the incidence of insects and pathogens all have a detrimental impact on agriculture, productivity, or quality. Changing climate has a considerable impact on production risk, particularly in areas where soil and water resources are few. Agriculture's capacity to adapt to changes in technology and food demand may influence how it adapts to climate change, in addition to altering weather patterns. Because of the utilization of contemporary biotechnology, the consequences of climate change have been mitigated. By adopting genetically altered seeds that are stress-tolerant or high producers, modern biotechnology has the potential to considerably mitigate the negative consequences of climate change. The author of this essay investigates energy-efficient agricultural systems, resilience to biotic or abiotic stress, as well as the role of biotechnology in climate change mitigation. In this paper, the author analyses the current situation of climate change, the contrast between adaptation and mitigation, and the challenges that mitigation and adaptation will face in the future.

## 2. LITERATURE REVIEW

Louis V. Verchot studied Climate change connected to adaptation or mitigation, and the authors of this paper gathered information on agroforestry's adaptation strategies in the humid or sub-humid tropics. Farming is the human industry most at risk from climatic changes.

Small-scale farmers lack the means to adapt to climate change, making tropical agriculture, especially subsistence agricultural production, particularly susceptible. The author next goes over the scientific data that supports the idea that agroforestry may help farmers adapt to climatic changes, especially small-scale farmers. The author finishes with a list of critical research questions about the function of agroforestry in adapting to climate change [13].

S. MARK HOWDEN STUDIED ADAPTING AGRICULTURE TO CLIMATE CHANGE, ACCORDING TO THE STUDY'S AUTHOR, THESE DECISIONS WILL BE PUT INTO PRACTICE UNDER MILD CLIMATE CHANGE AND ARE ANTICIPATED TO HAVE SIGNIFICANT POSITIVE EFFECTS ON A VARIETY OF AGRICULTURAL SYSTEMS. HOWEVER, IN THE FACE OF INCREASINGLY DRASTIC CLIMATIC CHANGES, THEIR UTILITY IS CONSTRAINED. THEY SUGGEST THAT INCREASING ADAPTATION ACTION WOULD REQUIRE INTEGRATING CLIMATE CHANGE-RELATED CONCERNS WITH OTHER RISK VARIABLES INCLUDING CLIMATIC CHANGES AND MARKET RISK, AS WELL AS OTHER POLICY AREAS LIKE ENVIRONMENTAL SUSTAINABILITY. INTERDISCIPLINARY PROBLEMS DEMAND INTERDISCIPLINARY SOLUTIONS, WHICH EMPHASIZE INTEGRATED SCIENCE RATHER THAN DISCIPLINARY SCIENCE AND INVOLVE CLOSER COLLABORATION WITH POLICYMAKERS. THIS PLAN MUST INCLUDE THE USE OF ADAPTATION EVALUATION CRITERIA THAT ARE SUITABLE, RELIABLE, OR SIMPLE FOR ALL STAKEHOLDERS, PRACTITIONERS, POLICYMAKERS, OR SCIENTISTS TO ADOPT. THEY CONTEND THAT FOR SCIENCE TO REMAIN RELEVANT, IT MUST EVALUATE RESEARCH NEEDS, BROADEN THE BASIC IDEA, AND INCORPORATE SCIENCE INTO CONVENTIONAL METHODS OF MANAGEMENT AND COMMUNICATION WITH DECISION-MAKERS**[14].**

Y. S. Mtui studied Biotechnology's role change in climate adaptation or mitigation This research examines how traditional and contemporary biotechnology may be used to address climatic change mitigation and adaptation for enhanced agricultural adaptability, production, and food and nutrition security while also helping to decrease greenhouse gas emissions. The current problems of biotechnology, as well as its promise for climate change mitigation and adaptation, are discussed. Low yields, hunger, or malnutrition as just a result of high heat, drought, salt, or infectious disease vectors are some of the negative effects of climate change on agricultural output and food safety. The scientific community believes that human activity has an influence on climate change, either directly or indirectly [15].

## 3. DISCUSSION

Environmental change is defined as a major and long-term shift in the observable aspects of climate change when observed over lengthy periods. It might be caused by natural cycles on the planet, such as sunlight-based radiation and continental drift, or by human mobility. Ozone-depleting compounds are natural and fake vaporous elements of the environment that store and transfer infrared radiation generated by the Earth's crust, air, or mists at certain frequencies. Water fume ($H_2O$), nitrous oxide ($N_2O$), carbon dioxide ($CO_2$), methane ($CH_4$), and ozone are the principal ozone-depleting chemicals in the atmosphere ($O_3$).

### *3.1. Biotechnology's Contribution to Climate Change Mitigation:*

Agricultural activities like as artificial fertilizers, overgrazing, rice crop cultivation, and deforestation account for 25.00% of greenhouse gas (carbon dioxide, nitrous oxide, or methane) emissions into the environment. Biotechnology is among the most dependable strategies for minimizing the use of synthetic fertilizers, sequestering carbon, and farming with less energy. The usage of genetically engineered plants has resulted in lower carbon

emissions. Farmers use less energy to power their equipment because genetically modified crops require less maintenance than normal crops. As a result, greenhouse gas emissions are reduced. This decrease in greenhouse gas emissions is considerable. The reduction in greenhouse gas emissions in 2012 was as effective as taking 11 million automobiles off the road for a year or removing 27 billion kilograms of $CO_2$ from the atmosphere. Farmers leave a lower carbon footprint because they consume less petroleum and do not need to spend as much time riding on farm equipment.

### *3.2. Using Energy-Efficient Agricultural Techniques:*

Green biotechnology (the use of specialized techniques to produce more productive or resistant plant nutrients) is now being used to eradicate world hunger by leveraging a variety of technologies that enable the development of more fertile or high resistance plants to both abiotic and biotic pressures. Through the use of less energy and environmentally friendly fertilizer, farmers may practice soil carbon sequestration. Oilseeds, rapeseed, sugarcane, and atrophy biofuels along with others generated from traditional or genetically modified organisms (GMO) crops will aid in reducing the detrimental impacts of pollution brought on by transportation. As a consequence, good farming will help to clean up the environment by planting perennial non-edible oil seeds. As a result, get deeply involved in the manufacturing of biodiesel for the energy industry. It is then mixed with fossil fuels to minimize carbon dioxide emissions [16].

### *3.3. Sequestration of Carbon Dioxide:*

Carbon capture or storage is the process of removing carbon-containing molecules from the environment, usually carbon dioxide. It helps with both the growth of soil organic carbon content and the collection of $CO_2$ from the atmosphere, both of which mitigate climate change. From this vantage point, storing increasing amounts of Carbon dioxide in the atmosphere through carbon sequestration is one of the best strategies to lessen the consequences of climate change. Conservation tillage refers to any tillage or planting practice that, after planting, covers more than 30% of the ground's surface with agricultural waste to prevent water erosion, increase methane consumption, as well as trap soil carbon.

The usage of genetically modified crops permits the removal of millions of tons of $CO_2$ from the environment. Roundup Ready TM, an herbicide-resistant soybean, was reported to store 63, 00 million tons of $CO_2$ in either the United States or Argentina. When their crops mature, farmers will be able to use no-till farming techniques. These strategies either increase soil quality or fix carbon in the soil, both of which help to mitigate global warming. The food and agriculture organization (FAO) has assessed how conservation tillage affects carbon sequestration. According to the FAO, increased nutrient cycling and a decrease in nutrient deficiencies were among the main benefits for farmers. Soil carbon capture and storage reduced $CO_2$ emissions by 1.8 tons per hectare annually in the first decade after farmers adopted the best conservation agriculture practices.

### *3.4.Reduced Synthetic Fertilizer Use:*

The use of synthetic fertilizers in agriculture has resulted in the release of potentially hazardous substances into the environment. When synthetic fertilizers interact with typical soil bacteria, some greenhouse gases ($N_2O$) are transferred from the soil to the environment, where they participate in the production and secretion of inorganic fertilizers, such as ammonium chloride, ammonium sulfate, calcium nitrate, and sodium nitrate, cause the release or production of greenhouse gases. Reduced usage of synthetic fertilizers is a benefit of the biotechnology alternative. Rhizobium inoculants' capacity to fix nitrogen was improved by genetic engineering. Growing crops that utilize nitrogen more effectively is another option.

Genetically modified canola is one example of a crop that has been demonstrated to drastically minimize the quantity of nitrogen fertilizer lost to the environment, rivers, and soil, as well as optimize farmer economics through increased profitability.

### *3.5. Crops Adaptation to Climate Stress Using Biotechnology*:

Crop output decline owing to rainfall, severe temperatures, the spread of weeds, and the presence of pests and disease are the ultimate climatic changes consequences on agriculture. Utilizing agricultural biotech to address the harmful impacts of such changes is one strategy to adapt to such a worldwide challenge. Genetic engineering offers new potential for boosting stress tolerance.

### *3.6. Adaptation to Biotic or Abiotic Stress:*

Concerning the amount of water used by agricultural land, climate change is bringing several issues. These challenges that hurt agricultural output include abiotic stressors such as salt, drought, extreme temperatures, or chemical toxicity. Climate change poses a significant problem in the case of cropland availability and freshwater utilization. The farming industry consumes over 70.00% of all available fresh water, a figure that is expected to climb as temperatures rise. Furthermore, every year, 25 million acres of land are lost to salt owing to insufficient irrigation measures. It is also estimated that increased salt in arable land will cause 32% of arable land to become uncultivated within 25 years, increasing to 50.00% by 2050. Certain stress-related genes are now either activated or controlled as molecular regulatory systems for abiotic stress responses. Plants that can endure salt are usually able to withstand additional challenges, such as freezing temperatures, cold, or drought. A wide range of GM agricultural plants that can withstand abiotic stress have previously been developed. Examples are the tobacco plants Arabidopsis thaliana and Brassica napus. Among the crops cultivated in the US are tomatoes, rice, cotton, wheat, maize, or oilseed rape [17].

The photosynthetic capability of these transgenic plants was intact, as was the level of photosynthetic bacteria enzyme. Recently, the tobacco plant Nicotiana Tabassum's aquaporin gene (NtAQP1) and its ability to guard transgenic tomato seeds from salt stress were both found (Solanum Lycopersicum). NtAQP1 is important for reducing root/shoot hydraulic failure, increasing water efficiency, or increasing salt tolerance. A significant amount of recent research suggests that plant polyamines (PAs) have a role in the achievement of resistance to stressors like greater or lower temperature, hyper osmosis, and salt, hypoxia, or air pollutants. Additionally, plants can be genetically altered to produce less poly (ADP ribose polymerize), a crucial stress-related enzyme, allowing them to withstand drought better than non-GM plants. According to field trial findings, these GM agricultural plants boost production by 44.00%. The availability of whole crop genetic sequences, genetics, physical mapping, and functional genomics technology has opened up new potential for integrative techniques employing molecular breeders or genetic modification to improve stress tolerance.

### *3.7. Mycobiotechnology:*

Climate change is a major issue that is already affecting people and the environment. Increased global average temperature mitigates the negative consequences of excessive precipitation or temperature, reducing farmer or ecological vulnerability as well as improving agroecological resilience. Mycobiotechnology is a type of fungal biotechnology that is used to tackle environmental problems or restore damaged ecosystems. These methods aim to employ fungus to help restore ecosystems that have been destroyed. Mycobiotechnology is part of a bigger movement in which biological systems are being used to tackle ecological issues and rehabilitate damaged environments. Mycoforestry or my restoration science

nowadays is part of a growing field of study and implementation for the regeneration of damaged ecological systems. My restoration is a method of using fungus to aid in the recovery of environmentally damaged habitats. Saprophytic or mycorrhizal fungi can assist in the recovery of habitats that have been devastated by manmade or natural disasters [18]. Trees provide micro-climates that promote available rainfall, forests will indirectly contribute to positive agricultural production and food security. In addition, trees serve as carbon sinks, helping with the effects of carbon sequestration and greenhouse gas reduction in the fight against climate change. As a result, forestry, as well as agroforestry, have the potential to create synergies between efforts to reduce global warming or initiatives to assist disadvantaged communities in adapting to its negative impacts. Food security, health, and security are all significantly affected by climate change, thus requiring strategies to adapt to the changing climate. There is no indication that the effects of climate change will be reversed shortly, so action must be taken to adapt quickly and avoid unexpected and adverse consequences.

## 4. CONCLUSION

In conclusion, access to information and skills will be crucial in enabling biotechnology deployment to sustain productivity in poorer countries, where there is a greater need to reduce climate change and increase food security. By reducing carbon emissions, enhancing carbon sequestration, decreasing the use of fossil fuels, boosting agricultural productivity, and reducing the demand for synthetic fertilizers, plant biotechnology can aid in climate change adaptation. These actions serve to safeguard the environment from harsh weather while also enhancing agricultural yield. Modern biotechnology will be used effectively to combat climate-related issues, ensuring agricultural production for a rapidly rising population. A strategy for the safe use of contemporary agricultural modern biotechnology will help boost production and food security, as well as contribute considerably to climatic change adaptation or mitigation efforts. Finally, the study examined the difficulties facing biotechnology now and its potential for future climate change adaptation and mitigation. This study aims to learn more about how biotechnological approaches can aid in the adaptation or mitigation of climate change. Using biotechnology to improve understanding of future climate change adaptation or mitigation measures.

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# CHAPTER 16

# EXPLORING THE TARGETING ABILITY OF CORDIOSIDE AGAINST PROGESTERONE RECEPTOR IN BREAST CANCER TREATMENT WITH AN *IN-SILICO* APPROACH

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***ABSTRACT:*** ***Breast cancer is defined as the uncontrolled proliferation of cells present in breast tissue. Breast cancers are induced by the upregulation of genes that promote proliferation in breast cells. BC is a severe disease that affects women worldwide, posing a huge healthcare burden in both developing and developed countries. It has demonstrated resistance to radiation therapy, chemotherapy, and hormone therapy in several cases stressing the need to develop effective inhibitors. In this research, the aim is to find out the potential of phytocompound from a famous medicinal plant against progesterone receptor, the most important hotspot target for breast cancer. To carry out the research, a docking process between the target structure, progesterone receptor, and selected phytocompound, cordioside was performed using a computational study. The affinity was determined and then the complex was visualized to check the interaction as well as its potential to be used as an anti-breast cancer agent. The binding energy of -6.43 kcal/mol was observed between the first pose of protein and ligand complex which can further pave the path for its deployment in breast cancer treatment in the future. However, further research including in-vitro studies, as well as larger trials, is needed to check its drug-likeliness.***

***KEYWORDS:*** ***Breast Cancer, Cordioside, Docking, Progesterone Receptor, Tinospora Cordifolia.***

## 1. INTRODUCTION

Cancer is a multifactorial genetic illness that causes uncontrolled division and multiplication of aberrant cells in the body, as well as travel towards other body areas [1]. Breast cancer is the most frequent type of cancer in women across the world, and in many countries, it is the major cause of death. It is a cancer that can spread to many organ systems of the body, including the lung, liver, and brain, rendering it incurable [2]–[4].Breast carcinogenesis goes unnoticed due to several risk factors related to biomolecular interplay. Breast cancer has been more common in the last 50 years, accounting for 23% of all cancer cases in Asia, as per WHO statistics [5]. Cancer is induced by genetic changes in cells, that result in incorrect cell division and mutation. Breast cancer is classified as hormonal cancer. Breast glandular tissues are particularly sensitive to changes in hormones. Mammary gland development starts during embryogenesis and continues during puberty and pregnancy as the gland matures and differentiates. Female sex hormones, estradiol, and progesterone stimulate the embryonic development of ductules, buds, and breast ducts [6]–[8]. During puberty, estrogen levels are increased, resulting in higher production of progesterone and estrogen receptor production in the mammary glands [9]. These female sex hormones can contribute to the risk factors development for breast cancer. Pregnancy and breastfeeding, on the other hand, help to lower the risk of cancer [10]. Higher breast mass has been linked to an increased chance of getting breast cancer. Increased breast mass is linked to elevated number of fat cells that produce estrogen from cholesterol, which elevates the breast cancer risk. According to studies, the synthesis of estradiol from cholesterol is a major factor in the breast cancer

development in postmenopausal women who have an overabundance of fat cells in their breasts. Additional breast cancer risk factors include childbearing beyond the age of 30, overweight, lack of sun exposure, presence of benign tumors in the breast vitamin D insufficiency, and so on [11].

Despite advances in early detection and various targeted therapeutics, the death rate from breast cancer has not declined. Furthermore, the medicines that are now accessible are pricey, insufficient, and have some major adverse effects. Tamoxifen (Nolvadex R ) is an estrogen-blocking drug that is taken as a tablet in the oral route of administration. Strokes, blood clots, uterine cancer, and cataracts are just a few of the significant adverse effects of Tamoxifen. Raloxifene has the potential to induce significant blood clots in the lungs, eyes or legs. Difficulty breathing, chest discomfort, leg swelling/pain, and eyesight problems are some of the other side effects [12]. As a result of these adverse effects, these drugs are unsuitable for treatment, necessitating research into a better alternative. Furthermore, acquired resistance that is sensitive to cancer-related mutations and resistance due to minor heterogeneity in subpopulations may improve treatment inefficiency. As a result, it is critical to developing a disease treatment that is acceptable, efficacious, and cost-effective, either delaying or reversing the metastatic process.

Phytocompounds are now being researched for use in modern medicine. These compounds are important components in the production of a variety of medicinal agents against a variety of diseases. Phytocompounds have been shown to have a variety of beneficial effects on human cancer models. Here, in this research study, the aim is to find out the interaction and potential of phytocompound against the target protein of breast cancer using a tool of molecular docking as a methodology. Molecular docking helps in studying the molecular dynamics of target proteins when they interact. It is a technology that is widely used in drug development. AutoDock, Vina, MOE-Dock, FLexX, and GOLD are the leading docking software used to perform the docking process to investigate the interaction.

## 2. LITERATURE REVIEW

Swargiary & Mani investigated and evaluated the targeting ability of phytoligands from "*Centella asiatica"* and "*Andrographis paniculate"* against estrogen and progesterone receptor using an in-silico approach and analysis of ADMET study. The interaction of selected compounds Andrographolide and Asiatic acid with targeted protein was found to be -8.7 and -6.7Kcal/mol. Other compounds such as apigenin, Bayogenin, kaemferol, andrographin, and others from selected species also demonstrated good binding energy. Phytocompounds against ER also demonstrated significant affinity but were then discarded because of their properties. Therefore, the study suggested the potential of asiatic acid and andrographolide for targeting the progesterone receptor involved in breast cancer development. However, the study needs more verification using in vitro experiments.

Another research by Isamail et al. investigated the 20 phytocompounds against progesterone receptor and estrogen receptors as anti-breast cancer using a molecular docking study. Out of those 20 phytocompounds, they found that Gabridin demonstrates binding energy for estrogen receptors which is the highest among all coming -10.3kcal/mol with a total number of 12 interactions with the selected protein. Apart from that Quercetin was the phytocompound which demonstrated the highest binding energy against the progesterone receptor. They also carried out a pharmacokinetic study which demonstrated the adherence of the lead compounds with the Lipinski rule of 5. Therefore, the study provides Gabridin and Quercetin as potential candidates that can be further taken for future studies as treatment drugs for breast cancer, however much larger studies are still a limitation.

Parhar et al. carried out another research to investigate the potential of phytochemicals as a therapeutic approach against breast cancer. They tested 1064 phytochemical compounds derived from plants against five possible hotspot targets: the human estrogen receptor alpha ligand-binding domain, the Progesterone receptor, the FRB portion of mTOR, and the breast cancer NUDT5 and the EGFR kinase domain. Their findings suggested that 25 phytoligands out of 1064 compounds studied have therapeutic promise in breast cancer treatment. In their study of virtual screening of phytoligands, the compounds Baicalein, morphine, 3,5-diglucoside, delphidin, and IsoSkimmiwalin demonstrated a higher affinity against the selected protein structure of EGFR, PR, ER, and NUDT5 as effective drug treatments. In addition to that more future studies are needed to explore the anti-cancer activity of certain compounds investigated in the study.

Alamri et al. investigated marine compounds as potential anti-breast cancer compounds targeting the estrogen receptor in breast cancer. They used virtual screening (VS) using docking and 450 marine compounds were investigated. The estrogen receptor with a 4-OHT X-ray-assisted structure was selected and the molecular operating environment (MOE) dock program was then used to perform the screening of the selected marine compounds. The results were then evaluated in terms of the protein inhibitor interaction (PII) and binding energy. GBVI/WAS binding-free energy evaluation (in kcal/mol) was used by researchers to rank the compounds for their targeting ability to inhibit the selected protein. The most efficient inhibitors were identified as those with a BE of less than -9.500 kcal/mol. Significant numbers of compounds were found to have a good binding affinity with estrogen receptors demonstrating the inhibitory potential of marine compounds against breast cancer in patients.

Another research by Zerezade undertook the screening of 20,000 natural compounds and screened their potential against the progesterone receptor of breast cancer followed by ADMET analysis and pharmacodynamics study of the interaction. Apart from that they also undertook principal component analysis (PCA) against the protein motions and then the MMPBSA method was used to evaluate the binding free energy which demonstrated that three out of the selected compounds namely “ZINC00869973”, “ZINC01020370”, and, “ZINC00936598” have the highest binding energy for the target protein when compared to the positive control which was taken as Levonorgestrel. The study and the analysis of H-bond formation, RMSD as well as Rg were then performed for lead compounds and their stability in the complex. This finding paves the path for the development of innovative PR-targeting drugs derived from natural sources [13]. The aim of this study renders to the potential of phytoligand from *Tinospora cordifolia* to fight against breast cancer for which a molecular docking study for interaction has been conducted in this research paper.

## 3. METHODOLOGY

### *3.1. Design*

Autodock 1.5.7 was used for virtual screening and molecular docking. The structure of the progesterone receptor was obtained in .pdb format from Protein Data Bank RCSB with PDB ID 4OAR in this investigation. The three-dimensional structure of cordioside, a natural product from *Tinospora cordifolia* was obtained in XML format from Pubchem and then converted into .pdb using a tool known as the open babel tool. The further process was done using .pdb files for both the protein and the ligand. The docking is done by Autodock and the results are displayed with Pymol and Biovia drug discovery studio.

### *3.2. Sample Collection*

#### *3.2.1. Target protein*

The key target for breast cancer was selected based on an existing review of literature which was taken as progesterone receptor that plays a significant role in breast physiology and the development of breast cancer. The retrieved structure of a protein is presented in Figure 1 below where the helical structures in cyan color represent the alpha-helices whereas the flat sheets in magenta color represent beta-pleated sheets.

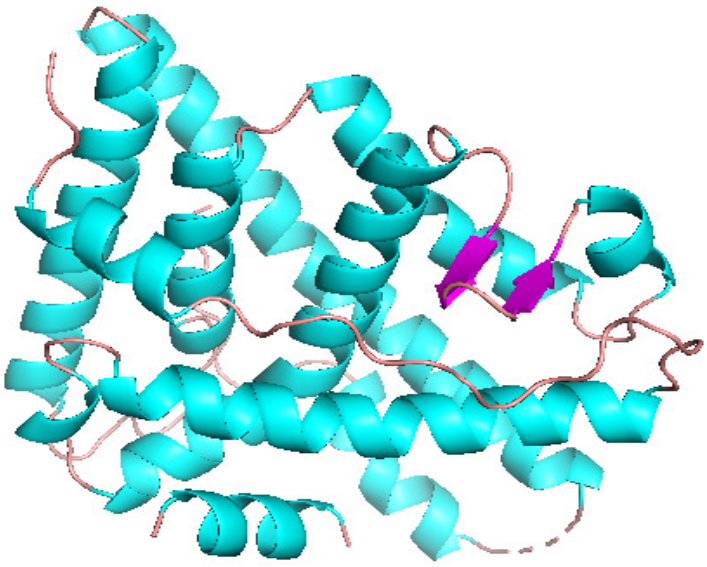

**Figure 3: Illustrates the three-dimensional structure of progesterone receptor where cyan-colored spirals are alpha-helices and the flat structures are beta-pleated sheets.**

*3.2.2. Ligand selection*

There is a large body of natural products found on the planet. Out of that vast array of natural products, the compounds from medicinal plants have demonstrated promising characteristics for various types of diseases which can range from small skin injuries to conditions like cancer. In this study, to select a potential ligand against the targeted protein progesterone receptor, a thorough review of existing literature about the potential anti-cancer compounds from Tinospora was performed. The compound cordioside was selected after reviewing its properties. The ball and stick structure of cordioside is given in Figure 2 below where green balls are representing carbon, and red oxygen and white balls are representing hydrogen molecules.

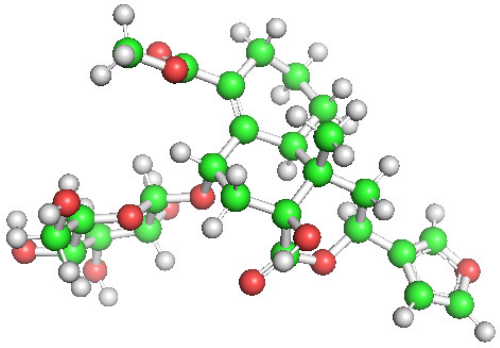

**Figure 4: Illustrates the ball and stick structure of cordioside present naturally in Tinospora cordifolia.**

*3.3. Instrument*

The structure of progesterone receptor with bound ulipristal acetate and a peptide from the co-repressor SMRT was downloaded from "Research Collaboratory for Structural Bioinformatics Protein Data Bank", maintained by the "Worldwide Protein Data Bank (wwPDB)", most commonly known by its short-form RCSB PDB. The download of protein was performed in .pdb format under the ID of 4OAR. This database contains 3 D structural data of nucleic acids and proteins collected and generated from different techniques such as spectroscopy, cryo-electron microscopy, and X-ray crystallography and is freely available. On the other hand, Pubchem was used to retrieve the structure of cordioside. PubChem is known as the biggest collection and database of compounds, chemicals, and their experimental test activity. The "National Center for Biotechnology Information (NCBI)," which is part of the "National Library of Medicine (NLM)," hosts and maintains this resource.

The autodock tool was then used to prepare the protein and ligand. First, the protein was prepared in pymol deleting all unwanted molecules which can interfere with the docking procedure. Then, further preparation was carried out by Autodock4. Firstly, the water molecules were deleted from the unwanted structure, after that, the addition of polar hydrogens was performed to find out the hydrogen bond interactions between the amino acids of the protein and ligand molecule. Finally, the Kollman charges were added that are essential for electrostatic calculations.

Following the preparation of the ligand and protein, grid assembly and docking were carried out using Autodock4. It is a free and simple tool for measuring a binding affinity between protein-ligand molecules as well as the protein-protein interaction. Drug Discovery Studio was used to analyze the docked complex once it was retrieved. The distance between bonds, the type of bonds produced, and the binding energy were all measured and computed using BIOVIA Drug Discovery software.

*3.4. Data Collection*

The output file was received in DLG format after performing the Autodock run. The minimum binding energy between the protein and ligand was then checked in the output file in DLG format and then the complex was visualized and analyzed using BIOVIA drug discovery studio. In the below Figure 3, the three-dimensional structure of the docked complex between progesterone receptor and cordioside is given which represents the type of bond formed between the complex. On the other hand, the length between the bonds was also noted represented in Figure 4.

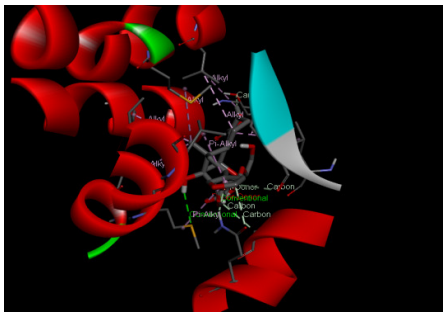

**Figure 5: Illustrates the type of bonds formed between the amino acids of progesteron receptor and cordioside.**

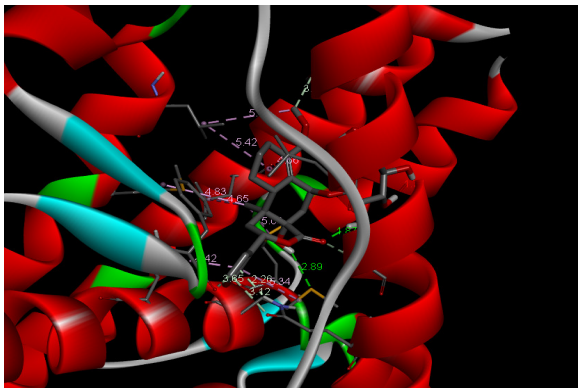

**Figure 6: Illustrates the distance between the bonds formed in the cordioside-progesterone receptor complex.**

*3.5. Analysis*

The 2-D structure view of a docked complex between protein and ligand was then constructed to have more clarification about the amino acids involved in complex formation. It was noted that the selected ligand formed hydrogen bonds and hydrophobic bonds with the amino acids of the targeted protein which makes the complex stable, a critical requirement in the drug discovery. Amino acids involved in hydrogen bonding were found to be MET759, GLY722, THR894, GLN725, PHE778, and GLN725. On the other hand, amino acids involved in hydrophobic interaction with ligand molecules were LEU797, MET756, MET801, LEU887, LEU797, MET759, and LEU763 demonstrated in the Figure 5.

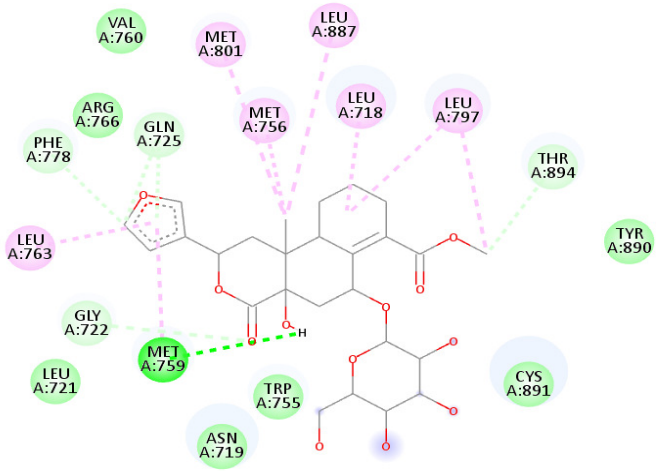


**Figure 7: Illustrates the two-dimensional structure of the docked complex between the progesterone receptor and the cordioside.**

## 4. RESULTS AND DISCUSSION

Tinospora cordifolia is one of the famous researched plants for its medicinal values with genetically diverse characteristics. One of the natural products of Tinospora cordifolia "cordioside" was used in silico study against the progesterone receptor involved in the development of breast cancer using a molecular dynamic study. To evaluate the protein and ligand interaction in terms of binding energy, which is simply defined as the total intermolecular energy displayed by receptor and ligand. After docking using Autodock4, the

output file was retrieved and utilized to determine the ligand's greatest affinity posture with amino acids present in the receptor. It should be emphasized that the greater negative the energy between protein and ligand, the stronger the stabilization of protein and ligand. It was discovered that the binding energy of the very first position was -6.43 kcal/mol, implying the stability of the docked structures. It was observed that 6 hydrogen bonds were formed with MET759, GLY722, THR894, GLN725, PHE778, and GLN725 amino acids with a distance of 2.88764, 3.53313, 3.00685, 3.12093, 3.64645, 2.26022 Å respectively. Apart from that eight hydrophobic bonds were observed with amino acids LEU718, LEU797, MET756, MET801, LEU887, LEU797, MET759, and LEU763 with the distance of 4.65544, 5.4218, 5.02585, 4.83493, 4.64795, 5.12814, 5.33764 and 4.42431 Å respectively. The different types of the bond involved in the complex formation and their category, as well as the distance bvetween them, are enlisted in Table 1.

**Table 2: Enlists the protein-ligand interactions, amino acids, distance, bond category, and types.**

| Protein: Ligand | Distance (Å) | Bond Category | Types |
|---|---|---|---|
| UNL1 - GLY722 | 3.53313 | H-Bond | C–H bond |
| UNL1 - MET759 | 2.88764 | H-Bond | Conventional Hydrogen Bond |
| UNL1- PHE778 | 3.64645 | H-Bond | C–H bond |
| UNL1 - GLN725 | 2.26022 | H-Bond | Pi-Donor Hydrogen Bond |
| UNL1 - LEU718 | 4.65544 | Hydrophobic | Alkyl |
| UNL1 - LEU797 | 5.4218 | Hydrophobic | Alkyl |
| UNL1 - MET756 | 5.02585 | Hydrophobic | Alkyl |
| UNL1 - THR894 | 3.00685 | H-Bond | C–H bond |
| UNL1 - MET801 | 4.83493 | Hydrophobic | Alkyl |
| UNL1 - LEU887 | 4.64795 | Hydrophobic | Alkyl |
| UNL1 - LEU797 | 5.12814 | Hydrophobic | Alkyl |
| UNL1 - GLN725 | 3.12093 | H-Bond | C–H bond |
| UNL1 - MET759 | 5.33764 | Hydrophobic | Pi-Alkyl |
| UNL1 - LEU763 | 4.42431 | Hydrophobic | Pi-Alkyl |

Phytochemicals provide us with several challenges. The primary problem is enhancing phytocompound bioavailability. Because many of these phytocompounds are naturally present in the food, they are easily metabolized and eliminated by the body. They do not stay in systems of the body for long, hence their therapeutic potential is limited. The advancement of research will aid in increasing the stability of these compounds. These include the incorporation of stabilizers or the encapsulation of phytochemicals into specific systems, such as microparticles or nanoparticles, which can greatly improve their stability and antioxidant capacity. The lack of target specificity is another disadvantage of employing natural bioactive phytocompounds in the treatment of cancer. It is usually believed that these drugs have a multi-targeted ability; as a result, cancer cells frequently activate alternative signaling cascades, leading to the failure of targeted treatment.

Phytocompounds become more potent under these settings due to their ability to trigger a variety of alternative survival signaling pathways. There are numerous techniques to address this issue, including (a) to improve bioavailability and effectiveness, semi-synthetic drugs can be formulated using phytocompounds (b) New methods for targeted delivery of selected phytocompounds need to be explored. (c) employment of novel formulation technologies.

## 5. CONCLUSION

Breast cancer is one of the most frequent malignancies in women across the world. Dry lab study-like computational approaches have been widely employed in drug development, particularly in the identification of phytoligands against many overexpressed proteins in breast cancer. In several in vitro and in vivo investigations, green phytocompounds derived from various medicinal plants have demonstrated a high potential for fighting BC cells. In this research, the interaction between one of the natural products "cordioside" from a medicinal plant, and one of the most important hotspot targets of breast cancer progesterone receptor was observed which demonstrated the significant binding energy of -6.35 kcal/mol. Therefore, the study suggests the targeting ability of cordioside against progesterone receptors which can be further improved with the help of novel formulations, chemical modifications, and drug delivery systems.

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# CHAPTER 17

# IMPACT OF NUTRITIONAL DEFICIENCY IN FETUS AND ITS HARMFUL EFFECTS ON HUMAN BODY

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***ABSTRACT:*** ***Malnutrition is a dangerous condition that arises when an individual's diet lacks the necessary nutrients. Maternal malnutrition, low weight gain throughout pregnancy, or notably maternal anemia are all modifiable variables linked to fetal development limitations. Children who are malnourished as infants are more likely to have poor physical health than adults and the author explores more emotional issues such as anxiety and obsessive-compulsive disorder due to malnourishment. Even as experts learn more about the long-term effects of neonatal malnutrition. The main problem mother faced due to malnutrition are hidden hunger, under-nutrition, and overweight. The main objective of this paper is to learn more about the effects of malnutrition on the development of a fetus. In the future, this paper will help to understand the impact of malnutrition on the development of the fetus, and once people understand the effects of malnutrition, they can overcome the problem caused by malnutrition.***

***KEYWORDS:****Fetus, Under-Nutrition, Malnutrition, Minerals, Vitamin.*

## 1. INTRODUCTION

Malnutrition is defined as inequality between the nutrition your body requires as well as the nutrients it receives. It might indicate malnutrition or over-nutrition, and the individuals may be malnourished due to a calorie shortfall, or you could have a vitamin, protein, or mineral deficiency. People may potentially have a surplus of calories that their body is unable to process [1], [2]. To sustain its tissues and activities, the body requires a wide range of nutrients in specific quantities. Malnutrition occurs when nutrients are insufficient to satisfy these requirements. People might be malnourished due to a lack of nutrition in general, and you might be malnourished due to the abundance of some nutrients but a deficiency in others. Even a solitary mineral or vitamin deficiency might have major health implications for your body. An overabundance of nutrients, on the other hand, might produce issues [3], [4].

### *1.1. Types of Malnutrition:*

Malnutrition can be defined as either under-nutrition or over-nutrition. It can also refer to a macronutrient (proteins, carbs, and fats) or micronutrient imbalance (vitamins and minerals), which is shown in Table 1.

**Table 1: Illustrate the Major types of Malnutrition and how they affect the growth of newborn babies.**

| Types of Malnutrition | Description |
|---|---|
| ***Under-nutrition*** | The majority of people associate malnutrition with under-nutrition. A lack of nutrients is known as under-nutrition. If people don't eat enough because their bodies can't absorb enough nutrition from their meals, they may be undernourished. Under-nutrition can result in fat and muscle loss that is obvious but can |

| | |
|---|---|
| | also be undetectable. It's possible to be overweight or malnourished at the same time. |
| ***Under nutrition in macronutrients*** | This is a lack of macronutrients such as proteins, carbs, and lipids, also known as protein's energy undernourishment. Macronutrient composition is the most important nutrient in your diet, as they are the ones that your body needs to create energy and stay alive. Your body starts to decline apart without them or even just one of them breaking down tissues or closing down non-essential activities to preserve energy. |
| ***Under nutrition in micronutrients*** | Vitamins and nutrients are micronutrients and the human body needs them at lower levels, yet they are required for a variety of processes. Due to a lack of diversity in their diet, several people are moderately deficient in various vitamins or minerals. People may not even notice if you have a moderate vitamin deficit, but if micronutrient malnutrition worsens, it could have major and long-term consequences. |
| ***Over-nutrition*** | To reflect the negative health implications that excessive nutrient consumption might have, the World Health Organization has included nutritional deficiencies in its description of malnutrition. This includes the consequences of being overweight or obese, both of which are strongly linked to a slew of non-communicable illnesses (NCDs). It also encompasses the poisoning that can occur when some micronutrients have overdosed. |
| ***Over-nutrition of macronutrients*** | When the human body has more carbohydrate, protein, or fat calories than it can need, it stores them in fat tissue in fatty tissue. Whenever your body runs out of tissue to store fat, the fat cells must develop to compensate. Fat cell enlargement is linked to persistent inflammation or, as a result, a slew of metabolic diseases. NCDs including coronary heart disease, diabetes, or stroke can result from these factors. |
| ***Over-nutrition in micronutrients*** | Vitamin / mineral supplements can overdose, and more study is needed to understand how this occurs and how much of a vitamin or mineral is too much. Micronutrient over nutrition is unusual and does not develop only as a result of diet. However, taking excessive amounts of some supplements might be hazardous. It's a good idea to double-check with your doctor beforehand. |

### *1.2. Malnutrition's Affects:*

Malnutrition, in its widest meaning, may impact everyone, and the malnutrition is caused by insufficient nutrition education, a shortage of access to a range of foods, sedentary contemporary lives, or economic disparities. Specific groups are more vulnerable to certain forms of malnutrition. Poor or low-income people are more vulnerable to malnutrition. Poorer groups have much less access to proper nutrition, whether in a wealthy country like the United States or undeveloped countries with limited resources overall [5], [6].

- To fully develop, children require more nutrients than adults, and the nutrition as well as its repercussions are especially dangerous for underprivileged youth.

- Many chronic conditions directly alter appetite or calorie intake. Some of these raise your calorie requirements. Under- nutrition is also a health risk for those who spend time in hospital.
- Adults' nutrition might decline as they get older for a variety of reasons, including limited mobility, institutionalization, decreased appetite, or lower vitamin absorption.

➢ *Over-nutrition is more common in some populations:*

- *Poor or low-income individuals:* Poorer populations in wealthy nations frequently have easier access to quick meals, which are heavy in calories but low in nutritional content, than healthy whole foods. This can result in macronutrient excess and micronutrient deficiency.
- *Sedentary:* Desk employment, family commitments, healthcare, and social issues can all contribute to major weight gain by keeping individuals sitting all day instead of getting out now and moving about.

### *1.3. What type of problem does the body face during malnutrition?*

Under nutrition in macronutrients (protein-energy under nutrition) precludes your body of the energy it needs to function. As a result, it begins to break down its very own tissues or shut down its activities to compensate. This starts with the body's fat storage, then moves on to skin, hair, muscle, or nails. Protein-energy malnutrition causes people to seem gaunt and the children's growth may well be stunted. The immune response is among the first systems to shut down. As a result, undernourished persons are more susceptible to disease and infection, and they recover more slowly. Wounds lead to more chronic pain than other types of injuries. Cardiac activity lowers as well, resulting in a reduced heart rate, blood pressure, or skin temperature. People would feel dizzy, weak, or uninterested in life. They could lose regular appetite, or sections of their digestive tract might deteriorate [7], [8].

People who are malnourished in macronutrients are more likely to be malnourished in micronutrients as well. Vitamin / mineral levels are affected when total calories are insufficient. Vitamin deficiencies are responsible for some of the consequences of severe malnutrition, such as marasmus and kwashiorkor. Vitamin A insufficiency, for example, can cause visual issues, while vitamin D shortage can lead to soft bones. Some people consume a huge amount of calories yet lack the vitamins and minerals they need. Malnutrition's consequences may be less visible in some circumstances. People might well be overweight or obese as a result of macronutrient over- nutrition, but they may also have anemia, weakness, faintness, and weariness as a result of a mineral as well as vitamin deficiency. Overeating can result in metabolic syndrome signs including insulin resistance or high blood pressure [9], [10].

### *1.4. Diagnosed Malnutrition:*

Protein-energy under nutrition and over nutrition may typically be diagnosed based on physical examination and a history of your food and health problems. To determine the severity of the condition, healthcare experts may take your BMI or even a child's arm circumference. They will collect a blood sample if feasible to check for particular vitamin abnormalities. Micronutrient malnutrition frequently occurs alongside macronutrient malnutrition, and it can also occur alongside macronutrient over nutrition. Whether people have similar symptoms, a blood test can also detect the unusual occurrence of micronutrients over nutrition.

### *1.5. Treatment malnutrition:*

Nutritional supplements are used to treat malnutrition, and the individual micronutrients may be prescribed, or you may be advised to the reef with a tailored, high-calorie nutritional mix designed to replenish what your body has lost. Reseeding might take weeks to treat severe malnutrition. Refeeding, on the other hand, might be risky, particularly in the first several days. To adapt to malnutrition, your body adjusts in a variety of ways. Refeeding forces it to revert to its previous mode of operation, which might be more than it can manage at times. To avoid and treat the significant and even life-threatening consequences of Refeeding syndrome, it's preferable to start Refeeding under constant medical supervision.

Overeating is usually addressed with losing weight, a change in food, and a lifestyle change. Losing weight can help you avoid secondary diseases like diabetes and high blood pressure by lowering your chance of getting them. Diet and exercise routines, drugs, and medical procedures may all be used to help you lose weight. An underlying issue, including thyroid disease or a mental health concern, might even require treatment. Depending on the road you pursue, weight reduction might be quick or slow and steady. But it's the lifestyle adjustments you commit to after humans lose the weight that will help you keep it off. Long-term welfare systems, including counseling, behavioral therapy, support groups, or dietary instruction, may be used.

### *1.6. Malnutrition prevention:*

Malnutrition is a worldwide issue, and the poverty as well as a lack of awareness of nutrition are the major factors in both the developing and developed economies. With greater global knowledge and assistance for the poor, especially access to clean drinking water, nutritious entire foods, or medicine, we can help manage the illness of malnutrition. Children and the elderly, who may be unable to advocate for themselves, are particularly vulnerable and may require extra care for their food and health. The greatest method for preventing malnutrition is to consume a very well diet with a range of healthy whole foods in it. If you have enough of all the nutrients your body needs, you will be less likely to overeat trying to satisfy those needs. Even with a pretty normal diet, some micronutrient shortages are frequent. A blood test is one approach to see if micronutrient supplements may help people. Human healthcare practitioner can assist you in determining the appropriate dose.

## 2. LITERATURE REVIEW

Neha et al. investigated and evaluated iron deficiency and growth retardation of newborns born by malnourished mothers using a nested case-control study. The research was carried out at Kasturba Medical College in Mangalore, which is affiliated with the Maniple Academy of Higher Education in India, as a subgroup analysis of participants in a randomized controlled experiment. There were 88 infants delivered through vaginal birth to malnourished primigravidae. The cord blood ferritin and neonatal anthropometry were assessed. Apart from that the maternal hemoglobin and ferritin were also assessed and investigated. Pre-pregnancy body mass index (BMI) or gestational weight increase were calculated using the mother's pre-pregnancy height, weight, or current weight. At birth, the average weight was 2.6 kg. Fifty-eight percent of babies were delivered at the appropriate gestational age (AGA). The percentage of AGA kids born to moms who gained 7.00 kg more was significantly higher. Nine babies were found to have low iron levels, three of whom were undersized for their gestational age (SGA). Therefore, the study suggested that the appropriate weight gain can prove to help achieve the desired growth of a fetus from malnourished mothers [11].

Xue et al. researched to investigate the effect of under-nutrition in mothers and its impact on fetal development and growth. The researchers used a sheep model to see if maternal

starvation affects fetal lipid metabolism or impedes fetal hepatic growth. For 15 days, 20 pregnant lambs were fed regularly or with a 30% fat restriction, after which fetal liver tissues were taken for metabolite, histomorphology, transcriptase, or biochemical analysis. The researchers discovered that maternal malnutrition altered the fetal liver's overall metabolic mode or transcriptase profile. Ketogenesis but also fatty acid oxidation was boosted in impoverished ewes' fetal livers, perhaps due to an active peroxisome proliferator-activated receptor signaling pathway, whereas fatty acid steroids, as well as cholesterol, were repressed. Furthermore, we discovered that maternal malnutrition inhibited DNA replication and cell differentiation, but also anti-apoptosis in the fetal liver, but also disrupted the balance between apoptosis and cell proliferation, implying that maternal malnutrition has an impact on fetal liver growth and development. In general, their findings show that maternal starvation throughout pregnancy inhibits fetal hepatic growth and changes fetal lipid metabolism in sheep, which will greatly aid future studies into fetal metabolism or development in humans [12].

Molly Rainforth investigated the effects of maternal under-nutrition or malnutritions on the performance or growth of offspring. Their research looked at pregnant cows' nutritional needs and reviewed the effects of maternal food restriction on calf growth throughout pregnancy. It also considers the many periods of development where food restriction will have the most impact. While early gestation is critical for the creation of the placenta or the growth of the first organs, it has been revealed that the fetus can adapt to developmental restrictions induced by maternal malnutrition during this period. During the mid-to-late stages of pregnancy, however, maternal food restriction has the greatest impact on child growth. Maternal malnutrition during this vital period might result in lower birth weight, which is linked to a lifetime's worth of health as well as growth efficiency [13].

## 3. DISCUSSION

Nutrition is critical for the maturation or psychological and social functioning of the nervous system in both children and mothers. During pregnancy, a lack of proper quality and quantity diet can create health issues for both the mother and the child. Mother nutrition has been linked to a beneficial relationship with newborn birth weight, maternal BMI before pregnancy, and weight increase throughout pregnancy. Preterm birth or intrauterine growth retardation (IUGR) is more likely in women with a low BMI before pregnancy if they do not gain enough weight [14], [15]. The nutritional state of the mother at conception is a significant element in the development and fetal growth a healthy. It is critical to provide a healthy diet to the mother before and through pregnancy for her to have enough energy and nutrients for herself, her fetus's growth, and future lactation. There is a slew of negative health consequences linked to maternal malnutrition. In the short and long term, it can have an impact on both the pregnant female and the developing baby, and there are two types of under-nutrition:

- Malnutrition
- Deficiency in micronutrients

When a person consistently consumes less protein or carbohydrate-based energy, malnutrition results. Underweight and other health issues result from malnutrition in people. Micronutrient deficiency occurs when a person consumes an adequate amount of food overall but not enough of the specific micronutrients necessary for the growth as well as operation of certain bodily systems and components. Calcium deficiency, for example, might affect bone or teeth development. The growth of the fetus is affected by maternal weight increases during pregnancy. Poor development and shorter gestation cause small neonate sizes at delivery, as well as the most adverse outcomes, occur in even the most immature newborns. Low first-

trimester weight gain has been linked to a higher risk of preterm birth, while low second and third-trimester excess weight has been linked to a higher chance of spontaneous preterm birth.

A mother's nutritional insufficiency has been related to a bad birth result. The relationship between maternal nutrition as well as birth outcome is complex, and it is influenced by several physiologic, social, but also demographic factors that differ widely between populations. Assessing the link between maternal nutrition but also birth outcomes may open the way for nutritional interventions that improve birth outcomes including long-term quality of life while decreasing mortality, health-care costs, morbidity, and spending [16]. The birth weight that is too low in which Low birth weight (LBW) is a primary driver of death, morbidity, or impairment in childhood and adolescence, as well as having a long-term influence on adult health outcomes.

Birth weight of less than 2,500 g is considered a lower birth weight. Premature birth, intrauterine growth failure, or disturbance can all cause it. Low birth weight is a contributing factor in 40%–80% of newborn mortality, with 98 percent of deaths occurring in underdeveloped nations. Low birth weight is significantly linked to prenatal morbidity and an elevated risk of long-term impairment in both advanced and developing world's [17]. Preterm delivery, which is described as a gestational age of fewer than 37 weeks, contributes significantly to birth weight and is the major cause of infant death among newborns with congenital abnormalities. For low birth weight or preterm newborns, the expenses of postpartum hospitalization and care are particularly high.

### *3.1. Health consequences of malnutrition during pregnancy*:

Pregnant women who consume insufficient nutrition have an increased risk of maternal morbidity including poor pregnancy outcomes, such as early birth and miscarriage. They are also more likely to suffer the following conditions: anemia, lethargy, infection, or weakness, as well as lower productivity [18], [19]. Risks to the mother's health and the health of the newborn infant Under nutrition during pregnancy has been related to a variety of detrimental effects on the developing fetus, such as intrauterine growth retardation and low birth weight. Low birth weight, paternal malnutrition throughout pregnancy, and intrauterine growth restriction (IUGR) are all associated with several unfavorable implications for the growing fetus or newborn infant, including a higher risk of birth abnormalities:

- Stillbirth - IUGR is responsible for around half of all stillbirths in properly developed fetuses.
- Perinatal mortality
- Premature birth (infant's death within seven days after birth) children who are overweight.
- Some organs are underdeveloped.
- Cretinism is a kind of conservatism (Thyroid disease is a hereditary illness that causes a lack of collaboration, a dull face expression, or dry skin).
- Damage to the brain.

Long-term hazards to the child's health shortly after birth, the fetus has metabolic and other alterations as a result of maternal malnutrition. A starving fetus, for example, responds by lowering insulin and glucose synthesis. Changes in a person's glucose tolerance or insulin metabolism over time, increase the risk of chronic nutritional ailments such as type 2 diabetes, and metabolic disorders, including obesity. According to one study, the lower an infant's birth weight, the higher the risk of developing type 2 diabetes. When comparing men born with low body weight to men born with high body weight, those born with low body

weight are seven times more likely to develop diabetes. Maternal malnutrition has different repercussions depending on the stage of pregnancy at which it occurs. As per one study, maternal malnutrition in the first three months of pregnancy is connected to an increased risk of obesity and coronary heart disease, whereas malnutrition in the third trimester of pregnancy is linked to impaired glucose metabolism [20].

### *3.2. Micronutrient Deficiency and Its Related Diseases:*

Throughout Pregnancy, there are several maternal or fetal health hazards linked with micronutrient deficit pregnancy, namely low in micronutrient like folate or vitamin B12. Many chronic disorders, including osteoporosis, colorectal cancer, osteomalacia, hypothyroidism, or heart diseases, are associated with micronutrient deficiency conditions. Fortification has a track record of efficacy or safety for more than a century, but it has proven successful in preventing certain disorders, including birth abnormalities.

#### *3.2.1. The mother's health is at stake:*

The following are some of the maternal health hazards that may occur as a result of micronutrient deficiency: For pregnant women, vitamin B12 insufficiency is linked to the following hazards:

- The signs and symptoms of anemia
- Complications of the nervous system

Lack of Vitamin K is linked to blood clotting issues, such as a longer clotting time, which is especially dangerous during pregnancy when women lose a great deal of blood, even if blood clotting operates correctly. Anemia due to iron insufficiency is connected to an iron shortage during pregnancy. Iodine deficiency has been associated with several adverse birth outcomes, including:

- Stillbirth
- Miscarriage

### *3.3. Nutrition's Function:*

A balanced and healthy diet benefits both the unborn baby and the mother throughout pregnancy. It has a direct influence on the weight of both the baby at delivery. It also assists in the protection of illnesses such as heart disease and obesity in children later in life. According to a study, women who eat poorly before pregnancy are much more likely to have a child prematurely than those who eat well. As a result, it's always a good idea to eat well, stay active, as well as drink enough water. All of this is critical for the mother's or unborn baby's health. To decrease the risk of maternal-fetal or newborn problems, nutritional counseling should be a part of prenatal treatment.

#### *3.3.1. Pregnancy Nutritional Requirements and Birth Outcomes:*

The consumption of sufficient nutrition to fulfill maternal and fetal requirements is critical to the success of a pregnancy. Malnutrition is caused by insufficient food intake, resulting in growth failure and protein-energy malnutrition, particularly during the fast growth periods of pregnancy. It was later shown that poor growth is caused not only by a lack of protein and energy but also by an insufficient intake of micronutrients that are essential during rapid development periods.

#### *3.3.2. Dietary Consumption, Nutritional Status, or Pregnancy Outcomes:*

The nutritional needs of pregnancy necessitate a well-balanced diet to promote healthy fetal learning and expansion. Birth weight is also influenced by maternal nutrition. Diets that are

low in protein should be avoided. Breast-feeding mothers have higher nutritional demands than pregnant mothers, and their energy requirements are higher during nursing than during pregnancy. For adequate nutrition as well as the greatest results, pregnant as well as breastfeeding females should consume a healthy diet that includes a variety of nutritious foods. Lack of nutrition in the fetus is being observed nowadays, so in this paper, the author talks about the importance of proper nutrition for the fetus during pregnancy and also talks about the different types of malnutrition. The author also discusses the consequences of malnutrition on the fetus.

## 4. CONCLUSION

Many variables, including poor socioeconomic position, increased parity, and a short inter-pregnancy gap, might alter a pregnant woman's nutritional state. Women of poor socioeconomic position are more likely to have insufficient food intake, unsanitary housing, or lack of sanitation, as well as a lower capacity to seek medical treatment and purchase medicine/supplements, all of which have an impact on their infants' birth weight. High parity increases the risk of placenta previa due to malnutrition, and these problems can predispose a woman to give birth to a low-birth-weight baby (LBW). Researchers have proved clinically in individuals that the use of new information can reduce the onset of many diseases. As a consequence, it's critical to comprehend both the components that impact fetal growth and the conditions that limit the fetus's access to nutrients as well as oxygen from the mother. Despite the progress made, additional study is needed to better understand how the fetus adjusts to the woman's limited availability of nutrients, how these adaptations influence the body's structure and function, and what molecular pathways nutrition and hormones may utilize to modify gene expression. People think that developing optimal nutrition food planning is vital to enhance pregnancy outcomes, healthy child development, promote growth lower the risk of chronic illnesses, and delay the metabolic decline associated with aging. The mother's nutritional state has a big influence on how the fetus develops and how the birth goes. It is a modifiable risk factor that has significance for public health in the avoidance of negative birth outcomes, particularly among populations that are still in the process of developing. The information in this essay will be useful in the future for understanding how starvation affects the growth of the fetus. Once people are aware of these consequences, they can work to solve the problem that malnutrition causes.

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# CHAPTER 18

# APPLICATION OF SIX SIGMA TECHNIQUES TOREDUCE PACKAGING PROCESS FOR LARGE-SCALEFOOD PROCESSING UNITS

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***ABSTRACT:*** ***Six Sigma has evolved as one of the most successful breakthrough improvement tactics among other performance and productivity improvement techniques.This study focuses on the different critical aspects of Lean Six Sigma (LSS). LSS is a method for minimizing or removing operations that do not bring value to the process. It emphasizes eliminating redundant processes in a process and only performing value-added activities. Some service businesses use Six Sigma to demonstrate the efficacy of Define, Measure, Analyze, Improve, and Control (DMAIC) in increasing quality in this sort of company. This study also explores several key elements of LSS. This study is an attempt to demonstrate the implementation of the Six Sigma quality improvement initiative in one of India's large-scale food-processing industries.These approaches need monetary resources for learning that may be difficult in a low-margin business that largely concentrates on reducing costs, as they necessitate statistical methods and expertise that are often seen as sophisticated and too technical in the food business. These results imply that when managers become more aware of the importance of LSS practices in improving food sector performance, they would promote staff to get expertise with the tools.***

***KEYWORDS:*** ***DMAIC, Food Industry, Lean Six Sigma (LSS), Lean Six Sigma Initiatives (LSSI), Lean Tools, Six Sigma.***

## 1. INTRODUCTION

Six Sigma (SS) is a powerful tool for achieving operational efficiencies at all levels. An organization's performance and efficiency may be improved using this quantitative research method. Industry in India requires total operational efficiencies if it is to compete globally, and has tried a variety of quality improvements like quality management, Total Quality Management (TQM), International Organization for Standardization (ISO) certification, as well as other similar initiatives. All of these methods are effective, but putting them into practice may be difficult, and the time it takes to see results can be discouraging. This calls for an innovative technique that may provide several advantages in a short amount of time, like Six Sigma [1].

A paradigm shift has occurred in the world's industrial industry since the 1980s. The key to a company's longevity is the realization of the significance of user satisfaction in light of rising consumer expectations. As a result, businesses have been compelled to improve the performance of either their operations or their goods [2]. The food sector, which is the subject of this investigation, has likewise become more complex and challenging in recent years. Directors of food companies face a variety of challenges in today's market. Operating expenses are rising as sales are declining and consumers become choosier or more demanding [3]. Six Sigma is a well-known approach to improving the quality of work in today's business environment. To reduce operational waste and redundancy, as well as mistakes, flaws, or waste, it establishes a set of standards that enterprises must adhere to.

Industrial and public sector organizations alike have adopted Lean Six Sigma as their standard operating procedure for implementing a culture of continual quality development.

Every company in the world has a primary goal of continuous improvement to assist them to attain qualitative or operational effectiveness and also enhance their productivity [4]. Many people believe that Lean and Six Sigma approaches can only be used in the production and supply chain sectors of an organization. It's important to note, though, that these technologies may be used across the board in a company. Lean or Lean Six Sigma (LSS) approaches need a firm's capacity to spot waste, reduce waste, or aggressively try to remove any operations which do not add value or improve customer satisfaction within and without the organization [5].

During industrial reforms, Indian industries have faced periodic effects of change. Large-scale governments and companies, primarily in core infrastructure production organizations have been the first emphasis. Performance and productivity have emerged as important concerns after liberalization and globalization [6]. With LSS, you get the best of both worlds: Lean and Six Sigma. It aims to build strong procedures, maintain a quality culture, enhance KPIs, or enhance consumer happiness. It reduces the amount of waste and unpredictability in the production of products and services while maximizing the use of resources without sacrificing performance.

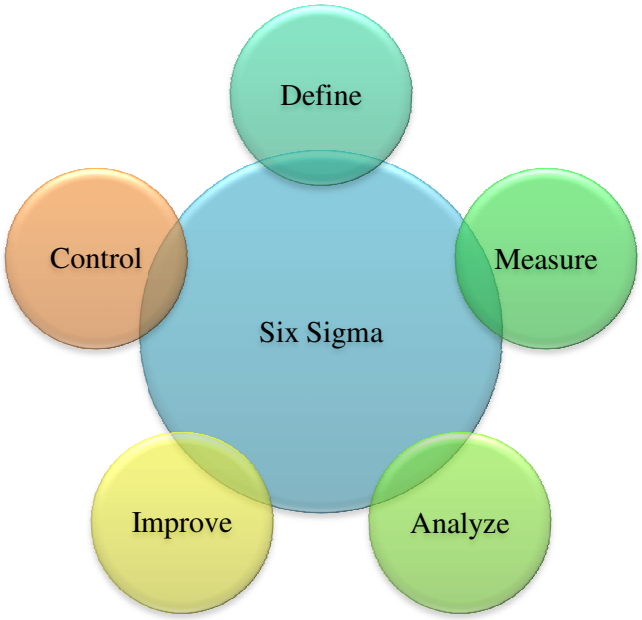


**Figure 1: Represents the Six Sigma DMAIC (Define, Measure, Analyze, Improve, and Control) Process.**

To enhance the production or transactional processes with Lean Six Sigma methods in the healthcare or other service sectors, manufacturing or administration, you may. DMAIC, a five-step problem-solving process, is emphasized throughout shown in Figure 1. It's a blend of well-known waste removal and process optimization methodologies, such as Lean Six Sigma. Six Sigma and Lean Manufacturing. LSS is widely used in high-performing firms because of its effectiveness in continuous improvement. A product of production, it has now made its way into the service sector [7]. The hybrid has some advantages, including:

- Front-Line Workers' Participation
- Customer-Centeredness
- Gain In Terms of Time and Money Saved

- Enhancements To the Process
- Eliminating Waste and Reducing Errors.

The DMAIC cycle, which stands for "define, measure, analyze, improve, and control," is a data-driven quality improvement technique. The abbreviation stands for the five stages of the process and the resources needed to fulfill each stage.

- Define: Customers, Critical Quality Concerns (CTQ), and also the main business procedures related should be defined.
- Measure: To determine the efficiency of the underlying business process, execute a test.
- Analyze: To identify the fundamental causes of faults and possibilities for improvements, evaluate the data gathered and the flowchart.
- Improve: The inventive ideas to cure and avoid difficulties in the goal process.
- Control: Keeping the Operation on the New Path by Making Changes.

This paper aims to explain how Six Sigma can be used to improve quality in one of India's largest food-processing industries. In this study, we explain the phase application of DMAIC on one of the persistent issues, the variance in milk powder packet weight. The study concluded with an estimate of the company's expected bottom-line gain from implementing the Six Sigma methodology and also the rate at which it has been accomplished.

## 2. LITERATURE REVIEW

Luana Bonome et al. stated in a study that producing a broad variety of goods in short delivery periods and at reasonable prices is a major problem for the food business, which is an essential part of the world economy. As the global economy and political issues continue to wreak havoc, this business might benefit from Continuous Improvement (CI) projects. It is common for Lean and Six Sigma projects to be combined under the term Lean Six Sigma. This strategy's applicability in the food business is less evident.The results indicated that Lean and Lean Six Sigma Initiatives (L&SSi) may be useful in the food business. Their use in the industry is still increasing. It was discovered that using them helped save expenses while also boosting output. Obstacles to these initiatives' execution have been discovered in human factors and food industry aspects. Further studies are required to determine the level of L&SSi adoption in the food industry and identify the most efficient implementation strategies [8].

The research was carried out at a particular industrial company by Brian Byrne et al., and the findings and applications it produced may be applied to other businesses in the same industry. As a result of the increasing demand, the production facility was experiencing bottlenecks in customer orders. Product effectiveness, brand awareness, and the COVID-19 epidemic all contributed to the high demand for pain management pills from an already busy location. There was a need for a problem-solving approach to be developed to decrease manufacturing site downtime while also improving product quality to meet consumer demands. According to the study, a seven-step problem-solving process was used to identify, strategize, and successfully remove waste activities, resulting in the entire removal of the issue under inquiry and savings of less than half a million dollars. The lessons learned are being implemented and utilized throughout the parent and sibling sites of pharmaceutical companies all around the globe. The author discovered that Lean Six Sigma tools and methods are successful in identifying the fundamental causes of issues and facilitating the execution of continuous improvement initiatives [9].

In their research, Harsimran Singh Sodhi examined the application of Lean Six Sigma in the food business to improve operations and quality. Studying Lean Six Sigma in a medium-sized confectionery is the goal of this study. There are data to back up the company's use of Lean Six Sigma, which resulted in a significant reduction in the overfilling of the finished product (gingerbread). The author presents a Lean Six Sigma methodology for reducing overfill and rework in the production process. For better bottom-line outcomes, the suggested framework blends lean tools with the Six Sigma methodology to define, measure, analyze and improve. The author revealed that food firms of all sizes adopt Lean Six Sigma more efficiently to minimize waste and unpredictability in their manufacturing processes [10].

A. Maleszka and M. Linke proposed in a study that Lean Six Sigma techniques are used regularly by some Polish manufacturing organizations as part of the study. The goal was to see how Lean Six Sigma technologies affected a specific management process to decrease or eliminate waste. To study, Lean Six Sigma methods have a good influence on the strategic plan since they help limit operational costs, according to the findings of the study the ability to raise profit margins by cutting costs is one way to gain a competitive edge [11].

Ming-Chang Lee stated in a study that Six-sigma systems may increase an organization's competitiveness by helping it reduce costs, increase performance, satisfy customers, or execute a sound plan. As a consequence of this study, the authors created a comprehensive SCOR methodology that incorporates lean and design for Six Sigma approaches to enhance and redesign service productivity improvements or design/or resign. A lean layout for six-sigma using a distribution network model was developed using the ideas of distribution network operations' advantages and disadvantages, lean, & designed for six-sigma [5].

Idrissi, Ismail, and Benazzouz, Bouchra proposed in a study that for the food industry's pursuit of process excellence, we've written this article to demonstrate the basic and crucial contrasts among 2 of the most effective techniques. At possible to correlate the outcomes of two case studies conducted in a fish canning firm in Moroccan by professionals in the fields of both Lean or Six Sigma techniques, top scholars and practitioners have provided their viewpoints. When it comes to process and operational improvement, Lean is simpler to implement since it doesn't include the use of sophisticated statistics, while Six Sigma necessitates a deep understanding of statistical tools—a skill that is woefully lacking in Morocco's food manufacturing sector. The findings and opinions presented in this article are based on two case studies conducted at a fish canning business in Morocco. To reach solid and reliable conclusions, it is necessary to include the perspectives of other academics and practitioners. Academics and professionals involved in the study and use of the two most potent approaches for establishing and maintaining operational efficiencies would greatly benefit from this work. Understanding the basic contrasts between these two approaches is also essential [12].

Nurul Najihah Azalanzazllay and Sarina Abdul Halim-Lim stated in a study that LSS is an organized constant improvement approach extensively used to promote the performance of the firm and reputation in the market because of its capacity to eliminate variation or improve quality performance that ultimately optimizes bottom-line savings. It has been extensively deployed in the industrial and service industries over the last 2 decades, but LSS in the food business is still lagging. Using Lewin's Change Model, to bridge a knowledge gap by focusing on the LSS criterion during the unfreezing stage of food production, as well as the key success factors (CSFs) during the change stage. As a result of the semi-structured conversations, 12 food sector quality management specialists provided their insights. A total of 29 nodes and 17 CSFs out of 31 LSS implementation success factors were identified in the food manufacturing industry, resulting in six ready constructs. The author revealed in their

study that the growing body of information about LSS's change model makes it easier for professionals in the food sector to plan for LSS implementation [13].

Saeid Hakimi et al. discussed in a study that Company A's standard plain yogurt manufacturing method has been investigated to enhance the product's acidity by altering several parameters that impact the yogurts acidic. An Iranian milk products manufacturing firm (company A) is utilizing Six Sigma-based DMAIC approaches to enhance the quality of the plain yogurt process of production by focusing on customer needs via the use of experiment design.Researchers found that a long incubation period or fat percentage is an important determinant of yogurt's pH, with 12 hours of incubation time and fat content of 1.5% being the optimal parameters. – It is widely accepted that Six Sigma is an excellent way for improving and developing new products via the use of efficient mathematical and descriptive tools and techniques. The author discusses the quality of plain yogurt produced by a dairy products manufacturing firm be improved by utilizing a Six Sigma framework based on the DMAIC methodology. Using the Six Sigma DMAIC technique to enhance the effectiveness of yogurt manufacturing is shown to be a viable option in this research. This situation may inspire firm leaders to adopt the Six Sigma technique to tackle more complex issues in those other processes when the underlying reasons are unclear [14].

## 3. DISCUSSION

A combination of processes and techniques known as Six Sigma is used by businesses to enhance the quality of the output of their processes and reduce the unpredictability of their operations. Production or business operations are examined to identify and remove the root causes of problems and to reduce variability. It employs a variety of quality management techniques, including experimental and statistical methodologies, and establishes a dedicated team of specialists inside the firm. Projects implemented by a company's Six Sigma team follow a predetermined sequence of actions and have specified value goals, such as decreasing process cycle time, decreasing pollutants, and lowering costs.A sigma rating, which indicates the productivity or the proportion of defect-free items produced, may characterize the maturity of a production process. Six Sigma is enhanced by providing:

- The significance of ongoing efforts to establish consistent and reliable process outcomes (for example by minimizing process variances) to the success of a business cannot be overstated.
- The features of manufacturing and commercial procedures may be examined, studied, regulated, and enhanced.
- Creating sustainable improvement activities demands the dedication of the whole company, especially the leadership.

Characteristics of the Six Sigma Method:

- Any Six Sigma project must emphasize obtaining measurable and quantifiable financial statements.
- A greater focus on powerful and ardent managerial leadership and assistance.
- Verifiable facts or analytical measures, instead of preconceptions and guesswork, will be used in making judgments.

The fundamentals of Six Sigma are aimed at enhancing production. Large-scale manufacturers were the first to use this standard of quality control. To improve and reduce the number of errors detected in the processes, it was designed. It's all about removing waste and giving the greatest possible consumer experience using the Lean approach to business.

Lean manufacturing experts believe that there are eight waste products: faults; oversupply; delay; non-utilized talent; transport; stock; movement; as well as excessive process [15].

*3.1.An introduction to Six Sigma:*

Many experts have defined Six Sigma in a variety of ways. Six Sigma was first described by Tomkins (1997) [16], as a program aiming at reducing the number of faults in all goods, processes, and transactions to near zero. Six Sigma, as described by Harry (1998) [17], is a deliberate program to enhance the existing, share price, as well as customer experience through statistical techniques that may result in quantum leaps in improvement.The DMAIC and also the DMADV are the two main methods employed in the execution.DMAIC is a process for the progressive improvement of existing processes that fall short of specifications, whereas DMADV (Define, Measure, Analyze, Define and Verify) is a process for the development of new processes and services at Six Sigma quality levels, or when a current procedure necessitates something more than just gradual improvements.There are five data-driven steps in the Six Sigma methodology: Define, Measure, Analyze, Improve, and Control (DMAIC). With DMAIC completely implemented, a company's problem-solving strategy is standardized, and it affects how it generates ideas for new improvements the quality.

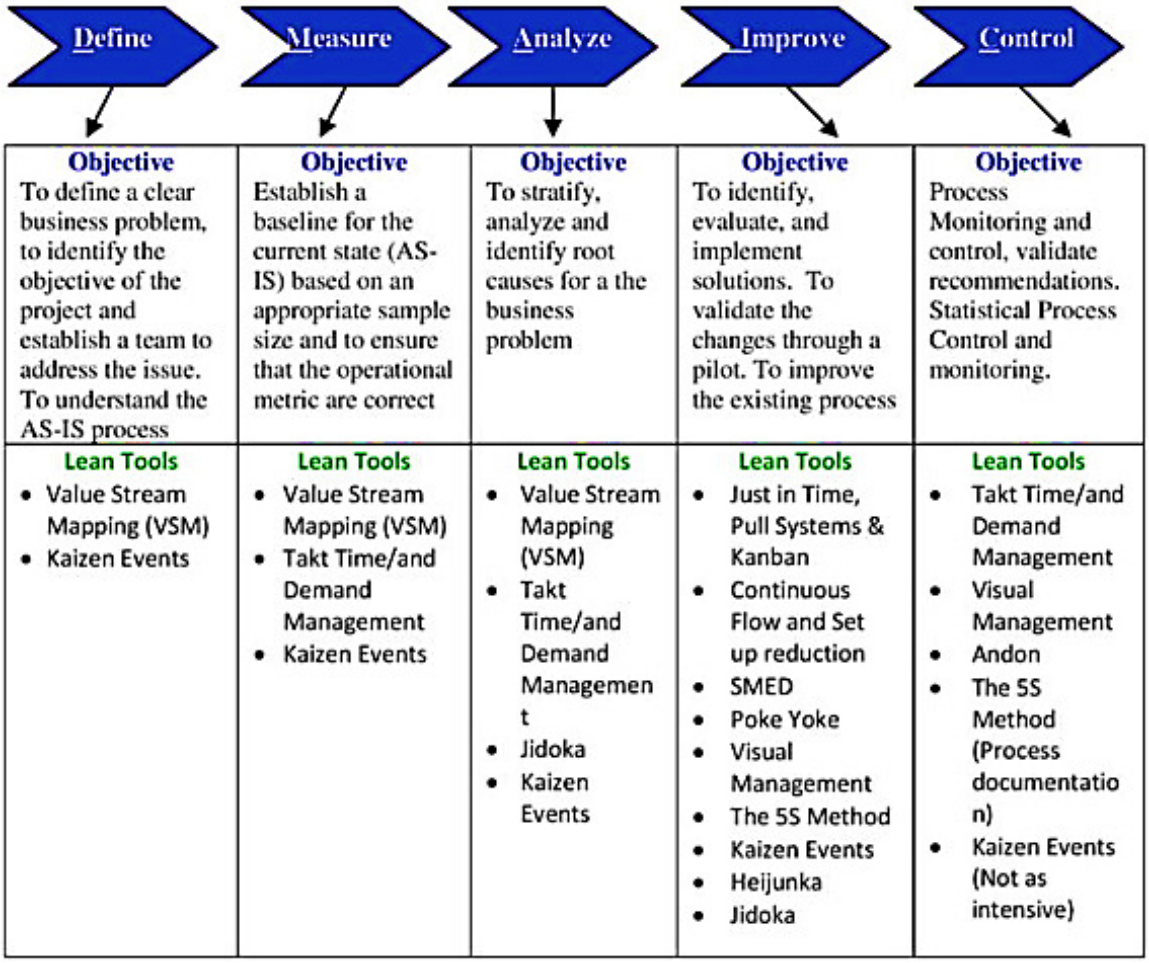


**Figure 2: Shows the DMAIC (Define, Measure, Analyze, Improve, and Control) Cycle of Six Sigma as well as Lean Tools** [18]**.**

It is important to note that Lean Six Sigma has gained widespread acceptance as a process improvement approach, and many well-known firms have already embraced it. The Lean Six

Sigma approach is now used by 35 percent of Fortune 500 organizations [18]. Figure 2 illustrates the DMAIC (Define, Measure, Analyze, Improve, and Control) is a six-step process that may combine Lean methods.

- Define: The issue will be identified by the senior management based on input from customers, the company's purpose and purpose, and the needs of customers and a target will be created.This is a critical step of the DMAIC approach. It identifies the issue in precise detail and therefore makes the next step clearer in respect of what has to be done.This should assist in identifying the product's most significant quality needs. In addition, this will aid in determining how a product can fail.
- Measure: This is essentially a data-collecting phase in which an existing set of circumstances data is gathered and the project's baseline sigma level is computed. In DMAIC, the Analyze phase is all about finding the core causes of problems, and measurement is a vital transitional stage in the Six Sigma process. Since the development team wants to validate and refine the issue and process, as well as evaluate important input/steps.
- Analyze: At this point, the team will utilize data analysis techniques or process analytical approaches to find and confirm the underlying reasons for the issue. It's for this reason that the project team must come up with causal hypotheses, select a few critical root causes, and test them for validity. To find the most important reasons, the following methods and instruments were used.The analysis involves utilizing statistical methods such as testing hypotheses to examine the process map and discover possible root causes and crucial factors/inputs that have a significant influence on the outcome. The Analyze phase of six sigma includes a wide range of tools and ideas to choose from.
- Improve:As a result of the enhance stage, we are trying to develop or implement solutions that can help remove issues, decrease variance in a procedure, or prevent future problems from occurring in the same location again. To address the fundamental causes of the problem, the project team will need to come up with new ideas and then test them.Six Sigma's DMAIC cycle has four phases: Improve is the fourth stage. This phase is all about finding a solution to the situation that was discovered in the preceding three stages. This stage necessitates the identification of the Key Process Input Variable (KPIV's) responsible for the result.
- Control: Continue to monitor and alter the functioning of the process once the enhancement has been achieved and the outcomes reported. To execute the project team to sustain performance and rectify issues as they arise, it is critical to creating standard metrics. Improvements to the process might well be lost if they aren't closely monitored.As part of this phase, need to monitor and supervise the modifications you've made to the physical or behavioral aspects of the process; retrain your personnel on the new processes; set mechanisms in place to keep up to date with changes, and write a plan of action.

The primary focus of lean six sigma concepts is on continuous improvement, even though the actual application of these concepts varies depending on the type of organization. Leadership at all levels is actively pursuing the integration of lean and Six Sigma ideas into every aspect of the organization, from product research and development to supply chain integration to sales and marketing to customer support shown in Figure 3 [19].

*3.2.Systems for implementing lean six-sigma improvements:*

Lean six sigma is a data-driven methodology based on reducing waste, minimizing variation in the process, and offering the greatest customer satisfaction. The Lean methodology identifies eight types of waste: faults, oversupply, delay, underutilized ability, transport, inventories, movement, as well as excessive processes.The purpose of the lean six sigma architecture is to provide an efficient method for combining lean and six sigma. Using the DMAIC paradigm, lean Six Sigma issue-solving follows the same steps as Six Sigma's ability to solve problems. It has been applied and validated at one UAE engineering firm [21].Utilizing the framework technique, the customer would have a methodical approach to continuous development. Because this architecture also enables the user to discover process problems and successfully resolve problems[21]. This structure has five steps. Define, Measure, Analyze, Improve, and Control are the five phases shown in Figure 4.

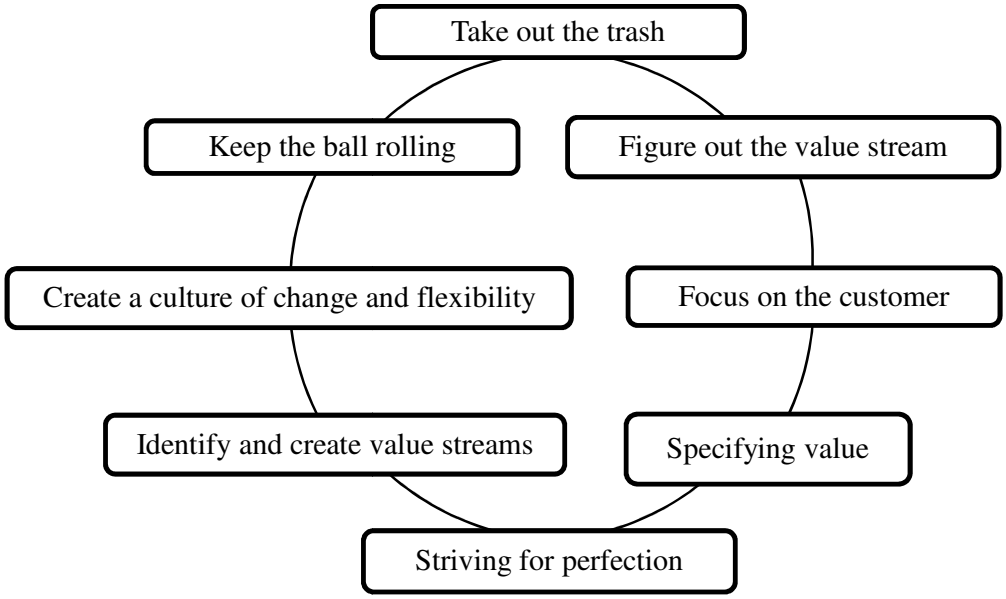


**Figure 3: Illustrating the Known 8 Major Principles of Lean Six Sigma** [20]**.**

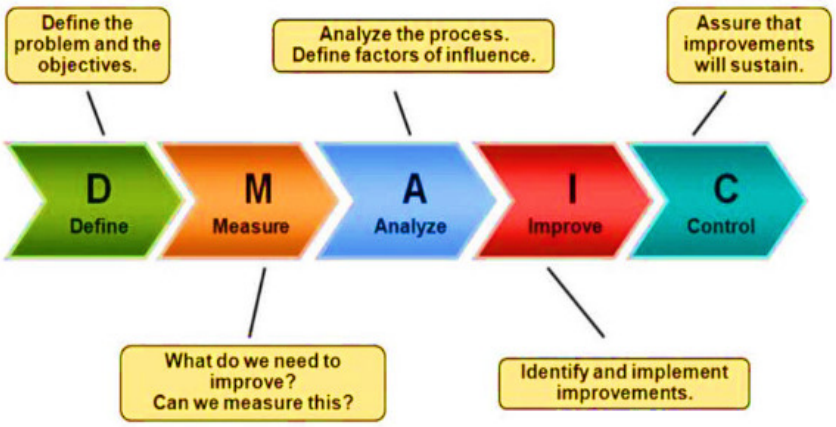


**Figure 4: Represents the Lean six sigma approach and framework i.e., DMAIC (Define, Measure, Analyze, Improve, and Control)** [22]**.**

Lean and Six Sigma efforts are used by the food sector primarily to boost productivity and minimize costs and inventories. By adopting Lean and Six Sigma efforts, the Food Industry has a significant chance to enhance its productivity.Customer satisfaction, as well as cost

savings, are two major advantages of the Lean Six Sigma method. It results in increased income and productivity. Lean Six Sigma is a methodology that imparts valuable analytical and procedural capabilities. These talents apply to positions of authority.

## 4. CONCLUSION

This study's primary purpose was to deploy the Six Sigma improvement technique in one of the most important food-processing sector units and also to highlight the advantages derived from doing so.The use of the DMAIC approach results in a comprehensive grasp of the difficulties, both qualitative as well as quantitative, and the elaboration of improvements based on an effective investigation of the problem's root causes.Lean focuses on optimizing process flow and reducing waste and variability, while Six Sigma focuses on improving processes by detecting issues and data collection and data analysis to determine and remove their core causes. Six Sigma has undergone a tremendous transformation, and it will certainly continue to expand in the next years. It is a vast sector rife with prospects for people who want to improve the workflow of their businesses and minimize wastage, and contemporary technological advances have made it much more advantageous in many ways. This study also concludes that The Analyze Phase is frequently undervalued, but without it, teams tend to leap to remedies before understanding the true core causes of the issues. This is the most significant of the five DMAIC stages. Six Sigma has evolved dramatically, and it is expected to expand much more in the next years. It's a vast sector full of potential for people looking to improve their businesses' workflow and eliminate wastage, and technological development has made it more interesting in many ways.Further, for future trends, academics and professionals might concentrate more on prioritizing important hurdles described in the chapter and addressing them during LSS deployment in production so that improvement can be readily realized.

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# CHAPTER 19

# AN IN SILICOAPPROACH TO EXPLORE TARGETING ABILITY OF BENZAVIR-2 ON VIRAL PROTEIN 35 OF EBOLA VIRUS (EBOV)

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***ABSTRACT:*** ***Ebola virus (EBOV), a Filovirus is classified as a risk group 4 pathogen, which presents considerable challenges for the development of antiviral therapeutics. EBOV has spread to several countries during the 2014–2016 outbreak and it has become a serious threat to world health. Anti-EBOV immunizations and therapeutics have been developed, with two antibody-based treatments and three vaccines approved in recent years. Despite this, the high mortality rate of Ebola virus disease emphasizes the necessity to continue developing antiviral treatments and therapeutics for the management of future EBOV outbreaks. The focus of this research study is to explore the targeting ability of Benzavir-2 which is a potent antiviral inhibitor of flavivirus infections against the EBOV VP35 Interferon inhibitory domain. The research was carried out with the help of a computational study using Autodock software which predicted the binding energy of -5.11kcal/mol between EBOV VP35 and Benzavir-2. Therefore, this research paves the path for the future research of Benzavir-2 as the potential compound against EBOV infections, however, more research is needed to increase the drug-likeliness and antiviral effect of Benzavir-2.***

***KEYWORDS:*** ***Benzavir-2, Docking, Ebola Virus (EBOV), EBOV VP35, Interferon Inhibitory Domain.***

## 1. INTRODUCTION

Ebola viruses (EBOVs) are Filoviridae viruses with a negative-stranded RNA genome. It must be emphasized that filovirus disease categorization and nomenclature have been hotly debated, as discussed elsewhere. Because of the rapidity with which EBOV causes acute hemorrhagic disease and the contagious virus nature, it has received worldwide attention. These viruses were discovered in 1976 during the first reported EBOV outbreak in the village of Yambuku in the Democratic Republic of Congo (DRC), and have since induced outbreaks of varying severity in several equatorial and West African nations [1]. The majority of outbreaks are traceable back to a particular EBOV dissemination into the human species from an unidentified source via unknown mechanisms. Following that, the virus is spread via contact with body fluids, infected tissues, or infected fomites or direct human-to-human contact.

The 2013–2016 Western African outbreak and, to a lesser degree, the subsequent EVD outbreaks in the Democratic Republic of the Congo have provided specialists with information that has improved their ability to define the usual stages of EVD in humans [2]. EVD usually starts with a nonspecific febrile condition and then progresses to major gastrointestinal manifestations and indications. EVD evolves into complex multiple organ dysfunction conditions that can be deadly in severely viraemic individuals who also have dysregulated immunological responses [3], [4]. Age, gender, and race are not very well demographic risk factors for EBOV infection and further EVD development. Sex variations in susceptibility have not been established based on existing (although incomplete) knowledge, however, women who care for others may be at increased risk of being exposed to EBOV, and the prevalence of EVD rises almost steadily with age, spiking at thirty five to

fourty four years [5]. Children have a quicker incubation time and a faster disease course than adults, even though they account for a disproportionately tiny number of cases. Children have a greater mortality rate than older people, with children under the age of five being the most vulnerable [6]. In addition to its direct incidence and death caused by EVD, the illness has indirect effects on public health because assets are funneled away from the public health care programs designed to control other serious and major diseases such as malaria, tuberculosis, human African trypanosomiasis, HIV infections, and improving maternal and neonatal health as well as primary care.

### *1.1. Ebola Virus and essential proteins*

The EBOV particle has a consistent diameter of around 80 nm and a length ranging from 970 to 1200 nm [7]. The core is made up of a single unit of non-segmented, single-stranded, linear, negative-sense RNA. This RNA segment is helically wrapped and attached to L proteins, viral protein 35 (VP35), VP30, and nucleoprotein (NP), the sequence of proteins is illustrated in Figure 1 below. This helical structure is encircled by an outer envelope of specialized glycoprotein (GP) spikes. The viral matrix proteins VP40 and VP24 are found between the outer envelope and nucleocapsid (NC). The genome of the virus is roughly a 19-kilo base pair in length. It is the longest virus in the Mononegavirales order [2], [8].


**Figure 8: Illustrates the sequence of viral proteins present in the Ebola Virus RNA genome.**

### *1.2. Life Cycle of Ebola Virus and importance of Viral Protein-35*

In humans, EBOVs infection occurs when contaminated bodily fluids interact with mucosal surfaces or skin lesions, allowing viruses to enter via direct contact with target sites. Furthermore, research in nonhuman primates (NHP) systems have shown that EBOVs infiltrate adrenal cells, monocytes, macrophages, endothelium cells, Kupffer cells and immature dendritic cells (DCs) in the liver. The ability of Glycoprotein-1 to bind with a multitude of host-cell proteins is primarily responsible for such diversity in target cells. Even though no particular receptor has been discovered, numerous adhesion factors have been discovered that may be responsible for EBOV binding [9].

The EBOV VP35 is of great interest since it carries out multiple functions that are thought to be essential for viral replication and pathogenicity. It functions as an a cofactor for viral polymerase, RNA interference inhibitor, an inhibitor of protein kinase R (PKR), and an inhibitor of IFN-production [10]. By disrupting the RIG-I pathway, VP35 reduces IFN production, and investigations on recombinant EBOVs producing mutant VP35 proteins show that this IFN antagonistic activity is crucial for effective viral replication and pathogenicity in vivo [11], [12]. Several operationally major areas within the VP35 carboxy-terminal domain, termed the interferon-inhibitory domain, were recently found by structural research [13]. These include regions important for VP35 interactions with dsRNA, IFN-production inhibition, and NP interaction.

### *1.3. Available Therapeutics and their inefficacy*

The goal of experimental therapeutics has mostly been to reduce viral replication quickly to prevent the worsening of the inflammation generated by viral growth. It would enable the illness to be overcome by efficient innate and adaptive immune systems. During the 2013-2016 West African Ebola outbreak, expedited clinical studies failed to prove the unquestionable effectiveness of any treatment, despite the identification of exciting and compelling experimental intervention strategies and the ease of implementation of these trials emerging as a crucial aspect of the global response. In 2018, no Ebola medical countermeasure has proven effective in treating Ebola virus infections. However, in September 2014, the WHO published a list of prospective therapeutic candidates that have shown antiviral activity in in-vitro or animal models [14]. Therapeutics with promising early efficacy or safety features require more research to determine their efficacy in people, define optimal dosages, and explore therapy combinations for targeting various pathways in the viral life cycle. This research, therefore, focuses on the VP35 Interferon Inhibitory domain as a potential target to explore the targeting ability of Benzavir-2. Apart from that, the VP35 of the Ebola virus as a potential target for drug diocovery was also reviewed via a thorough review of existing literature. In addition to that, the research studies which focus on the said domain are also discussed.

## 2. LITERATURE REVIEW

Nag & Chowdhury carried out research based on in silico molecular docking of Piperine, an alkaloid of black pepper seeds against the Ebola Virus Interferon Inhibitory domain. The ADME Study was carried out to check the fitness of the selected compound to be acceptable as a lead compound and they found the compound is safe and also possesses significant drug-likeliness. A molecular docking study was also performed to check the interaction as well as the binding energy between the protein and ligand. The results of dynamic simulation between the protein and ligand demonstrated significant binding energy in comparison to the positive control which was taken as Ribavirin. They also carried out statistical analysis using clustering approaches and multivariate analysis which revealed that the selected alkaloid can present as one of the potent compounds against VP35 protein [15].

Brown et al. carried out a study for the identification of novel leads against the EPOV VP35 Interferon inhibitory domain. They performed screening of 7675 natural products of African origin and performed filtering using ADMET studies with using 7 as a threshold value and obtained a total of 1470 compounds for further molecular docking study and used five known compounds as a standard control for the study. They obtained 0.72 as the value of the area under the curve which was evaluated using the receiver operating characteristic value. They found that NANPDB2476, ZINC000095486250, NANPDB2412, and NANPDB4048 are the four lead molecules that demonstrated significant binding energy of −8.0, −8.1, −8.2, −8.2kcal/mol which was acceptable for antiviral activity. Therefore, their study suggested the promising antiviral activity of the four identified compounds for future employment, however, there is still a lack of experimental studies to confirm the same [16].

Bhowmik et al. presented a virtual screening of 10,829 compounds based on molecular docking against EBOV VP35 using Autodock Vina as a docking software tool. They noted that irinotecan and fexofenadine were the two top drugs that demonstrated the high binding affinities against the selected target -8.2 and -8.0kJ/mol respectively which is regarded as acceptable for the development of the antiviral lead compound. Therefore, their study demonstrated that compounds with the highest affinity can prove to be viable avenues to develop an effective drug molecule against EBOLA V35 Interferon inhibitory domain.

The above studies harness the potential of targeting VP-35 to explore different natural and synthetic compounds from variety of sources. However, there is strill a lack of exploring the anti-Ebola activity of existing agents that are approved or are in a row to be approved which will further help in the reduction of time taken to search for novel compounds. Therefore, the present research focuses on the broad spectrum of anti-viral agent "Benzavir-2" against the potential tareget EBOV VP35 of EBOV.

*Research Question*

- What is the targeting ability of Benzavir-2 against EBOV VP-35?

## 3. METHODOLOGY

### *3.1. Design*

A computational method with Autodoc4 was used for evaluating the interaction between the selected compounds and the target of EBOV which was set selected to be the EBOV VP35 Interferon Inhibitory domain, a good research domain to develop the anti-EBOV inhibitors. The receptor, as well as the target, were obtained from PDB and PubChem respectively which were then used in the pdbqt format for the further procedure required for docking. After performing docking the results were then Visualized and analyzed with the help of BIOVIA Drug Discovery Studio and Pymol.

### *3.2.Sample Collection*

EBOV Viral Proteins 35 is the core of the inhibitory function by which EBOV sets its highly efficient barrier against cellular immune responses, and it is one of the EBOV encoded proteins that are responsible for the exceptional virulence of the virus. The initiation of the cascade that leads to IFN production is inhibited by VP35. Therefore, the EBOV V35 was selected as the receptor because of the above roles that are promising for the discovery of drug agents against the deadly virus. The three-dimensional structure of EBOV VP35 is given in Figure 2 where the helical structure represents the alpha helices and the flat ribbons represent the beta-pleated sheets.

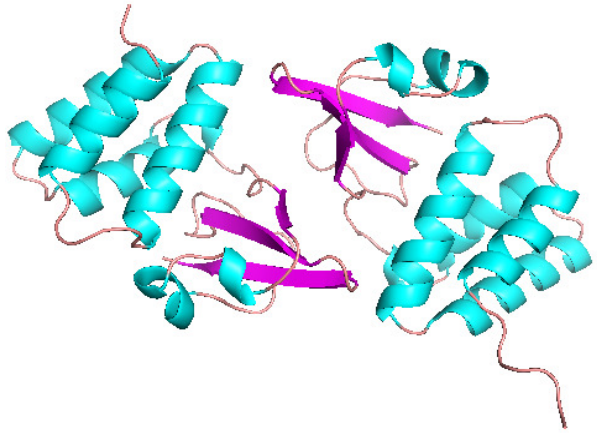

**Figure 9: Illustrates the three-dimensional structure of EBOLA VP35.**

A thorough review of the literature was performed to select the ligand against the targeted protein EBOV V35. After performing a meta-analysis on the different kinds of potential inhibitors, Benzavir-2 was selected to explore its targeting ability against the EBOV VP35. Benzavir-2 is previously discovered to have a broad spectrum of antiviral activity against the

Flaviviruses such as Zika virus as well as other viral agents like HIV, and Herpes simplex virus. Figure 3 below illustrates the ball and stick representation of Benzavir-2 where blue color represents nitrogen, the grey color represents carbon, red color represents oxygen, and the green color represents Florine.

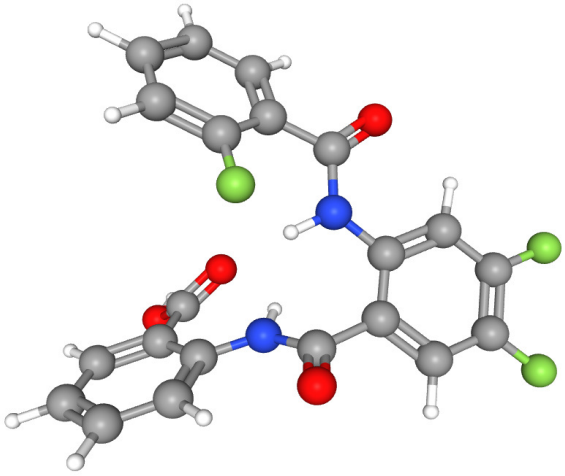

**Figure 10: Illustrates the ball and stick structure of Benzavir-2.**

*3.3. Instrumentation*

The protein structure of EBOV VP35 was downloaded from PDB RCSB with the PDB ID of 3FKE (PDB DOI: 10.2210/pdb3FKE/pdb). PDB RCSB stands for Protein Data Bank Research Collaboratory for Structural Bioinformatics) which helps in accessing the information about the 3-D structure of the proteins present in a variety of organisms ranging from bacteria to humans. This webpage also helps in acquiring essential data on the available proteins as well as the associated research studies that further assist in investigating the probable interaction between the protein and ligand. In this research, Benzavir-2 was selected and structure retrieval was performed from PubChem. PubChem is a database to search chemical structures in 2-D, 3-D as well as in crystal form which can be further retrieved for a variety of analyses and research. This database also helps in the visualization of chemicals in different patterns such as wire-frame, sticks, ball and stick, and many others. In addition to that, it plays a significant role in the retrieval of chemicals of interest for further molecular modeling and computational studies.

Furthermore, Autodock4 was then used to determine the interaction between the protein and ligand structure. Autodock4 is one of the bioinformatics tools that is commonly being employed in computational studies to search novel compounds against target proteins or to investigate the protein-protein interaction. Therefore, this tool or the software predicts how a small molecule or the selected compound will attach and interact with a protein in the living system without using any wet laboratory experiments. After performing the docking between the protein and ligand, the complex was then analyzed and visualized with the help of Pymol and BIOVIA Drug Discovery studio which helps in determining the specific amino acids of proteins involved in the interaction with ligand to make the complex stable.

*3.4. Data Collection*

The docking file is extracted as a DLG file, which is a docking log file with docking outcomes. Following the reading of this file, the interaction between the VP35 and the Benzavir-2 formed a three-dimensional complex structure, which was visualized using BIOVIA Drug Discovery Studio, as shown in Figure 4. Figure 5 depicts the interaction between proteins and compounds, with water and hydrogen atoms removed to reveal the particular amino acids engaged in bond formation.

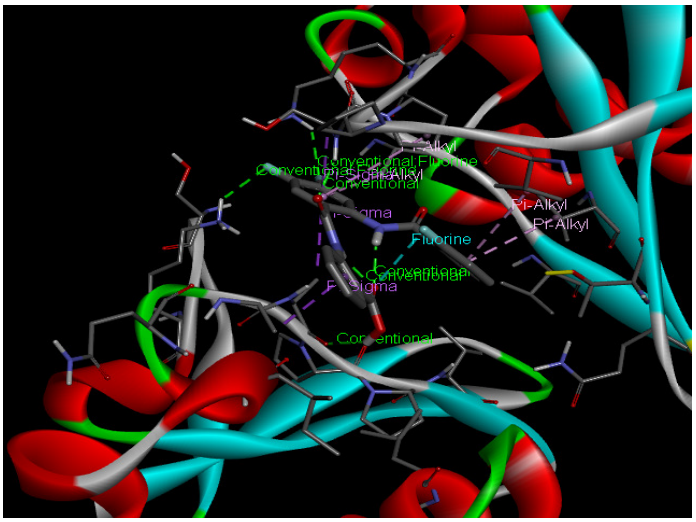


**Figure 11: Illustrates the Type of Bonds Formed Between Benzavir-2 and the Amino Acids Present in EBOV VP35.**

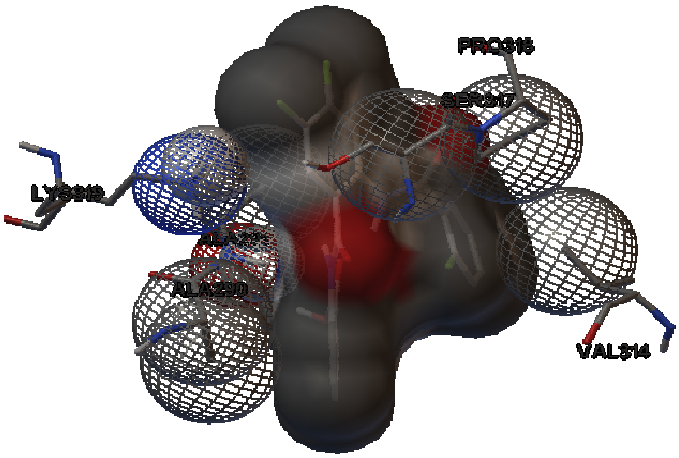


**Figure 12: Illustrates the Amino Acids Involved in the Intearaction with Benzavir-2.**

*3.5. Data analysis*

To more detailed information about the amino acids involved in the interaction with ligand, a two-dimensional structure was constructed which is represented in Figure 6. It has been noted that different kinds of amino acids were involved in different kinds of bond formation namely hydrogen bonds; halogen and hydrophobic bonds. Hydrogen bond formation was observed with amino acid SER317 and ALA291, whereas the Hydrogen Bond; halogen was observed interacting with A chain of LYS319 and B chain of LYS319. In addition to that, seven

hydrophobic bonds were observed with amino acids SER317, ALA290, ALA291, ALA291, PRO318, VAL314, and VAL327 as provided in Table 1 below.

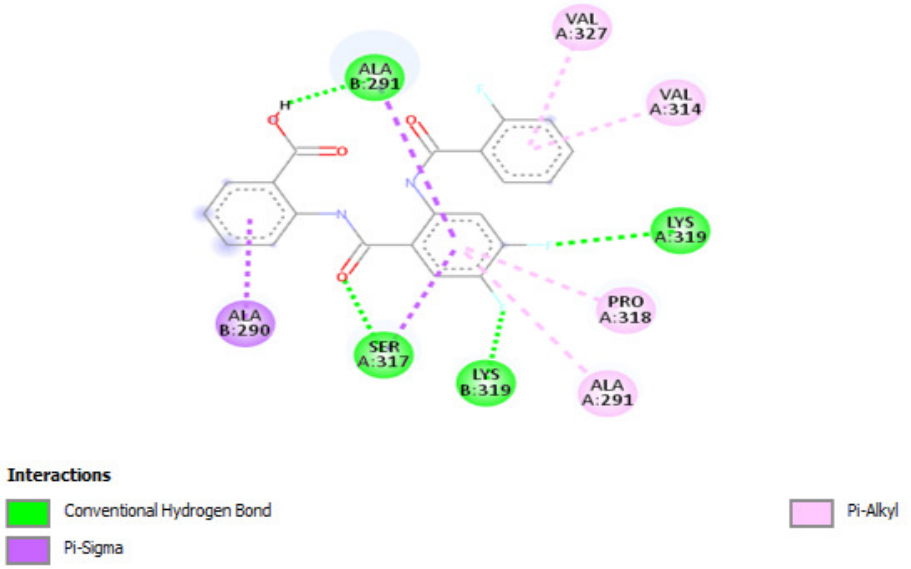


**Figure 13: Illustrates the Two-Dimensional Structure of the Docked Complex.**

**Table 3: Illustrates the Amino Acids Interacting with the Ligand Molecule, and the Distance Between the Bond Formation and Bond Specificity.**

| Sr. No | Protein: Ligand | Distance(Å) | Bond Category | Types |
|---|---|---|---|---|
| **1** | UNL1 - SER317 | 2.01183 | H-Bond | Conventional H-Bond |
| **2** | UNL1- LYS319 | 2.59252 | H-Bond; Halogen | Conventional H-Bond; Halogen (Fluorine) |
| **3** | UNL1-LYS319 | 2.76874 | H-Bond; Halogen | Conventional H-Bond; Halogen (Fluorine) |
| **4** | UNL1-ALA291 | 2.30294 | Hydrogen Bond | Conventional H-bond |
| **5** | UNL1 -SER317 | 3.69943 | Hydrophobic | Pi-Sigma |
| **6** | UNL1 - ALA290 | 3.66099 | Hydrophobic | Pi-Sigma |
| **7** | UNL1 -ALA291 | 3.99822 | Hydrophobic | Pi-Sigma |
| **8** | UNL1 - ALA291 | 5.2489 | Hydrophobic | Pi-Alkyl |
| **9** | UNL1 - PRO318 | 5.0176 | Hydrophobic | Pi-Alkyl |
| **10** | UNL1 - VAL314 | 4.69155 | Hydrophobic | Pi-Alkyl |

| | | | | |
|---|---|---|---|---|
| **11** | UNL1 - VAL327 | 4.53777 | Hydrophobic | Pi-Alkyl |

## 4. RESULTS AND DISCUSSION

Binding energy is the amount of energy required to separate particles or scatter all of the particles in a system. Total Intermolecular -Energy, Final Total- Internal -Energy, or Torsional -Free -Energy is added together to get Binding Energy, which is then deducted from the energy of the Unbound-System. These data would offer a list of ligands together along with their binding energy or affinity after docking. According to a common rule of thumb, the higher the negative value for binding energy, the better the will be a ligand. The binding energy was determined to be -5.11 Kcal/mol which depicts that Benzavir-2 can be enhanced for its antiviral activity with the help of different modifications.

As the development of effective therapeutic drugs seems far away from the current practices, there is still a need to be cautious about its prevention. Researchers are working hard to find viral control methods in locations where there are lack of health infrastructure and food instability. Infection control is centered on limiting the spread and transmission of the virus through a multimodal strategy that involves early case detection, prompt isolation of potentially infected individuals, competent clinical practice, appropriate burial practices, and education programs. Cases of the EBOV disease must be quickly identified in high-risk areas utilising surveillance systems. Each area has to have easy access to specialized labs with equipment for quick virus identification and isolation. Additionally, in times of public health emergency, strong relationships with the local populace must be built through efficient communication tactics. The validity and assessment of the information must be reviewed using biological and scientific methods and expertise that is provided to the local population for prophylaxis.

## 5. CONCLUSION

Filovirus infections are a serious public health concern. Millions of individuals are infected each year, and filoviruses pose enormous health, economic, and social consequences across the world. Although the virus is common in many regions, it can trigger disastrous outbreaks**.** Ebola virus disease is one of the diseases that is caused by the Ebola virus. Ebola virus induces severe hemorrhagic fever which needs immediate attention to find drug agents against it. EBOV V35 is a multifunctional protein that presents as a potential therapeutic target due to its involvement in the cell cycle and viral replication. In this research, the VP35 of the Ebola virus was used to explore the interaction of the broad spectrum anti-viral agent “Benzavir-2”. The interaction and binding energy demonstrated its significance to be in a row for the development of lead compounds in treating the Ebola virus infections.

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# CHAPTER 20

# A COHERENT STUDY ON BRAIN TUMOR DETECTION USING IMAGE PROCESSING TECHNIQUE

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***ABSTRACT:Cancer of the brain is a disease that cannot be cured. PET-CT (Positron Emission Tomography-Computed Tomography) and MRI (Magnetic Resonance Imaging) scans have been created to find the brain tumor; however, they do not generate the requisite accuracy and take additional time for physicians to diagnose the brain tumor for successful therapy. This study discusses the need for medical information that is needed by doctors to cure patients accurately and quickly, an image processing technique as well as a technique for reading MRI images are formed, the purpose is to support the processing of medical images, and this study also reviews various techniques and methodologies that were used to discover brain tumors on MRI images, and therefore are expected to provide information about different methods and technologies in image processing as a basis for image analysis. The study has concluded that magnetic resonance imaging (MRI), is a technological advancement in the field of medicine. MRI is capable of producing images with a high resolution, allowing for the detection and subsequent classification of diseases that are located in the patient's organs***.

***KEYWORDS: Brain Tumor, Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Preprocessing Stage, Support Vector Machine (SVM).***

## 1. INTRODUCTION

Brain tumors form as a result of abnormal cell proliferation inside the brain. Brain tumors are typically categorized into two types: malignant or benign tumors [1]. When it comes to cancerous tissue, a malignant tumor is the fastest-growing, while a benign tumor is the slowest-growing one. A brain tumor is one of the most lethal of all malignancies. Like other cancers, brain or spinal cord tumors are caused by alterations in DNA. Cancerous tumors are abnormal collections of cells that form in the brain.

They come in a wide variety of shapes and sizes and are treated in a variety of ways. Brain tumors are now divided into primary and metastatic types. Brain tumors come in a variety of forms and sizes, and they may be found in different parts of the brain. Images are created using radio wave pulses as well as a magnet in MRI. This is where all of the internal organs and structures are housed. An MRI test image is taken by placing a specific area of the body within a machine that generates a strong magnetic field. This is because an MRI may reveal a great deal about the cellular structure, vascular supply, or overall anatomy. As a result of these factors, it is an essential and effective diagnostic, screening, and therapy tool [2].

Oncologists must frequently do the time-consuming as well as the challenging process of manually segmenting tumors using MRI data. Segmenting medical pictures takes a significant amount of time for radiologists and some other medical specialists. It is, though, a time-consuming job to precisely identify and categorize brain tumors [3]. Brain tumors are amongst the most widespread brain diseases, and they have ruined the lives of many people. The International Agency for Research on Cancer (IARC) estimates that more than 126,000 individuals are diagnosed with brain tumors annually, and also more than 97,000 of those people ultimately die of their condition[4].

A brain tumor is a group of malignant cells that have accumulated in the brain. Both primary brain tumors and metastatic cancers from other parts of the body might metastasize to the brain. Seizures, severe headaches, double vision, loss of balance, and disorientation are among the symptoms of this condition. When detected at an early stage and entirely removed by surgery, level 1 brain tumors have a chance of being cured. The growth and spread of level 2 tumor cells are slower than those of level 3 and level 4. They have the potential to recur and perhaps spread to the surrounding tissue. Tumors at this level are very difficult to treat successfully. A person may think that there are no signs of discomfort. Radiation, surgery, and chemotherapy are all options for dealing with tumors. The automated system for detecting and curing tumors in the brain consists of a variety of methods and technologies that work together to achieve the desired outcome. When the surgeon or patient works together in this way, they are more likely to achieve better outcomes. In addition, this would enable the patient to feel more at ease since they will be able to comprehend the treatment-process sequence, which would in turn aid the medical practitioner in dealing with the situation calmly.

This study aims to identify the detection of brain tumors using image processing methods and increase patient survival. This study also discussed the Clinical symptoms vary according to on category, size, and place of the tumor, but the most frequent clinical signs include chronic headache, seizures, nausea and vomiting, loss of consciousness, dizziness, mood-fluctuations, cognitive issues, vision defects, weakening of body parts, problems with speech development, or hearing impairment. Other diagnostic methods include CT, MRI, as well as a biopsy. Surgery, radiation, chemotherapy, and indicated therapy are treatment options. This mini-review covers brain classification and treatment options.

## 2. LITERATURE REVIEW

Michael R. Kaus stated in a study thattime-efficient scan segmentation was a challenge. This work uses hierarchical segmentation of diffusion–filtering. Manual tumor segmentation takes 3-5 hours. So, author employs an algorithm to separate the brain tumor in 5-10 minutes. It checks the algorithm's speed. The clinical analysis uses it. It offers an intermediary response in 1–3 minutes [5].

N. Nabizadeh discussed in a study that just one of 3 algorithms may yield the desired outcome. Because of the uncertainty of brain sore region, size, shape, or surface, automated MR scanning separation is crucial. Skippy greedy snake algorithm is, Histogram-based Gravitational Optimization Algorithm (HGOA), whereas Skippy greedy snake algorithm segments tumors accurately and efficiently. Multispectral MR images are brighter. It has difficulties in separating lesions from healthy cells. This technique improves tumor segmentation accuracy (96.8 0.3% for simulated MR images or 93.8 0.1% for actual MR pictures) and reduces computing complexity [6].

J. Sachdeva et al. remove the tumor from the brain after detecting and reducing the segmented images Computer-aided detection (CAD) technology is being developed in this study to assist clinicians in the classification of multiclass brain tumors.The Genetic Algorithm (GA) is the foundation of artificial intelligence learning. Generalized additive models coupled with either Support Vector Machines (SVM) or Artificial Neural Networks (ANN) constitute the machine learning technique (i.e. GA-SVM and GA-ANN). Medulloblastoma (MED) and Low-Grade Glioma (LGG) are included in the dataset Large Granular Lymphocytic (LGL). There are no radiologist-related issues with GA-SVM, which is a time-saving tool. Only the general form of the object is considered. The gathered dataset's final result is shown using the GA optimization approach, resulting in classifiers as well as neural network accuracy increases of 79.3% and 91.7%, respectively [7].

Y. Chen et al. stated in a study that convolutional neural networks retrieved hyperspectral Images. This uses convolutions or convolutional to extract nonlinear, discriminating, invariant HSI deep aspects. Deep Feature Extraction (FE) uses a Convolution Neural Network to classify Hyper Spectral Images (HSI) (CNN). To get the spectrum of every pixel in a picture to find items, detect materials, and detect processes. Kennedy Space Center, Indian Pines, and the University of Pavia gathered the datasets. Image categorization and target identification benefit from these features. Experiment accuracy is 88.75% [8].

Devkota et al. created the full segmentation algorithm based on Mathematical Morphological Operations as well as a spatial Fuzzy Cognitive Maps (FCM) algorithm that increases computation time, however, results in the 86.6% precision of the classifier and also the 92% success rate in detecting cancer have not yet been validated.According to the findings of the research, the suggested approach has a good chance of being successful when used in the diagnosis of brain tumors in patients [9].

S.koley et al. proposed in a study that effective work on tumor identification and division of brain MRI was carried out to detect the specific position of a brain tumor through the use of a K-means clustering approach. When compared to other merging techniques, it has shown to be an effective self-merging algorithm, reducing the impact of noise and increasing the possibility of pinpointing the tumor's exact position. It is much simpler and faster to implement their approach on a computer [10].

Amsaveni, V. Singh, and N. Albert suggested a study on medical imaging information (area of interest) to increase tumor segmentation computing speed. The proposed is a feature-based method for segmenting basic brain cancer.Brain magnetic resonance images with T1 weighting applied to axial slices obtained after contrast enhancement is evaluated. To recover image feature points, researchers used a morphometric or wavelet fusion of edge maps. Geometric transformations and picture scaling use the collected key points. To isolate the tumor, a region-growing algorithm was used. The technique has shown excellent segmentation accuracy. This method reduces calculations. Future research will investigate the method's use in automated 3D segmentation methods, Region of Interest (ROI) clustering in these other medical pictures, and medical image retrieval systems [11].

R. Gurusamy and V. Subramaniam stated in a study that feature-extracted and classified pictures. These photos have a valuable collection of features that improve computer system performance. ML, Preprocessing, Feature Extraction (Wavelet transform), and SVM were employed. The technology helps clinicians evaluate alternative treatment opinions. Analyzing real-time MR images. Unwanted sounds are removed via pre-processing. The test is accurate 96% of the time [12].

## 3. DISCUSSION

The main organ of the nervous system is the Brain. The skull covers it in the head. The brain controls all bodily components. It's an organ that helps humans adapt to different environments. The human brain permits movement, thinking, and sensation. This section describes brain anatomy for fundamental comprehension shown in Figure 1 [13].The brain governs cognition, memories, emotions, sensation, motor function, visuals, respiration, temperatures, and appetite, as well as all physiological processes. The Central Nervous System is comprised of both the brain and the spinal cord.

Primary (benign) and secondary (malignant) brain tumors are the 2 main types (malignant tumors). Gliomas are a form of a brain tumor in which one type of cell develops gradually in the brain. It comes from astrocytes, which are non-neuronal brain cells. In general, original tumors are less aggressive than secondary tumors, although secondary tumors exert

significant pressure on the brain, causing malfunction [15].Secondary tumors seem to be more aggressive as well as proliferate more quickly. The primary source of a secondary brain tumor is somewhere else in the body. A metastatic cancer cell is present in these tumors, therefore means that the disease has migrated throughout the body, including the brain, lungs, and other vital organs. Secondary brain tumors are very dangerous. Cancers of the lungs, kidneys, bladder and other organs are the most common secondary causes of brain tumors [16].

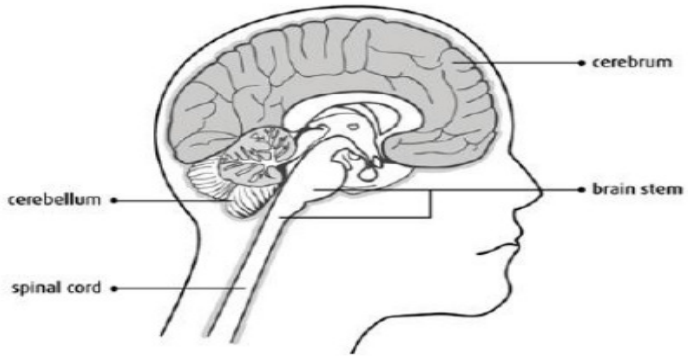


**Figure 1: Describes the structure of the human brain.** [14]**.**

An image is "processed" when it undergoes a sequence of processes to either enhance the original image or extract useful data from it. The image is generated and the result may be the resulting image or information on the image's attributes and features, making it a kind of signal analysis. Magnetic Resonance Imaging (MRI) produces higher-quality images of the brain and cancerous tissues than some other medical imaging techniques such as X-rays and Computed Tomography. This has led to its widespread use (CT). MRI is widely utilized since it is a technology that does not include any invasive procedures. The fundamental idea behind magnetic resonance imaging is to produce pictures of the body by scanning it with radio waves and a powerful magnetic field. These images may then be used to investigate the anatomy of the body [17].The continuing support for technological advancement is unquestionably helpful for many areas of life, not the least of which is the medical profession, which is responsible for the development of magnetic resonance imaging technology. The magnetic resonance imaging technique generates highly relational pictures of many interior organs of the human body, such as the brain, tissues, etc. To determine whether or not a disease is present, examinations of a number of the human body's internal organs are performed.

A brain tumor is one of the disorders that may be discovered using MRI imaging. The reading of MRI pictures is still a manual process in the field of medicine, which is used to diagnose brain cancers. Radiologists and physicians do this task. Because of this circumstance, the interpretation of MRI images became a challenging task, even though it was performed by qualified medical professionals. This is because it was determined that relying solely on human visual vision was inadequate to diagnose accurately and quickly from these MRI images. In most cases, the reading of MRI pictures by medical professionals such as radiologists and physicians takes at least a week to two weeks before the findings are communicated to patients. In addition to the difficulty that is already there, the intricacy of the anatomy of the human brain makes it exceedingly risky or delicate to physically identify brain cancers. This is due to the possibility that a brain tumor would balance in with the patient's brain health tissue [18].The advent of digital image processing and image processing, on the other hand, has made it feasible to assist medical professionals, such as radiologists and physicians, in reading MRI pictures more quickly,which could be useful in

making the detection of brain tumors. These goals can take the form of image enhancements, enhancements to image quality, or objects in the image that could be processed as required. Image processing is also known as picture processing. In this particular scenario, the MRI image processing that is being done to detect brain tumors could be further analyzed with a variety of techniques or techniques for the processing of images to gather significant amounts of information and data associated with the detection of brain tumors, which would subsequently be used to classify the tumors [19].

*3.1. Image Processing Stage of aMagnetic Resonance Imaging (MRI) Technique:*

To identify tumors, image processing methods are used. These approaches consist mostly of the following stages: preprocessing, segmentation, feature extraction, as well as classification. Figure 2, presents a flowchart illustrating the phases involved in the process of detecting and classifying tumors.Detection of brain tumors by the use of MRI image processing. Brain tumor diagnosis is required to find the abnormal tissue, hence the image's precise size and position must be determined. Because brain tumors vary in intensity, they may affect various areas of the brain [20].

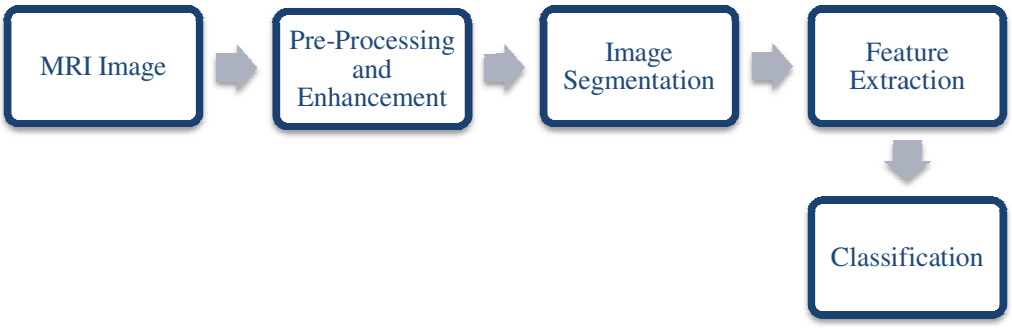


**Figure 2: Flowchart Displaying theImportant Image Processing Procedures for Brain Tumor Detection.**

*3.1.1. Image Enhancement and Pre-Processing:*

Detecting a dangerous area is made easier with this initial stage in image processing. The image's finer features are improved, while the image's noise is reduced. When clinical MRI images are tainted by noise, the images' precision is compromised. The noise is reduced using a variety of filters.An anisotropic filter is being utilized to lessen the amount of background noise, and a weighted median filter has been implemented to do away with salt and black pepper. De-noising methods based on wavelets are biased toward wavelets and scaling coefficients.Steps in preprocessing are outlined in Figure 3:

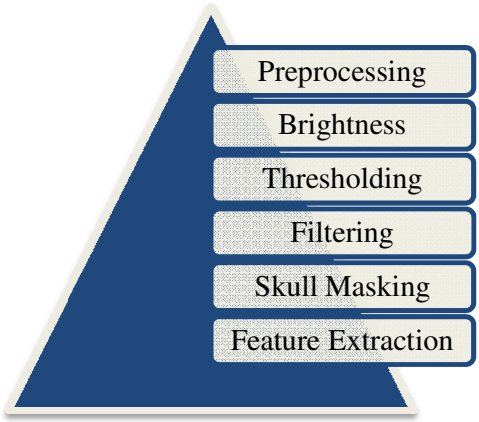

**Figure 3:Depicts the Preprocessing Stage of the MRI Processing Techniques.**

It is possible to illustrate the benefits and drawbacks of each kind of preprocessing that is employed in MRI pictures by looking at the image that is shown in Table 1, which has numerous steps of preprocessing [21].

**Table 1: Shows the Image Segmentation Using MRI Scan.**

| Preprocessing Stage | Goal | Merits |
|---|---|---|
| Brighter image | Aiming to enhance the visibility of dark MRI images | To make the MRI picture brighter for the following step. |
| Threshold Value | Make a binary image out of a grayscale picture | Determine which items and backgrounds were included in them, which are typically denoted by the pixel values 1 and 0 respectively. |
| R Filtering<br>- Median<br>- High Pass | Refine, sharpen, modify, and remove noise from pictures to obtain the information required to sharpen edges, compute particle-hole boundaries, and produce element-background contrast. | Can refine edges, compute particle whole boundaries, and also create element-background contrast. |
| Skull masking | Boundary-control objects | Know region edges (ROI). |
| Feature Extraction | The collection of picture data in the form of size, structure, color, or contrast is the goal of the process known as feature extraction. | Wavelet removes picture noise while preserving important details. |

*3.1.2. Segmentation of Image:*

Segmentation is necessary for the correct information to be obtained from MRI pictures. MRI pictures may also be separated into numerous segments so that the processed image could be more easily analyzed because of digital MRI image segmentation's ability to acquire pixels or objects based on their texture or color. According to the reason for which the digital picture is being analyzed and also the 3 methods of segmentation, the image may be segmented into pixel, edge, and region-based segments.Different image segmentation methods are available for MRI image segmentation, such as pixels, lines, and areas. Thresholding and Clustering are two of the most used pixel-based segmentation algorithms. A clustering separation is a collection of pixels that have a common feature. Those pixels that are nearer to the cluster's core indicate that it is possible to segment or identify the area as a tumor. However, if the region is not validated, the comparison may be resumed, and also the tumor could be divided if the images are not near the cluster center [22].

*3.1.3. Feature Extraction:*

Because of the brain's intricate anatomy, locating a particular tumor requires special expertise. The image's size, shape, composition, and placement are all taken into consideration while extracting features. The characteristics that are retrieved throughout the process of feature extraction are used to categorize the various types of tumors.

*3.2. Classification of MRI Images:*

On both practical and theoretical grounds,when it comes to evaluating MRI pictures, one of the most effective machine learning methods is called the Support Vector Machine (SVM). Large datasets may be used to train SVMs, and the data model can be explored. Using SVM, you can accurately anticipate future data.Both the Support Vector Machine (SVM) and also the Radial Basis Function Neural Network (RBFNN) are machine learning techniques that have been used to gather and display the findings of various studies using MRI image classifications (RBF NN). To put it another way, SVM is one of the most effective machine learning approaches, both in terms of practical application and theoretical analysis, whereas RBFNN is a training algorithm that is utilized for classification. This algorithm is often referred to as a hybrid algorithm as well. This is because it employs not one but two distinct learning strategies at the same time, namely guided and unguided learning. Additionally, the RBNFF is made up of layers that are input, hidden, or outputs.

Before processing the picture, the categorization must identify the pattern. Large datasets are used to train SVM, which then investigates these data models. SVM predicts data accurately, but RBNFF output depends on the activation function and also the weights of the hidden layer. It is also possible to segment MRI images or background pictures to make the final processed image simpler to examine. There are three basic ways to divide a picture: pixel-based, edge-based, or region-based. Each of these methods is used to extract different kinds of information about the digital photo being analyzed [23].The following information pertains to the accuracy of the Radial Basis Function (RBNFF) or Support Vector Machine in the categorization of MRI images to detect brain tumors shown in Table 2:

**Table 2: Shows the Classification and the Accuracy of Support Vector Machines.**

| **Segmentation** | **Extraction of Features** | **Accuracy and categorization.** |
|---|---|---|
| K-means clustering | The matrix of gray level co-occurrence. | With an SVM efficiency of 98.51% |
| Thresholding | Matrix of the GLCM | 83 percent of SVM |
| Histogram Gradian | Filter- median | 86.6 percent, as determined by the Support Vector Machine (SVM). |
| Fuzzy C-Means | Discrete Wavelet Transform (DWT) | Accuracy may be Expected from Radial Basis Function as well as Deep Neural Network |
| Wavelet Berkeley | Discrete Wavelet | 97.2 percent accuracy using the SVM (Support Vector Machine). |

According to table 2, the findings gained accuracy ranged from 83 percent to 98.86 percent, which indicates that the classification accuracy was achieved by a combining of segmented, extracting features, or classification algorithms.

*3.3. Clinical presentation:*

Brain tumor symptoms may range from mild to severe, depending on the specific tumor type, its location, its size, and how rapidly it is developing into a rise in intracranial pressure as well as a focus, all of which are characteristics of native tissue death. For the time being, headaches are the most often observed sign or symptom of the disease. Nearly half of all

sufferers report experiencing a headache as a common symptom of their condition. In the vast majority of instances, the onset of a headache is preceded by other symptoms. This would include nausea in addition to the more well-known symptoms of headache or seizures, such as vertigo, vision abnormalities (such as double vision), personality or behavioral changes (such as speaking difficulty), or impaired awareness.

*3.4. Identification:*

It is extremely important to have a comprehensive medical history as well as an access medical examination, a neurological investigation, pertinent imaging modalities, as well as a histological test before attempting to make a diagnosis. The neurosurgeon would test the patient's vision, coordination, reflex, auditory, direction, muscle definition, and other functions as part of the neurologic evaluation. Additional imaging techniques include Positron Emission Tomography (PET) -CT scans or Computed Tomography (CT) scans in addition to the standard MRI, which would be the principal instrument.Although MRI is the primary imaging method used in patients, there are situations in which MRI is not a viable option due to factors such as the patient's size, the presence of indwelling implants or catheters, or a fear of being confined in small spaces. In these scenarios, computed tomography could be used as an alternative. Histopathological examination is essential for the examination of various tumors, which is necessary to arrive at a definitive diagnosis. This is in addition to the diagnostic value that radiography, such as MRI and PET-CT scans, may provide [24].

*3.5. Treatment:*

The alternatives for treatment that are detailed here, like surgeries, radiation treatment, chemotherapeutics, as well as targeted therapy, are all examples of treatments that are available. Surgery has the potential to be the only therapy required for a low-grade brain tumor, particularly if the whole tumor could be removed surgically.The factors that influence the outcome There are a variety of therapy options based on the tumor's size, grading, and location, as well as the patient's age, fitness level, comorbidities, or treatment preferences.Surgery, radiation, chemotherapeutic, surgical intervention, and physiotherapy may be used alone or in conjunction with other treatments as part of a patient's regular treatment regimen. Surgery is the cornerstone of therapy in the vast majority of instances, and it is often necessary only in benign cases.

## 4. CONCLUSION

The abnormal growth of tissues inside the brain, known as a brain tumor, is characterized by the fact that it disrupts the brain's capacity to perform its typical functions. The primary objective of medical image analysis is to extract accurate and relevant information from pictures while introducing as little error as is practicable in the process.This may be difficult to detect brain cancers using MRI technology due to the complexity of the brain. These tumors can be segmented using a variety of image segmentation techniques. Detecting brain cancers from MRI images involves 4 steps: pre-processing, picture segmentation, feature extraction, and classification approaches. These stages are listed in this order: pre-processing, segmentation techniques, as well as extraction of features. To identify and categorize brain cancers, MRI image processing is the best option in medicine. An MRI scan may be used to assist doctors to discover and diagnose brain cancers. In addition, the Support Vector Machine (SVM) stands out as the most efficient method for detecting brain cancers in MRI scans, according to the most recent algorithms, especially when combined with preprocessing techniques such as feature extraction and the Radial basis function (RBNFF). This study concluded that the greatest results may be achieved in the future using MRI image processing by combining multiple current image processing algorithms to identify and classify brain

cancers.In the future, it would be better to improve the precision by combining real-world examples and clinical data from a large dataset that encompasses a variety of scenarios with segmentation and feature extraction methods that are more precise. This will allow the analysis to be applied to a wider range of scenarios.

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# CHAPTER 21

# OPTIMIZATION OF FERMENTATIVE PRODUCTION OF LACTOBACILLUS FOR ENRICHED FUNCTIONAL FOOD PROPERTIES

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***ABSTRACT:Fermented foods have been around since the dawn of human civilization and are among the oldest processed foods still being eaten today.Lactic Acid Bacteria (LAB) create lactic acid during the fermentation process, resulting in the end consequence of the fermentation. This study focuses on food fermentation which has been recognized as an application in this study to improve food attributes such as flavor, fragrance, shelf life, texture, and nutritional content. Fermented meals from the LAB improve the body's resilience to dangerous bacteria and stimulate the immune system. Bacteriocin generated by the LAB might one day replace antibiotics as a treatment option for pathogens with multiple drug resistance. As a result of advances in food/feed fermentation technology, certain chemicals may be extracted from microbial metabolism.As a result, LAB fermentation is not just economically important, but it also has a good influence on human well-being and contributes to feeding the rising population of the world. As a consequence of this, LAB fermentation has become more popular in recent years.***

***KEYWORDS: Fermentation, Fermented Foods, Lactic Acid Bacteria (LAB), Lactic Acid, Lactobacillus.***

## 1. INTRODUCTION

Biotechnology is the technical application for biological systems, live creatures, or their metabolites to produce or change items or processes for a particular purpose. Microbial inoculants are used in most developing nations' biotechnology food processing to improve qualities such as flavor and fragrance of foods, storage stability, consistency, or nutritional content [1].In recent years, customers who are health aware have increased their search for natural foods that do not include chemical preservatives and are compatible with their healthy lifestyle. One of the primary reasons for this condition is the rise in the consumption of pre-cooked meals, which are more likely to be subjected to improper temperature control, as well as the importation of raw foods from underdeveloped nations. The terms "longer shelf life" or "improved safety" refer to biopreservation, which is the process of preserving foods by employing microbes or their metabolites [2].

Foods that have undergone the process of fermentation are fundamental to human nutrition and it has been the subject of production and consumption ever since the dawn of human civilization. Fermentation is the term that refers to the process by which microbes and the enzymes that they produce cause beneficial changes to occur in dietary items. The principal metabolites and microorganisms that are involved in the fermenting of food may be characterized as follows: LAB from genera such as Streptococcus, Leuconostoc, and Lactobacillus [3].

Over the last several decades, there has been a rise in interest in the study, development, and use of alternative sources of energy and biochemical sources which are less detrimental to the environment as worries about the exhaustion of fossil fuels have increased worldwide. Since around the middle of the 19th century [4], the petroleum industry has been in charge of satisfying the energy and chemical requirements of the whole globe. Despite this, the fact that

oil is a finite resource, that its price is highly volatile, and that the use of oil affects the environment are a few considerations that have stimulated research intoalternative strategies for fulfilling a rising population's energy and chemical needs. The utilization of microorganisms in energy production and compounds from sustainable sources of energy as substrates is the primary emphasis of the rapidly developing discipline of biotechnological processes, which is a subset of biotechnology.

It is also possible to classify fermentations according todairy products, meats, and fish, vegetables, legumes, various grains, grapes, additional fruits, soybeans, different legumes, cereals, even starchy roots may be utilized as dietary substrates.Lactic acid bacteria digest the monosaccharides, disaccharides, or, in rare circumstances, starch present in raw materials. Lactic acid is produced as a result of fermentation. LAB are anaerobic, facultative aerobic cocci or rods, gram-positive, and non-sporulation. Even though they are sensitive to artificial media, these bacteria thrive on a wide variety of food substrates. They may also quickly reduce the pH to levels where competing organisms can no longer thrive. One of the most prominent byproducts of carbohydrate metabolism is lactic acid, which is produced during fermenting[5].

Fermentation is generally used in the preparation step of foods and beverages like chocolate and coffee. The fermentation of cocoa beans results in the production of chocolates. Acetic Acid Bacteria (AAB), Lactic Acid Bacteria (LAB), and yeast drive the fermentation process that converts the acetic acid and cocoa pulp substrates into ethanol and lactic acid, respectively. Enzymes including invertases, glycosidase, proteases, and polyphenol oxidase are in charge of the formation of taste and olfactory precursors as well as the degradation of pigments that occur throughout the fermentation process. The molecules, like amino acids simple sugars, or peptides that are turned into taste and fragrance characteristics during the roasted processes or the drying process of chocolate production are influenced by fermentation [6].

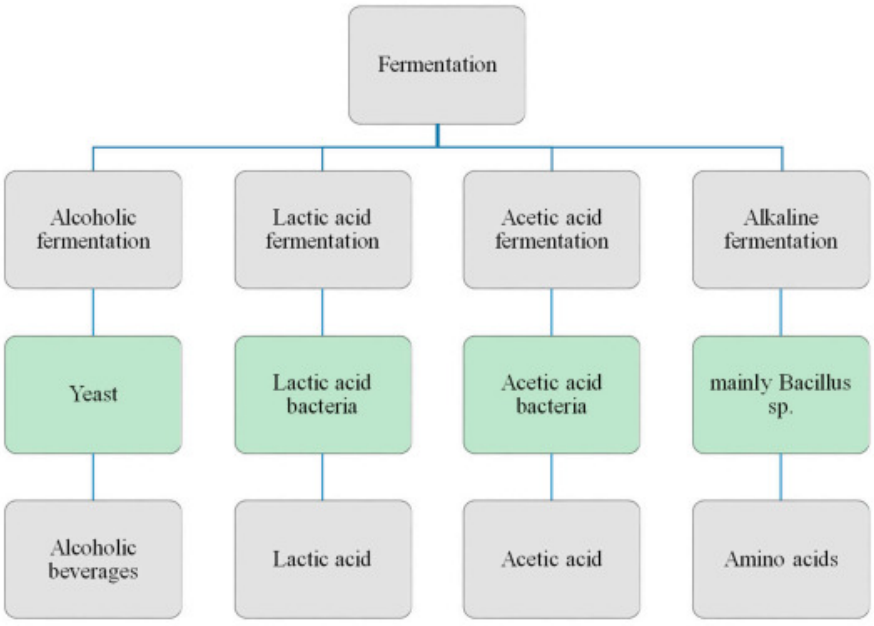


**Figure 1: The most prevalent forms of Fermentation, The microorganisms that are engaged, and their** [7]**.**

The lactic acid bacteria are primarily responsible for carrying out the lactic acid fermentation process. The fermentation process is aided by the availability of oxygen that produces acetic acid from alcohol and transforms alcohol into acetic acid produced by acetic acid manufacturers that belong to the species Acetobacter. Fermentation of fish or seeds, both of which are often used in culinary applications, frequently results in the production of alkaline byproducts [8].

## 2. LITERATURE REVIEW

P. Saranraj *et al.* stated in a study that Fermented foods are a favorable medium for microbial development and proliferation. LAB suppresses pathogenic, nonpathogenic, and spoilage organisms in fermented foods and drinks. Plant by-products are excellent preservatives. This article briefly reviews "microbial fermentation and fermented food production." Some bacteria generate bioactive chemicals as metabolites for self-defense against other germs. All fermented foods require lactic acid bacteria [9].

Rowaida Khalil *et al.* proposed in a study that a newly identified strain of Bacillus megaterium 19 was used to create a bacteriocin, which was then isolated and characterized by the researchers. The strain that was extracted from fermented vegetable wastes produced a bacteriocin with a bactericidal mechanism of action and demonstrated antimicrobial activity over a broad range against food-spoiling microbes [10].

Adetunji and Adegoke stated in a study thatlactic acid bacteria obtained from West African cream cheese have been shown to produce bacteriocin as well as cellulose. The cellulose was created by all of the LAB that was employed in this investigation. After an incubation period of 72 hours, a very substantial association could be shown between the creation of cellulose and the proliferation of bacteria. Bacteriocin, which is generated by the strains, has the potential to be useful for biopreservation [11].

Dickson *et al.* conducted a study thatwhich the researchers developed a species-specific PCR assay for identifying Lactobacillus fermentum.The synchronization of bacterial 16S rRNA genes, along with the identification of Lactobacillus fermentum-specific nucleotides at the 3' ends of the linked genetic makeupgenes allowed for the identification of Lactobacillus PCR primers. This polymerase chain reaction test offers a faster, more specific, and more sensitive alternative to the traditional culture-based techniques that are traditionally used to identify Lactobacillus fermentum [12].

## 3. DISCUSSION

Several well-established single-product bioreactors are now available, each of which demonstrates environmental and economic viability. Therefore, to enhance industrial single-product biotechnological processes, it has been suggested that multi-product methods, which include the valorization of residues and by-products, should be taken [13]. One instance of this would be the manufacture of value-added compounds using the cellulose and hemicellulose that are extracted from sugarcane bagasse (a waste that is typically utilized for the generating of energy and is acquired during the manufacturing of bioethanol) [14].Glycerol, for example, is a byproduct of the biodiesel business with minimal commercial value, we may use it in the fermentation process since it contains proteins of interest. Bio-refineries are multi-product processes noted for their reliance on the transformation of renewable feedstock into products that are based on biological material, as shown in Figure 2[15].

The research, production, and use of robust strains of bacteria that are capable of using a variety of substrates and also generating a broad range of products are regarded as significant milestones in the process of developing bioenergy. Lactic Acid bacteria are a varied group

that has been shown to have the potential to contribute to the creation of interconnected bio-refineries. LAB are catalase-negative, Gram-positive, coagulase-negative, non-motile, acid-tolerant, non-respiratory, aero-tolerant rods, or cocci. One of the distinguishing features of these bacteria is their ability to ferment carbohydrates into lactic acid (LA) [17]**.**

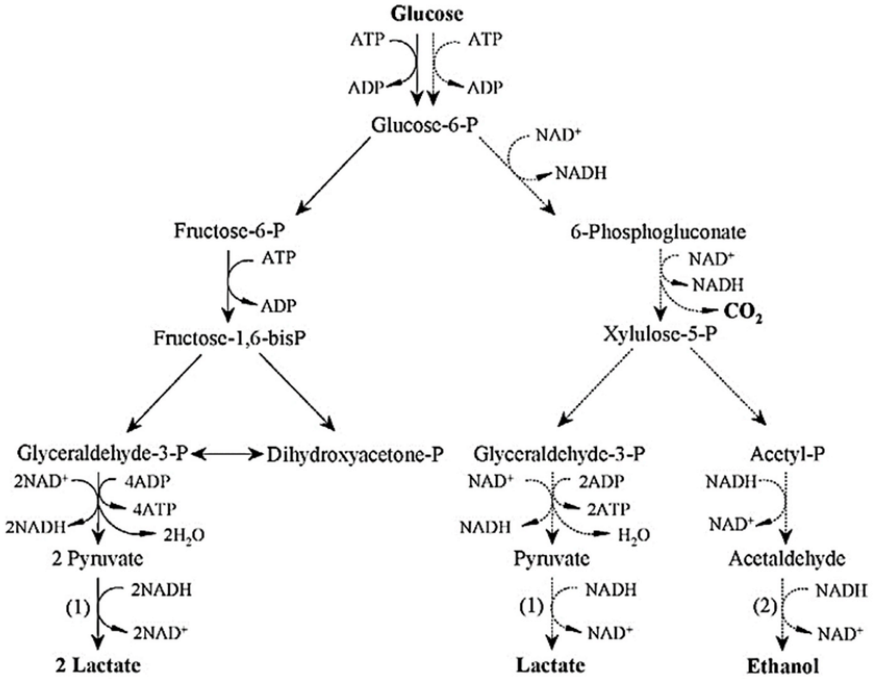


**Figure 2: Metabolic Pathway of homofermentative and heterofermentative Lactic Acid Bacteria** [16]**.**

Lactic acid (LA), the primary end-stage metabolism byproduct of carbohydrate fermentation, is produced by these microorganisms. This is one way to identify them. LAB are unable to produce their oxygen, therefore they receive the energy they need to survive via a process called substrate-level phosphorylation.This process takes place in conjunction with one of the two metabolic pathways for hexose fermentation: homogeneous or heterogeneous fermentation. Figure 2, depicts the first route, which is primarily responsible for the generation of LA and is based on glycolysis. The second mechanism termed the pentose phosphate route, differs from the first in that it produces carbon dioxide in addition to ethanol or acetate and LA and shows both possible routes [18].

*3.1.Utilization of Biotechnology in the Fermentation of Food:*

When fermented foods are being manufactured, the presence of microorganisms is an essential component of the manufacturing system. Both classical and molecular methods may be used to modify the genetic makeup of microbial cultures, and this genetic modification of bacteria is now the focus of a significant amount of study in both academic and commercial settings. For commercial food applications, sensory quality has been one of the factors considered in both developed and emerging nations ( texture, consistency, general acceptability, flavor, aroma, and visual appearance ) [1].

*3.2.The Fermentation Process and Its Significance in the Food Industry:*

*3.2.1. Enhancement of flavor:*

The scent and taste of the meal are both improved by the process of fermentation, which also makes the dish more appetizing. Because fermented foods possess certain organoleptic

qualities, consumer approval of fermented foods is higher than that of unfermented foods [19].

*3.2.2. Quality of the Foods Consumed:*

A variety of foods, particularly grains, are low in nutritional content; nonetheless, these items make up the majority of the staple diet of economically disadvantaged communities.Fermentation withLAB, on the other hand, has been shown to boost the nutrient benefit and digestibility of certain meals. The fermentation products have an acidic character, which boosts the activation of microbial enzymes when the temperatures are in the zone of 22-25 degrees Celsius [20].Amylases, peptidases, lipases, and phytases are among the enzymes that break down polysaccharides, proteins, and lipids in basic dietary items. As a result, LAB fermentation increases the availability of minerals like iron, protein, and simple carbohydrates by decreasinganti-nutrient levels in food, such as phytic acid or tannin. In the fermentation process, the concentration of nutrients is also enhanced [21].

*3.2.3. Properties of Preservatives*:

Some fermented foods, such as cereals and yogurt, have been shown to contain LAB, which has been shown to possess antimicrobial properties. The development of harmful bacteria, which may lead to food spoilage, food poisoning, or illness, is inhibited when the pH is lowered to a level lower than 4, which could be accomplished by acid production. LAB, for instance, has antifungal properties. The storage life of fermented foods may be increased significantly by carrying out these steps [22].

*3.2.4. Detoxification:*

Throughout the years, it has been established that mycotoxin detoxification in food may be achieved using LAB fermentation. The use of LAB fermentation as a kind of detoxification is more favorable than other methods since it is a gentler process that maintains the nutritious content and taste of food after it has been cleansed of contaminants. In contrast to this, LAB fermentation is capable of degrading mycotoxins irreversibly while leaving no hazardous residues behind. It is hypothesized that the binding action on toxins is responsible for the detoxifying effect.

*3.2.5. Antibiotic Activities*:

LAB is used as a barrier to prevent the growth of bacteria that are unable to survive in acidic environments. These bacteria are environmentally removed from the medium because of their susceptibility to acidic conditions. Additionally, it has been established that fermentation is more efficient in removinggram-negative bacteria is substantially more resistant to fermentation processes than gram-positive bacteria. Thus, fermented foods may help prevent and treat diarrhea in youngsters. Bacteriocin, for example, are protein antibacterial compound produced by LAB. There are peptides known as Bacteriocin that have antibacterial action towards spoilage food-borne diseases or microorganisms' organisms, however, they do not affect the microorganisms that make them. Reutericyclin, hydrogen peroxide, and reuter in are some of the various anti-microbial chemicals that may be produced by LAB. Additionally, LAB may be used as a probiotic to help individuals with diarrhea whose gut flora has been depleted by antibiotic treatment [23].

*3.3.Applications That Could Be Found for Lactic Acid Bacteria:*

After the fermentation process is complete, the chemicals need to go through the procedures of isolating and purifying before they can be extractedfrom the biomass phase or the supernatant.However, in contrast to the supernatant products, it is possible that some LAB biomass, which is normally employed in single-product fermentation procedures, also has

probiotic potential. Therefore, isolating them and finding additional uses for them, while keeping in mind the possibility of a multi-product processing strategy, can add value to the product shown in Figure 3.

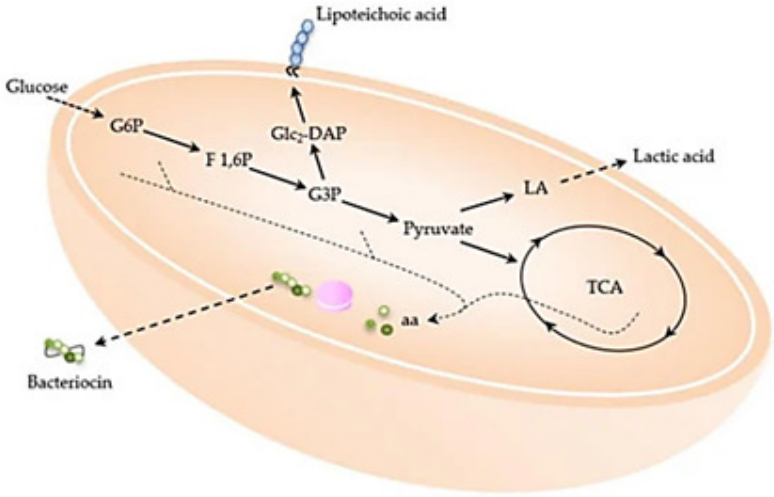


**Figure 3: A Lactic Acid Bacterium's (LAB) Co-Production of Several Chemicals is Represented** [24]**.**

For example, bacteriocin and LA, two LAB metabolites, are examples of continually generated extracellular bacterial products which stay in the fermented broth after growth. Biomass is often thrown away after the fermentation process; however, lately,there is growing interest in biomass as a high-value product. Probiotic application in the food sector could also be created if the bacterial species exploited is identified as a biomass source of protein for the supplementing of green process by-products. The cell wall of LAB is composed of peptidoglycan, Lipoteichoic acid (LTA) and LTA-rich sacculus, and cell-wall polysaccharides, including cell-surface proteins, most of which would have been used in biotechnological processes.

*3.4.Food industry LAB:*

Food preservation by fermentation is an old practice. LAB, or lactic acid bacteria, is essential to this process and makes it possible. In the absence of oxygen, carbon dioxide as well as other organic molecules like mannitol and dextran are used to fuel the processes that convert carbohydrates to lactic acid. It also influences the food's aroma, flavor, structure, or color.The research emphasized the influence of the possible biotechnological uses of LAB in the food manufacturing process. This was done because of the many uses that it has in the food business. Strategies based on genetic engineering improve fermentation efficiency while also enhancing the product's shelf life, nutritional value, and sensory qualities. When food items are manufactured, the presence of unwanted contaminants may result in diminished flavor, decreased production, and even food poisoning.LAB can be genetically altered to continue growing pollutants, while it can also create antimicrobial chemicals that could inhibit or eliminate the growth of contamination, especially infections.

Cultures are being maintained since LAB may be used for a wide variety of purposes. Freezing is a common practice that is used in the preservation of LAB for later applications. When it is thawed, however, the analytical method of preservation causes a reduction in both the capacity to acidify and the viability of the substance.However, scientists are working to

create genetically modified (GM) LAB that can continue to operate normally under these extreme circumstances. On the other hand, neither the European Union nor Ethiopia does not recognize GM goods in a widespread manner.

*3.4.1. Bacteria in Yogurt Production:*

In the production of yogurt and some types of cheese, the thermophilic LAB, *Streptococcus thermophilus*, and *Lactobacillus delbrueckiisubsp. bulgaricus* are used together as crucial beginning microorganisms. The term "proto-cooperation" refers to an indirect but favorable connection between two bacteria since both bacteria can flourish in milk. It is common for this favorable interaction to have a good influence on the development of bacteria as well as the creation of lactic acid or aromatic chemicals.The production of lactic acid causes a drop in pH, which, in turn, renders the environment unfit for the proliferation of bacteria that cause spoilage or disease.

*3.4.2. Meat Products Value or Safety Increased by Lactic Acid Bacteria:*

The nutritional approaches that aim to enhance the quality of food items derived from animals are a relatively recent kind of approach. According to the findings of the study, the use of tomato powder that has been fermented with specific LAB strains may be suggested not only as a coloring agent but also as a supply oflycopene produced during the preparation of ready-to-cook minced hog meat.The inexpensive natural marinades that are fermented with chosen LAB and based on potato juice might be advised for the treatment of hog meat to increase its softness and color, hence boosting its overall attractiveness. Aside from Polycyclic aromatic hydrocarbons (PAHs) or Biogenic amines (BA's) creation, the surface treatment of chosen LAB previously grown in potato juice had an impact on the development of these two compounds in cold-smoked pig meat sausage. In addition, the effects of treating sausages with LAB after smoking on changes in PAHs concentration were studied. Another discovery was related to how LAB metabolites affect the growth of food-sabotaging bacteria.

*3.5. The nutritional value of Tomatoes may be enhanced by Lactic Acid Bacteria*:

The use of certain LAB strains in the processing of tomatoes resulted in some significant shifts in the concentration of carotenoids as well as the lycopene isomer profile. Because the tomato cell matrix was broken down by the LAB treatment, the carotenoids were made more readily accessible,this resulted in a rise in the overall number of carotenoids. Furthermore, tomato fermentation with lactic acid caused a significant rise in the quantity of cis-lycopene present as well as the bioavailability of lycopene. Some laboratories suggest it might aid in tomato preservation, suggesting it as a method for amassing more biologically valuable tomatoes.

*3.6.LAB's Important Metabolites and Their Importance:*

LAB are well-known for their antifungal activity which would be connected to the production of a wide variety of chemicals including acids, alcohols, carbon dioxide cycle peptides, diacetyl, hydrogen peroxide, phenylacetic acid, and bacteriocin. These compounds were added to a range of meals to protect them against microorganisms that could cause foodborne disease or food degradation.The raw components are fermented in fermentation systems, and the most important product that LAB produces is organic acids. Lactic acid or acetic acid are the two primary acids that are created by LAB; however, depending on the strain of LAB, several additional acids may also be formed.

To protect the target organisms from food-borne as well as spoilage microorganisms, these acids would be dispersed through to the membrane organisms when they are in their un-dissociated hydrophobic state they will then be employed tolower cytosolic pH and stop

metabolism rate. When compared to chemical preservatives, that are frequently activated at low pH values (around 3 and 4.5), this could be regarded to be one of the most important reasons why LAB is utilized in the process of food preservation. In addition, LAB provides a wide range of anti-fungal activities that is effective against a variety of fungi that cause food spoiling and produce mycotoxins, whereas commercial stabilizers are often used to control just one or a small number of fungi.

### *3.7. Lactic Acid Bacteria for the Production of Feed with a Higher Value:*

For 14 days, calves were fed fermenting potatoes juices having 9.6 log10 colony-forming units per milliliter of LAB to reduce the chance of acidosis (blood pH was stabilized), reduce lactate and carbon dioxide concentrations, as well as reduce the probability of a skin lesion in plasma and E. coli in the stool.As a result, one might make a compelling case that these foods need to be deemed to have comparable health advantages to those that are provided by probiotic lactobacilli of the same genus. This would be a valid argument to make. It is important to take note of the fact that the regulatory standards of certain countries, such as Italy or Canada, provide a list of the species that are regarded as probiotics. Conversely, it is illegal for packaged foods to use the name "probiotics" across most of Europe and Ethiopia.

## 4. CONCLUSION

The microorganisms that are most often used for food fermentation or storage are called lactic acid bacteria (LAB). Their significance is mostly based on the fact that their metabolic activity is completely risk-free while they are growing in meals and using the sugar that is accessible for the creation of organic acids as well as other metabolites. It is difficult to make foods that have the same sensory qualities and nutritional value as conventional goods, and much more difficult to develop meals that have specific health-promoting features, all while maintaining a process that is regulated, secure, and under management. In terms of nutrition, health, or nutrition, fermented foods are essential. LAB-produced Bacteriocin could be a future medication contender for replacing antibiotics in the treatment of infections with multiple drug resistance because of the improved life span, sustainability, and nutritional value that fermentation provides. To enhance the performance of LAB genetic modification (GM) LAB, additional study is needed in Africa, notably in Ethiopia, where several LAB strains are currently being developed. This study would shed light on the importance of fermentation in human nutrition and also the rationale for their incorporation in Ethiopian national dietary recommendations.

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