A RESEARCH PROJECT

ON

Plant mediated biological synthesis of Copper Nanoparticles using peeled leaf extract of *Aloe barbadensis* and its Antimicrobial effect on Skin flora

In partial fulfillment of the requirements

For the degree of

M.Sc. BIOTECHNOLOGY

BY

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SUBMITTED TO

PANJAB UNIVERSITY

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2019

DECLARATION

I hereby declare that the research project entitled "**Plant mediated biological synthesis of copper nanoparticles using peeled leaf extract of *Aloe barbadensis* and its antimicrobial effect on Skin flora"** submitted for partial fulfillment the degree of M.Sc. Biotechnology from Panjab University, Chandigarh is my original research work and that no part of this desitation has been submitted for award of any other degree.

Date:

Place: Ludhiana **Harsharan Kaur**

CERTIFICATE

This is to certify that the desitation entitled "**Plant mediated biological synthesis of copper nanoparticles using peeled leaf extract of *Aloe barbadensis* and its antimicrobial effect on Skin flora"** submitted for partial fulfillment the degree of M.Sc. Biotechnology from Panjab University, Chandigarh, is a bonafide research work carried out by Ms. Harsharan Kaur under my supervision and that no part of work has been submitted for any other degree

This assistance and help received during the course of investigation have been fully acknowledged.

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Harsharan Kaur

ABSTRACT

There is worldwide interest in the synthesis of nanoparticles. In this study the copper nanoparticle synthesized by biological method, provides a feasible alternative as compared to chemical and physical methods that are expensive and can involve the use of toxic, hazardous chemicals, which may pose biological and environmental risk. To avoid the chemical toxicity, biosynthesis (green synthesis) of metal nanoparticles is proposed as cost-effective. Aloe vera (*Aloe barbadensis)* leaf extract is a medicinal agent with multiple properties other than applications exploiting their antibacterial activity.

This work reports the biosynthesis of Copper nanoparticle using *Aloe barbadensis.* The plant extract was prepared with triple distilled water by cold percolation method. The sample was maintained at pH 9 and 100^0 C temperature. The preliminary characterization of nanoparticles was done by using Colorimeter at 540nm with the regular 1 hour interval. Confirmatory analyses of synthesized copper nanoparticles were done by using TEM (Transmission electron microscopy), SEM (Scanning electron microscopy) and EDS (Energy Dispersion X-ray Spectrometer). These biosynthesized copper nanoparticles were used in the evaluation of antimicrobial activity that was done by Turbidimetric analysis method against facial skin flora. The detection of the synthesized nanoparticles is observed by Colorimeter at 540 nm.

Keywords: Green synthesis, Copper nanoparticles, Cold Percolation, TEM (Transmission Electron microscopy), SEM (Scanning Electron microscopy), EDS (Energy Dispersion X-ray Spectrometer), Antibacterial activity, Skin flora.

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CHAPTER-1

INTRODUCTION

Nanotechnology is enabling technology that deals with Nano-meter sized objects. It is expected that nanotechnology will be developed at several levels: materials, devices and systems. Nanotechnology is an important field of modern research dealing with synthesis, strategy and manipulation of particle's structure ranging from approximately 1 to 100 nm in size. On the scale of natural sciences, nanotechnology is a young field, which emerged in the second half of the 20th century and has been developing with superfast pace ever since. Nanoparticles have attracted the attention of an increasing number of researchers from several disciplines in the past 10 years [1]. The term nanoparticle came into frequent use in the early 1990s together with the related concepts of "nanoscaled" or "nanosized" particles. Until then, the more general terms submicron and ultrafine particles were used. The discovery of the fullerenes, carbon-based structures with a 3-D hollow shape, and the invention of high-resolution microscopes (atomic force microscope and the scanning tunnel microscope) are often considered the cornerstones of the field [2]. Within this size range all the properties (chemical, physical and biological) changes in fundamental ways of both individual atoms/molecules and their corresponding bulk. Novel applications of nanoparticles and nanomaterials are growing rapidly on various fronts due to their completely new or enhanced properties based on size, their distribution and morphology. It is swiftly gaining renovation in a large number of fields such as health care, cosmetics, biomedical, food and feed, drug-gene delivery, environment, health, mechanics, optics, chemical industries, electronics, space industries, energy science, catalysis, light emitters, single electron transistors, nonlinear optical devices and photo-electrochemical applications[3]. Tremendous growth includes the production of nanoscale materials afterwards in investigation or utilization of their mysterious physicochemical and optoelectronic properties. The nanoparticles used for all the aforesaid purposes, the metallic nanoparticles considered as the most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains [4]. Among the all noble metal nanoparticles, copper nanoparticle are an arch product from the field of nanotechnology which has gained boundless interests because of their unique

properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal in addition to anti-inflammatory activities which can be incorporated into composite fibres, cryogenic superconducting materials, cosmetic products, food industry and electronic components.[5]

Nanobiotechnology is a new field of science that introduces special physicochemical and biological properties of nanostructures and their applications in various areas such as medicine and agriculture. Green nanotechnology integrates the principles of green chemistry and green engineering to produce eco-friendly, safe, nanostructures that do not use toxic chemicals in the synthesis protocol. The parallel development of nanotechnology with green chemistry and potential synergism between the two fields can lead to sustainable methods with reduced environmental impacts, protection of resources and human health. With recent developments in nanostructures, concerns of uncertainty and risk regarding environment, health, and safety (EHS) cannot be ignored. Thus the concept of "green nanotechnology" comes to rescue. The main goal of green nanotechnology is to produce nanostructures without affecting the environment or human health. This can be a viable substitute to the conventional physical and chemical methods of synthesizing nanostructures. [6]

Nanoparticles (also known as ultrafine particles) are defined as having one structural dimension of less than 100nm, making them comparable in size to subcellular structures, including cell organelles or biological macromolecules, thereby enabling their ready incorporation into biological systems.

Nanoparticles synthesized from living systems are highly dynamic in nature. Plant-extracted metabolites that can act as reducing and stabilizing agents for the synthesis of nanoparticles are highly dependent on plant species, plant parts (root, stem, leaf, seed, etc.) and methods/conditions used for extraction. In addition, the different types of nanoparticles require a different purification method that also controls the quality and quantity of synthesized nanoparticles. Moreover, the behavior of synthesized nanoparticles is highly affected by experimental conditions. Previous studies have shown that stability, reactivity, and physicochemical properties are influenced by reaction conditions. In a study it was observed that the crystalline nature of zinc sulfide nanoparticles changed immediately when its environment was changed from a wet to a dry condition.[7]

Nanoparticles have various properties that differ from the corresponding bulk material, and this makes them attractive for many new electronic, optical, or magnetic applications. Nanoparticles administered to the lung produce more potent adverse effects in the form of inflammation and subsequent tumors compared to larger-sized particles of identical chemical composition. They are broadly classified into three classes:

• One dimension nanoparticles

One dimensional system (thin film or manufactured surfaces) has been used for decades. Thin films (sizes 1–100 nm) or monolayer is now common place in the field of solar cells offering, different technological applications, such as chemical and biological sensors, information storage systems, magneto-optic and optical device, fiber-optic systems.

• Two dimension nanoparticles

Carbon nanotubes

• Three dimension nanoparticles

Dendrimers, Quantum Dots, Fullerenes (Carbon 60), (Qds)

Intentionally produced nanoparticles are oraganized into following classes:

- carbon-based nanoparticles
- ceramic nanoparticles
- metal nanoparticles
- semiconductor nanoparticles
- polymeric nanoparticles
- lipid-based nanoparticles.[8]

Properties of Nanoparticles

Physical properties of nanoparticles

Nanoparticles consist of three layers: the surface layer, the shell layer, and the core. The surface layer usually consists of a variety of molecules such as metal ion, surfactants, and polymers. Nanoparticles may contain a single material or maybe consist of a combination of several materials. The properties of nanoparticles are dependent their

size. For instance, copper nanoparticles than are smaller than 50 nm are super hard materials and do not exhibit the properties of malleability or ductility of bulk copper. Other changes that are dependent on the size of nanoparticles are superparamagnetism exhibited by magnetic materials, quantum confinement by semiconductor Q-particles, and surface plasmon resonance in some metal particles.

Chemical properties of nanoparticles

The chemical processing and synthesis of high-performance technological components for the private, industrial, and military sectors requires the use of high-purity ceramics ,polymers, glass-ceramics, and composite materials, as metal carbides (SiC), nitrides, metals (Al, Cu), non-metals (graphite, carbon nanotubes) and layered (Al + Aluminium carbonate, Cu + C). In condensed bodies formed from fine powders, the irregular particle sizes and shapes in a typical powder often lead to non-uniform packing morphologies that result in packing density variations in the powder compact.

Uncontrolled agglomeration of powders due to attractive van der Waals forces can also give rise to microstructural heterogeneity. Differential stresses that develop as a result of non-uniform drying shrinkage are directly related to the rate at which the solvent can be removed, and thus highly dependent upon the distribution of porosity. Such stresses have been associated with a plastic-to-brittle transition in consolidated bodies, and can yield to crack propagation in the unfired body if not relieved.

Antimicrobial properties

Nanoparticles are potential broad-spectrum antibiotic agents, and they can overcome evolved resistances to conventional antibiotics. The over-use of regular antibiotics has created ‘superbugs’, bacteria that are resistant to almost all types of antibiotics. Nanoparticles may present a promising solution to this public health hazard.[10]

Nano copper is effective killing agent against Gram-positive and Gram negative bacteria such as *Streptococcus, Micrococcus, Propionibacterium, Dermabacter hominis*

Applications of nanoparticles [11]

Nanoparticles are used, or being evaluated for use, in many fields. The list below introduces several of the uses under development.

- Fluorescent biological labels

- Drug and gene delivery
- Bio detection of pathogens
- Detection of proteins
- Probing of DNA structure
- Tissue engineering
- Tumour destruction via heating (hyperthermia)
- Separation and purification of biological molecules and cells
- MRI contrast enhancement
- Phagokinetic studies
- Stem cell therapy

APPLICATIONS OF COPPER NANOPARTICLES

Copper nanoparticles with great catalytic activities can be applied to biosensors and electrochemical sensors. Redox reactions utilized in those sensors are generally irreversible and also require high overpotentials (more energy) to run. In fact, the nanoparticles have the ability to make the redox reactions reversible and to lower the overpotentials when applied to the sensors.[12]

One of the examples is a glucose sensor. With the use of copper nanoparticles, the sensor does not require any enzyme and therefore has no need to deal with enzyme degradation and denaturation[13]. Depending on the level of glucose, the nanoparticles in the sensor diffract the incident light at a different angle. Consequently, the resulting diffracted light gives a different color based on the level of glucose. In fact, the nanoparticles enable the sensor to be more stable at high temperatures and varying pH, and more resistant to toxic chemicals. Moreover, using nanoparticles, native amino acids can be detected. A copper nanoparticle-plated screen-printed carbon electrode functions as a stable and effective sensing system for all 20 amino acid detection.[14]

Skin flora

The term skin flora (also commonly referred to as skin microbiota) refers to the microorganisms which reside on the skin, typically human skin. Many of them are

bacteria of which there are around 1000 species upon human skin. Most are found in the superficial layers of the epidermis and the upper parts of hair follicles.

Skin flora is usually non-pathogenic, and either commensal (are not harmful to their host) or mutualistic (offer a benefit). The benefits bacteria can offer include preventing transient pathogenic organisms from colonizing the skin surface, either by competing for nutrients, secreting chemicals against them, or stimulating the skin's immune system. However, resident microbes can cause skin diseases and enter the blood system, creating life-threatening diseases, particularly in immunosuppressed people.[16]

Commonly found flora on facial skin of humans is either Gram-positive or Gram-negative few of them are *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Micrococcus. Corynebacterium, Propionibacterium, Brevibacterium, Dermabacter hominis, Diphtheroids, Acinetobacter, Escherichia coli, Pseudomonas aeruginosa, Bacteroidetyes etc.*

***Aloe barbadensis* (Aloe vera)**

Scientific Classification:

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Liliopsida

Subclass: Liliidae

Order: Liliales

Family: Aloeaceae

Genus: Aloe

Species: barbadensis

Common name of *Aloe barbadensis is* **Gwarpatha or Aloe vera.** It originates from the Arabian Peninsula but grows wild in tropical climates around the world and is cultivated for agricultural and medicinal uses. The species is also used for decorative purposes and grows successfully indoors as a potted plant. Vascular plants are catagorised in the Tracheobionta subkingdom. Lilliidae order of plant shows that *Aloe babrbadensis*

monocotyledons. There are over 500 species of Aloes, mostly in Africa, but only a few have medicinal uses and only Aloe Vera has culinary uses. Not all Aloe Vera varieties are considered edible, the main edible one being var Chinensis.

Uses and health benefits:[17]

The medicinal claims made about Aloe vera, as with many herbs and plants, are endless. Some are backed by rigorous scientific studies while others are not. This article focuses mainly on those that are backed by research.

- Aloe vera in tooth gels is as effective as toothpaste in fighting cavities.
- Aloe vera for the treatment of constipation. Dosages of 50-200 milligrams of Aloe latex are commonly taken in liquid or capsule form once daily for up to 10 days.
- Aloe's ability to treat ulcers due to the acidity
- Aloe vera used on skin conditions or superficial cuts for its antimicrobial and antioxidant properties. Mycoplasma is a type of bacteria that lack a cell wall; they are unaffected by many common antibiotics. Antimycoplasmic substances destroy these bacteria.
- Baby Aloe shoot extract and adult Aloe shoot extract might have a protective effect on UVB-induced skin photoaging; in other words, whether they could protect the skin from the aging effects of sunlight.
- Different topical creams to see how effective they might be in protecting the skin of breast cancer patients receiving radiation therapy. One of these creams contained Aloe.
- Nutritional Neuroscience found that Aloe vera reduced depression and improved memory in mice.
- The burn wounds among the patients treated with Aloe vera healed significantly quicker compared with those treated with 1 percent silver sulfadiazine (SSD).

Nanoparticles offer a wide range of applications in a plrthora field but the scope and intent of this project is to focua on the following objectives.

Objective

The objectives of our study are as follows:

- To study the biological synthesis of Copper nanoparticles usimg plant material *Aloe barbadensis*
- To prepare raw extract from *Aloe barbadensis* using Cold percolation Method.
- To study and analyze the biological synthesis of copper nanoparticles using triple distilles water ans ethanol as a solvent.
- To study the biological synthesis of copper nanoparticles from plant extract of *Aloe barbadensis* subjected at pH-9 and 100^{o} C temperature.
- To preliminary characterise copper nanoparticles obtained from plant extract using Colorimeter."
- To confirm the Characterization nanoparticles using Transmission Eclectron Microscopy.
- To confirm the Characterization nanoparticles using Scanning Eclectron Microscopy.
- To confirm the synthesized nanoparticles as being copper nanoparticles through Energy Dispersion X-ray specrometer.
- To analyze the Antimicrobial avtivity using Turbidimetric analysis method against skin flora (face of human).
- To detect, study and analyze the presence of metal ions in the synthesized copper nanoparticles.

CHAPTER-2

REVIEW OF LITERATURE

2.1 Nanotechnology

Nanoscience primarily deals with synthesis, characterisation, exploration, nanostructured materials. Science of Nanotechnology is supposed to have started by lecture of Richard Feyman on there is plenty of room at annual meeting of American physical Society of California Institute of Technology in 1959. The term nanotchnology was coined in 1974 by Norio Taniguichi of Tokyo Science University to describe semiconductor processes such as thin-film deposition that deal with control on the order of nanometers. His definition still stands as the basic statement today: "*Nano-technology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule* "[18]

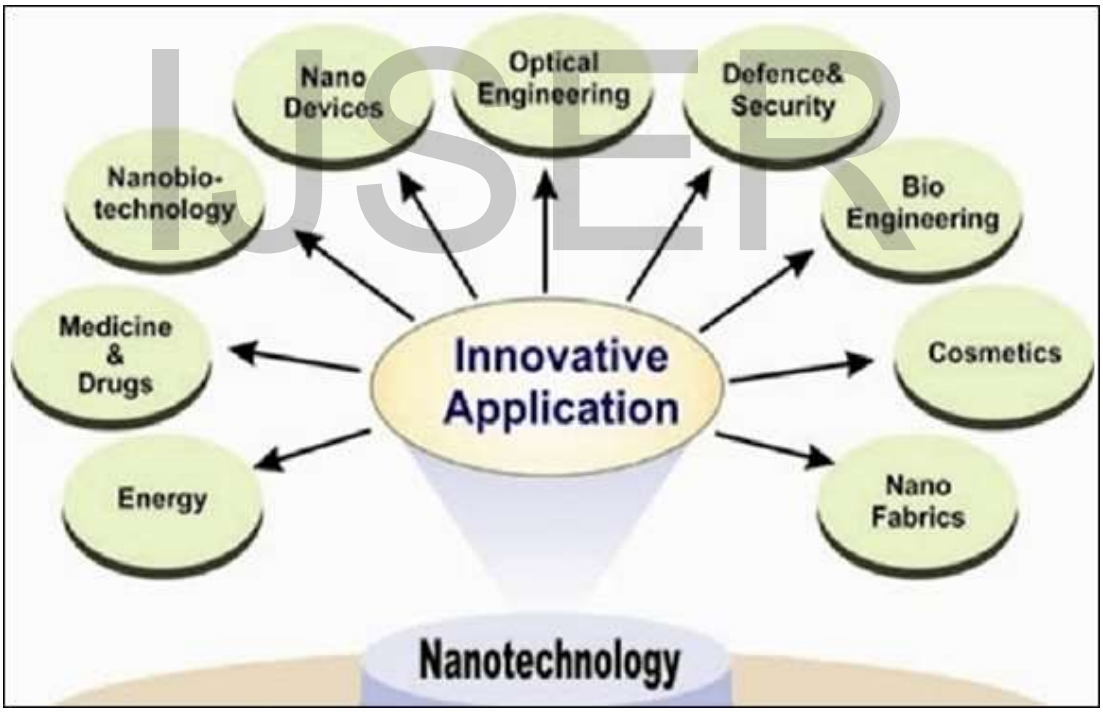

Figure 1: Showing the applications of Nanobiotechnology (source-Awareness in the fields of Nano-technology)

Nanotechnology, the manipulation and manufacture of materials and devices on the scale of atoms or small groups of atoms. The “nanoscale” is typically measured in nanometres, or billionths of a metre (nanos, the Greek word for “dwarf,”), and materials built at this scale often exhibit distinctive physical and chemical properties due to

quantum mechanical effects. Although usable devices this small may be decades away (see microelectromechanical system), techniques for working at the nanoscale have become essential to electronic engineering, and nanoengineered materials have begun to appear in consumer products. For example, billions of microscopic “nanowhiskers,” each about 10 nanometres in length, have been molecularly hooked onto natural and synthetic fibres to impart stain resistance to clothing and other fabrics; zinc oxide nanocrystals have been used to create invisible sunscreens that block ultraviolet light; and silver nanocrystals have been embedded in bandages to kill bacteria and prevent infection[19].

Possibilities for the future are numerous. Nanotechnology may make it possible to manufacture lighter, stronger, and programmable materials that require less energy to produce than conventional materials, that produce less waste than with conventional manufacturing, and that promise greater fuel in land transportation, ships, aircraft, and space vehicles. Nanocoatings for both crimy and translucent surfaces may render them resistant to corrosion, scratches, and radiation. Nanoscale electronic, magnetic, and mechanical devices and systems with unprecedented levels of information processing may be fabricated, as may chemical, photochemical, and biological sensors for protection, health care, manufacturing, and the environment; new photoelectric materials that will enable the manufacture of cost-efficient solar-energy panels; and molecular-semiconductor hybrid devices that may become engines for the next revolution in the information age. The potential for improvements in health, safety, quality of life, and conservation of the surrounding are vast.[20]

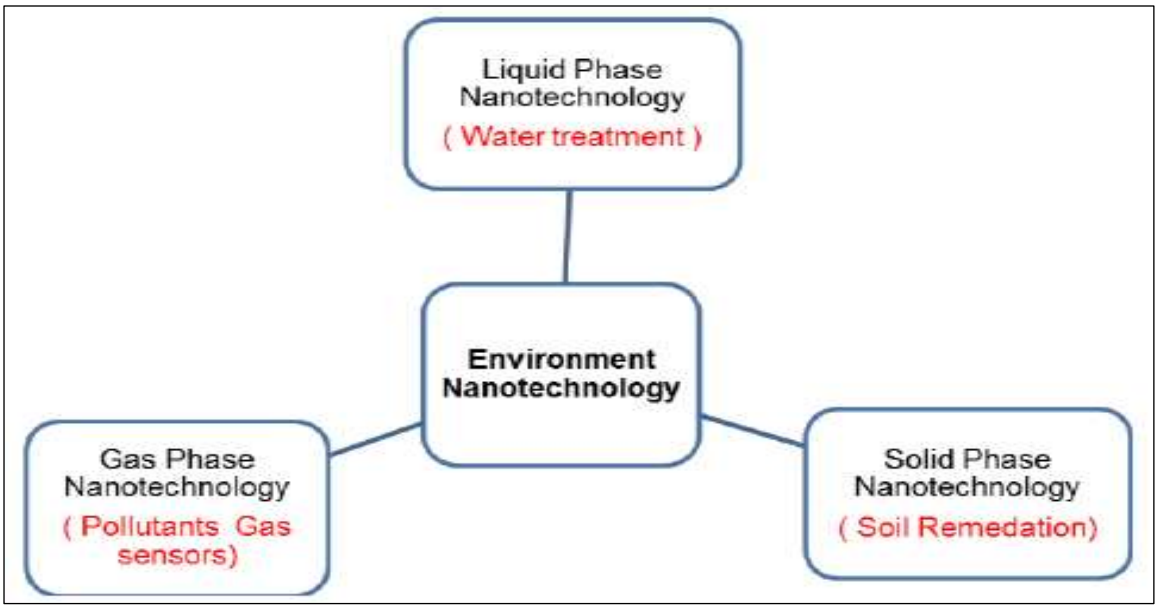

Figure 2: Showing the applications of nanoparticles to environment

At the same time, significant challenges must be overcome for the benefits of nanotechnology to be realized. Scientists must learn how to manipulate and characterize individual atoms and small groups of atoms reliably. New and improved tools are needed to control the properties and structure of materials at the nanoscale; significant improvements in computer simulations of atomic and molecular structures are essential to the understanding of this realm. Next, new tools and approaches are needed for assembling atoms and molecules into nanoscale systems and for the further assembly of small systems into more-complex objects. Furthermore, nanotechnology products must provide not only improved performance but also lower cost. Finally, without mutually depended of nanoscale objects with systems at the micro- and macroscale (that is, from millionths of a metre up to the millimetre scale), it will be very difficult to exploit many of the unique properties found at the nanoscale.

Nanoscience expands the borders of material science by adding biology and biochemistry to the mix. Nanosciences is thus a horizontal integrating interdisciplinary science that cuts across all vertical sciences and engineering disciplines. These materials are characterized by a least one dimension in the nanometer range. On nanoscale, nanotechnology is a new area of research that are known to be formed from centuries. Nanotechnology is the art and science of manipulating matter at nanoscale to create new and unique materials and products with enormous potential to charge society. Nanotechnology literally means and technology on the nanoscale that has application in the real world. Nanotechnology encompasses the production and application of physical, chemical and biological at scale ranging from individual atoms or molecules to submicron dimension, as well as the interaction of the resulting nanostructures into larger systems[21]. Science and technology research in nanotechnology break through out in areas such as material and manufacturing, nanoelectronics, medicine and healthcare, energy, biotechnology, information technology. Nanoscience expands the border of material science by adding biological and biochemistry to the mix. Nanoscience is thus a horizontal integrated interdisciplinary science that cuts across all vertical sciences and engineering engineering disciplines. The application of nanosciences to practical devices is called nanotechnology. Nanotechnology as defined by size is naturally very broad, including fields of science as diverse as surface science, organic chemistry, molecular biology, semiconductor physics, energy storage, micro fabrication, molecular

engineering etc. The associated research and applications are equally diverse, ranging from extensions of conventional devices physics to completely new approach is based upon molecular self-assembly, from development new material with dimension on the nanoscale to direct control of matter on the atomic scale. It has the ability to control or manipulate on the atomic scale. Nanotechnology is proposed presently to defined as a complex of fundamental and engineering science that integrates a chemistry, physics, biology of nanostructures with the material science, electronics and possess technologies focused on a development of self and automatic assembly of nanoparticles using complex probes microbes and manufacturing of nanodevices, nanomachines, ultra-low integrated circuits, nanobiorobots etc [22].

The nanotechnology field, in addition to the fabrication and Nano system provides impetus for the development of experimental and computational tools, nanosciences and nanotechnology depends on the exceptional properties of the matter and enabling convergent technologies which cross all vertical industrial vectors. All industrial sectors rely on materials and devices made of atoms and molecules does in principle all material can be improved with Nano material and all industrial can benefit from nanotechnologies. Basically, it is the engineering of functional systems at the molecular scale. This covers both current work and concepts that are more advanced. In its original sense, nanotechnology refers to the projected ability to construct items from the bottoms up, using techniques and tools spring developed today to make complete, high performance products. Nanotechnology combines solid state physics, chemistry, electrical engineering, chemical engineering, biochemistry, biophysics and material science. It is thus a highly interdisciplinary area integrating ideas and techniques from a wide array of traditional disciplines[23].

It has the potential to revolutionize a series of medical and biotechnology tools and procedures so that they are portable, cheaper, safer and easier to administer. Nanotechnology has created potential impact in various fields like medicine including immunology, cardiology, endocrinology, ophthalmology, oncology, pulmology etc. In addition its highly utilised in specialised area like brain targeting, tumor targeting and gene delivery. Nanotechnologies also provide significant systems devices and materials for pharmaceutical applications[24].

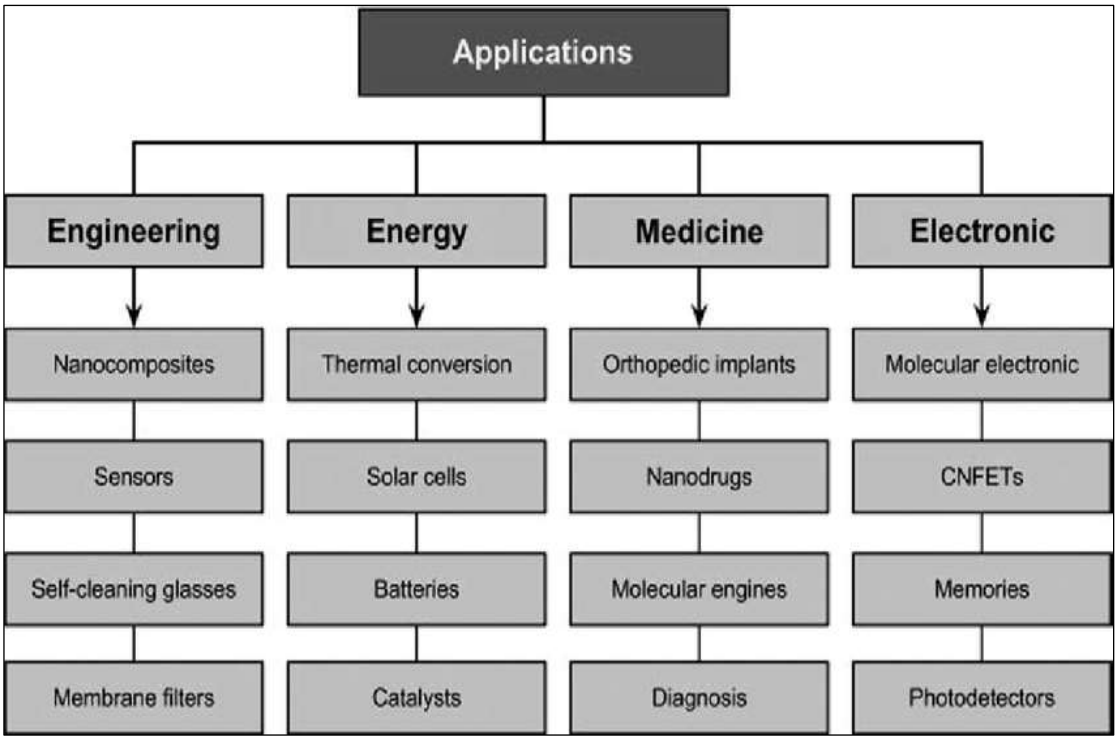

Figure 3: Showing the applications of nanoparticles

2.2 Nanobiotechnology

Nanobiotechnology is a discipline in which tools from nanotechnology are developed and applied to study biological phenomena. For example, nanoparticles can serve as probes, sensors or vehicles for biomolecule delivery in cellular systems[25].

Nanobiotechnology is the interface of nanotechnology and biotechnology and it includes the application of nanotechnology in the life sciences. This branch of nanotechnology is now widely applicable in:

- New molecular imaging techniques, which are used to diagnose the disease in early stages and to understand the effects of therapies.
- Quantitative analytical tools that give an idea of how does the cell act at the molecular level.
- Physical model of the cell as a machine, which can help us to understand the mechanism of the disease and target it effectively.
- Better ex vivo and improvement in current laboratory techniques.
- Better drug delivery systems.

Figure 4: Showing the applications of Green Nanotechnology/Nanobiotechnology

(source-Applications of green nanotechnology)

Nanobiotechnology are terms that refer to the intersection of the nanotechnology and biology. Nanobiotechnology is combination of engineering and molecular biology that is leading to a new class of multifuctional devices and systems for biological and chemical analysis. Nanobiotechnology is the field of science and technology providing tools serving as platform for the investigation and transformation of biological system and it offer inspiration models and bio assembles compnents of nanotechnology. It is the branch of nanotechnology is defined as field that applies the Nanosclae principle and techniques to understand and transform bio systems both living and nonliving and which uses biological princilpes and materials to create new devices and systems integrated from nanoscalecosmetic preparation, water filteration and catalytic system. Biomedical science, pharmaceutical this is playing an important field in bringing advances in scientific world. This technology has broad application in electronics, bionanotechnology generally refers to the study of how the goals of nanotechnology can be guided by how machines work and adapting the biological motifs into improving existing nanotechnologies or creating new ones. Analysis of signaling pathways by nanobiotechniques might provide new insight into disease process, thus identifying efficient biomarkers and understanding the mechanism of action an drugs.

Nanobiotechnology have profound impact on disease prevention, diagnosis and treatment. Recent advancement in nanotechnology has proven that nanoparticles acquire a great potential as drug carriers. It has the potential to revolutionize a series of medical and biotechnological tools and procedures.[26]

2.3 Nanoparticles

A nanoparticle (or nanopowder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100 nm. They can possess physical properties such as uniformity, conductance or special optical properties that make them desirable in materials science and biology.

The term nanoparticle is not usually appied to individual molecules; it usually refers to inorganic material. Presently, different net metallic nanomaterial are being produced using copper, zinc, titanium, magnesium, gold alginate, and silver nanoparticles are being used for diverse purposes, from medical treatments, using in various branches of industry production such as solar and oxide fuel batteries for energy storage to wide incorporation into diverse material of everyday use such as cosmetics for clothes. Nanoparticles are of great scientific interest as they are, in effect, a bridge between bulk materials and atomic on molecular size-related properties significantly different from those of either fine particle or bulk materials[25].

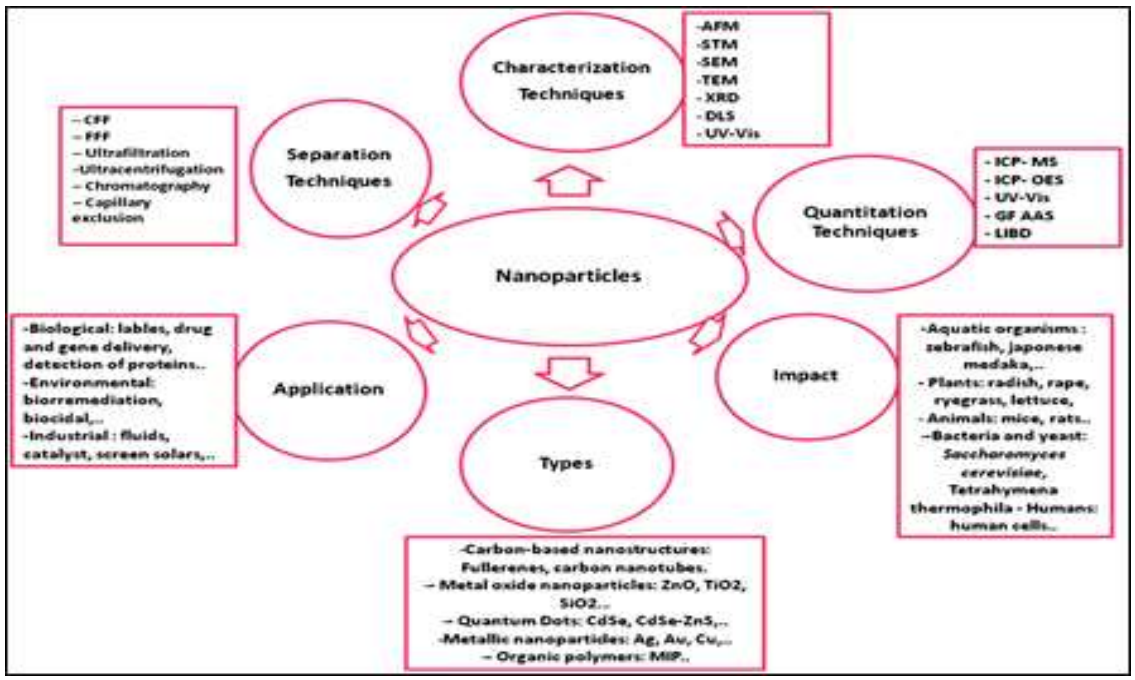

Figure 5: Showing the different types of nanoparticles (source- Nanoparticles: a global vision)

Nanoparticles research is currently and area of intense scientific interest due to a wide variety of potential application in biomedical, optical and electronic fields. For bulk materials larger than one micrometre or micron, the percentage of the surface is insignificant in relation to the volume in the bulk of the material. Nanoparticles which in general term are defined are as engineered structures with diameter of < 100 nm, are devices and system producing by physical and biological processes having specific properties. The term nanoparticles are attractive for such purposes is based on their important and unique features, such a surface to mass ratio, which is much larger than that of other particles and materials, allowing for catalytic promotion of reactions as well as their ability to observe and carry other compounds. Nanoparticles are small enough to confine their electrons produce quantum effects. Nanoparticles are being used for diverse purposes, from medical treatments, using in various branches of industrial production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes, optical devices, catalytic, bactericidal, electronic sensor Technology, biological labelling and treatment of some cancers. Due to their exceptional properties including antibacterial activity, high resistance to oxidation and high thermal conductivity, nanoparticles have attracted considerable attention in recent years. Metal, dielectric and semiconductor nanoparticles have been formed, as well as hybrid structure (e.g. core shell nanoparticles) [27]. Nanoparticles made of semiconducting material may also be labelled quantum dots if they are small enough (typically sub 10 nm) that quantization of electronic energy levels occurs. Such nanoscale particles are used in biomedical application as drug carriers or imaging agents semi-solid and soft nanoparticles have been manufactured. Nanoparticles have been found to impart some extra properties to various day to day products. For example the presence of titanium dioxide nanoparticles imparts on call self-cleaning effects and the size beingh nano-range, the particle cannot observed. zinc oxide particles have been found to have superior UV blocking properties compared to its bulk substitute. This is one of the reasons of used in the preparation of sunscreen lotion is completely stable and non-toxic. Nanoparticles have also been attached to textile fibres in order to create smart and functional particles.

2.3.1 Properties of nanoparticles

Two primary factors cause nanoparticles to behave significantly differently than bulk materials: surface effects. These factors affect the chemical reactivity of materials as well

as their mechanical, optical, electric and magnetic properties. Nano silver has chemical and biological properties that are appealing to the consumer products, food technology, textiles/fabrics, and medical industries. Nano silver also has unique optical and physical properties that are not present in bulk silver, and which are claimed to have great potential for medical applications [28].

(a) Surface to Volume Ratio

Nanoparticles have unique properties due to their small size. All nanoparticles regardless of their chemical constituents have surface area: volume ratios that are extremely high. Thus, many of the physical properties of the nanoparticles such as solubility and stability are dominated by nature of the nanoparticles surface.

(b) Surface plasmonic resonance

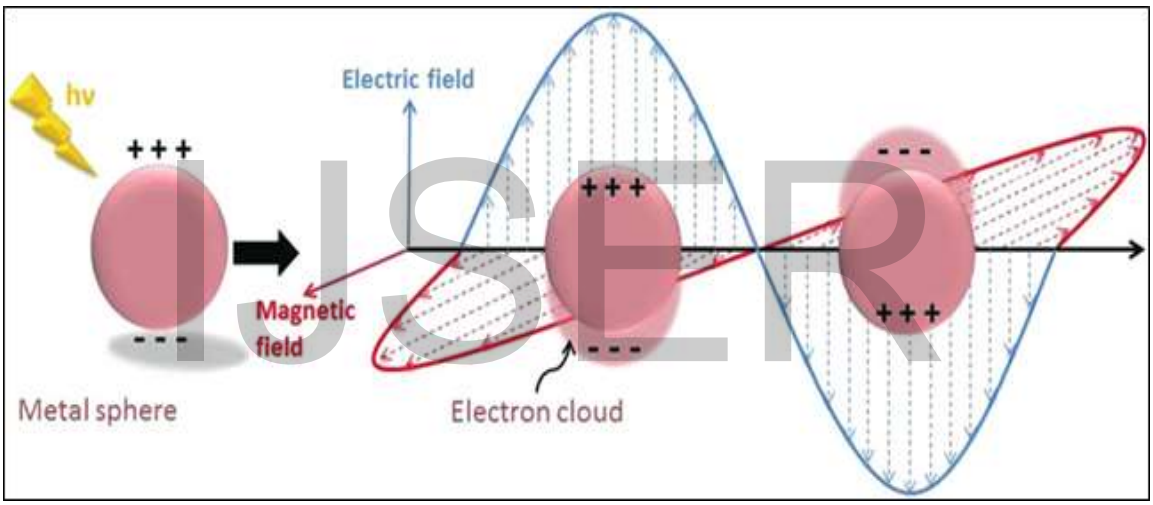

Figure 6: Showing illustration of Surface plasmonic resonance (source-collective oscillations of delocalized electrons in response to an external electric field)

The optical and electronic properties of nanoparticles are inter-dependent to greater extent. For onstance, noble metals nanoparticles have size dependent optical properties and exhibit a strong UV-visible extinction band that is not present in the spectrum of the bulk- metal. This excitation band results when the incident photon frequency is constant with the collective excitation of the conduction electrons and is known as the localized surface plasma resonance (LSPR). LSPR excitation results in the wavelength selection absorption with extremely large molar excitation coefficient resonance ray light scattering with efficiency equivalent to that of ten fluorophores and enhanced local electromagnetic fields near the surface of nanoparticles that enhanced spectroscopies. It is well established that the peak wavelength of the LSPR spectrum is dependent upon the

size, shape and antiparticle spacing of the nanoparticles as well as its own dielectric properties and those of its local environment including the substrate, solvents and absorbates.

(c) Shape and Size

The ability to produce metal nanoparticles (NPs) of controlled size and shape is important for the study of their physical properties. Among the various metallic nanoparticles, CuNPs have received great attention because Cu is much cheaper than Ag or Au.Very peculiar optical, electrical and thermal properties have been described for CuNPs. Indeed, the nanoscale properties of Cu have found application in catalysis, cooling fluids for the electronic system, and conductive inks. Furthermore, nanosized Cu exhibits a prominent Localized Surface Plasmon Resonance (LSPR) in the visible range as Ag and Au. Nevertheless, a major drawback limiting the use of CuNPs is their tendency to oxidize. The literature on the synthesis of CuNPs of controlled size and shape is thus less developed than that for Ag or AuNPs.

(d) Surface and size distribution of nanoparticles

A number of in vitro studies with different NPs have been published in which the effect of the different parameters such as dispersion, surface properties, and agglomeration and de-agglomeration can be controlled using ultrasonication, ionic strength and pH of aqueous solutions, physiological buffers, and cell culture media[30].

(e) Antimicrobial properties

Nanoparticles are potential broad-spectrum antibiotic agents, and they can overcome evolved resistances to conventional antibiotics. The over-use of regular antibiotics has created ‘superbugs’, bacteria that are resistant to almost all types of antibiotics. Nanoparticles may present a promising solution to this public health hazard.

Nano copper is effective killing agent against Gram-positive and Gram negative bacteria such as *Streptococcus, Micrococcus, Propionibacterium, Dermabacter hominis*

2.3.1.2 Chemical Properties of Nanoparticles

Chemical properties include the elemental composition of nanomaterials and its surface chemistry such as zeta potential and photocatalytic properties. The chemical properties of a material are determined by the type of motion of its electrons. There is a wide range of NPs contributing to many different chemical properties [31].

(a) Metallic nanoparticles

Compared with other nanostructures, metallic NPs have been proven to be the most flexible nanostructures owing to the synthetic control of their size, shape, composition, structure, assembly, and encapsulation, as well as the resulting tenability of their optical properties.

(b) Metal oxide nanoparticles

Metal oxide NPs can exhibit unique chemical properties due to their limited size and a high density of corner or edge surface sites. Particle size is expected to influence important groups of basic properties in any material.

(c) Quantum dots

The size effects in metal oxide chemistry have frequently two interrelated faces, structural/electronic quantum-size and size-defect or non-stoichiometry effects. Structurally quantum dots (QDs) consist of a variety of metal complexes such as semiconductors, metals, and magnetic transition metals. The bioactivity of QDs can be improved by suitable surface coating with biocompatible material and/or modification with desired functional groups.

(d) Ploymeric nanoparticles

Polymer NPs have attracted the interest of many plant research groups. The term polymer nanoparticle is given for any type of polymer NPs but specifically for nanospheres and nanocapsules. These are obtained from synthetic such as from synthetic polymers, such as polycaprolactone, polyacrylamide, and polyacrylate, or natural polymers, albumin, DNA and chitosan, gelatin, and poly (L-lactide) (PLA).

(e) Magnetic properties

Magnetic NPs are of great curiosity for investigators from an electric range of disciplines, which include heterogenous and homogenous catalysis, biomedicine, magnetic fluids, data storage magnetic resonance imaging (MRI), and environmental remediation such as water decontamination. The uneven electronic distribution in NPs leads to magnetic property.

(f) Thermal properties

It is well-known fact that metals NPs have thermal conductivities higher than those of

fluids in solid form This is partially due to the difficulties of experimentally measuring and controlling the thermal transport in nano scale dimensions .For macroscopic systems, the dimension is large enough to define a local temperature in each region within the materials and this local temperature will vary from region to region, so that one can study the thermal transport properties of the materials based on certain temperature distributions of the materials. But for nanomaterial systems, the dimensions may be too small to define a local temperature.

(g) Optical Properties

The optical properties of small particles have received considerable attention because of potential applications in optical sensors and lasing devices. Nano crystalline systems have attracted interest for their novel optical properties, which differ remarkably from bulk crystals. The linear and non-linear optical properties of such materials can be finely tailored by controlling the crystal dimensions and the chemistry of their surfaces, fabrication technology becomes a key factor for the applications.

(h) Electrical and Electronic Properties

According to the theory of electron scattering in solids, the electrical resistivity of nanocrystalline materials is expected to be higher than that in the corresponding coarse-grained polycrystalline ones due to the increased volume fraction of atoms lying on the grain boundaries. It is well known that the electrical conductivity of the solids is determined by its electronic structure. Generally in solids, the valence band is completely filled by electrons and separated from the empty conduction band with the energy gap of Eg (band gap). For metals, Eg =0, which results in the mixing of the valence and conduction bands. In the case of semiconductors, the value of Eg is small. The electrons can be excited from the valence band to conduction band using heat, light etc., which results in partial conductivity. aterials can be finely tailored by controlling the crystal dimensions and the chemistry of their surfaces, fabrication technology becomes a key factor for the applications.

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2.3.2 Sources of naoparticles:

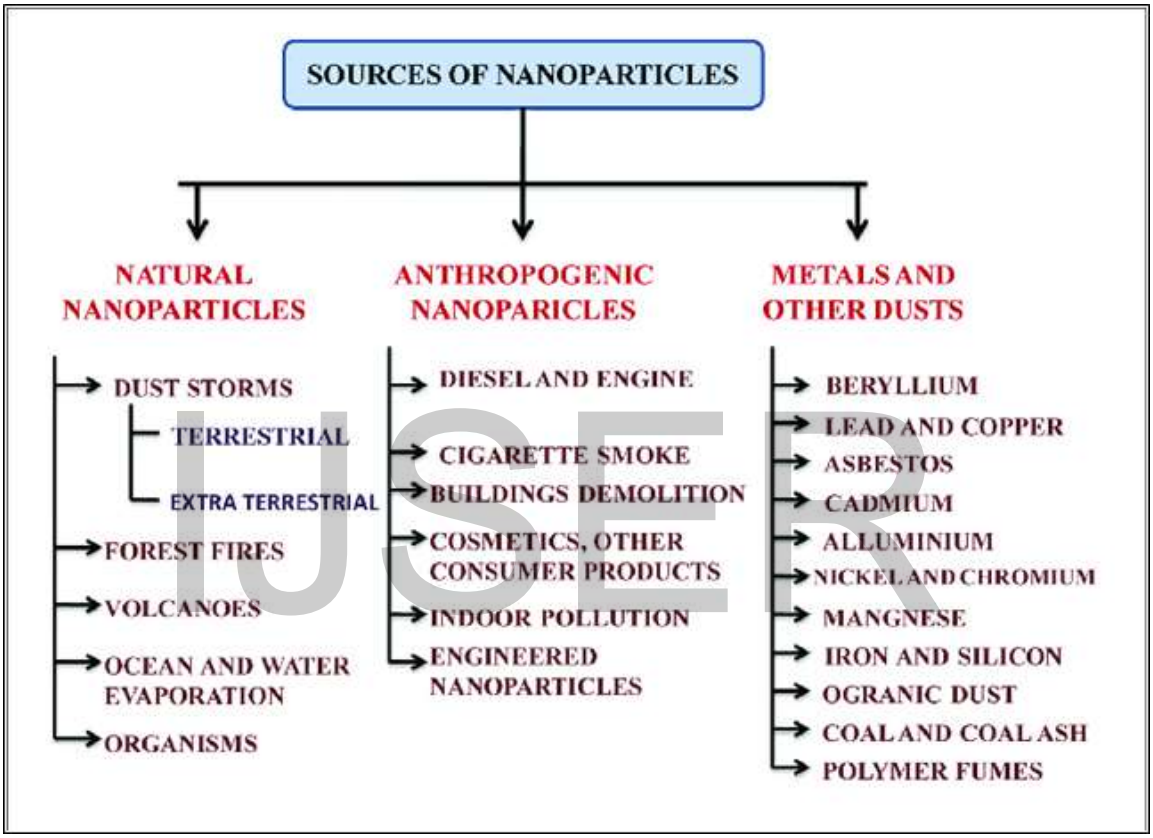

Figure 7: Showing different sources of nanoparticles (source- An outline of the various sources of nanoparticles in the environment)

2.3.2.1. Natural sources of nanoparticles

Nanoparticles are abundant in nature, as they are produced in many natural processes, including photochemical reactions, volcanic eruptions, forest fires, and simple erosion, and by plants and animals, e.g. shed skin and hair. Though we usually associate air pollution with human activities – cars, industry, and charcoal burning, natural events such as dust storms, volcanic eruptions and forest fires can produce such vast quantities of nanoparticulate matter that they profoundly affect air quality worldwide. The aerosols generated by human activities are estimated to be only about 10% of the total, the

remaining 90% having a natural origin. These large-scale phenomena are visible from satellites and produce particulate matter and airborne particles of dust and soot ranging from the micro- to nanoscales. Small particles suspended in the atmosphere, often known as aerosols, affect the entire planet's energy balance because they both absorb radiation from the sun and scatter it back to space. It has been estimated that the most significant components of total global atmospheric aerosols are, in decreasing mass abundance: mineral aerosols primarily from soil deflation (wind erosion) with a minor component (<1%) from volcanoes (16.8 Tg), sea salt (3.6 Tg), natural and anthropogenic sulfates (3.3 Tg), products of biomass burning excluding soot (1.8 Tg), and of industrial sources including soot (1.4 Tg), natural and anthropogenic nonmethane hydrocarbons (1.3 Tg), natural and anthropogenic nitrates (0.6 Tg), and biological debris (0.5 Tg) (note: 'Tg' here denotes terragram, equal to 1012 grams)[32].

2.3.2.2. Anthropogenic nanoparticles

Humans have created nanomaterials for millennia, as they are byproducts of simple combustion (with sizes down to several nm) and food cooking, and more recently, chemical manufacturing, welding, ore refining & smelting, combustion in vehicle and airplane engines, combustion of treated pulverized sewage sludge , and combustion of coal and fuel oil for power generation . While engineered nanoparticles have been on the market for some time and are commonly used in cosmetics, sporting goods, tires, stain-resistant clothing, sunscreens, toothpaste, food additives, etc., these nanomaterials, and new more deliberately fabricated nanoparticles, such as carbon nanotubes, constitute a small minority of environmental nanomaterials. The quantity of manmade nanoparticles ranges from well-established multi-ton per year production of carbon black (for car tires) to microgram quantities of fluorescent quantum dots (markers in biological imaging).

2.3.2.3 Metals and other dusts

Small quantities of many metals, including copper, magnesium, sodium, potassium, calcium, and iron are essential for proper functioning of biological systems. At higher doses, however, metals can have toxic effects and exposure to high levels of environmental metals causes disease in humans. The metals listed below in this paragraph are known to be toxic upon inhalation, ingestion or dermal exposure. Nanoparticles manufactured from these metals will have health effects not necessarily easily predicted from previous studies of non-nanoparticulate quantities of the same

metals. As it could easily expose workers to these toxic materials, manufacturing of metal nanoparticles should be considered a serious occupational hazard. The inhalation of metallic or other dusts is known to have negative health effects. The type of lung disease caused by dust inhalation depends on the nature of the material, exposure duration, and dose. The inhalation of some metal fumes (e.g. zinc, copper) may lead to metal fume fever, a influenza-like reaction. Several metal dusts (e.g. platinum, nickel, chromium, cobalt) can lead to asthma, while inhalation of other metallic dusts can cause pulmonary fibrosis, and ultimately lung cancer. The percentage of lung cancers attributable to occupational hazards is about 15%, with exposure to metals being a major cause [33].

2.3.3 Methods of nanoparticle synthesis

Nanosized metallic particles are unique and can considerably change, Physical, chemical and biological prpoerties due to their surface to volume ratio therwfore, these nanoparticles have been exploited for various purposes. In order to fulfill the requirements of CuNPs, various mathod has been adopted for synthesis of copper nanoparticles. Generally, conventional physical ans chemical methods seem to be very expensive and hazardous. Interestingly, biological- prepared CuNPs shows high stability. Among all the methods, biological method seems to be simple, rapid, non-toxic, dependable and green approches produce well-defines size and morphology under optimized conditions [34].

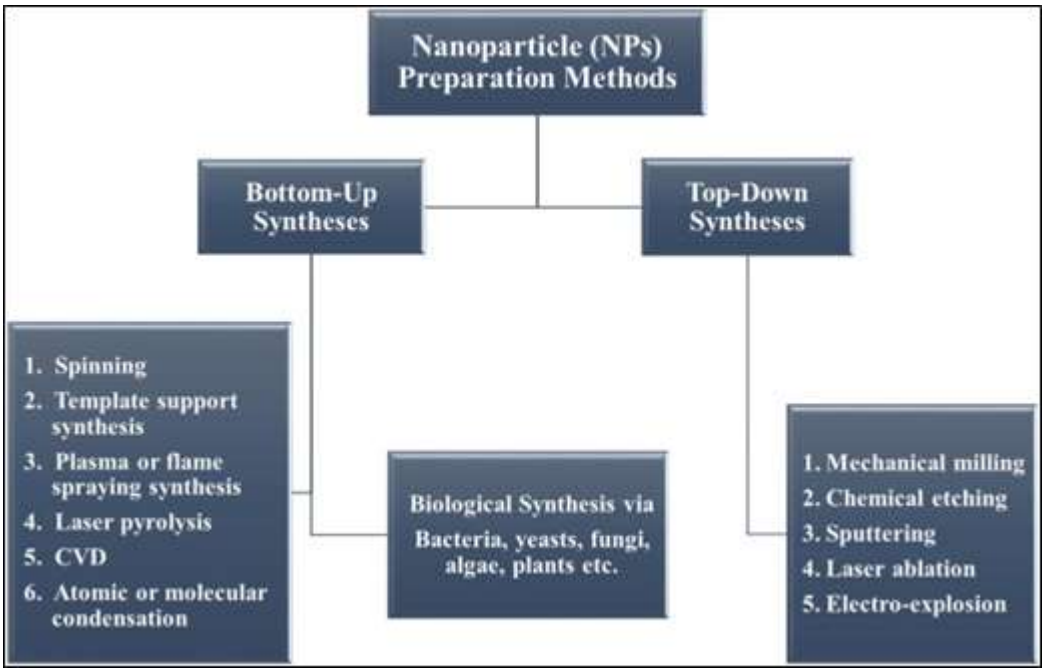

Figure 8: Showing the different approaches of nanotechnology

Top-down and bottom-up approaches

There are two types of approaches for synthesis of nano material and fabrication of nano structure.

- Top-Down approaches refers to slicing or successive cutting of a bulk material to get nano sized particles. there are two types *attrition ,* milling
- Bottom-up refers to methods where devices 'create themselves' by self-assembly. Chemical synthesis is a good example. Bottom-up should broadly speaking be able to produce devices in parallel and much cheaper than top-down methods, but getting control over the methods is difficult when things become larger and more bulky than what is normally made by chemical synthesis. Of course nature has had time to evolve and optimize self-assembly processes that can do wonders [35].

Bio-reduction mechanism

The biochemical reaction of $CuSO_4$.$5H_2O$ reacts with plant broth leads to the formation of CuNPs. Figure explains the proposed mechanism of biological synthesis of nanoparticles.

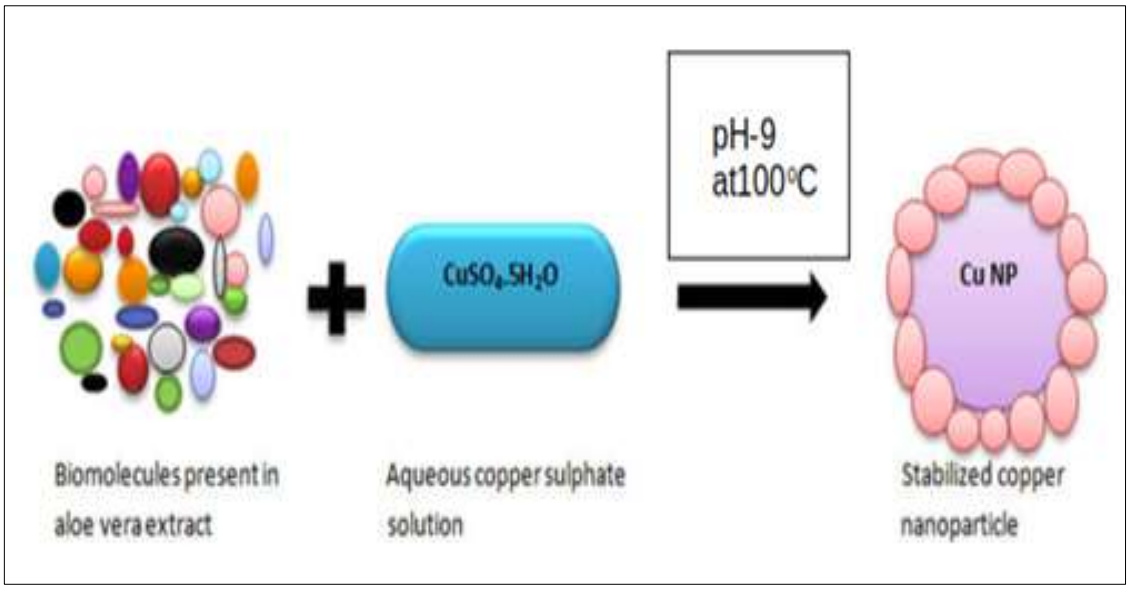

Figure 9: Showing schematic representation of bioreduction of aqueous copper sulphate solution by biomolecules in Aloe vera extract to stabilized copper nanoparticles

2.3.3.1 Synthesis of nanoparticles by various methods

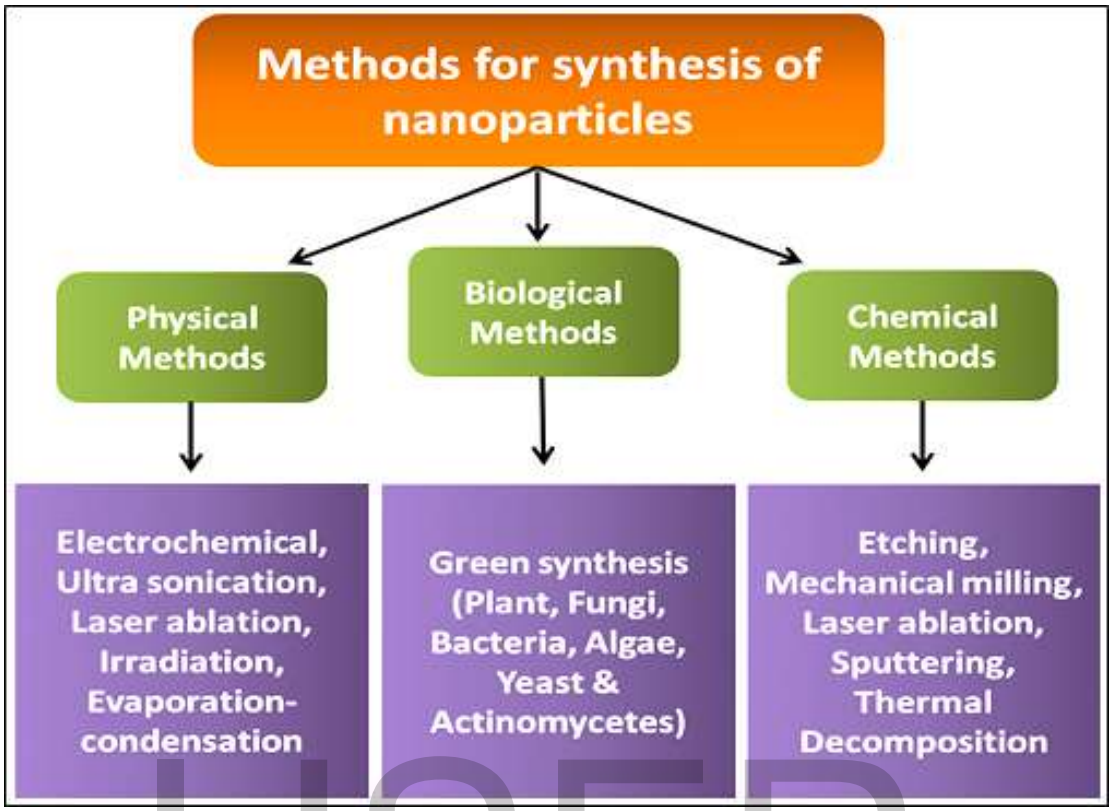

Figure 10: Showing the various methods of synthesis of nanoparticles

(a) Physical method

In physical processes, metal nanoparticles are generally synthesized by evaporation-condendsation, which could be carried out using a tube furnance at atmospheric pressure. The source material with in a boat centered at the furnance is vaporized into a carrrier gas. Nanoparticles of various materials, Cu, Ag, Au, PbS and fullerence, have previously been produced using the evaporation/condensation technique. However, the genration of copper nanoparticles [35].

Physical techniques are unconventional methods, such as those that require vacuum or plasma, sometimes obtaining nanoparticles with low quality. Several physical techniques are incorporated during or after a chemical process, for example, the laser ablation requires a colloidal solution, which minimizes the chances of oxidation on the surface of the nanoparticles, and it must be placed in a vacuum chamber in order to remove or extract atoms from a bulk surface through emission of laser beam; this method is not feasible due to the complexity of the equipment and the use of high energy for the laser. The number of pulses applied of the laser beam and the exposition time are important

parameters to define the particle size. These parameters are in the range from 6000 to 10000 pulses in periods of 10 and 30 minutes. The decomposition of volatile compounds inside a vacuum chamber or reactor is used for the deposition of atom by atom or molecule by molecule to form layers on a solid surface at subatmospheric pressure. Unlike other physical techniques, in the Pulsed Wire Discharge (PWD), the ions are implanted on solid substrate by pulse electrical current [36].

(b) Chemical method

Various methods have been described to chemicaly synthesize copper nanoparticles. An older method involves the reduction of copper hydrazine carboxylate in an aqueous solution using reflux or by heating through ultrasound under an inert argon atmosphere. This results in a combination of copper oxide and pure copper nanoparticle clusters, depending on the method used. A more modern synthesis utilizes copper chloride in a room temperature reaction with sodium citrate or myristic acid in an aqueous solution contain sodium formaldehyde sulfoxylate (SFS) to obtain a pure copper nanoparticle powder. While these syntheses generate fairly consistent copper nanoparticles, the possibility of controlling the sizes and shapes of copper nanoparticles has also been reported. The reduction of copper (II) acetylacetonate in organic solvent with oleyl amine and oleic acid causes the formation of rod and cube-shaped nanoparticles while variations in reaction temperature affect the size of the synthesized particles.

Another method of synthesis involves using copper hydrazine carboxylate salt with ultrasound or heat in water to generate a radical reaction, as shown in the figure to the right. Copper nanoparticles can also be synthesized using green chemistry to reduce the environmental impact of the reaction. Copper chloride can be reduced using only L-ascorbic acid in a heated aqueous solution to produce stable copper nanoparticles [37].

(c) Biological method or Green synthesis

Plants consist of large number of biologically active compounds and hence, most of the plants have proven record for their anthelmintic, antitumor, antimutagenic, antibacterial and fungicidal properties. The synthesis of metallic NPs involves simple mixing of metal solution with extract of plant. Nanoparticles are produced in the medium due to reduction of metal ions. Many earlier investigations revealed that Cu NPs can be synthesised by the application of most common precursor copper salts namely, cupric

acetate (monohydrate) ((CH_3 $COO)_2$ $Cu{\cdot}H_2O$) 19 , Copper chloride di-hydrate ($CuCl_2$. $2H_2O$) 20 and Copper sulfate pentahydrate ($CuSO_4$ .5H2O). 21 Various factors such as concentration, pH, temperature, influence the nature and properties of synthetic Cu NPs as well as CuO Nps [38].

Several studies reported the synthesis of nanoparticles using green, cost effective and biocompatible methods without the use of toxic chemicals in biological methods. The biological synthesis of nanoparticles depends on three factors, including (a) the solvent; (b) the reducing agent; and (c) The non-toxic material. The major advantages of biological methods is the availability of amino acids, proteins, or secondary metabolites present in the synthesis process, the elimination of the extra step required for the prevention of particle aggregation, and the use of biological molecules for the synthesis of nanoparticles is eco-friendly and pollution-free. Biological methods seem to provide controlled particle size and shape, which is an important factor for various biomedical applications. Using bacterial protein or plant extracts as reducing agents, we can control the shape, size, and monodispersity of the nanoparticles. The other advantages of biological methods are the availability of a vast array of biological resources, a decreased time requirement, high density, stability and the ready solubility of prepared nanoiparticles in water. Biological methods of nanoparticles provide a new possibility of conveniently synthesized nanoparticles using natural reducing and stabilizing agents [39].

- **Hot Percolation Method:**

The most common approach for synthesis Cu nanoparticles is Hot Percolation Method and Cold Percolation Method. Hot Percolation Methods is used for synthesis of nanoparticles using two different solvents i.e. triple distilled water and 70% ethnol at different reaction conditions like pH and temperature. In the Hot Percolation Method, the plant extract is treated with triple distilled water directly exposure to the heat i.e. on hot plate for sample preparation and for other solvent reflux condenser this is also a Hot Percolation Methods but not directly exposure to heat.

- **Cold Percolation Method:**

The biological activity of nanoparticles depends on the morphology and structure of nanparticles, controlled by size and shape of the particles. Compared to chemical

methods, biological methods allow for more ease in the control of shape, size, and distributed of the produced naboparticles by optimization of the synthesis methods, including the amount of precursors, temperature, pH ,and the amount of reducing and stbilizing factors.

2.3.4 Types of nanoparticles [40]

Silver: Silver nanoparticles have proved to be most effective because of it's good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms. They are undoubtedly the most widely used nanomaterials among all, thereby being used as antimicrobial agents, in textile industries, for water treatment, sunscreen lotions etc. Studies have already reported the successful biosynthesis of silver nanoparticles by plants such as Azadirachta indica20, Capsicum annuum 21 and Carica papaya

Gold: Gold nanoparticles (AuNPs) are used in immunochemical studies for identification of protein interactions. They are used as lab tracer in DNA fingerprinting to detect presence of DNA in a sample. They are also used for detection of aminoglycoside antibiotics like streptomycin, gentamycin and neomycin. Gold nanorods are being used to detect cancer stem cells, beneficial for cancer diagnosis and for identification of different classes of bacteria.

Alloy: Alloy nanoparticles exhibit structural properties that are different from their bulk samples. Since Ag has the highest electrical conductivity among metal fillers and, unlike many other metals, their oxides have relatively better conductivity, Ag flakes are most widely used. Bimetallic alloy nanoparticles properties are influenced by both metals and show more advantages over ordinary metallic Nps.

Magnetic: Magnetic nanoparticles like Fe3O4 (magnetite) and Fe2O3 (maghemite) are known to be biocompatible. They have been actively investigated for targeted cancer treatment (magnetic hyperthermia), stem cell sorting and manipulation, guided drug delivery, gene therapy, DNA analysis, and magnetic resonance imaging (MRI).

Copper: Copper is found to be too soft for some applications, and hence it is often combined with other metals to form numerous alloys such as brass, which is a copper-zinc alloy. Copper nanoparticles are graded as highly flammable solids, therefore they must be stored away from sources of ignition. The morphology of copper nanoparticles is round, and they appear as a brown to black powder.

Characteristics:

Copper is the most important trace element that plays the significant role in biological process. It is essential for proper functioning of human body. Hence, considering the importance of copper and its unique properties viz., potent catalytic, optical and thermal conduction ability, it is widely compared with other metal nanoparticles.

1. Copper nanoparticles display unique characteristics including catalytic and antifungal/antibacterial activities that are not observed in commercial copper.
2. Copper nanoparticles demonstrate a very strong catalytic activity, a property that can be attributed to their large catalytic surface area.
3. Copper nanoparticles have small size and great porosity, the nanoparticles are able to achieve a higher reaction yield and a shorter reaction time when utilized as reagents inorganic and organometallic synthesis.
4. Copper nanoparticles that are extremely small and have a high surface to volume ratio can also serve as antifungal/antibacterial agents.
5. The antimicrobial activity is induced by their close interaction with microbial membranes and their metal ions released in solutions.
6. Among all metallic particles copper is most widely used due to their superior optical, electrical, antifungal/antibacterial property.
7. Copper nanoparticles have low cost and high conductivity have wide applications as heat transfer system and catalyst.
8. Copper nanoparticles form copper oxide when they are exposed to air. To protect copper nanoparticles from oxidation with air, they are usually coated with organic and inorganic coating such as silica and carbon [45].

Applications:

Applications for copper nanocrystals include as an anti-microbial, anti-biotic and anti-fungal (fungicide) agent when incorporated in coatings, plastics, textiles, in copper diet supplements, in the interconnect for micro, integrated circuits, for its ability to absorb radioactive cesium, in super strong metals, alloys, in nanowire , nanofiber, in certain alloy and catalyst applications[46].

1. The use of silver and copper ions as superior disinfectants for wastewater generated from hospitals containing infectious microorganisms.
2. Cu NPs have superior antibacterial activity as compared to silver nanoparticles due to this Cu NPs are used in wound dressing.
3. Nobel metals powder like gold or silver are now replaced with copper in electronic industry.
4. Copper is highly toxic to microorganism and used for food packaging .Due to optical property of Cu NPs, it is used in optical devices and optical materials.
5. Copper nanoparticles with great catalytic activities can be applied to biosensors and electrochemical sensors. In fact, the nanoparticles have the ability to make the redox reactions reversible and to lower the over potentials when applied to the sensors.
6. Copper nanoparticles were used as an antimicrobial agent due to their high surface-to- volume ratio and easy interaction with other particles to enhance their antimicrobial efficiency. Nanocopper particles were used as the fuel additive for diesel engine with Soya bean biodiesel (B10).

2.3.5 Tools for Characterization of Nanoparticles

To evaluate the synthesized nanomaterials, many analytical techniques have been used, including **Colorimter, XRD (X-ray Diffrectometry), FTIR(Fourier-transform infrared spectroscopy), DLS (Dynamic Light Scattering), SEM (scanning Electron microscopy), TEM(Transmission Electron Microscopy), AFM (Atomic Force Microscopy), EDS(Energy Dispersion X-ray Spectrometer),** These techniques are used to determine the Different parametres. Moreover, Orientation, intercalation, and dispersion of nanoparticles and nanotubes in nanocomposite materials could be determined by these techniques [48].

(a) TEM (Transmission Electron Microscopy): The transmission electron microscope is a very powerful tool for material science. A high energy beam of electrons is shone through a very thin sample, and the interactions between the electrons and the atoms can be used to observe features such as the crystal structure and features in the structure like dislocations and grain boundaries. Chemical analysis can also be performed. TEM can be used to study the growth of layers, their composition and defects in semiconductors.

High resolution can be used to analyze the quality, shape, size and density of quantum wells, wires and dots.

The TEM operates on the same basic principles as the light microscope but uses electrons instead of light. Because the wavelength of electrons is much smaller than that of light, the optimal resolution attainable for TEM images is many orders of magnitude better than that from a light microscope. Thus, TEMs can reveal the finest details of internal structure in some cases as small as individual atoms[49].

Imaging

The beam of electrons from the electron gun is focused into a small, thin, coherent beam by the use of the condenser lens. This beam is restricted by the condenser aperture, which excludes high angle electrons. The beam then strikes the specimen and parts of it are transmitted depending upon the thickness and electron transparency of the specimen. This transmitted portion is focused by the objective lens into an image on phosphor screen or charge coupled device (CCD) camera. Optional objective apertures can be used toenhance the contrast by blocking out high-angle diffracted electrons. The image then passed down the column through the intermediate and projector lenses, is enlarged all the way.

The image strikes the phosphor screen and light is generated, allowing the user to see the image. The darker areas of the image represent those areas of the sample that fewer electrons are transmitted through while the lighter areas of the image represent those areas of the sample that more electrons were transmitted through.

Diffraction

As the electrons pass through the sample, they are scattered by the electrostatic potential set up by the constituent elements in the specimen. After passing through the specimen they pass through the electromagnetic objective lens which focuses all electrons scattered from one point of the specimen into one point in the image plane. the electrons scattered in the same direction by the sample are collected into a single point. This is the back focal plane of the objective lens and is where the diffraction pattern is formed[50].

(b) SEM (Scanning Electron Microscopy):

Scanning electron microscopy (SEM) has become a powerful and versatile tool for material characterization. This is especially so in recent years, due to the continuous

shrinking of the dimension of materials used in various applications. Electron microscopes use electrons for imaging, in a similar way that light microscopes use visible light. SEMs use a specific set of coils to scan the beam in a raster-like pattern and use the electrons that are reflected or knocked off the near-surface region of a sample to form an image. Since the wavelength of electrons is much smaller than the wavelength of light, the resolution of SEMs is superior to that of a light microscope.

In SEM, the electron beam scans the sample in a raster pattern. But first, electrons are generated at the top of the column by the electron source. These are emitted when their thermal energy overcomes the work function of the source material. They are then accelerated and attracted by the positively-charged anode. The entire electron column needs to be under vacuum. Like all the components of an electron microscope, the electron source is sealed inside a special chamber in order to preserve vacuum and protect it against contamination, vibrations or noise. Although the vacuum protects the electron source from being contaminated, it also allows the user to acquire a high-resolution image. In the absence of vacuum, other atoms and molecules can be present in the column. Their interaction with electrons causes the electron beam to deflect and reduces the image quality. Furthermore, high vacuum increases the collection efficiency of electrons by the detectors that are in the column [51].

(c) EDS(Energy Dispersion X-ray Spectrometer):

Energy Dispersive X-Ray Spectroscopy (EDS or EDX) is a chemical microanalysis technique used in conjunction with scanning electron microscopy (SEM).The EDS technique detects x-rays emitted from the sample during bombardment by an electron beam to characterize the elemental composition of the analyzed volume. Features or phases as small as 1 μm or less can be analyzed. When the sample is bombarded by the SEM's electron beam, electrons are ejected from the atoms comprising the sample's surface. The resulting electron vacancies are filled by electrons from a higher state, and an x-ray is emitted to balance the energy difference between the two electrons' states. The x-ray energy is characteristic of the element from which it was emitted. The EDS x-ray detector measures the relative abundance of emitted x-rays versus their energy. The detector is typically a lithium-drifted silicon, solid-state device. When an incident x-ray strikes the detector, it creates a charge pulse that is proportional to the energy of the x-ray. The charge pulse is converted to a voltage pulse (which remains proportional to the x-ray energy) by a charge-sensitive preamplifier. The signal is then sent to a

multichannel analyzer where the pulses are sorted by voltage. The energy, as determined from the voltage measurement, for each incident x-ray is sent to a computer for display and further data evaluation. The spectrum of x-ray energy versus counts is evaluated to determine the elemental composition of the sampled volume [52].

2.6 Aloe barbadensis

The leaves of ***Aloe barbadensis*** are succulent, erect, and form a dense rosette. Many uses are made of the gel obtained from the plant's leaves.

Botanical Name	*Aloe barbadensis*	
Common Name	Gwarpatha or Aloe vera	
Classification	**Kingdom:**	Plantae
	Subkingdom:	Tracheobionta
	Division:	Magnoliophyta
	Class:	Liliopsida
	Subclass:	Liliidae
	Order:	Liliales
	Family:	Aloeaceae
	Genus:	Aloe
	Species:	barbadensis
Part used	Fresh leaves and leaf-juice	
Medicinal Properties	Bitter, sweet, cooling, anthelmintic, carminative, diuretic, stomachic, depurative.	
Medicinal Use	The leaf-juice is used in dyspepsia, amenorrhoea, burns, colic, hyperadenosis, hepatopathy and skin diseases. Also used for abdominal tumours, dropsy, helminthiasis.	

Chemistry	Leaves: barbaloin, chrysophanol glycoside and aglycone, aloe-emodin, aloesone and aloesin.
Cultivation	Cultivated in drier areas.
Regional Habitat	Wildly found in drier part of Rajasthan.
Description	A perennial herb with short stem and shallow root system. Leaves: fleshy in rosettes, sessile, very much horny prickles on the margin, convex below, surface pale green; flowers: yellow or orange in racemes; fruits: loculicidal capsule.

Figure 11: Showing the leaves of the *Aloe barbadensis*

2.6.1 Uses and health benfits: [53]

The medicinal claims made about Aloe vera, as with many herbs and plants, are endless. Some are backed by rigorous scientific studies while others are not. This article focuses mainly on those that are backed by research.

1. Teeth and gums

A study published in General dentistry reported that Aloe vera in tooth gels is as effective as toothpaste in fighting cavities.

The researchers compared the germ-fighting ability of an Aloe vera tooth gel with two popular toothpastes. They found that the gel was just as good, and in some cases even

better than the commercial toothpastes at controlling cavity-causing oral bacteria.

The authors explain that Aloe latex contains anthraquinones, compounds that actively heal and reduce pain through natural anti-inflammatory effects.

The scientists warned that not all gels they analyzed contained the proper form of Aloe vera - they must contain the stabilized gel that exists in the centre of the plant to be effective.

2. Constipation

Germany's regulatory agency for herbs - Commission E - approved the use of Aloe vera for the treatment of constipation. Dosages of 50-200 milligrams of Aloe latex are commonly taken in liquid or capsule form once daily for up to 10 days.

The U.S. Food and Drug Administration (FDA) ruled in 2002 that there is not enough data on the safety and efficacy of Aloe products; so, in the U.S., they cannot be sold to treat constipation.

3. Diabetes-induced foot ulcers

A study carried out at the Sinhgad College of Pharmacy, India, and published in the International Wound journal looked at Aloe's ability to treat ulcers.

They reported that a "gel formed with carbopol 974p (1 percent) and Aloe vera promotes significant wound healing and closure in diabetic rats compared with the commercial product and provides a promising product to be used in diabetes-induced foot ulcers."

4. Antioxidant and possible antimicrobial properties

Aloe vera may be used on skin conditions or superficial cuts for its antimicrobial and antioxidant properties.

Researchers at the University of Las Palmas de Gran Canaria, Spain, published a study in the journal Molecules.

The team set out to determine whether the methanol extract of leaf skins and flowers of Aloe vera might have beneficial effects on human health. The scientists focused on the extract's possible antioxidant and antimycoplasmic activities.

Mycoplasma is a type of bacteria that lack a cell wall; they are unaffected by many common antibiotics. Antimycoplasmic substances destroy these bacteria.

They reported that both Aloe vera flower and leaf extracts had antioxidant properties, especially the leaf skin extract. The leaf skin extract also exhibited antimycoplasmic properties.

The authors concluded that "A. Vera extracts from leaf skin and flowers can be considered as good natural antioxidant sources."

5. Protection from ultraviolet (UV) irradiation

Scientists at Kyung Hee University Global Campus, South Korea, wanted to determine whether baby Aloe shoot extract and adult Aloe shoot extract might have a protective effect on UVB-induced skin photoaging; in other words, whether they could protect the skin from the aging effects of sunlight.

Baby Aloe shoot extract (BAE) comes from 1-month old shoots while adult Aloe shoot extract (AE) comes from 4-month old shoots.

In an article published in Phototherapy Research, the authors concluded: "Our results suggest that BAE may potentially protect the skin from UVB-induced damage more than AE."

6. Protection from skin damage after radiation therapy

A study carried out at the University of Naples, Italy, tested five different topical creams to see how effective they might be in protecting the skin of breast cancer patients receiving radiation therapy. One of these creams contained Aloe.

They divided 100 patients into five groups of 20; each was prescribed a different topical treatment. They applied the creams twice daily, starting 15 days before radiation therapy treatment, and carried on for 1 month afterward.

During the 6-week period, the participants underwent weekly skin assessments. In the journal Radiation Oncology, the scientists reported that the preventive use of the topical hydrating creams reduced the incidence of skin side effects in the women treated with radiation therapy for breast cancer, none performed significantly better.

"All moisturizing creams used in this study were equally valid in the treatment of skin damage induced by radiotherapy."

7. Depression, learning, and memory - an animal experiment

Nutritional Neuroscience found that Aloe vera reduced depression and improved

memory in mice.

After carrying out experiments on laboratory mice, they concluded: "Aloe vera enhances learning and memory, and also alleviates depression in mice."

Further studies are needed to establish whether humans might also receive the same benefits.

8. Wounds from second-degree burns

A team of plastic surgeons compared Aloe vera gel to 1 percent silver sulphadiazine cream for the treatment of second-degree burn wounds.

They reported in the Journal of Pakistan Medical Association that the burn wounds among the patients treated with Aloe vera healed significantly quicker compared with those treated with 1 percent silver sulfadiazine (SSD).

The researchers added that those in the Aloe vera group experienced significantly more and earlier pain relief than those in the SSD group.

The authors wrote: "Thermal burns patients dressed with Aloe vera gel showed advantage compared to those dressed with SSD regarding early wound epithelialization, earlier pain relief, and cost-effectiveness."

9. Irritable bowel syndrome (IBS)

A randomized, double-blind human trial carried out at St. George's Hospital Medical School, London, United Kingdom investigated Aloe and IBS. Their results were published in the International Journal of Clinical Practice. Participants with IBS were given either Aloe vera or a placebo. After 3 months, there were no significant differences in symptoms of diarrhea.

However, "There was no evidence that AV [Aloe vera] benefits patients with IBS. However, we could not rule out the possibility that improvement occurred in patients with diarrhea or alternating IBS whilst taking AV. Further investigations are warranted in patients with diarrhea predominant IBS, in a less complex group of patients."

2.7 Evaluation of Anti-microbial activity:

The resistance of microorganisms to the action of antimicrobial agents, especially antibiotics, is a serious public health problem, which has been a reason for the search and development of new antimicrobials through nanotechnology. The manipulation on a

nanoscale of metal oxide has provided new research in the pharmaceutical area due to the antimicrobial properties of these oxides, according to data revealed in in vitro studies. In this sense, the metal oxide NPs between 1 and 100 nm with different shapes allow their physical and chemical properties could become in some promise antimicrobial agents against infectious diseases for the recent findings about their interaction which has become of vital importance due to the increasing of infection diseases by bacterial resistance[54] .

Evaluation of antimicrobial is done by various methods including diffusion methods i.e. Agar well diffusion method and disc methods, minimum inhibitory concentration method in this study turbidimetric analysis method is used [55].

2.7.1 Turbidimetric analysis method

A scattered-light procedure for the determination of the weight concentration of particles in cloudy, dull, or muddysolutions; uses a device that measures the loss in intensity of a light beam as it passes through the solution. Also known as turbidimetry [56].

Turbidimetry (the name being derived from t*urbidity*) is the process of measuring the loss of intensity of transmitted light due to the scattrring effect of particles suspended in it. Light is passed through a filter creating a light of known wavelength which is then passed through a cuvette containing a solution. A phtoelectric cell collects the light which passes through the cuvette. A measurement is then given for the amount of absorbed light [57].

Turbidimetry can be used in biology to find the number of cells in a solution.

CHAPTER-3

MATERIALS AND METHODOLOGY

All the chemicals in this present study were analytical grade and pure.

Biosynthesis of copper Nanoparticles from *Aloe barbadensis* using triple distilled water as solvent

3.1 Plant Material:

Aloe barbadensis sample for the biosynthesis of copper nanoparticles were collected from the fields of Punjab Agriculture University Ludhiana.

Figure 12: Showing the plant of *Aloe barbadensis*

3.2 Preparation of $CuSO_4.5H_2O$ stock solution:

For each time experiment set, fresh $CuSO_4.5H_2O$ solution was prepared. 1mM $CuSO_4.5H_2O$ (MW 249.68) 0.249 gm was dissolved in 1000ml of triple distilled water resulting in 1000 ml $CuSO_4.5H_2O$ solution.

3.3 Preparation of *Aloe barbadensis* raw Extract:

Aloe barbadensis plants were taken and thoroughly washed with running tap water and distilled water. *Aloe barbadensis* leaves were peeled, used to make extract. Peeled leaves were allowed to dry at room temperature until the moisture of the leaves get dried .Dried leaves were crushed to make fine powder. 25 gm of powder was mixed with 5 times in

triple distilled water to get final volume of about 125ml and then was subjected to Cold Percolation process. In Cold Percolation method, mix of powder of Aloe Vera and triple distilled water was placed on rotatory shaker for 24 hours at room temperature (10^0 C). After 24 hours mixture was kept undisturbed for 10 minutes. Sample was filtered with Whatman™ 1001-125 Grade1 Qualitative filter paper, to obtain filtrate and was used as raw extract for the synthesis of copper nanoparticles.

Figure 13: Showing (left) unfiltered raw extract (right) filtered raw extract after 24 hour Cold percolation

3.4 Conditions for synthesis of Copper nanoparticles:

For each experimental set up, 2.5 ml of raw extract was augmented with 50 ml of $CuSO_4.5H_2O$ solution and maintained with pH 9 at 100^0 C. Optical density of resultant

was recorded after regular interval of 1 hour using Colorimeter. Change in colour was observed as preliminary observation for synthesis of copper nanoparticles

3.5 Confirmatory Analysis

Copper nanoparticles so obtained from raw extract of Aloe barbadensis leaf powder then were subjected to TEM (Transmission electron microscope) for confirmatory analysis, SEM (Scanning electron microscope) for surface features, shape, size and structure EDS (Energy Dispersive X-Ray Spectrometer) technique detects x-rays emitted from the sample during bombardment by an electron beam to characterize the elemental composition of the analyzed volume of copper nanoparticles prepared.

- **3.5.1 Characterization of Transmission electron microscope (TEM):**

Preparation of sample (peeled leaf) using 70% ethanol as a solvent:

- Sample at pH-9 with maximum optical density at 540nm was subjected at temperature 100^0 C and was selected for the characterization of synthesized nanoparticles.
- The synthesized nanoparticle at pH-9 at temperature 100°C was centrifuged at 18,000 rpm for 15 minutes.
- Clear pellet was observed after centrifugation of sample.
- Washing of the pellet was done with triple distilled water and process was repeated 3-4 times.
- The pellet so obtained was preserved in solvent used in the sample.

- **Transmission electron microscopy (TEM):**

It is a microscopy technique whereby a beam of electrons transmitted through a sample interacting with the copper nanoparticles as it passes through. An image is formed from the interaction of electrons transmitted through the sample; the image is magnified and focuses onto an imaging device such as fluorescent screen, on a layer photographic film, or to be detected by sensor such as a CCD camera.

Procedure of Transmission electron microscopy (TEM) analysis:

- At the time of characterization, pellet dissolved in a particular solvent was again

centrifuged then again dissolved in 70% ethanol.

- Aliquots of nanoparticles dissolved in solvent used were placed on a carbon coated copper grid and allow to dry under ambient conditions for 30 minutes.
- Then this carbon coated grid of nanoparticles are placed inside a partly evacuated chamber connected to power supply.
- Preparing TEM samples achieved the dilution ratio of nanoparticles to achieve a monolayer of nanoparticles visible on the sample grid when viewing in the microscope.
- Nanoparticles were identified at areas of higher particle density to be viewed as images in order to collect more information possible from each image.

Figure 14: Showing Instrumentation of Transmission Electron Microscopy

3.5.2 Characterization of Scanning electron microscope (SEM):

Scanning electron microscopy is a technique is used to produces images of sample by scanning the surface with a focused beam of electrons. The electron beam is scanned in a raster scan pattern, and the position of the beam is combined with the intensity of the detected signal to produce an image. Specimens are observed in high vacuum in conventional SEM, or in low vacuum or wet conditions in variable pressure or environmental SEM.

Figure 15: Showing Instrumentation of Scanning Electron Microscopy

Sample Preparation:

Sample preparation can be minimal or elaborate for SEM analysis, depending on the nature of the samples and the data required. Minimal preparation includes acquisition of a sample that will fit into the SEM chamber and some accommodation to prevent charge build-up on electrically insulating samples. Most electrically insulating samples are coated with a thin layer of conducting material, commonly carbon, gold, or some other metal or alloy. The choice of material for conductive coatings depends on the data to be acquired: carbon is most desirable if elemental analysis is a priority, while metal coatings are most effective for high resolution electron imaging applications. Alternatively, an electrically insulating sample can be examined without a conductive coating in an

instrument capable of "low vacuum" operation.

Procedure of Scanning electron microscope (SEM):

- At the time of characterization, sample should be in powder form or in dry form. If the sample is solvent, place the drops of solvent on the glass slide.
- Allow it to dry at room temperature until sample will get completely in dry state.
- With the help of ion sputter sample is coated with gold.
- Gold coated sample is placed in the vaccum sample chamber of Scanning Electron Microscope.
- After the 5 minutes, electron beam was turned on and sample is observed on the monitor.
- Nanoparticles were identified at areas of higher particle density.

3.5.3 Characterization of Energy Dispersion X-Ray Spectrometer (EDS):

Energy Dispersive X-Ray Spectrometer (EDS):

Energy Dispersive X-Ray Spectroscopy (EDS, EDX, EDXS or XEDS), sometimes called Energy Dispersive X-Ray Analysis (EDXA) or Energy Dispersive X-Ray Microanalysis (EDXMA), is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on an interaction of some source of X-Ray excitation and a sample. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing a unique set of peaks on its electromagnetic emission spectrum and shows which type of nanoparticles are synthesized.

Procedure of Energy Dispersive X-Ray Spectrometer (EDS):

- At the time of characterization, pellet dissolved in a particular solvent was again centrifuged and dissolved in 70% ethanol.
- Aliquots of nanoparticles dissolved in solvent used were placed on a carbon coated grid and were allowed to dry under ambient conditions for approximately 30 minutes.
- Then this carbon coated grid of nanoparticles was placed inside a partly

evacuated chamber connected to power supply.

- High electron beam bombarded the sample placed on a grid, depending on the amount of energy absorbed by a sample. In TEM, and EDS detector is usually located at an angle of 10^0-20^0 with regard to sample.
- The sample was surrounded by the furnance without any direct line of sight from the sample to the EDS detector.
- Then the EDS spectrum was shown on TEM's peripheral monitor.

3.6 Identification of micro-organisms from different skin types:

Microbial swab sample was taken from 5 different skin types:

(a) Combination skin

(b) Oily skin

(c) Scalp

(d) Acne skin

(e) Sensitive skin

Skin flora present on different skin types was identified using different identification method:

3.6.1 Staining

3.6.2 Differential Media

3.6.3 Biochemical Test

3.6.1 Staining

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. Two types of staining methods were used to identify microbes.

3.6.1.1 Gram Staining

3.6.1.2 Endospore Staining

3.6.1.1 Gram Staining:

Gram Staining is also known as Gram's Method. This method is used to identify

bacterial species. Gram staining is used to differentiate between gram positive and gram negative bacteria on the basis of differential staining with crystal violet, and a safrain counterstain. Name of this method comes from “Hans Christian Gram” because he developed this technique. He discovered gram staining technique in 1884. According to this method bacteria that retain crystal violet stain are said to be “gram positive” and those retain red stain are said to be “gram negative” . The staining response is based on the chemical and structural make up of cell wall of both these type of bacteria. According to Hans Christian Gram, gram positive bacteria have a mesh like thick cell wall made up of peptidoglycan, which stain purple because of high content of peptidoglycan. Also, gram positive bacteria have a additional outer mambrane which contains lipids and periplasmic space is present between lipid content and cell wall. Whereas, gram negative bacteria have thinner layer of peptidoglycan which stains red and pink.

Reagents used for Gram staining are:

- Crystal violet (primary stain)
- Iodine solution/Gram's Iodine (mordant that fixes crystal violet to cell wall)
- Decolorizer (e.g. ethanol)
- Safranin (secondary stain)
- Water (preferably in a squirt bottle)

Procedure:

- Firstly, sterile the glass slide with ethanol and dry it near spirit lamp.
- Preparation of smear- with a sterile cooled loop, place a drop of sterile water on the slide. Sterile and cool the loop again and pick up very small amount of microbial colony from petriplate and gently stir into the drop of water on the slide to create emulsion. Now with the help of another glass slide prepare a smear of the sample.
- Heat fixing- allow the slide to air dry. After smear have air dried, hold the slide at one end and pass the entire slide through the flame of Bunsen burner two or three times. Now, the smear is ready to be stained.
- Place the heat fixing slide over the staining tray. Gently, flood smear with crystal

violet dye and let it stand for 1 minute.

- Tilt the slide slightly and gently rinse with distilled water.
- Gently flood the smear with safranin and let in stand for 45 seconds.
- Let the slide to air dry.
- View the slide under light microscope.
- Observe bacteria from their colour.

Gram-Positive Bacteria

Cocci- Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Micrococcus.

Rods - Corynebacterium, Propionibacterium, Brevibacterium, Dermabacter hominis, Diphtheroids

Gram-Negative Bacteria

Rods- *Acinetobacter, Escherichia coli, Pseudomonas aeruginosa, Bacteroidetyes*

3.6.1.2 Endospore staining

Endospore staining is used to recognize the presence of spore in bacterial vegetative cells. The bacterial endospores need staining which can penetrate wal thickness of spore bacteria. There are two methods used In Schaeffer Fulton method, malachite green is alkaline substance used to stain endospores of bacteria. Whereas, in Klein method, methylene blue dye are used.

Reagents used: -malachite green.

Procedure:

- Firstly, sterile the slide properly with ethanol.
- Then, prepare a smear of microbial culture and let it stand for air drying.
- Now, add malachite green dye over the smear.
- After 2 minutes, wash slide with distilled water to remove extra stain from slide.
- After drying the slide, visualize it under light microscope.

Endospore forming bacteria are *Bacillus and Clostridium*

Non-spore forming bacteria are *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Micrococcus, Diphtheroids, Corynebacterium, Propionibacterium, Brevibacterium, Dermabacter hominis, Acinetobacter, Escherichia coli, Pseudomonas aeruginosa, Bacteroidetyes.*

3.6.2 Differential Media

Differential media distinguish one microbe from another growing on the same media. This type of media uses biochemical characteristics of microorganism growing in the presence of specific nutrients or indicators. This method is used by biologists to detect recombinant strains of bacteria. Types of differential media are:

3.6.2.1 MacConky Agar Media

3.6.2.2 EMB (Eosin methylene blue)

3.6.2.1 MacConkey agar

MacConkey agar was the first solid differential media to be formulated. MacConkey agar is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. The inclusion of crystal violet and bile salts in the media prevent of growth of Gram positive bacteria and fastidious Gram-negative bacteria. It has also become common to use the media to differentiate bacteria by their abilities to ferment sugars other than lactose.

Composition of MacConkey agar media is given below

Table 1: Composition of MacConkey agar media

Components	Amount(g/L)
Peptone	17g
Polypeptone	3g
Lactose	10g
Sodium chloride	5g
Crystal violet	1mg
Neutral red	30mg
Bile salts	1.5g
Agar	13.5g

Procedure:

- Firstly, pour MacConkey agar in Petri-plates and let it solidify.
- Streak the plates of MacConkey agar with the desired pure culture.
- Incubate the plates in for 24 hours at 37 C Temperatures.
- Observe the plates.

Microbes that can grow on MacConkey agar media are: -*Campylobacter, Escherichia coli, Streptococcus and Staphylococcus.*

3.6.2.2 EMB

EMB (Eosin-Methylene blue) is a differential media used to bacterial

Colonies. The mechanism involved in the action of EMB in differentiating bacterial species was investigated using prototrophic strains of micro-organisms. The final pH of EMB agar resulting from growth of microbes at the expense of lactose was shown to play a critical role in the differential action. An EMB complex, which formed under acidic conditions, appeared to be involved in the differential action of the medium.

Composition of EMB agar is given below:

Table 2: Composition of EMB agar media

Components	Amount (g/L)
Peptone	10g
Lactose	10g
Dipotassium hydrogen phosphate	2g
Eosin Y	0.4g
Methylene blue	0.065g
Agar	15g
pH	6.8

Procedure:

- Pour prepared EMB agar media in petri-plates and let them solidify.
- Now, streak the sample on EMB media plate.
- Incubate tubes in incubatoe for 24 hours at 37^0C temperature.
- After 24 hours of incubation observe microorganism present in the sample of skin flora.

Bacterial species grow on EMB are- *Escherichia coli*

3.6.3 Biochemical Test

Biochemical tests are used for the identification of bacterial species based on the difference in the biochemical activities of different bacteria. Bacterial physiology differs from one species to another. Various biochemical tests are:

3.6.3.1 Potassiumhydroxide Test

3.6.3.2 Glucose and Gas Fermenting Test

3.6.3.3 Lactose Fermenting Test

3.6.3.4 Sucrose Fermenting Test

3.6.3.5 Catalse Test

3.6.3.6 Oxidase Test

3.6.3.7 Motility Test

3.6.3.8 Urease Test

3.6.3.9 Methyl Red Test

3.6.3.1 Potassium hydroxide Test

It is a non-staining method used for the determination of Gram positive and Gram negative bacteria. According to this test, if microbial and KOH suspension becomes viscous or thick within 1 minute, then isolate bacteria is Gram negative. If suspension is not thick or viscous, then isolated bacteria are Gram positive.

Procedure:

- Collect microbial sample from petriplate or broth culture with the help of inoculum loop.

- Place the sample on sterile glass slide.
- Now, add 10% or 20% potassium hydroxide over the glass slide.
- The slide is left to stand for 5 to 15 minutes.
- Observe the slide for viscosity.

If suspension becomes thick, microbial species are Gram negative. If suspension does not become viscous, microbial species are Gram positive.

3.6.3.2 Glucose and Gas Fermenting Test

This test is used to determine that microbes can utilizes carbohydrates such as glucose as a carbon source or not. It test for the presence of acid or gas produced from carbohydrates fermentation. Phenol red glucose broth media is used for glucose fermenting test.

Table 3: Composition of Phenol red glucose broth

Components	Amount(g/L)
Peptone	10g
Beef Extract	1g
Sodium chloride	5g
Phenol Red	0.08g
Glucose	5g
pH	7.2

Procedure:

- Firstly, inoculum from a pure culture is transferred aseptically to a sterile tube of phenol red lactose broth.
- The inoculated tubes were incubated at 37^0 C temperatures for 24 hours.
- After 24 hours observe it for colour change.

Glucose fermenting bacteria are: *Neisseria, Proteus vulgaris, Proteus mirabilis, Cornebacterium.*

3.6.3.3 Lactose Fermenting Test

The purpose of lactose fermenting test is to see if the microbes can ferment the lactose as the carbon source. If the lactose is fermented to produce acid end product, thr pH of medium will drop. The pH indicator in media changes colour to indicate acid production. Phenol red lactose broth is most commonly used media for lactose fermenting test.

Table 4: Composition of phenol red lactose broth

Components	Amount(g/L)
Peptone	10g
Beef Extract	1g
Sodium chloride	5g
Phenol Red	0.018g
Lactose	5g
pH	7.4

Procedure:

- Firstly, inoculum from a pure culture is transferred aseptically to a sterile tube of phenol red lactose broth.
- The inoculated tubes were incubated at 37^0 C temperatures for 24 hours.
- After 24 hours observe it for colour change.

Lactose fermenting bacteria are: *E.coli, Shigella sonnei, Klebsiella pneumo, serratia.*

3.6.3.4 Sucrose Fermenting Test

Sucrose Fermenting Test is used to identify the microbes that can ferment the carbohydrates sucrose as carbon source. If sucrose is fermented, pH of media will decline. pH indicator change colour to indicate acid production. Phenol red sucrose broth

is used for sucrose fermenting test.

Table 5: Composition of phenol red sucrose broth

Components	Amount(g/L)
Casein peptone	10g
Sucrose	5g
Sodium chloride	5g
Phenol red	0.018g

Final pH of media is 7.4 at 25^0 C temperatures.

Procedure:

- Pour the media into test tubes.
- With the help of sterile inoculum loop pick up an inoculum from the culture tube.
- Immediately transfer the inoculum into fresh sterile media.
- After inoculation, place the test tube into incubator for 24 hours at 37^0 C temperature.
- Observe test results, culture will change to yellow in presence of acid and pink in the presence of bases.

Sucrose fermenting bacteria- *E.Coli, Enterobacter.*

3.6.3.5 Catalse Test

The catalase test facilitates the detection of enzyme catalase. The catalase is a heme containing enzyme whose role is to neutralize bactericidal effect of hydrogen peroxide Catalase expedites the breakdown of hydrogen peroxide enzyme into water and oxygen. And, this reaction is recognized by formation of bubbles.

Reagent: 3% hydrogen peroxide.

Procedure:

- Pick the inoculum from a cultured plate and place it on a sterile glass slide.

- Add one drop of hydrogen peroxide and look for immediate bubbling.

Catalse Positive species are *Staphylococcus*

3.6.3.6 Oxidase Test

The oxidase test was performed with the help of Kovac's reagent. The oxidation reaction is based on the ability of some microbes to produced indophenol by the oxidation of dimethyl-p-phenylenediamine and α-naphthol.

Reagent: Kovac's reagent.

Procedure:

- Firstly, cut small piece of Whatman filter paper and put it on a sterile glass slide.
- Use sterile loop to transfer microbial culture to Whatman filter paper.
- Observe it for almost three minutes, if the area of inoculation turned purple or maroon. Then, the result is positive. If colour change does not occur within three minutes, then, result is negative.

Oxidase positive bacteria- *Pseudomonas, Neisseria, Vibrio, Camphylobacter, Aeromonas, Alcaligenes, Brucella, Pasturella, Eikenella, Kingella, Moraxella, Legionella, Helicobacter, Chromobacter.*

3.6.3.7 Motility Test:

Motility Test is used to differentiate bacteria on the basis of motile and non-motile. The motility of bacterial species is measured in test tube by determining the distribution of bacteria throughout the tube. In semisolid agar media motile bacteria swarm and give a diffuse spreading growth that can be easily recognized by naked eyes.

Procedure:

- Prepare a semi solid agar media in test tubes.
- Inoculate the microbial sample with the help of inoculum loop at the centre of the tube.
- Incubate the tubes in incubator at 37^0C temperature for 24 hours.
- To observe motility of microbes, hold the tube up to the light and look at the stab line.

- Non-motile bacteria generally grow near the stab line, whereas, motile bacteria give diffused growth throughout the media.

Non-motile bacteria: *E.faecalis, Enterococcus faecium.*

3.6.3.8 Urease Test:

The urease test used to identify the microbes that are capable of hydrolysing urea to produce ammonia and carbondioxide. Urease media is required to perform urease test.

Table 6: Composition of urease broth media

Components	Amount(g/L)
Urea	20g
Na_2HPO_4	9.5g
KH_2PO_4	9.1g
Yeast Extract	0.1g
Phenol red	0.1g
pH	6.8

Procedure:

- Sterile the inoculation loop, and then take out the micro-organisms from tryptic broth culture tube.
- Then, take sterile urea broth tube, remove cotton plug and sterile the neck of the tube.
- Inoculate the urea broth with the inoculation loop containing the organism from the tryptic broth culture.
- Again flame the neck of the test tube for sterilization, cover with cotton plug and place it in the test tube rack.
- Incubate the tubes in incubator at 37^0C temperature for 24 to 48 hours.
- After incubation observe the broth for colour change.

- If colour turns pink, microbial species are urease positive, if colour does not change, then the species are urease negative.

Urease positive Organisms: *Proteus, Cryptococcus, Corynebacterium, Helicobacter pylori, Yersinia, Brucella etc.*

3.6.3.9 Methyl red test:

The methyl red test is used to detect the production of acid during the fermentation of glucose. According to this test, microbes initially metabolise glucose to pyruvic acid and then produce the stable acid. This type of acid produced varies from species to species depends upon the specific pathway present in microbes. The acids decrease the pH to 4.5 or below and hence color of methyl red changes from yellow to red.

Table 7: Composition of methyl red broth

Components	Amount(g/L)
Buffered Peptone	7g
Glucose	5g
Dipotassium Phosphate	5g
pH	6.9

Reagent used: Methyl red.

Procedure:

- Before inoculation, allow to equilibrate to room temperature.
- Take microbial culture with the help of inoculum loop and lightly inoculate the media.
- After 24 hours incubation, pour 1ml of broth to a clean test tube.
- Reincubate the broth for addition 24 hours.
- After 24 hours, add 2-3 drops of the methyl red indicator to the tube.
- Observe the red colour immediately.

Methyl red positive bacteria: *E. coli, Yersinia* species present.

3.7 Anti-microbial Test:

Antimicrobial susceptibility tests are used to determine which specific antibiotics a particular bacteria or fungus is sensitive. Most often, this testing complements a Gram stain and culture, the results of which are obtained much sooner. Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. Microbial and plant products occupy the major part of the antimicrobial compounds discovered. The most known and basic methods are the disk-diffusion and broth or agar dilution methods. The method used for analysis is Turbidimetric analysis.

3.7.1 Turbidimetric analysis:

Turbidimetry or Turbidimetric analysis is the process of measuring the loss of intensity of transmitted light due to the scattering effect of particles suspended in it. Light is passed through a filter creating a light of known wavelength which is then passed through a cuvette containing a solution. A photoelectric cell collects the light which passes through the cuvette. Scattering of the incident light leads to increased extinction of the transmitted light and is recorded by the photodetector. A measurement is then given for the amount of absorbed light.

To follow this method, fresh sterile nutrient broth media is prepared. Bacterial culture is revived in peptone water and incubated at 37^0 C for 24 hours. **Amoxycillin** is used as antibiotic to observe the antimicrobial effect on the bacteria. Then synthesised copper nanoparticles (at pH-9, and 100^0 C) were used to determine the effect on bacteria.

Table 8: Composition of Nutrient Broth Media

Components	Amount(g/L)
Peptone	5g
Beef Extract	3g
Sodium Chloride	5g
pH	6.8

Autoclaved at 121^0 C for 15 minutes.

Dilution of Amoxycillin

To make the dilution of amoxycillin, 1g of amoxycillin (powdered form) is diluted in 100 ml (i.e. 10%) of distilled water and mix it thoroughly.

***** Do not heat the mixture it may cause the denaturation. Because amoxycillin is heat sensitive compound.

Procedure of studying antimicrobial effect of nanoparticles using Turbidimetric analysis:

- Before inoculation, allow to equilibrate to room temperature, make sure the temperature is not too hot. The media container should be comfortable to the touch.
- 10ml of media is taken and poured in the vial. Vial should be steriled by ethanol wash or autoclaving. Otherwise it may effect the results.
- Take 2.5 ml of bacterial culture with the help of micropipette and poured in the cool media.
- Take 2.5 ml of antibiotic mixture or synthesised nanoparticles and add it into the media.
- In one vial add media and antibiotic or nanoparticles. Do not add bacterial culture. Mark it as blank.
- Observe the initial turbidity by calculating optical density (O.D) on colorimeter.
- After 24 hour incubation at 37^0 C, observe the final turbidity on colorimeter.

If turbidity or optical density will increase or remain same then no antimicrobial effect occurs. If turbidity or optical density will decrease then antimicrobial effect occurs.

CHAPTER-4

RESULTS AND DISCUSSION

4. To study the p**lant mediated biological synthesis of copper nanoparticles from *Aloe barbadensis*:**

4.1 PRELIMINARY TEST

4.1.1 Preparation of Aloe barbadensis raw Extract

Aloe barbadensis leaves were peeled and allowed to dry at room temperature until the moisture of the leaves get dried .Dried leaves were crushed to make fine powder. 25 gm of powder was mixed with about 125ml of triple distilled water and then was subjected to Cold Percolation process. In Cold Percolation method, powder of *Aloe barbadensis* and triple distilled water was placed on rotatory shaker for 24 hours at room temperature (10^0 C). After 24 hours mixture was kept undisturbed for 10 minutes. Sample was filtered with Whatman™ 1001-125 Grade1 Qualitative filter paper, to obtain filtrate and was used as raw extract for the synthesis of copper nanoparticles.

Figure 16: Showing raw extract of *Aloe barbadensis* after Cold Percolation

4.1.2 Conditions for the biosynthesis of copper nanoparticles using peeled leaf (only leaf powder) of *Aloe barbadensis*:

2.5 ml raw extract was augmented with 50ml of $CuSO_4.5H_2O$ solution. This reaction mixture was subjected to pH-9 and temperature 100^0 C. The optical density of sample at 540nm on regular interval of 1 hour was recorded using Colorimeter. Change in colour i.e. light green to dark green was observed as preliminary observation.

Figure 17: Showing the change in colour from light green to dark green

Table 9: Observation of optical density (O.D.) at pH-9 and temperature 100^0 C after 1 hour interval at 540nm using colorimeter

Time	Optical density at 540nm			
	1st attempt	2nd attempt	3rd attempt	Mean
initial	1.28	1.32	1.27	1.29
After 1 hour	1.60	1.41	1.28	1.43
After 2 hours	1.75	1.80	1.29	1.61
After 3 hours	1.88	1.80	1.31	1.66

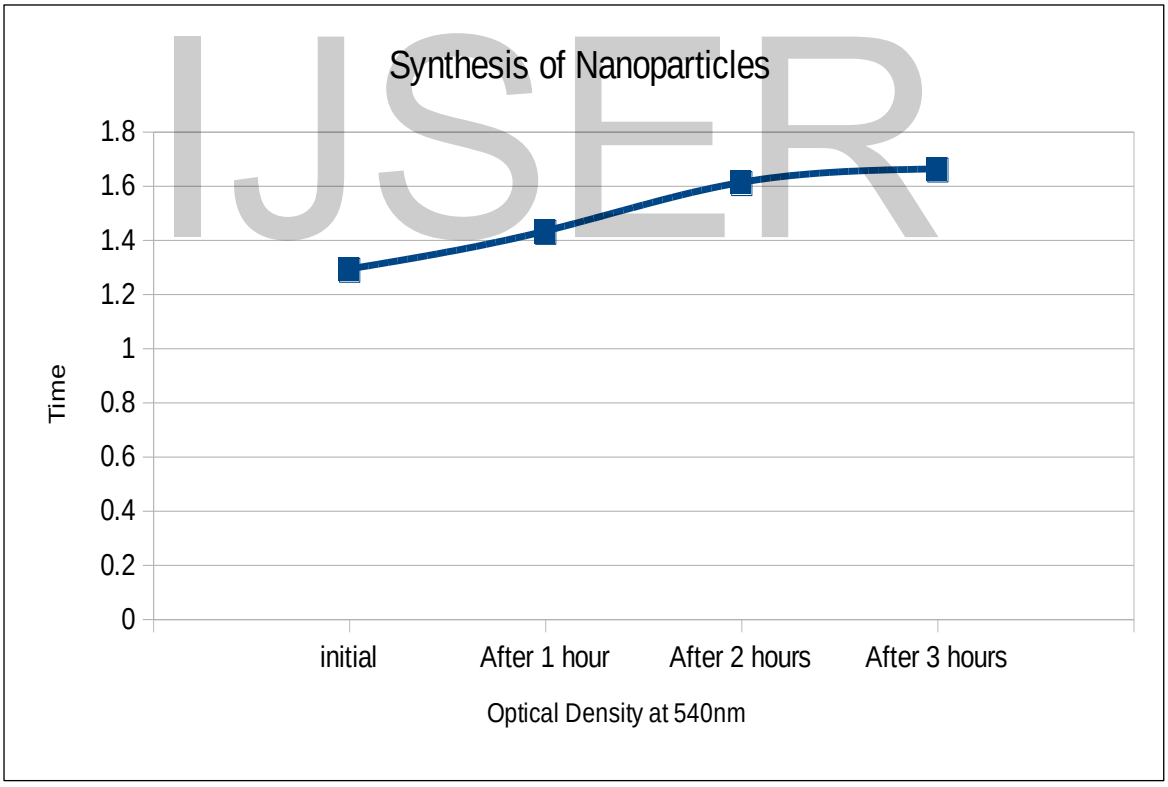

Figure 18: Showing the graph of increase in optical density of synthesized copper nanoparticles at pH-9 and temperature 100^0 C after 1 hour interval

4.2 Confirmatory Analysis

4.2.1 CHARACTERIZATION OF COPPER NANOPARTICLES USING TRANSMISSION ELECTRON MICROSCOPE (TEM)

Raw extract of *Aloe barbadensis* is made by using triple distilled water by cold percolation method. optical density is observed with pH-9 at temperature 100^0 C at 540nm using Colorimeter in plant material *Aloe barbadensis* peeled leaf for confirmation of presence of copper (Cu) as a true metal ion.

70% Ethanol as a solvent for sample preparation for TEM (Transmission Electron Microscopy).

TRANSMISSION ELECTRON MICROSCOPE (TEM) ANALYSIS:

For the confirmatory analysis, characterisation of synthesized copper nanoparticles from Aloe *barbadensis* was performed using TEM (transmission electron microscope). The shape and size on high resolution of the resultant nanoparticles in plant material Aloe *barbadensis* peel were elucidated with the help of Transmission elctron microscopy. Aliquots of nanoparticles solution were placed on a carbon coated copper grid and allowed to dry under ambient conditions and washing of the sample is given by triple distilled water and TEM images were recorded which confirms the synthesis of nanoparticles in the plant extract . TEM micrographs showed that nanoparticles produced are mostly spherical in shape and size ranging from 27nm to 35nm. The Copper nanoparticles so formed were shown in the figure:

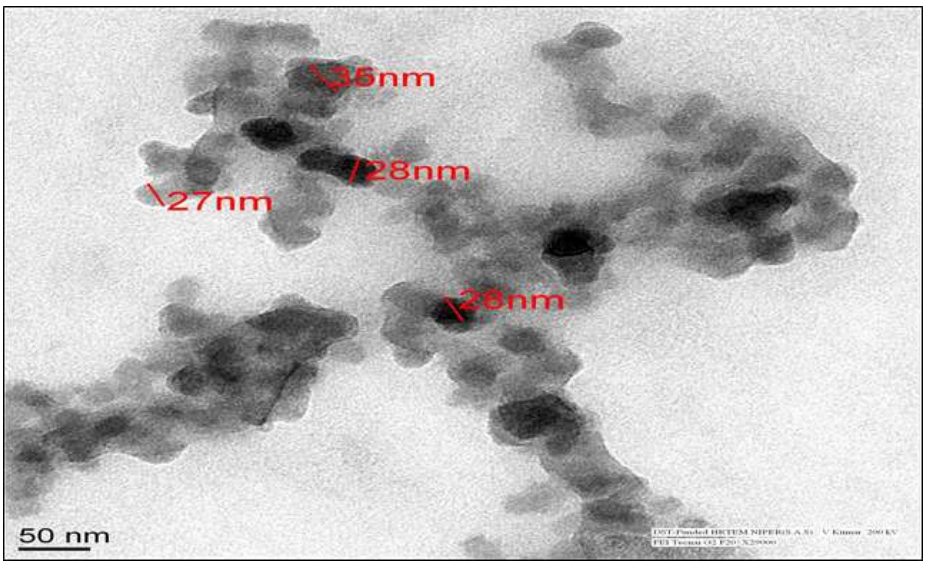

Figure 19: Showing Confirmatory analysis of nanoparticles of size ranging from 27nm to 35nm using Transmission Electron Microscopy

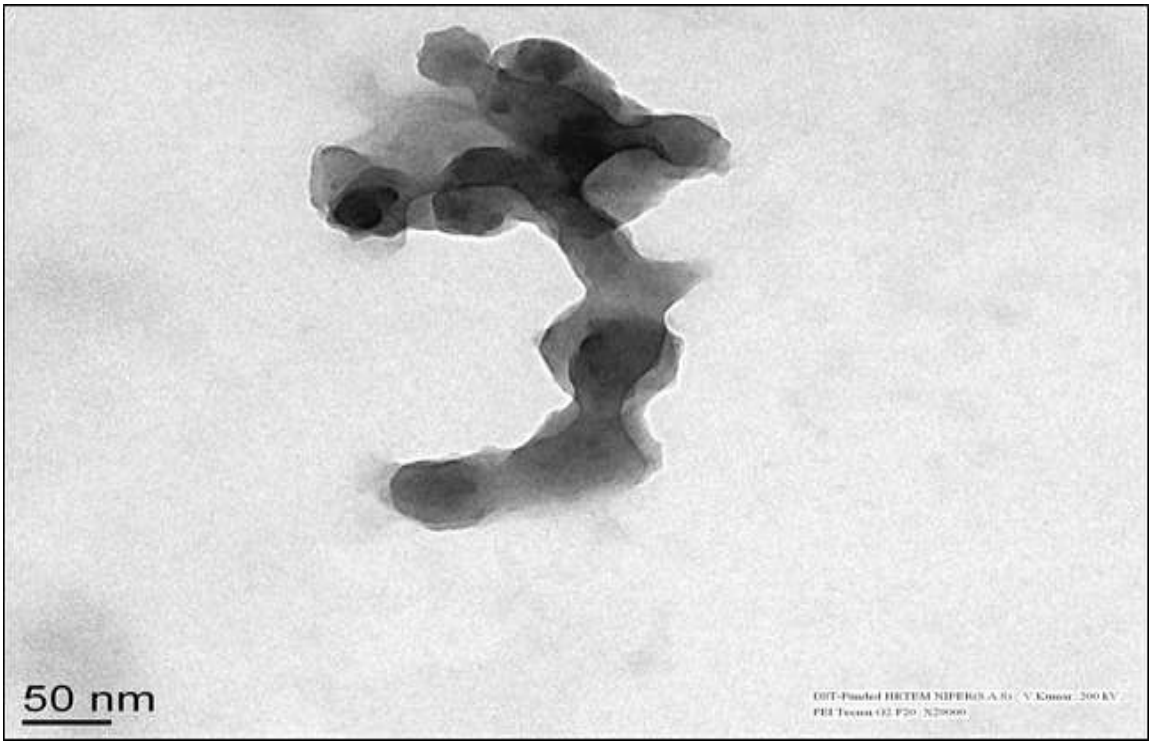

Figure 20: Showing the nanoparticles using Transmission Electron Microscopy

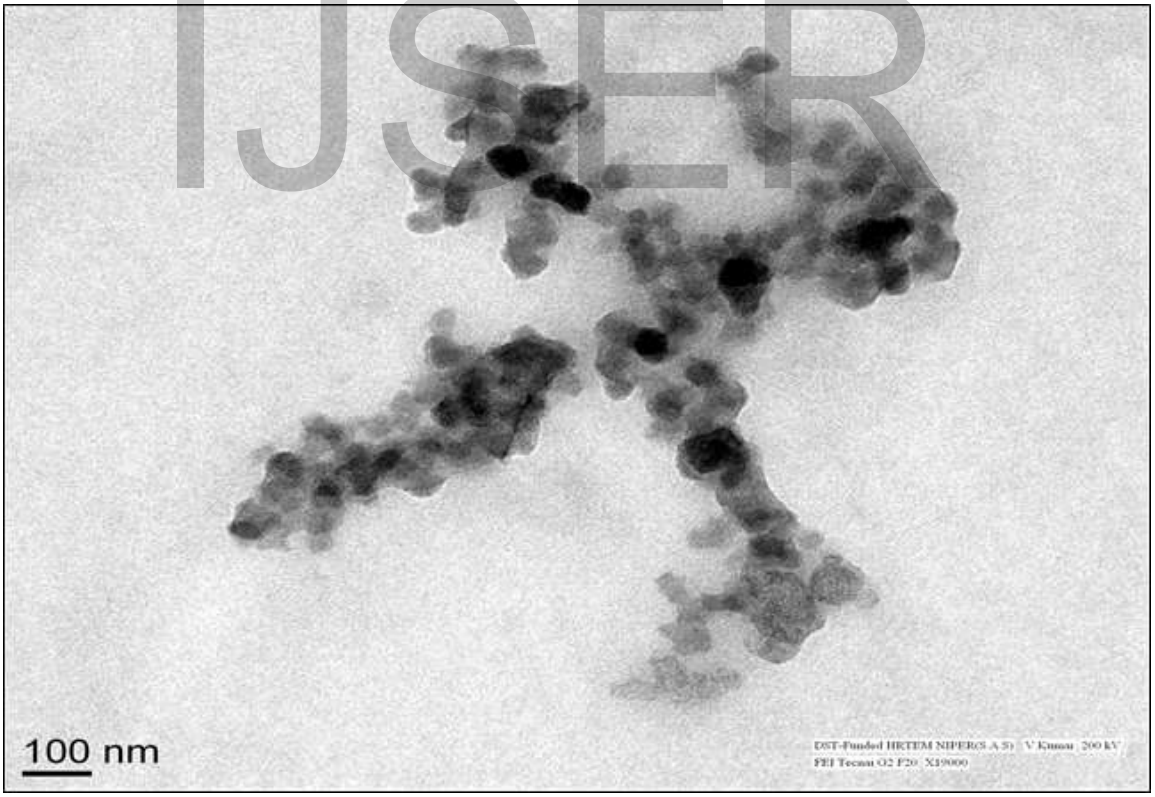

Figure 21: Showing the nanoparticles using Transmission Electron Microscopy

4.2.2 SCANNING ELECTRON MICROSCOPE (SEM) ANALYSIS:

Scanning Electron Microscope (SEM) was performed for the confirmatory analysis of morphology of synthesized copper nanoparticles from *Aloe barbadensis.* SEM provided the images of the surface of the particles and its composition. Aliquots of nanoparticles solution placed on the thin glass slide and allowed to dry under ambient conditions. Dried sample was coated with the thin layer of the heavy metal i.e. Gold. SEM images were recorded which confirms the morphology of synthesized copper nanoparticles in the plant extract. SEM shows the spherical shape of the nanoparticles and sizes ranging from 160nm to 210nm.The Copper nanoparticles so formed were shown in the figure:

Figure 22: Showing Confirmatory analysis of nanoparticles of size ranging from 113nm to 164nm using Scanning Electron Microscopy

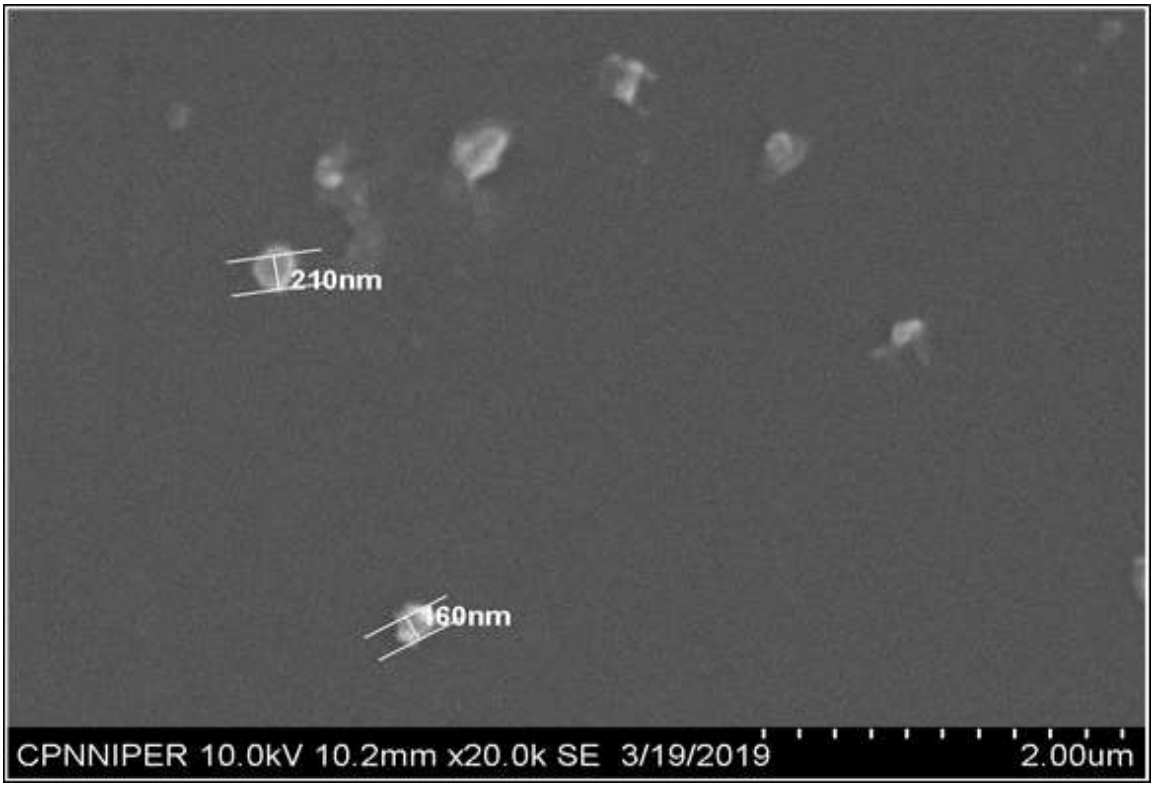

Figure 23: Showing the size of nanoparticles ranging from 160nm to 210nm using Scanning Electron Microscopy

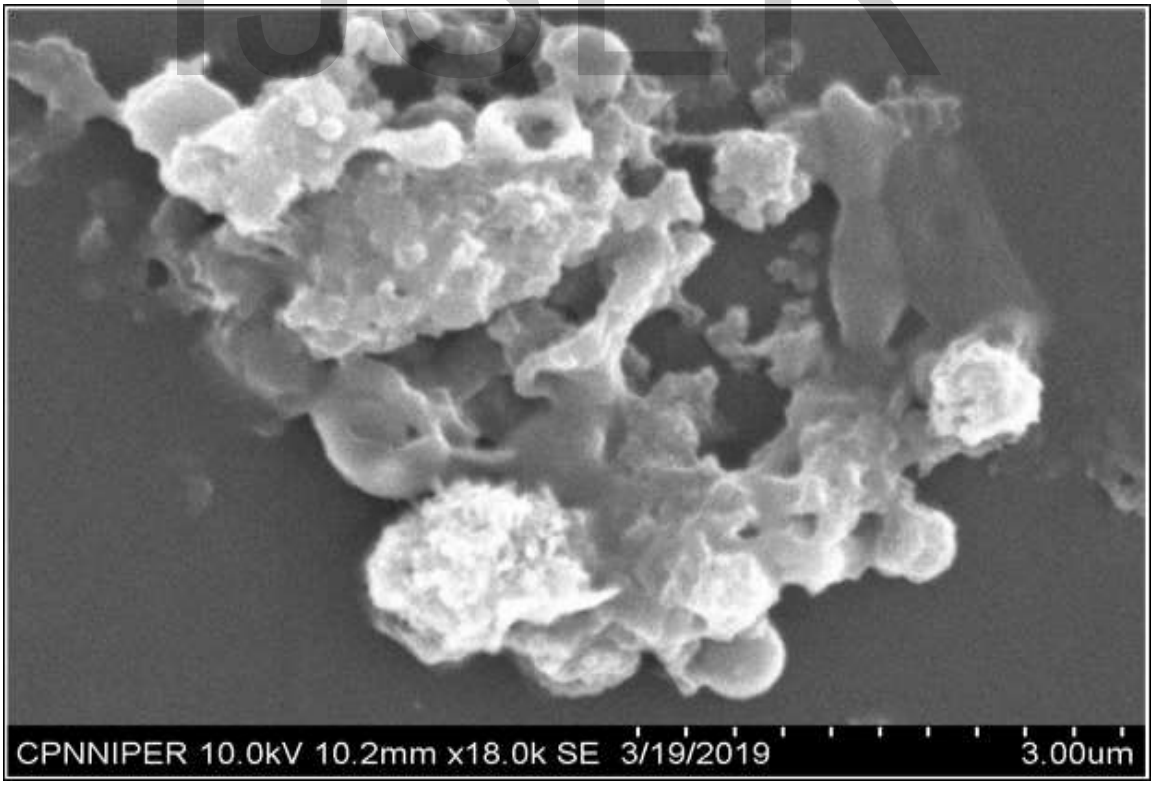

Figure 24: Showing the nanoparticles using Scanning Electron Microscopy

4.2.3 ENERGY DISPERSION X-RAY SPECTROMETER (EDS) ANALYSIS:

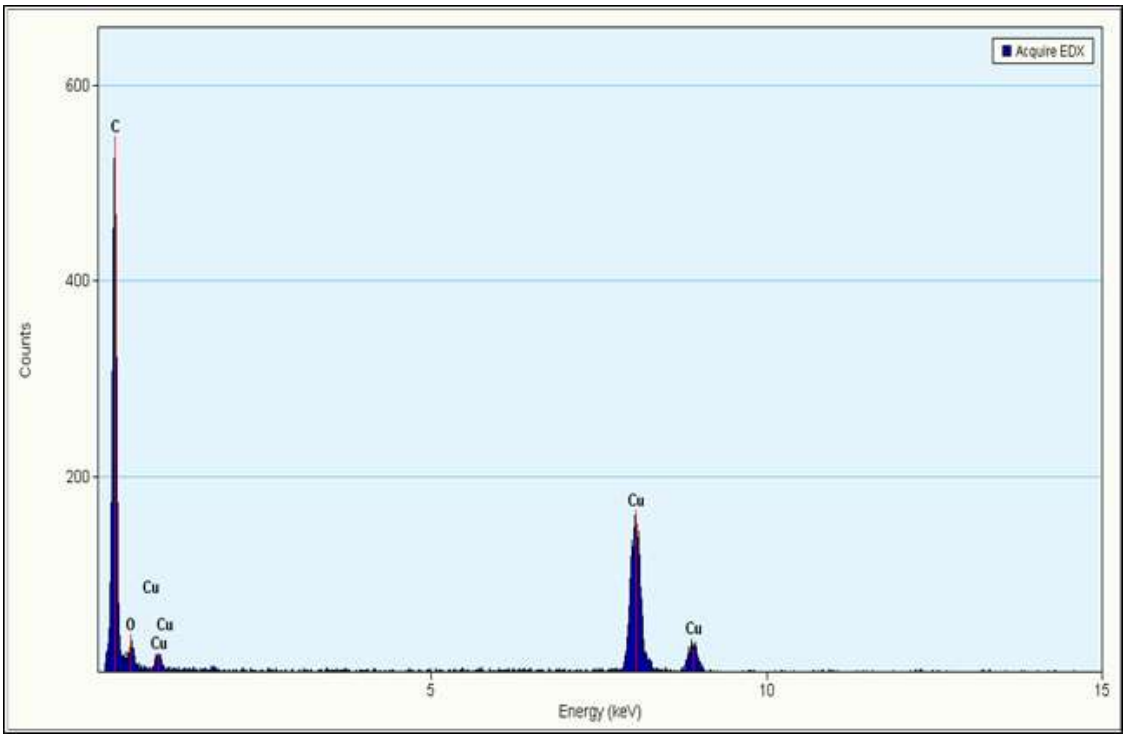

Figure 25: Showing the confirmatory analyse of Copper as a metal ion

Energy Dispersion X-Ray Spectrometer (EDS) is an analytical technique **used** for the elemental analysis or chemical characterization of a sample. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing a unique set of peaks on its electromagnetic emission spectrum.

Above picture shows the confirmatory analysis for presence of copper being as a metal ion in the biological source *Aloe barbadensis* sample. The peaks in the image shows that, in this amount copper particles are formed in the sample.

4.3 Identification of micro-organisms from different skin types:

Microbial swab sample was taken from 5 different skin types:

(a) Combination skin (oily+dry)

(b) Oily skin

(c) Scalp

(d) Acne skin

(e) Sensitive skin

Figure 26: NAM (Nutrient agar media) showing the growth of bacteria present on the Combination skin

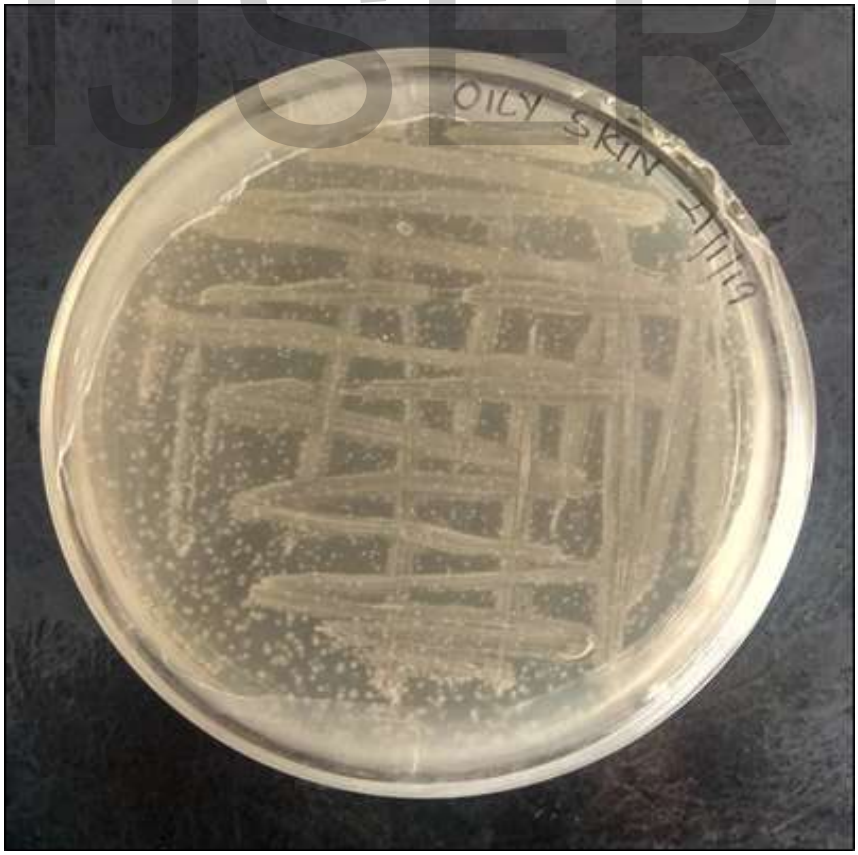

Figure 27: NAM (Nutrient agar media) showing the growth of bacteria present on the Oily skin

Figure 28: NAM (Nutrient agar media) showing the growth of bacteria present on the Scalp

Figure 29: NAM (Nutrient agar media) showing the growth of bacteria present on the Acne skin

Figure 30: NAM (Nutrient agar media) showing the growth of bacteria present on the Sensitive skin

Skin flora present on different skin types was identified using different identification method:

2.3.1 Staining

2.3.2 Differential Media

2.3.3 Biochemical Test

4.3.1 Staining

Staining is an auxiliary technique used in microscopy contrast in the microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biology and medicines to highlight structures in biological tissues for viewing often with the aid of different microscopes.

4.3.1.1 Gram Staining

Gram staining is a method of staining used to distinguish and classified bacterial species in to two large: groups Gram-positive and Gram-negative. **Gram positive** bacteria retain

the crystal violet dye, and thus are stained violet dye. **Gram negative** bacteria do not retain the crystal violet dye, after washing; a counter stain is added (commonly safranin or fuchsine) that will stain these a Gram-negative bacterium a pink colour.

On the various skin types Staining is performed three times, Gram-positive and Gram negative both bacteria are present but Gram-positive bacteria is prominently present.

Table 10: Observation of Skin flora samples by Gram Staining

Sample	Gram positive	Gram Negative
Combination skin	✔	✘
Oily skin	✔	✘
Scalp	✔	✘
Acne skin	✔	✘
Sensitive skin	✔	✘

Gram-Positive Bacteria

Cocci- Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Micrococcus. Rods-Corynebacterium, Propionibacterium, Brevibacterium, Dermabacter hominis, Diphtheroids

Gram-Negative Bacteria

Rods- *Acinetobacter, Escherichia coli, Pseudomonas aeruginosa, Bacteroidetyes*

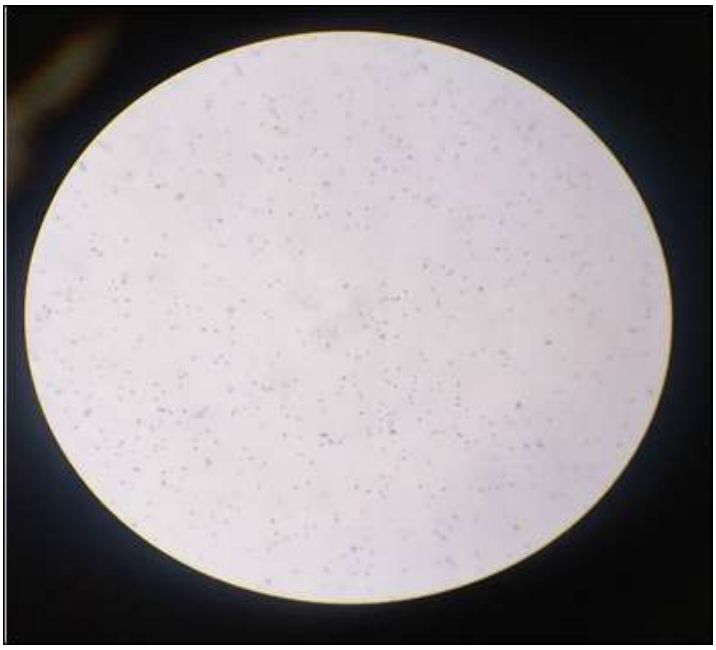

Figure 31: Observation of the growth of the Gram positive bacteria on Combination Skin

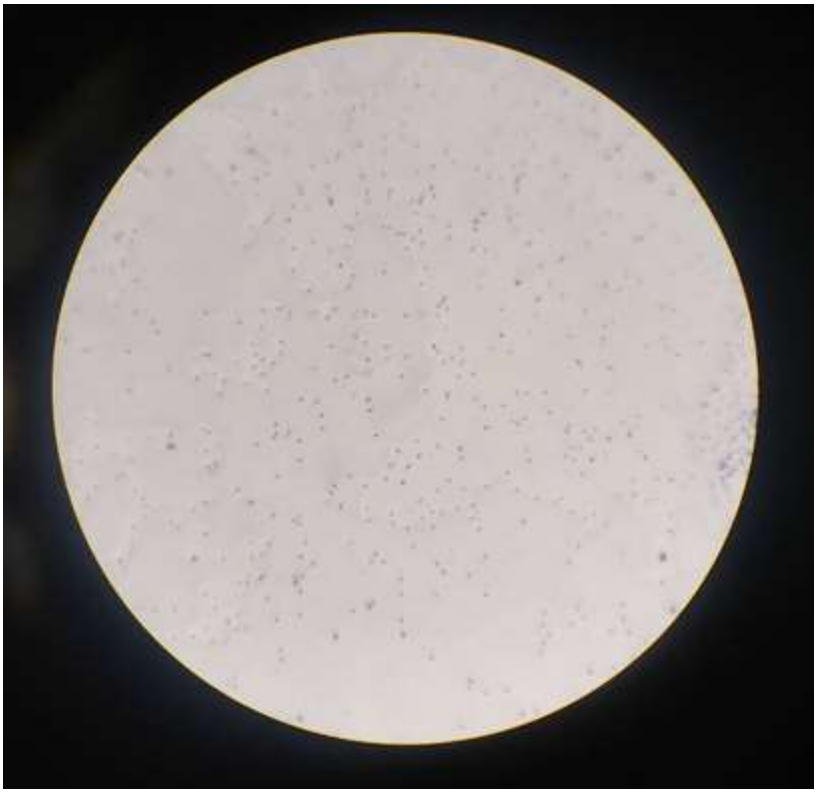

Figure 32: Observation of the growth of the Gram positive bacteria on Oily Skin

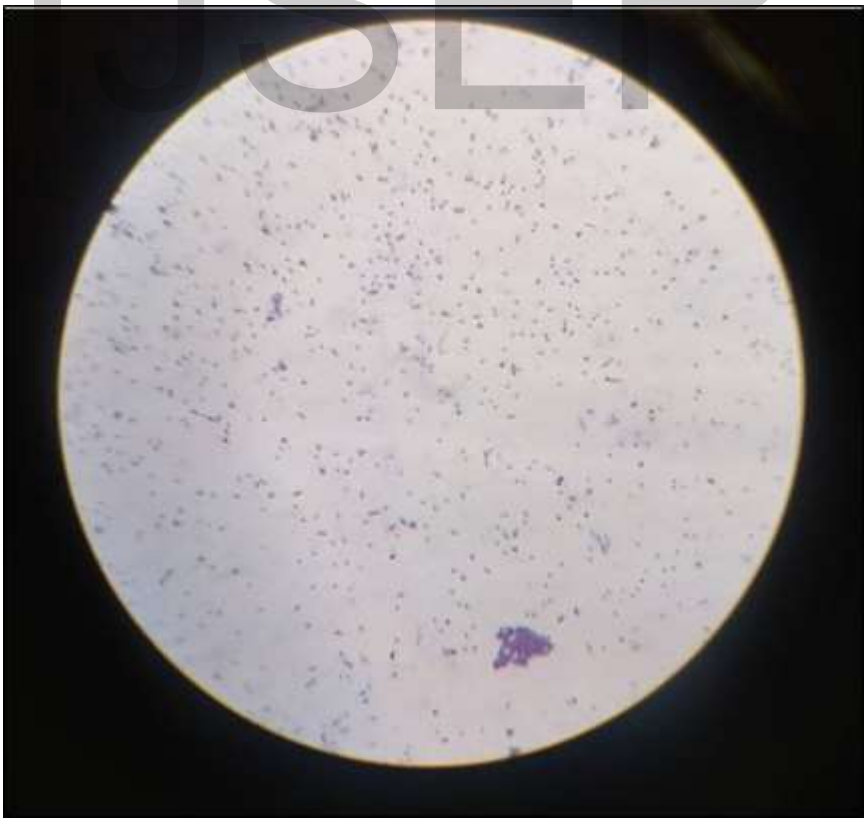

Figure 33: Observation of the growth of the Gram positive bacteria on Scalp

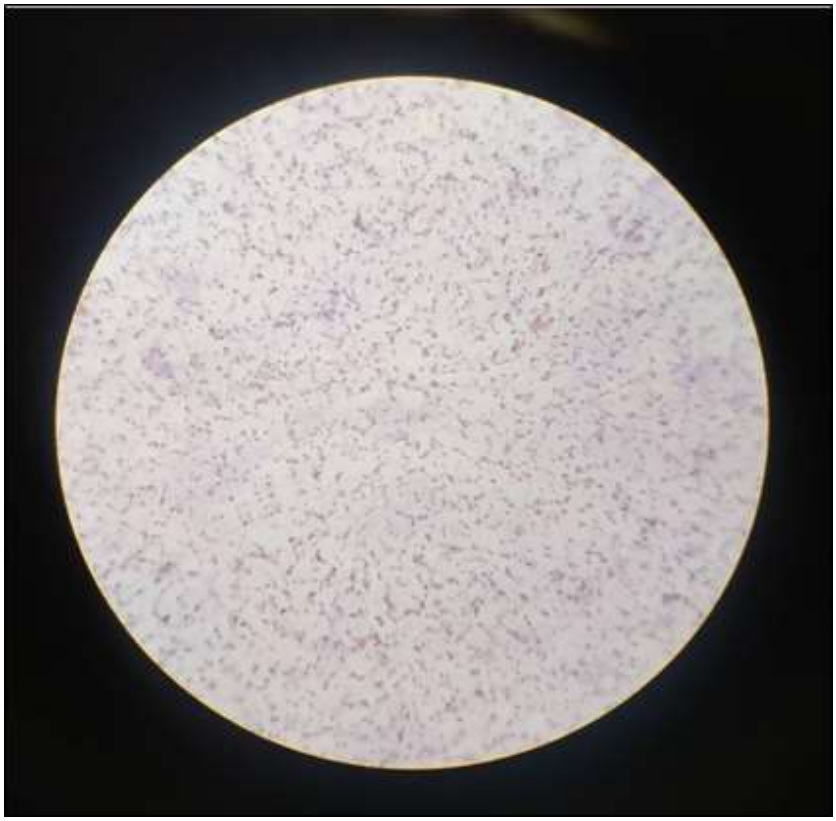

Figure 34: Observation of the growth of the Gram positive bacteria on Acne Skin

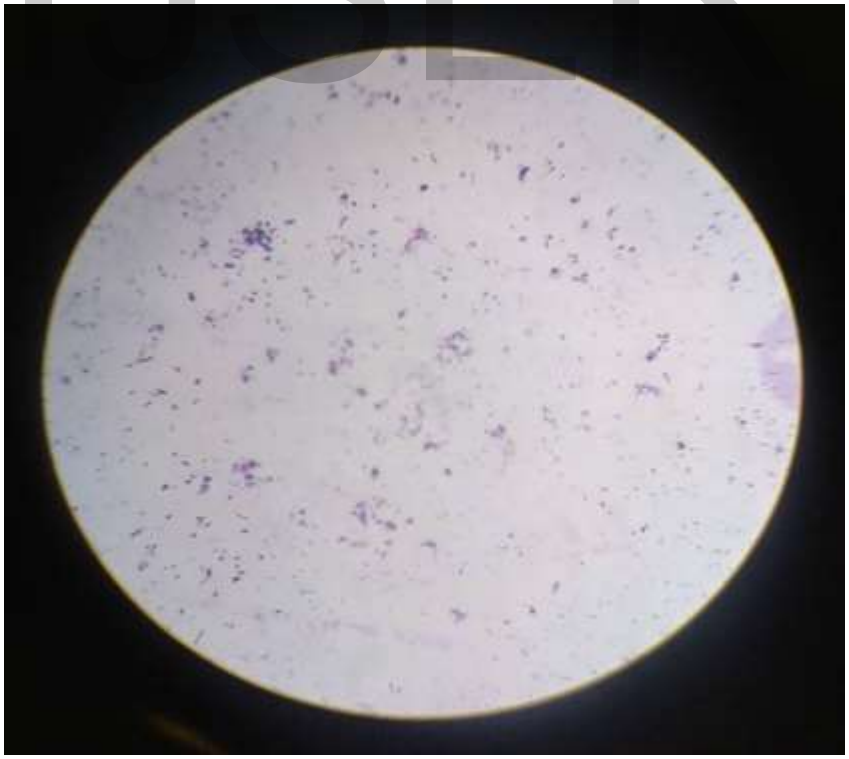

Figure 35: Observation of the growth of the Gram positive bacteria on Sensitive Skin

4.3.1.2 Endospore staining

It is a differential stain used to visulize bacterial spores

Table 11: Observation of Skin flora sample by Endospore staining

Sample	**Observation**
Combination skin	No endospore formed
Oily skin	No endospore formed
Scalp	No endospore formed
Acne skin	No endospore formed
Sensitive skin	No endospore formed

Result: non-spore forming bacteria are *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Micrococcus, Diphtheroids, Corynebacterium, Propionibacterium, Brevibacterium, Dermabacter hominis, Acinetobacter, Escherichia coli, Pseudomonas aeruginosa, Bacteroidetyes.*

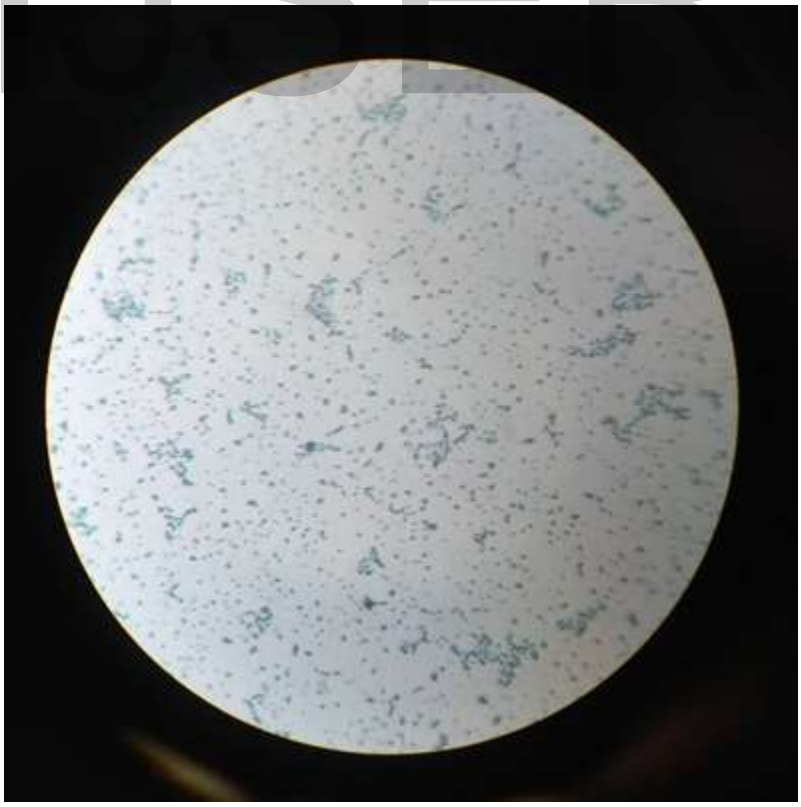

Figure 36: Observation of the growth of the Non-endosporing bacteria on Combination Skin

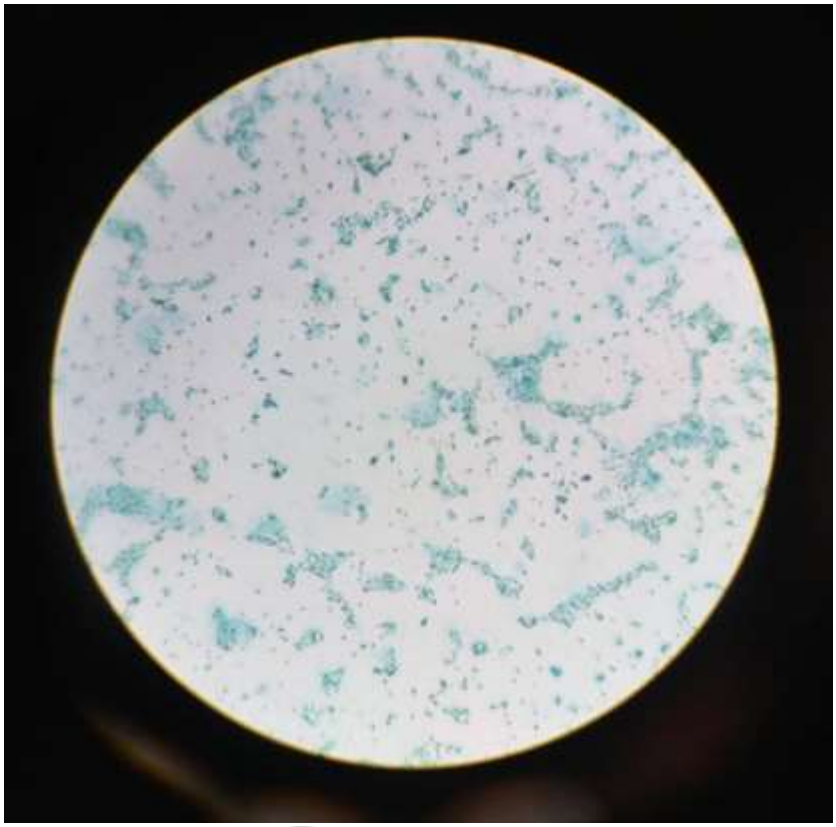

Figure 37: Observation of the growth of the Non-endosporing bacteria on Oily Skin

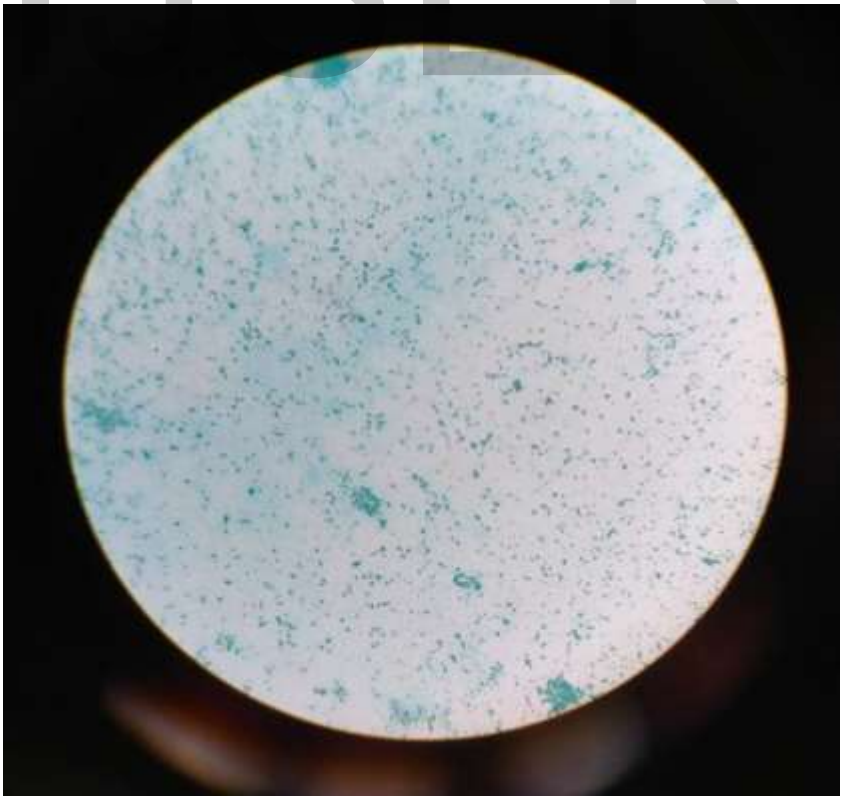

Figure 38: Observation of the growth of the Non-endosporing bacteria on Scalp

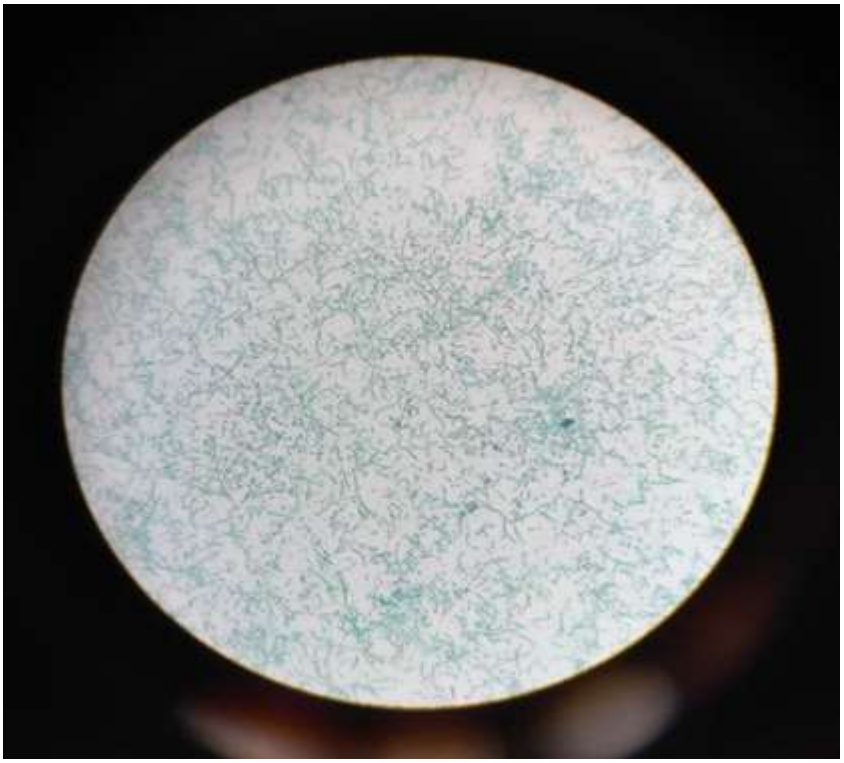

Figure 39: Observation of the growth of the Non-endosporing bacteria on Acne skin

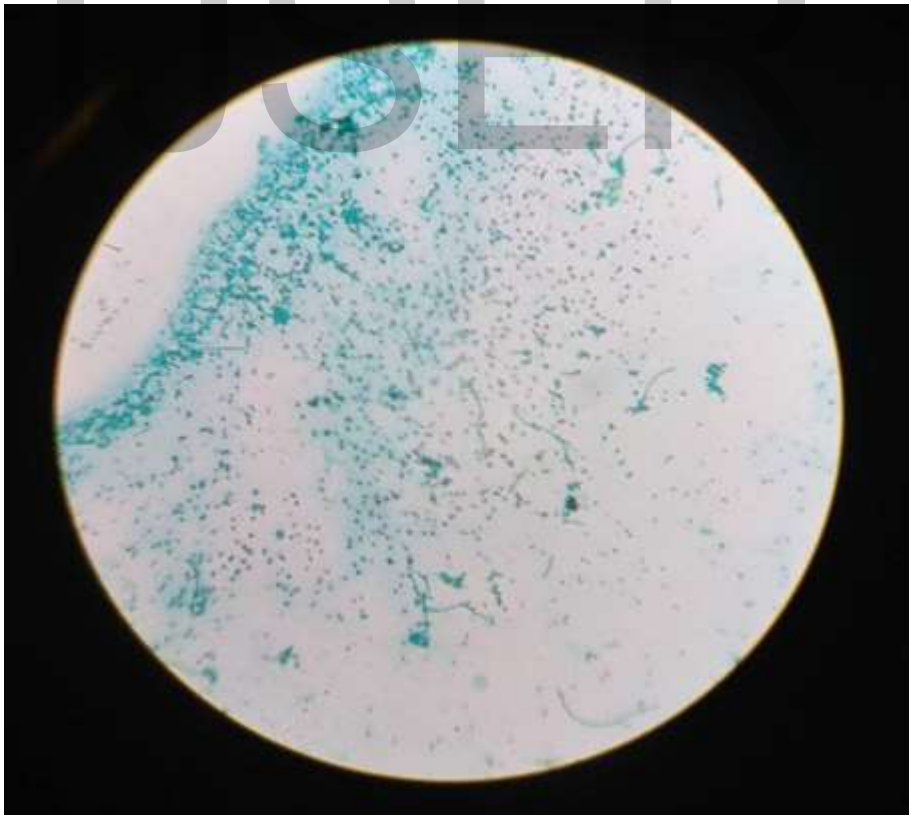

Figure 40: Observation of the growth of the Non-endosporing bacteria on Sensitive skin

4.3.2 Differential media

4.3.2.1 MacConkey agar

It is a selective and differential media for the isolation of Gram-negative bacteria.

Table 12: Observation of Skin flora sample by MacConkey agar

Sample	**Observation**
Combination skin	No growth
Oily skin	No growth
Scalp	No growth
Acne skin	No growth
Sensitive skin	No growth

Result: No results are observed that shows Gram-positive bacteria present.

Bacteria present are Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Micrococcus, Corynebacterium, Propionibacterium, Brevibacterium, Dermabacter hominis, Diphtheroids.

Figure 41: Observation of No bacterial Growth on MacConky agar media of Combination Skin

Figure 42: Observation of No bacterial Growth on MacConky agar media of Oily Skin

Figure 43: Observation of No bacterial Growth on MacConky agar media of Scalp

Figure 44: Observation of No bacterial Growth on MacConky agar media of Acne Skin

Figure 45: Observation of No bacterial Growth on MacConky agar media of Sensitive Skin

4.3.2.2 EMB (Eosin-Methylene blue) agar

Eosin methylene blue agar (EMB) is a selective and differential medium used to isolate fecal coliforms. Eosin Y and methylene blue are pH indicator dyes which combine to form a dark purple precipitate at low pH; they also serve to inhibit the growth of most Gram positive bacteria.

Table 13: Observation of Skin flora sample by EMB agar

Sample	**Observation**
Combination skin	Growth is observed & prominent pink colour is observed
Oily skin	No growth
Scalp	No growth
Acne skin	No growth
Sensitive skin	No growth

Result: Positive result shows the presence *Escherichia coli*

Negative result shows *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Micrococcus, Diphtheroids, Corynebacterium, Propionibacterium, Brevibacterium, Dermabacter hominis, Acinetobacter, Pseudomonas aeruginosa, Bacteroidetyes.*

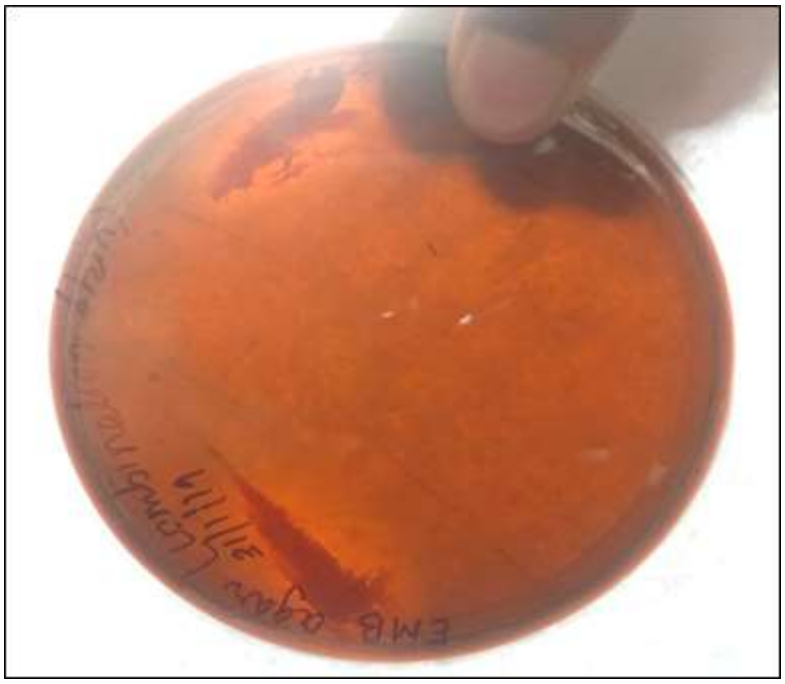

Figure 46: Observation of pink coloured bacterial growth on EMB of Combination skin

Figure 47: Observation of no bacterial growth on EMB of Oily skin

Figure 48: Observation of no bacterial growth on EMB of Scalp

Figure 49: Observation of no bacterial growth on EMB of Acne skin

Figure 50: Observation of no bacterial growth on EMB of Sensitive skin

4.3.3 Biochemical Test

Test used for identification of bacterial species based on the difference in the biochemical activities of different bacteria.

4.3.3.1 Potassium hydroxide Test (KOH)

KOH test is to identify gram-negative bacteria, but do not effect gram-positive bacteria. Positive result shows no viscous formation in sample, negative result show viscous formation.

Table 14: Observation of Skin flora sample by Potassium hydroxide Test

Sample	Observation
Combination skin	No viscocity
Oily skin	No viscocity
Scalp	No viscocity
Acne skin	No viscocity
Sensitive skin	No viscocity

Result: No viscosity shows the presence of *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Micrococcus, Corynebacterium, Propionibacterium, Brevibacterium, Dermabacter hominis, Diphtheroids*

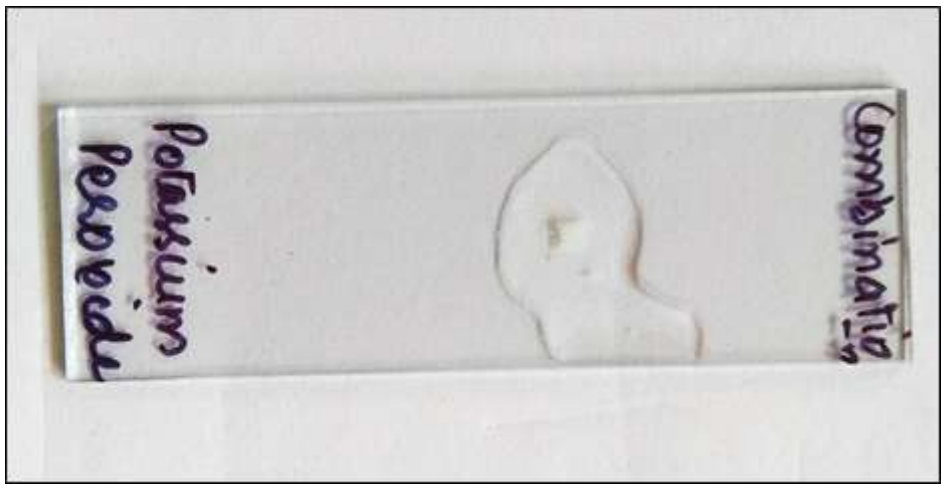

Figure 51: Observation of No viscosity on Combination skin

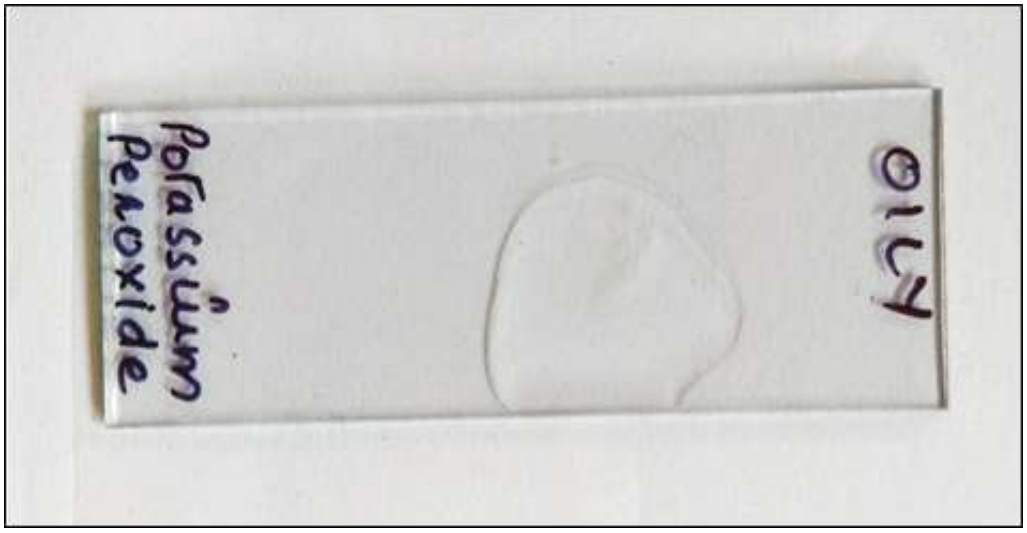

Figure 52: Observation of No viscosity on Oily skin

Figure 53: Observation of No viscosity on Scalp

Figure 54: Observation of No viscosity on Acne skin

Figure 55: Observation of No viscosity on Sensitive skin

4.3.3.2 Glucose and Gas Fermenting Test

This tests for the ability of bacteria to ferment glucose and produce gas or an acid end product. A positive result for acid is yellow after indicator is added (indicating glucose fermentation). Completely negative result has no colour change or raddish colour.

Figure 56: Observation of no colour change after 24 hour incubation in Combination skin

Table 15: Observation of Skin flora sample by Glucose and Gas Fermenting Test

Sample	Observation
Combination skin	No colour changes
Oily skin	No colour changes
Scalp	No colour changes
Acne skin	No colour changes
Sensitive skin	No colour changes

Result: Non glucose fermenting bacteria are *Acinetobacter, Pseudomonas aeruginosa*

Figure 57: Observation of no colour change after 24 hour incubation in Oily skin

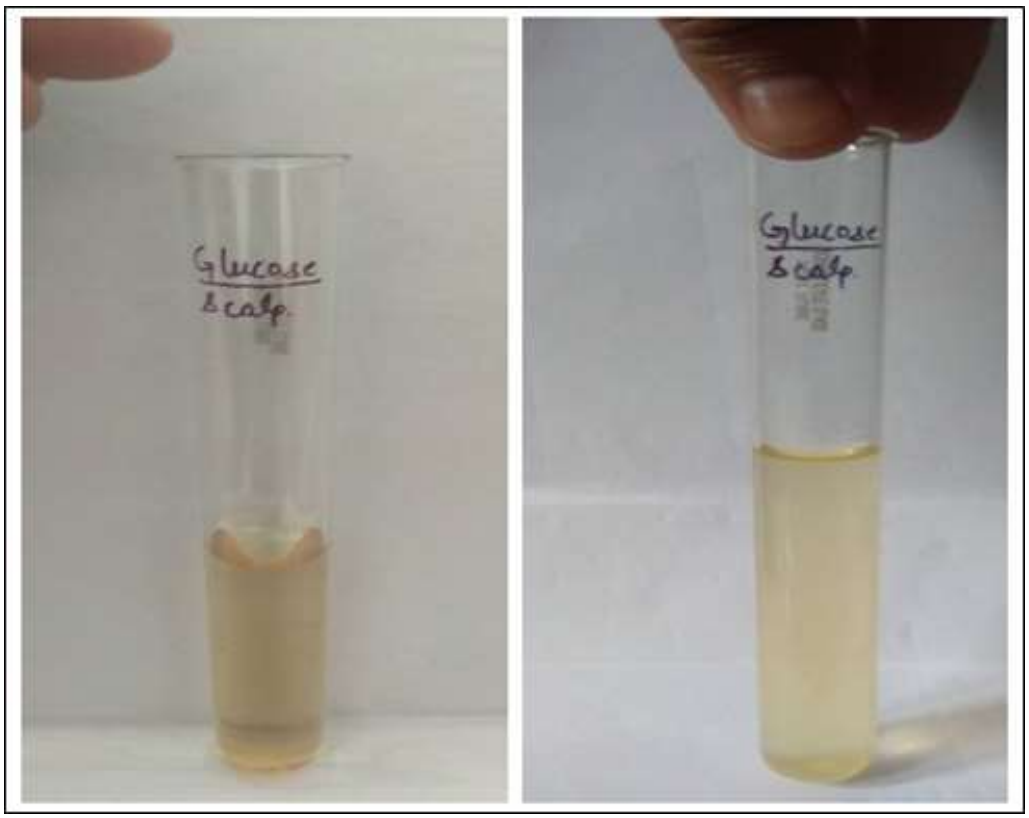

Figure 58: Observation of no colour change after 24 hour incubation in Scalp

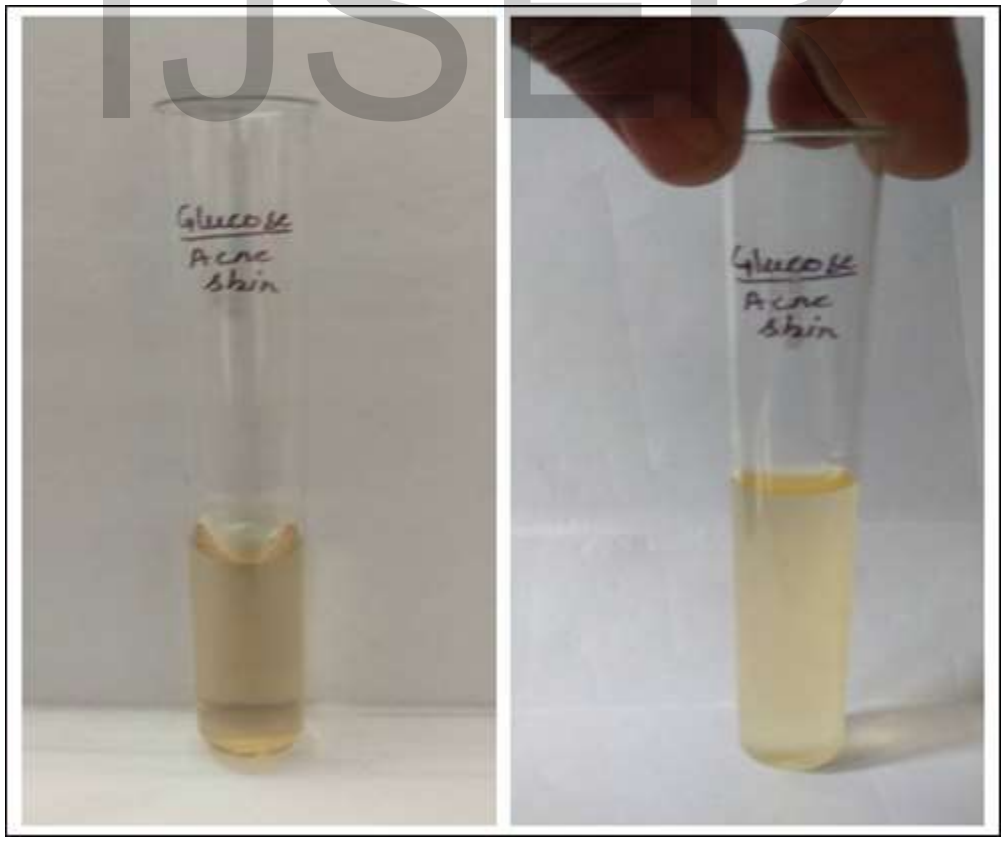

Figure 59: Observation of no colour change after 24 hour incubation in Acne skin

Figure 60: Observation of no colour change after 24 hour incubation in Sensitive skin

4.3.3.3 Lactose Fermenting Test

This tests is used to check the ability of bacteria to ferment lactose. A positive result is yellow after indicator is added (indicating lactose fermentation). A negative result will have no change in colour or will be reddish.

Table 16: Observation of Skin flora sample by Lactose Fermenting Test

Sample	Observation
Combination skin	No colour changes
Oily skin	No colour changes
Scalp	No colour changes
Acne skin	No colour changes
Sensitive skin	No colour changes

Result:

Non-lactose fermenting bacteria is *Pseudomonas aeruginosa*

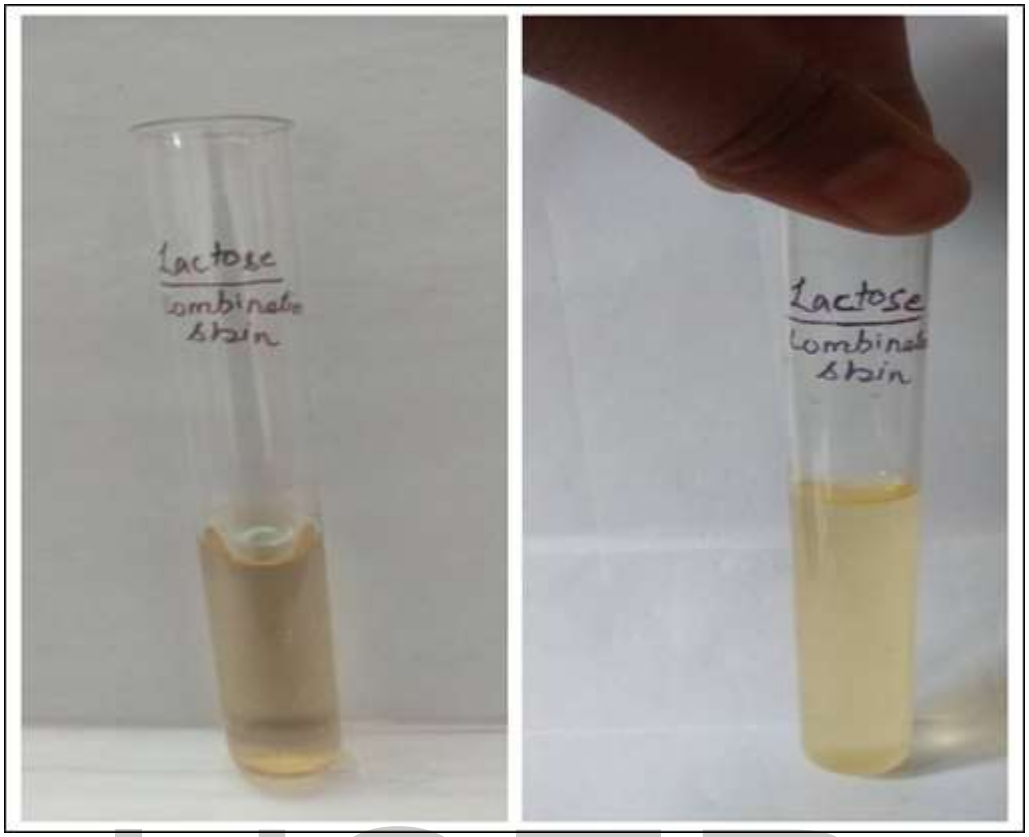

Figure 61: Observation of no colour change after 24 hour incubation in Combination skin

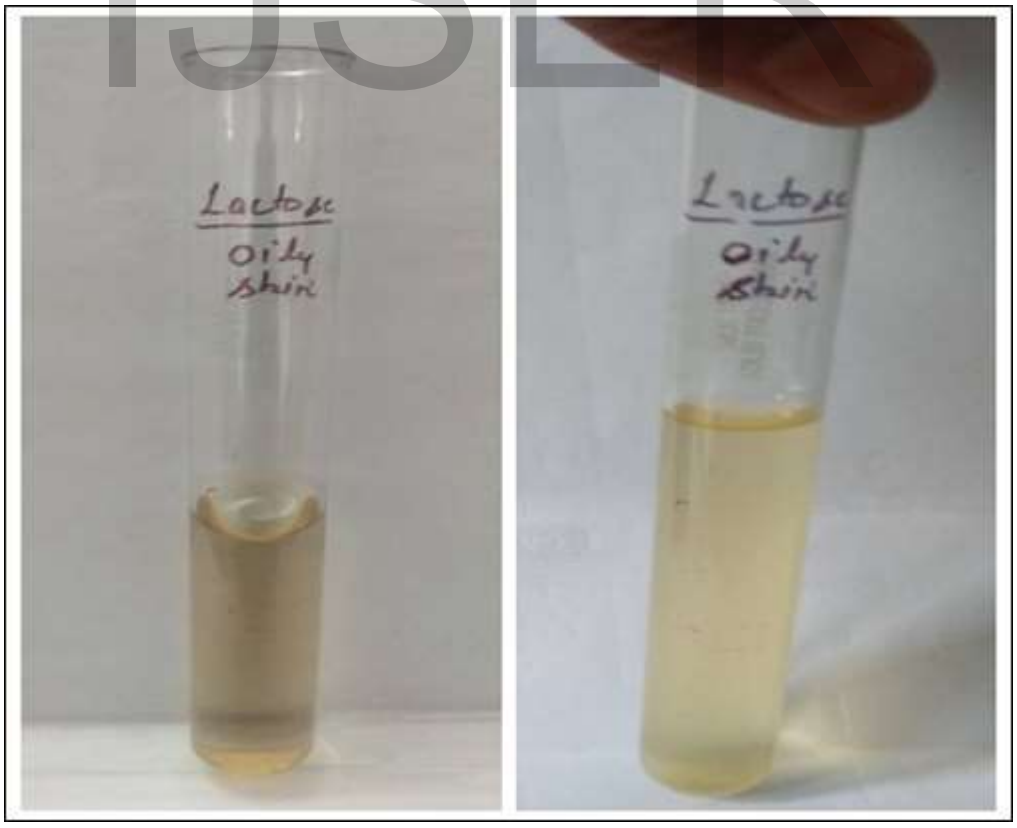

Figure 62: Observation of no colour change after 24 hour incubation in Oily skin

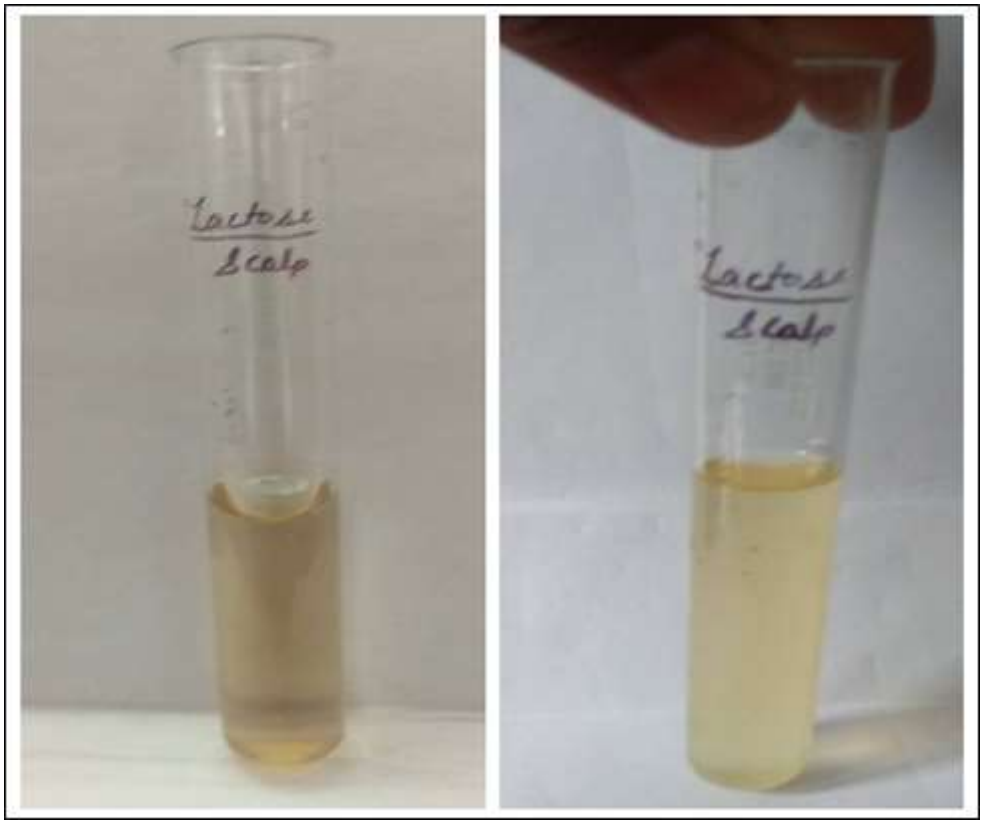

Figure 63: Observation of no colour change after 24 hour incubation in Scalp

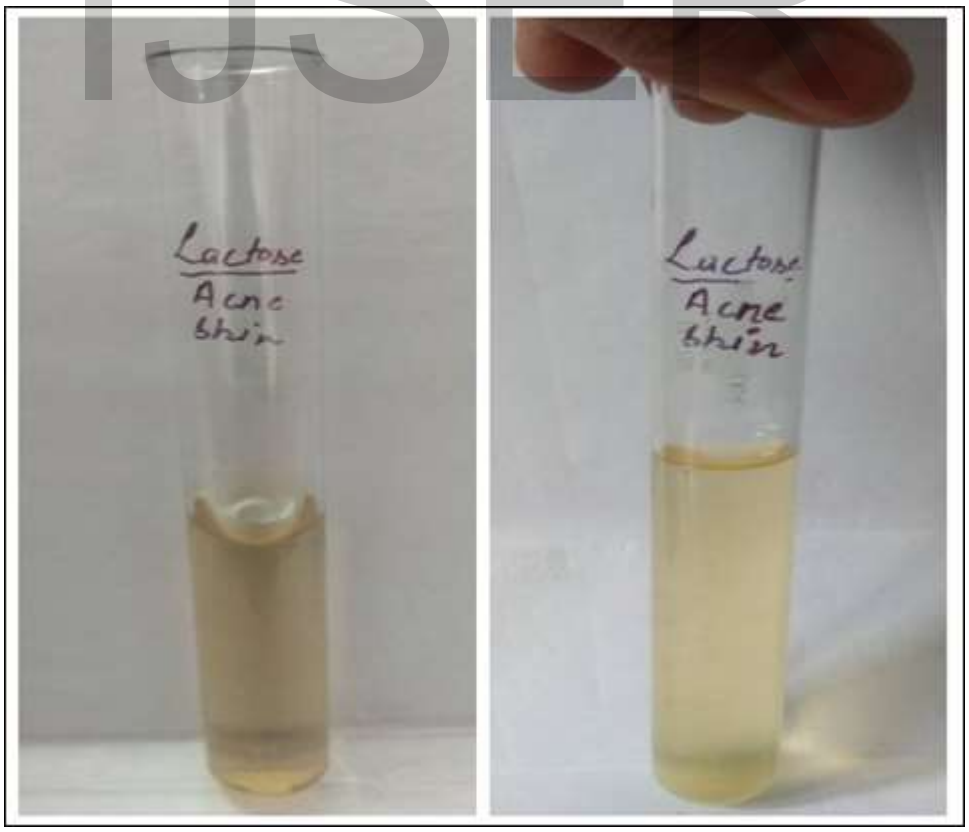

Figure 64: Observation of no colour change after 24 hour incubation in Acne skin

Figure 65: Observation of no colour change after 24 hour incubation in Sensitive skin

4.3.3.4 Sucrose Fermenting Test

This tests for the ability of bacteria to ferment sucrose and production of acid end products. A positive result is yellow after indicator is added (indicating sucrose fermentation). Negative result has no colour change or is reddish.

Table 17: Observation of Skin flora sample by Sucrose Fermenting Test

Sample	**Observation**
Combination skin	No colour change
Oily skin	No colour changes
Scalp	No colour changes
Acne skin	No colour changes
Sensitive skin	No colour changes

Result: Non-sucrose fermenting bacteria- *Acinetobacter*

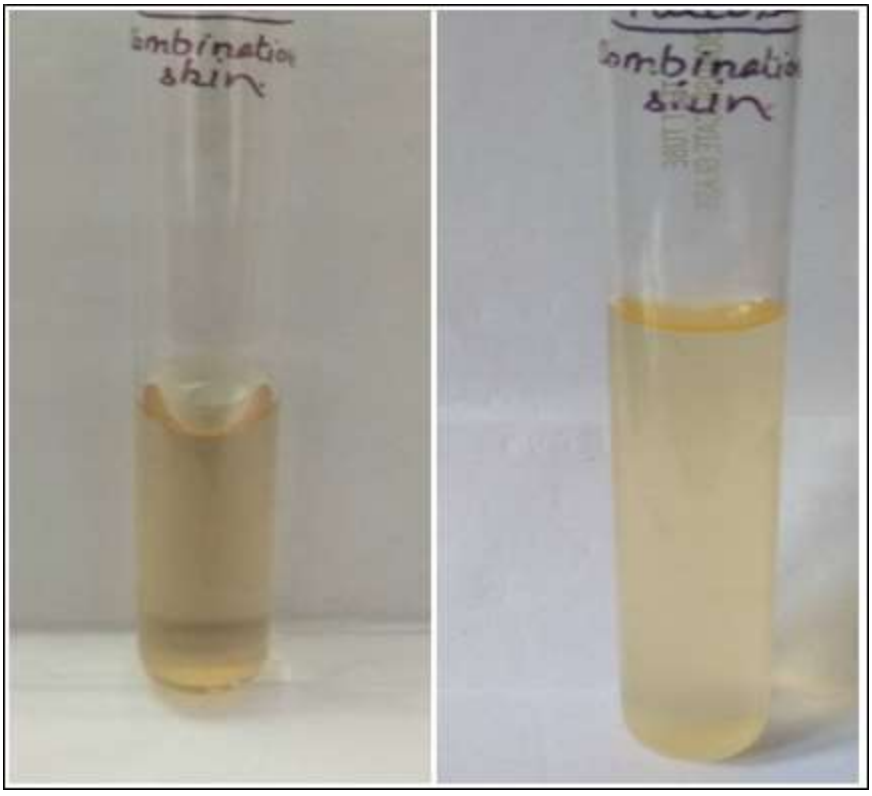

Figure 66: Observation of no colour change after 24 hour incubation in Combination skin

Figure 67: Observation of no colour change after 24 hour incubation in Oily skin

Figure 68: Observation of no colour change after 24 hour incubation in Scalp

Figure 69: Observation of no colour change after 24 hour incubation in Acne skin

Figure 70: Observation of no colour change after 24 hour incubation in Sensitive skin

4.3.3.5 Catalse Test

This test demonstrates the presence of enzyme catalase in the organism. Positive result shows growth of *Staphylococcus, Enterobacteriacae.*

Table 18: Observation of Skin flora sample by Catalase Test

Sample	**Observation**
Combination skin	No bubbling
Oily skin	No bubbling
Scalp	No bubbling
Acne skin	No bubbling
Sensitive skin	No bubbling

Result: Catalase negative bacteria are *Streptococcus, Brevibacterium*

Figure 71: Observation of No bubbling is observed in Combination skin

Figure 72: Observation of No bubbling is observed in Oily skin

Figure 73: Observation of No bubbling is observed in Scalp

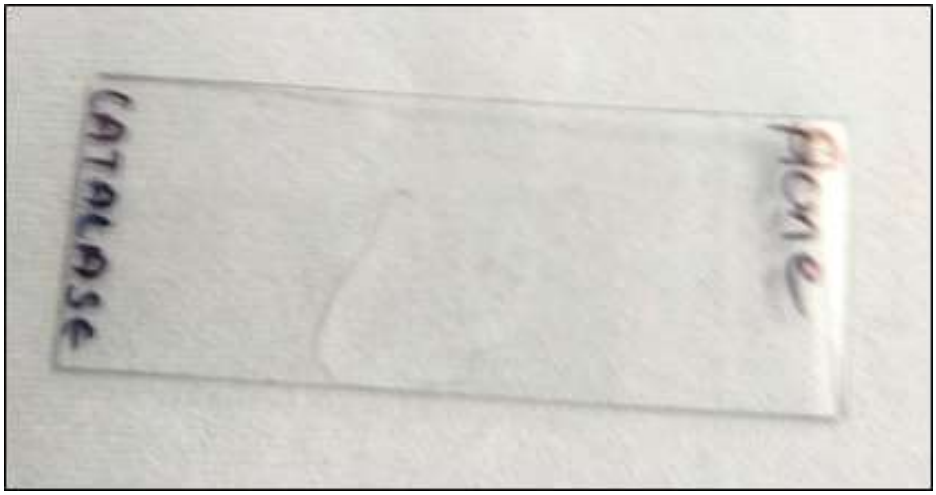

Figure 74: Observation of No bubbling is observed in Combination skin

Figure 75: 1Observation of No bubbling is observed in Sensitive skin

Table 19: Observation of Skin flora sample by Oxidase Test

Sample	**Observation**
Combination skin	No colour changes
Oily skin	No colour changes
Scalp	No colour changes
Acne skin	No colour changes
Sensitive skin	No colour changes

Result: Oxidase negative bacteria are *Corynebacterium, Propionibacterium, Diphtheroids, Micrococcus, Escherichia coli, Dermabacter hominis, Bacteroidetyes.*

4.3.3.6 Oxidase Test

The oxidase test is used to identify bacteria that produce cytochrome C oxidase, an enzyme of the bacterial electron transport chain. Bacteria that are oxidase positive are aerobic; it has oxygen as a terminal electron acceptor in respiration.

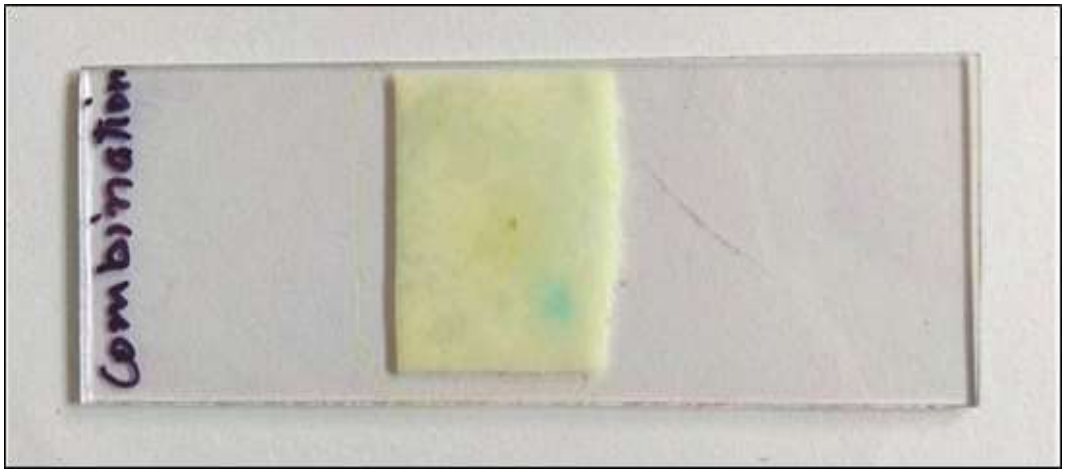

Figure 76: Observation of no purple or maroon colour observed on Combination skin

Figure 77: Observation of no purple or maroon colour observed on Oily skin

Figure 78: Observation of no purple or maroon colour observed on Scalp

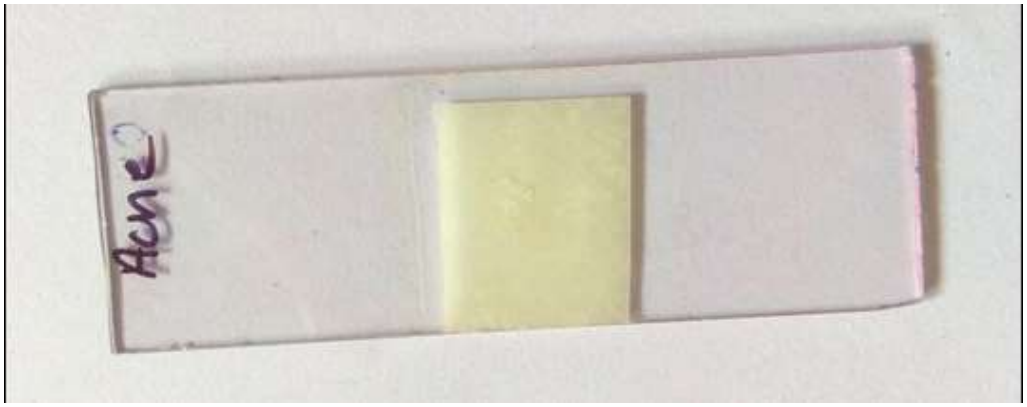

Figure 79: Observation of no purple or maroon colour observed on Acne skin

Figure 80: Observation of no purple or maroon colour observed on Sensitive skin

4.3.3.7 Motility Test:

Motility Test is useful for identification of bacteria which possess similar biochemical reactions, if microbe is swimming bt mean flagella.

Table 20: Observation of Skin flora sample by Motility Test

Sample	**Observation**	**Type of bacteria**
Combination skin	No stab line	Non-motile
Oily skin	Diffused growth throughout media	motile
Scalp	No stab line	Non-motile
Acne skin	Diffused growth throughout media	motile
Sensitive skin	Diffused growth throughout media	Motile

Result: **Motile Bacteria are** *Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa*

Non-motile Bacteria are *Corynebacterium, Propionibacterium, Staphylococcus aureus,*

Streptococcus, Micrococcus, Diphtheroids, Acinetobacter, Bacteroidetyes, Dermabacter hominis

Figure 81: Observation of non-motility of bacteria in Combination skin

Figure 82: Observation of motility of bacteria in Oily skin

Figure 83: Observation of non-motility of bacteria in Scalp

Figure 84: Observation of motility of bacteria in Acne skin

Figure 85: Observation of motility of bacteria in Sensitive skin

4.3.3.8 Urease Test*:*

Urease test is used for the presumptive evidence of the presence of Helicobacter pylori the sample.

Table 21: Observation of Skin flora sample by Urease Test

Sample	**Observation**
Combination skin	No colour change
Oily skin	No colour change
Scalp	No colour change
Acne skin	No colour change
Sensitive skin	No colour change

Result: Negative result shows the presence of *Propionibacterium, Brevibacterium, Dermabacter hominis, Diphtheroids, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Micrococcus, Acinetobacter, Escherichia coli, Bacteroidetyes.*

Figure 86: Observation of no colour change observed in Combination skin

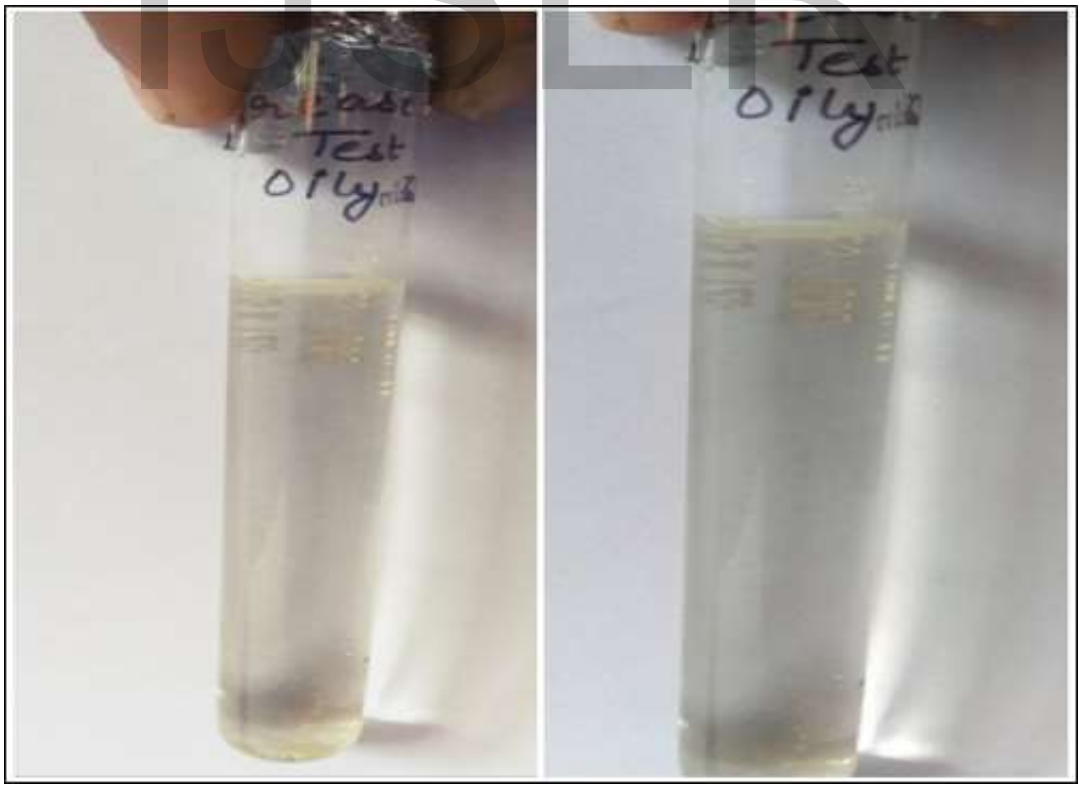

Figure 87: Observation of no colour change observed in Oily skin

Figure 88: Observation of no colour change observed in Scalp

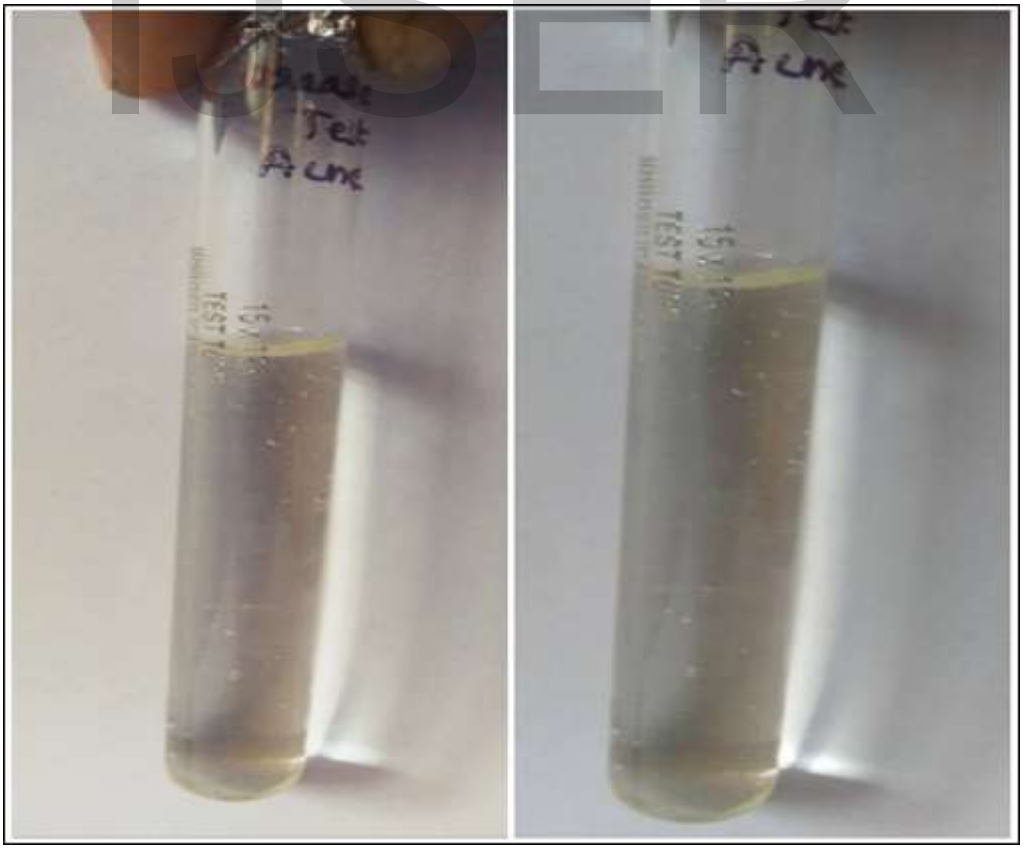

Figure 89: Observation of no colour change observed in Acne skin

Figure 90: Observation of no colour change observed in Sensitive skin

4.3.3.9 Methyl red test:

To test the ability of the organism to produce and maintain stable acid end products from glucose fermentation and to overcome the buffering capacity of the system. This is a qualitative test for acid production. Change in colour from yellow to red after indicator is added.

Table 22: Observation of Skin flora sample by Methyl red Test

Sample	**Observation**
Combination skin	Colour turns into **red**
Oily skin	Colour not changed
Scalp	Colour turns into **red**
Acne skin	Colour not changed
Sensitive skin	Colour turns into **red**

Result:

Positive result shows: *Escherichia coli*

Figure 91: Observation of the change in colour into pink (red) in Combination skin

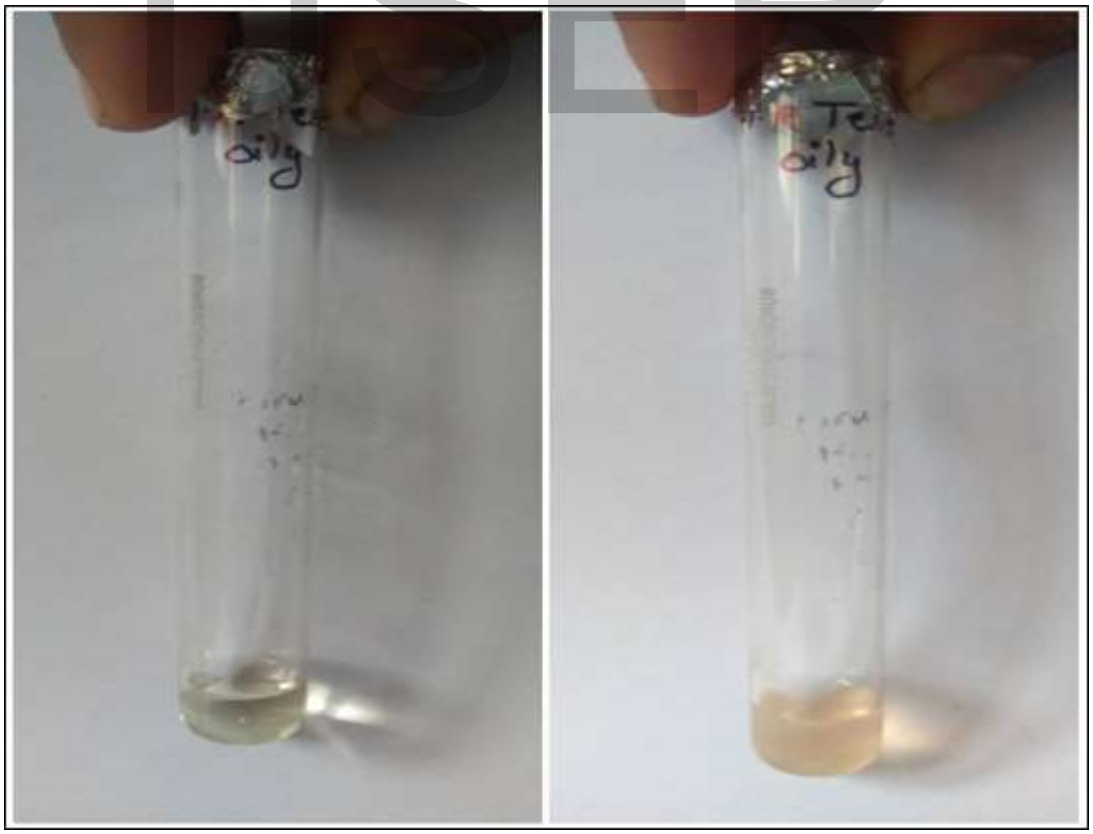

Figure 92: Observation of the No colour change in Oily skin

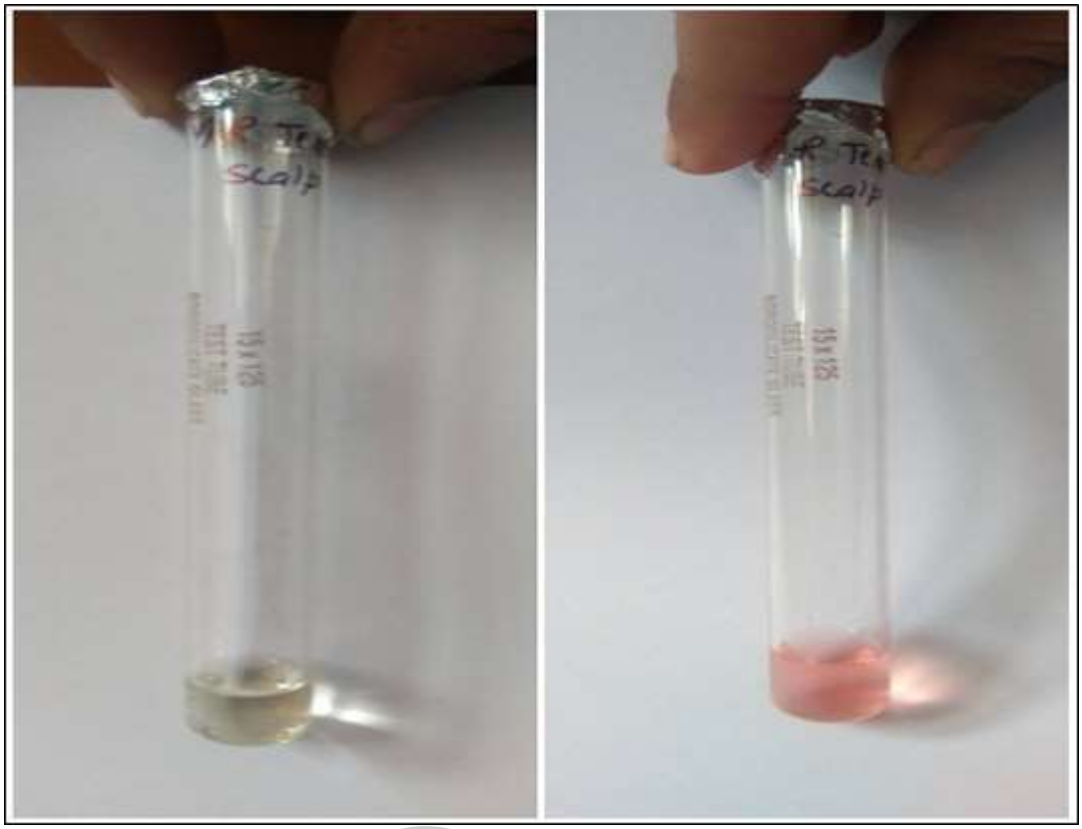

Figure 93: Observation of the change in colour into pink (red) in Scalp

Figure 94: Observation of the no change in colour in Acne skin

Figure 95: Observation of the change in colour into pink (red) in Sensitive skin

From all above identification methods it is shown that *Streptococcus, Micrococcus, Propionibacterium, Dermabacter hominis* are present prominently as skin flora on the face of human.

- *Staphylococcus aureus, Staphylococcus epidermidis, Corynebacterium, Propionibacterium* are present prominently on the **Combination skin**
- *Corynebacterium, Propionibacterium* are present prominently on the **Oily skin**
- *Escherichia coli* is present on the **Scalp**
- *Corynebacterium, Propionibacterium* are present prominently on the **Acne skin**
- *Staphylococcus aureus, Staphylococcus epidermidis* are present prominently on the **Sensitive skin**

Table: 23: Summary of identification methods

Combination Skin	Oily Skin	Scalp	Acne Skin	Sensitive Skin
Gram Staining				
Gram-positive	Gram-positive	Gram-positive	Gram-positive	Gram-positive
Endospore Staining				
Non-spore Forming	Non-spore Forming	Non-spore Forming	Non-spore Forming	Non-spore Forming
MacConky Agar Media				
No colour change	No colour change	No colour change	No colour change	No colour change
EMB Agar Media				
Growth is observed & prominent pink colour is observed	No Growth	No Growth	No Growth	No Growth
Potassium hydroxide Test (KOH)				
No viscosity	No viscosity	No viscosity	No viscosity	No viscosity
Glucose and Gas Fermenting Test				
No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change
Lactose Fermenting Test				
No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change
Sucrose Fermenting Test				
No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change
Catalse Test				
No bubbling	No bubbling	No bubbling	No bubbling	No bubbling

Oxidase Test				
No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change
Motility Test				
No stab line	Diffused growth throughout media	No stab line	Diffused growth throughout media	Diffused growth throughout media
Urease Test				
No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change
Methyl red test				
Colour turns into **red**	Colour not changed	Colour turns into **red**	Colour not changed	Colour turns into **red**

4.4 Anti-microbial Test:

The most known and basic methods are the **disk-diffusion** and broth or **agar dilution** methods but to determine the antimicrobial activity of copper nanoparticles **turbidimetric analysis** is used.

Turbidimetric analysis

The turbidity of the sample is observed by concentration of that sample using colorimeter.

Table 24: Observation of antimicrobial against copper nanoparticles

Sample	Optical density at 630nm							
	1st attempt		2nd attempt		3rd attempt		4th attempt	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Blank	0	0	0	0	0	0	0	0
Combination skin	0.07	0.06	0.05	0.04	0.04	0.02	0.06	0.05
Oily skin	0.02	0.01	0.02	0.02	0.02	0.01	0.10	0.07
Scalp	0.21	0.15	0.06	0.05	0.04	0.03	0.20	0.19
Acne skin	0.13	0.12	0.06	0.06	0.04	0.01	0.17	0.14
Sensitive skin	0.09	0.10	0.08	0.12	0.04	0.06	0.15	0.21

Table 24(a): Mean of observation of antimicrobial against copper nanoparticles

Mean Value		
Sample	Intial	Final
Blank	0	0
Combination skin	0.05	0.04
Oily skin	0.04	0.02
Scalp	0.12	0.10
Acne skin	0.10	0.08
Sensitive skin	0.09	0.12

Copper nanoparticles show the antimicrobial effect in every skin type except Sensitive skin. The turbidity of the sanative skin sample is increased after 24 hour incubation at 37^0 C.

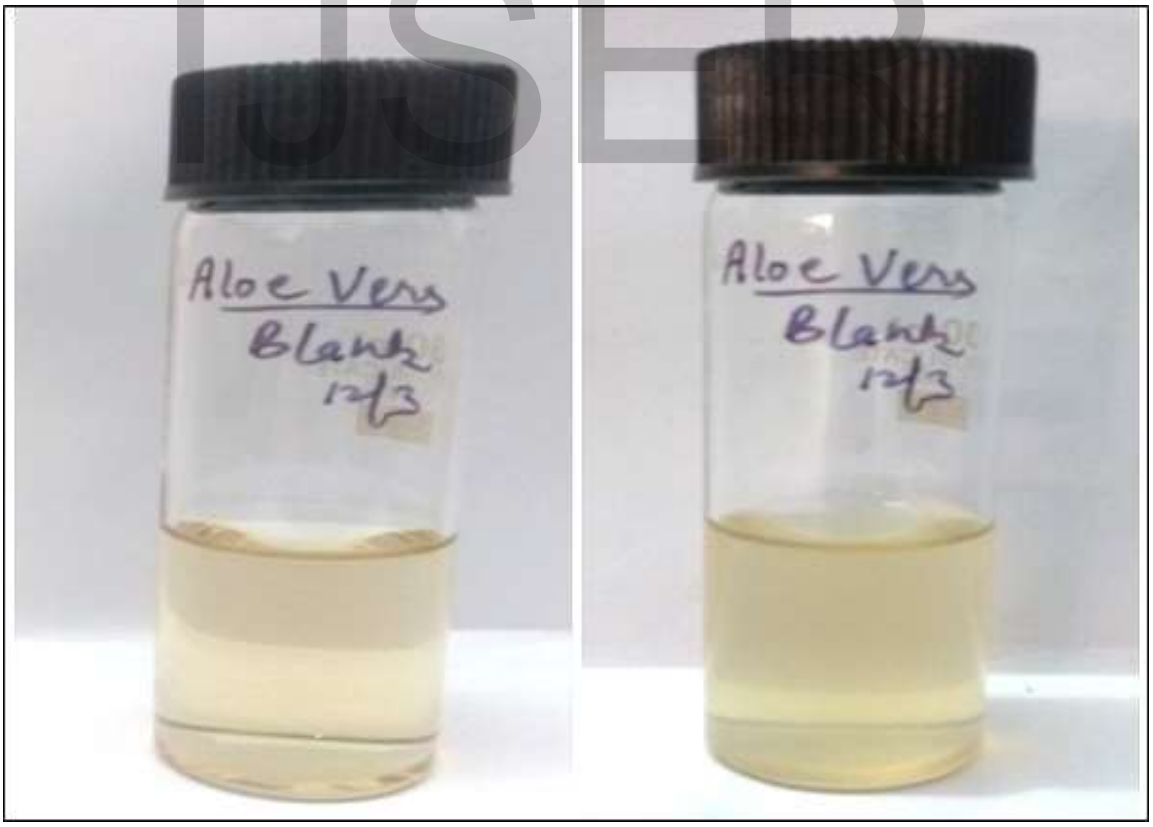

Figure 96: Observation of the decrease in turbidity (transparency) of Blank sample after 24 hour incubation at 37^0 C

Figure 97: Observation of the decrease in turbidity (transparency) of sample in Combination skin after 24 hour incubation at 37^0 C

Figure 98: Observation of the decrease in turbidity (transparency) of sample in Oily skin after 24 hour incubation at 37^0 C

Figure 99: Observation of the decrease in turbidity (transparency) of sample in Scalp after 24 hour incubation at 37^0 C

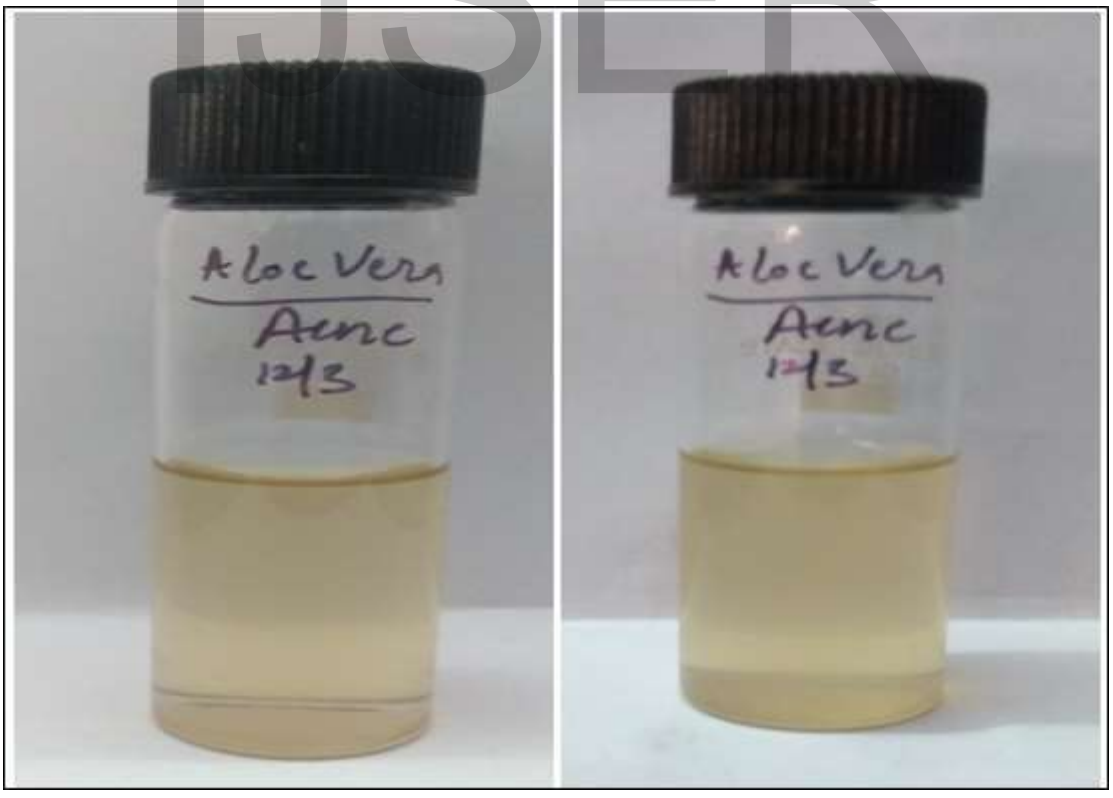

Figure 100: Observation of the decrease in turbidity (transparency) of sample in Acne skin after 24 hour incubation at 37^0 C

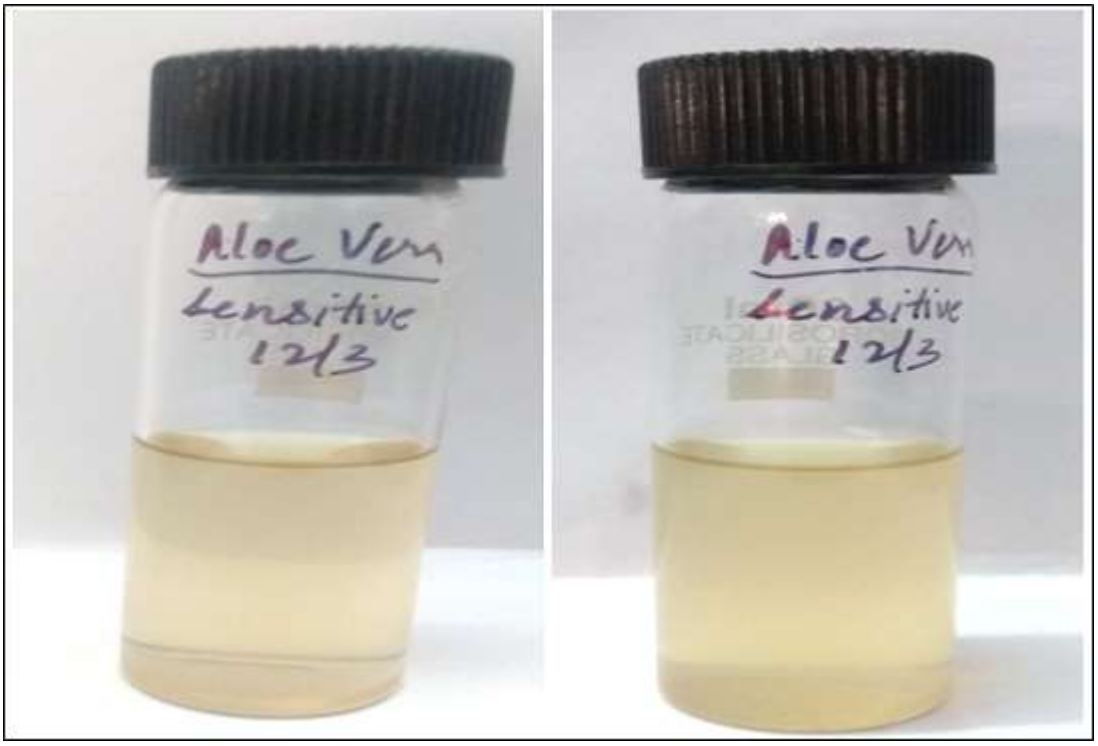

Figure 101: Observation of the increase in turbidity (transparency) of sample in Sensitive skin after 24 hour incubation at 37^0 C

CHAPTER-5

CONCLUSION

Nanobiotechnology has emerged up as integrated between biotechnology and nanotechnology for developing biosynthesis and environment friendly technology for the synthesis of nanomaterial. In this study copper nanoparticles was synthesized from the plant extract of *Aloe barbadensis*. This plant has been used extensively in curing wide variety of health problems. Biological synthesis of nanoparticles involves natural phenomenon that takes place in biological systems. Biological method is cheap, fast and eco-friendly method as compared to the physical and chemical method and evaluated as very good choice of antimicrobial agents due to continuous increase in emergence and re-emergence of multidrug resistance pathogens. In the present study, sample of extract was prepared by treating it by Cold Percolation method and maintained at pH 9 and 100°C. Bioreduction of Cu was observed when 2.5 ml of extract was augmented with CuSO4.5H2O and kept at pH 9 and 100° C.

It was concluded that plant extract of *Aloe barbadensis* peel obtained by Cold Percolation Method using triple distilled water as a solvent at pH 9 and 100°C, uniform number of Copper nanoparticles were synthesized and Optical density is observed at 540 nm and Optical Density increase from 1.29 nm initially to 1.66 nm finally. Colour of the nanoparticles is changed from light green to dark green after regular interval of 1 hour. These synthesized copper nanoparticles were first preliminary confirmation by Colorimeter.

It was concluded that after regular interval of 1 hour, number of copper nanoparticles synthesized increases due to Surface Plasmon resonance.

Since the Optical Density of sample increase with increase in time with double distilled water and 70% ethanol as a solvent; these samples were used for the confirmatory analysis for the presence of copper being a metal ion and copper nanoparticles was performed with the help of pellets developed from extract suspension and was performed using Energy Dispersion X-Ray Spectrometer (EDS), Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) that showed topper as a true metal ion and size of the copper nanoparticles with extract respectively. As confirmed the TEM test, the size of the synthesized copper nanoparticles is ranging from

27 nm to 35 nm and with test of SEM, the size of synthesized nanoparticles is ranging from 113 nm to 210 nm.

In order to determine the antimicrobial activity by Turbidimetric Analysis againt Skin Flora of human face by taking swabs from Combination skin (oily+Dry), Oily skin, Scalp, Acne skin, Sensitive skin. Microbes are identified using different identification method (i.e. staining, differential media and biochemical test). Possible bacteria are:

- *Staphylococcus aureus, Staphylococcus epidermidis, Corynebacterium, Propionibacterium* are present prominently on the **Combination skin**
- *Corynebacterium, Propionibacterium* are present prominently on the **Oily skin**
- *Escherichia coli* is present on the **Scalp**
- *Corynebacterium, Propionibacterium* are present prominently on the **Acne skin**
- *Staphylococcus aureus, Staphylococcus epidermidis* are present prominently on the **Sensitive skin**

For Turbidimetric analysis 10 ml of Nutrient broth Media is augmented with 2.5 ml of bacterial culture and 2.5 ml of synthesized nanoparticles mixed and optical density is observed at 540 nm using Colorimeter and incubate for 24 hour at 37^0 C and final optical density is observed. Optical density decreased that shows the bacterial growth is reduced in all skin types except Sensitive Skin, because the Optical Density is increased in Sensitive Skin.

CHAPTER-5

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