Kasdi Merbah University – Ouargla

Faculty of Natural and Life Sciences

Department of Biological Sciences

Final Year Thesis

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Field: Natural and Life Sciences

Major: Biology

Specialty: Applied Microbiology

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Theme:

Rapide biosynthesis of silver nanoparticle using bacteria :Characterization ,optimization , antioxydant and antimicrobial activity

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In front of the jury composed of:

Mr HENNI Abdellah	**Pr**	**President**
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Appreciate

We would like to express our gratitude to:
God for assisting us in completing this work.
God Almighty for granting us good health and the determination to carry out this project.

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Greeting

I have the honor to dedicate this modest work accomplished with the help of the Almighty God.

*To my beloved **grandparents**, may God grant you health and a long life*

*To my dear **father** and my dear **mothe**r.*

*To my aunt **Nesrine**.*

*To my aunt **Hassiba**.*

*To my dear sisters **Mayar, Aya, Meriem**, and **Nora**. To my dear brother **Yahia**, and to my dear cousin **Omar***

*To all the members of my family **DJAFRI** and **HANINE**.*

*To my dear aunt **Djahida**.*

*To my friend and partner: **Amina.***

*To my friends **Maria, Loubna, Roua ,Wail***

To my colleagues from the 2022 Applied Microbiology M2.

Rayan

Greeting

I dedicate this humble work to:

*My dear **parents**, for all their sacrifices, love, support, and prayers throughout my academic and university life. May God grant your health and a long life**.***

*To my sisters **Sabrina, Meriem, Nariman**, and my little sister **Nessma**, and my brother **Amine**.*

*To my dear **Hamza**, **Dania**, **Eline**, and **Islam**.*

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*To my aunt **Samira**.*

*To my friend and my partner **Rayan**.*

*To my friends **Maria, Loubna, Roua ,Wail***

To my colleagues from the 2022 Applied Microbiology M2

Amina

List of abbreviations

Ag	Silver
Ag^+	Silver ions
$AgNO_3$	Silver nitrate
AgNPs	Silver nanoparticle
AuNPs	Gold nanoparticles
CFU	Colony Forming unit
°C	Degrees Celsius
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDX	Energy-dispersive X-ray analysis
E.coli	*Escherichia coli*
Fe_3O_4	Iron Oxide
FTIR	Fourier-transform infrared spectroscopy
HIV-1	Human immunodeficiency virus-1
H_3N_2	Influenza A virus
HSV-2	Human herpes simplex virus 2
IC_{50}	Half maximal inhibitory concentration
K^+	Potassium ions
KeV	kiloelectron volts
K.pneumoniae	*Klebsiella pneumonia*
LPS	Lipopolysaccharide
LSPR	Localized surface plasmon resonance

MBC	Minimum bactericidal concentrations
mg	Milligram
MH	Muller-Hinton
MIC	Minimum inhibitory concentration
Ml	Milliliter
mM	Millimolar
mm	Millimeters
MRSA	Methicillin-resistant Staphylococcus aureus
MRSE	Methicillin-resistantStaphylococcus epidermidis
NADH	Nicotinamide adénine dinucléotide (NAD) + hydrogène (H)
NB	Nutritive Bouillon
nm	Nanometer
NO^-_2	Nitrite ion
NO^-_3	Nitrate ion
NPs	Nanoparticles
OD	Optical Density
PEG	Polyethylene glycol
P.aeruginosa	*Pseudomonas aeruginos*
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
rpm	Revolutions per minute
SEM	Scanning electron microscope
S.aureus	Staphylococcus aureus
SiO_2	Silicon dioxide
SPR	Surface Plasmon Resonance
TiO_2	Titanium dioxide

Ul	Microliter
μM	Micromolar
μm	Micrometer
UV	Ultraviolet
XRD	X-ray diffractometry
ZnO	Zinc oxide
Λ	Wavelength
%	Percent
Θ	Theta angle
°	Degrees

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General Introduction

Nanotechnology, introduced by Nobel laureate Richard P. Feynman in 1959, has emerged as an interdisciplinary science focusing on the fundamental properties of nano-sized objects. Nano refers to one billionth or 10^9 units, indicating the small scale of these objects. In the context of nanoscience and nanotechnology, nanoparticles are clusters of atoms ranging in size from 1 to 100 nm. Nanotechnology has found applications in various fields such as biomedicine, renewable energy, cosmetics, and mechanics**(Sanchez and Sobolev 2010; Abou El-Nour et al. 2010)**

Metallic nanoparticles, such as silver nanoparticles (AgNPs), exhibit unique physical, chemical, and biological properties due to their high surface-to-volume ratio. Silver nanoparticles have gained significant attention due to their biomedical applications, including antimicrobial properties and potential uses in food packaging, biosensors, and water decontamination. Nanoparticle synthesis can be achieved through physical, chemical, and biological approaches. Among these, green synthesis using biological methods has become increasingly popular due to its environmental friendliness, cost-efficiency, and scalability. The synthesis and characterization of nanoparticles, including AgNPs, play a crucial role in exploring their unique properties and potential applications. The biological mechanisms and cytotoxicity of AgNPs are important considerations for their medical applications. Overall, nanomaterials, with their distinct properties and advancements, have revolutionized various industries and are at the forefront of nanotechnology research **(Santos et al. 2021)**.

Nanoparticles can be synthesized using plants and microbes, with intracellular ectracellular nanoparticle formation occurring in bacteria and fungi . Nanomedicine holds great potential for transforming global disease treatment, particularly in the context of animal classification. Silver nanoparticles (AgNPs) possess unique physical and chemical properties, including optical, electrical, thermal, and biological characteristics. These properties have led to their widespread use in various industries, such as medicine, food, consumer products, and manufacturing. AgNPs have diverse applications as antibacterial agents, medical device coatings, optical sensors, cosmetics, drug delivery systems, and anticancer agents. They are extensively employed in textiles, wound dressings, keyboards, and biomedical devices **(Carrapiço et al. 2023; Xu et al. 2020).**

Silver nanoparticles (AgNPs) possess unique physical and chemical properties, making them highly valuable in healthcare, medicine, and industry. Evaluating AgNPs involves analytical techniques such as X-ray diffractometry (XRD), ultraviolet-visible spectroscopy (UV-vis spectroscopy), scanning electron microscopy (SEM), and localized surface plasma resonance (LSPR) **(Salleh et al. 2020a)** .These analyses provide insights into

the behavior, bio-distribution, and reactivity of AgNPs. The physicochemical properties of AgNPs, including size, composition, crystallinity, shape, and structure, can be modified through synthesis methods, reducing agents, and stabilizers **(Pourali et al. 2013).** Size plays a crucial role in the biological properties of AgNPs and can be tailored for specific applications. The toxicity of AgNPs is size-dependent, with smaller particles exhibiting higher toxicity due to increased reactivity and ion release in cells **(Huang et al. 2020)**. The shape of AgNPs also influences their toxicity, with different nanostructures like nanoplates, nanospheres, nanorods, and flower-like nanoparticles exhibiting varying effects. AgNPs are widely used in antimicrobial applications due to their proven antimicrobial characteristics. Understanding the physicochemical properties of AgNPs is vital for their cellular uptake, distribution, and therapeutic effects. Developing AgNPs with uniform morphology and functionality is crucial for diverse biomedical applications. However, it is important to consider the potential nano-toxicity of silver nanoparticles, as further studies are needed to fully understand their environmental and health impacts. Overall, the controlled development of AgNPs with consistent structures and properties holds significant potential for various biomedical applications, enhancing the bioavailability of therapeutic agents and influencing cellular uptake, distribution, and therapeutic outcomes **(A. S. Kumar et al. 2020; Tashi, Gupta, and Mbuya 2016).**

L'objectif de cette étude est de caractériser les propriétés des nanoparticules d'argent biosynthétisées à partir du surnageant de culture des bactéries Staphylococcus aureus, en mettant l'accent sur l'optimisation et l'étude de leurs activités antibactérienne et antioxydante.

Chapter I: general view

I.1.Nanotechnology

Dreams and human imagination often give rise to new science and technology. Nanotechnology, which is the latest technology in the 21st century, was born out of this dream. Although human exposure to nanoparticles has continued throughout human history, it increased dramatically during the Industrial Revolution. The search for nanoparticles is not new. The concept of "nano" was first proposed by Richard Zigmondi, a Nobel Prize winner in chemistry in 1925. He coined the term "nano," which clearly refers to the size of particles measured in one-billionth or 10^9 units, and was the first to use a microscope to measure The particle size is like gold colloids **(Hulla, Sahu, and Hayes 2015).**

Nanotechnology can achieve material characteristics by size control. The understanding and control of the material between 1 and 100 nanometres have caused unique phenomena and new applications of nanomaterials. Metal nanoparticles have extraordinary optical, thermal, chemical and physical characteristics. The decrease in material size has a significant impact on physical characteristics, and these characteristics are significantly different from the corresponding large amounts of materials **(Hulla, Sahu, and Hayes 2015)**.

Some physical properties displayed by nanomaterial return to larger surface, surface energy, spatial restrictions and the reduction of defects. The biological characteristics of these particles provide researchers with the ability to reasonably design and use nanoparticles as drugs, as image comparison media and diagnostic purposes **(Pande and Bhaskarwar 2016).**

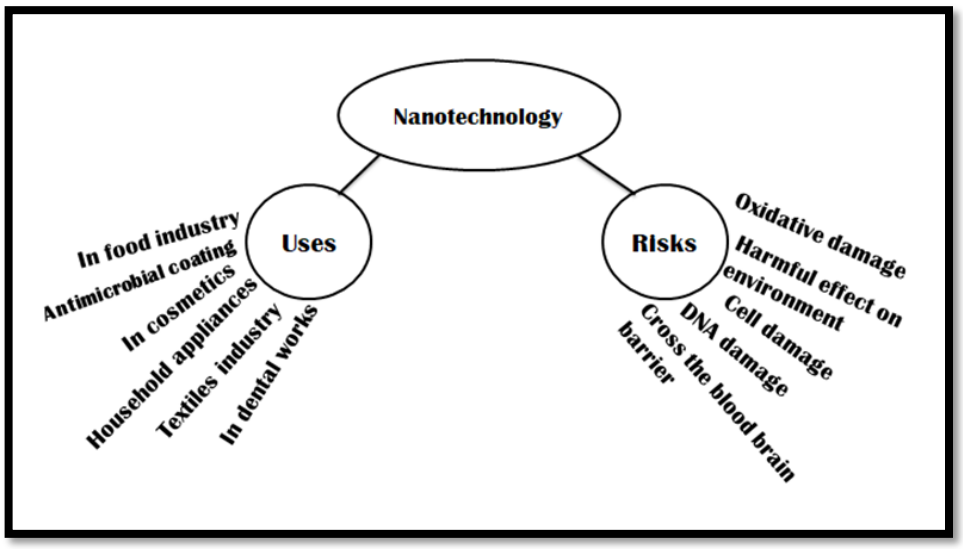


Figure 1*:** Uses and risks of nanotechnology(Jaswal and Gupta 2021)***

I.1.1. Applications of green nanotechnology

A. Application of nanotechnology in industrial wastewater treatment

Nanotechnology is used in the treatment of wastewater, drinking water, and groundwater remediation. However, potential risks to the environment and health have led to the emergence of green nanotechnology, which aims to manage these risks while promoting responsible development and application of nano-enabled products. Nanomaterials are small in size and can consist of various structures. Green nano-products are used to prevent harm from known pollutants and are integrated into environmental technologies to remediate hazardous waste sites, clean up polluted streams, and desalinate water. It is important for technological vision and scientific understanding to continue to progress to meet the challenges of the future **(Kanchi and Ahmed 2018).**

B.The vision of renewable energy technologies

Renewable energy technologies such as solar, wind, and wave energy are considered visionary technologies for a sustainable and cleaner future. However, the pursuit of these technologies faces challenges such as industrial pollution, environmental disasters, and loss of biodiversity. To address these challenges, science must be re-envisioned using approaches such as green nanoscience and nanotechnology. The true emancipation of renewable energy technology requires a wider vision and a deep understanding of the challenges it faces **(Kanchi and Ahmed 2018).**

C.Future research trends and flow of thoughts

Green nanotechnology and renewable energy technologies offer sustainable solutions for our planet. Through sustainable chemistry and engineering, these technologies are set to revolutionize our way of life. Future research will focus on opening up new areas of green nanotechnology, creating more efficient renewable energy technologies, and solving global water issus. By continuing to advance our understanding of nanotechnology, we can adress the most pressing environmental challenges and create a more sustainable future **(Kanchi and Ahmed 2018).**

I.2. Nanoparticles

The term "nanoparticles" generally refers to materials with all external dimensions in the nanoscale range, typically between 1 and 100 nm. However, if the dimensions of a nanomaterials differ significantly, terms such as "nanofibers" or "nanoplates" may be used instead of "nanoparticles" **(Joudeh and Linke 2022; Mohanraj and Chen 2007).**The International Organization for Standardization (ISO) defines nanoparticles as nano-objects with all three external dimensions in the nanoscale range, where the longest and shortest dimensions do not differ significantly. This definition is important for standardizing the use and characterization of nanoparticles in various applications, including medicine, energy, and electronics **(Joudeh and Linke 2022).**

Nanoparticles have unique properties due to their size and surface area, and they can be used in various drug delivery systems. Nanoparticles can be used to improve drug solubility, protect the drug from degradation, and target specific cells or organs. Additionally, nanoparticles can be engineered to release drugs in a controlled manner, which can improve the efficacy and reduce side effects of the drug. Biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymers such as polyethylene glycol PEG , have shown promise as drug delivery systems due to their ability to circulate in the body for a prolonged period of time and target specific organs **(Mohanraj and Chen 2007).**

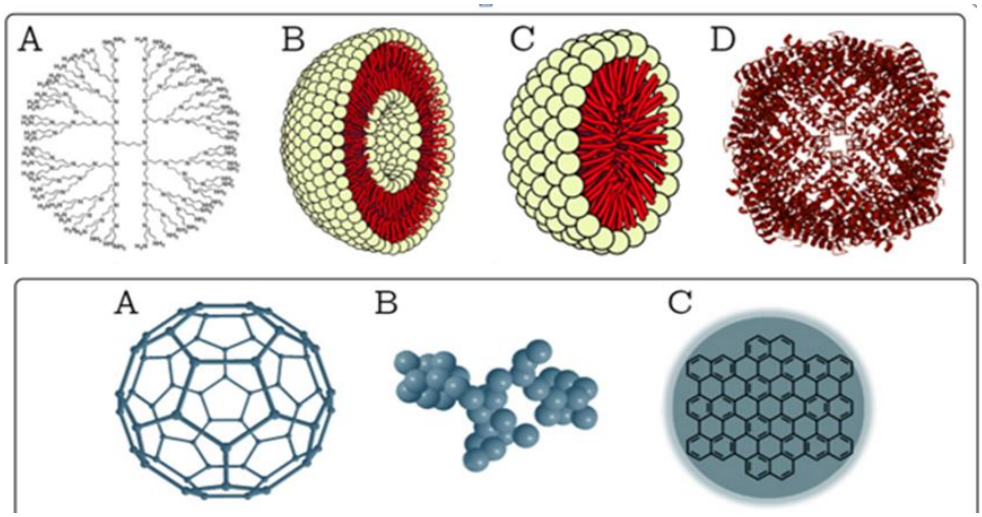


Figure 2: The different types of NPs ***(Joudeh and Linke 2022)***

I.2.1. Proprieties of NPs

A. Physicochemical properties of NPs

As discussed earlier, various physicochemical properties such as large surface area, , mechanical strength, optical and chemical activity make NPs unique and suitable condidate for various applications**. (Joudeh and Linke 2022)**

B. Electronic and optical properties

Noble metal nanoparticles (NPs) exhibit interdependent optical and electronic properties. Due to their size, these NPs have a strong UV-visible extinction band which is not present in the bulk-metal spectrum. This phenomenon is called localized surface plasmon resonance (LSPR) and occurs when the incident photon frequency matches the collective excitation of the conduction electrons. LSPR excitation results in wavelength-selective absorption, resonance Rayleigh scattering, and enhanced local electromagnetic fields near the surface of NPs. The peak wavelength of the LSPR spectrum depends on the size, shape, and interparticle spacing of the NPs, as well as its own dielectric properties and those of its local environment. Gold and silver colloidal NPs are examples of noble metal NPs that have different colors due to the properties of the free electrons on their surface. Noble metal NPs are widely used in nanotechnology for their unique optical, electrical, and catalytic properties **(Joudeh and Linke 2022).**

C. Magnetic properties

Magnetic NPs have unique properties due to their size and composition, making them suitable for various applications. The magnetic properties of NPs are dominated by the size of the particle, with particles smaller than 10-20 nm being the most effective. **(Joudeh and Linke 2022).** The electronic distribution in NPs contributes to their magnetic properties, and different synthetic methods can be used to prepare them. Some applications of magnetic NPs include catalysis, biomedicine, magnetic fluids, data storage, MRI, and environmental remediation **(Joudeh and Linke 2022).**

D. Mechanical properties

The mechanical properties of NPs play a important role in biomedical applications such as drug delivery, tissue engineering, and diagnostic imaging. The rigidity of NPs can affect their cellular uptake, and their adhesion properties can impact their interaction with biological tissues **(Joudeh and Linke 2022)..** For instance, NPs with a softer surface are believed to be more easily taken up by cells than stiffer ones. Furthermore, the mechanical properties of NPs can be engineered by altering their composition, size, and shape, making them promising candidates for biomedical applications. Overall, the unique mechanical properties of NPs make them very important for various fields and can be refined for specific needs **(Joudeh and Linke 2022).**

E. Thermal properties

In addition to their enhanced thermal conductivity, the use of nanofluids in heat transfer applications offers several advantages such as lower pumping power requirements, reduced pressure drop, improved heat transfer coefficient, and increased critical heat flux. These advantages make nanofluids suitable for various applications such as nuclear reactors, electronics cooling, automotive engines, and solar collectors. However, there are also some challenges associated with the use of nanofluids such as the agglomeration of nanoparticles, instability of suspensions, and potential for erosion of heat transfer surfaces. Therefore, careful design and selection of nanofluids and their application conditions are required to realize their full potential in heat transfer applications **(Joudeh and Linke 2022).**

I.2.2. Application of nanoparticle

NPs, due to their above-mentioned unique or enhanced physicochemical properties, are used in a wide range of applications in different fields. In addition, several potential applications are in research and development. Here we present some examples of these applications **(Joudeh and Linke 2022).**

A. Medicine and pharmaceutical

Metallic and semiconductor NPs have great potential for various medical applications such as cancer therapy, targeted drug delivery, cellular imaging, biosensors, medical diagnostics, and as antimicrobial and antibacterial agents in medical products. Further research and development in this area could lead to more efficient and effective medical treatments and products **(Joudeh and Linke 2022).**

B. Electronics

Nanoparticles have potential applications in optoelectronics, especially in the realm of solar cells. Dye-sensitized solar cells (DSSCs) offer a promising alternative to traditional silicon-based solar cells due to their lower cost and simpler manufacturing process. TiO2 and ZnO nanoparticles are frequently utilized as electron acceptors in DSSCs owing to their high electron mobility and low recombination rates. Plasmonic nanoparticles like silver and gold can also be used to boost light absorption in solar cells through the localized surface plasmon resonance (LSPR) effect **(Joudeh and Linke 2022).**

C. Agriculture

Nanoparticles can also play a crucial role in the field of environmental remediation in agriculture, particularly in addressing the issue of heavy metal contamination in soil, which can lead to lower crop yields and pose potential health hazards to consumers. Research has shown that nanoparticles such as iron oxide (Fe_3O_4) and titanium dioxide (TiO_2) are effective in removing heavy metals from contaminated soil through phytoremediation. **(Joudeh and Linke 2022).**

D. Food industry

NPs have potential applications in food production and processing. For example, TiO_2 NPs have been used to remove unwanted compounds such as pesticides, herbicides, and heavy metals from food products, such as fruits, vegetables, and tea leaves. This is achieved through the photocatalytic degradation of these compounds by TiO_2 NPs. Similarly, Fe_3O_4 NPs have been used to remove aflatoxins, toxic fungal metabolites that contaminate food products such as peanuts, maize, and cereals, through magnetic separation. Furthermore, the use of NPs such as ZnO and SiO_2 in food processing can enhance the functional properties of food, such as improving the texture, stability, and shelf-life of products such as cheese, yogurt, and baked goods. However, it is important to ensure the safety of NPs in food-related applications, as there are concerns about their potential toxicity and long-term effects on human health. **(Joudeh and Linke 2022).**

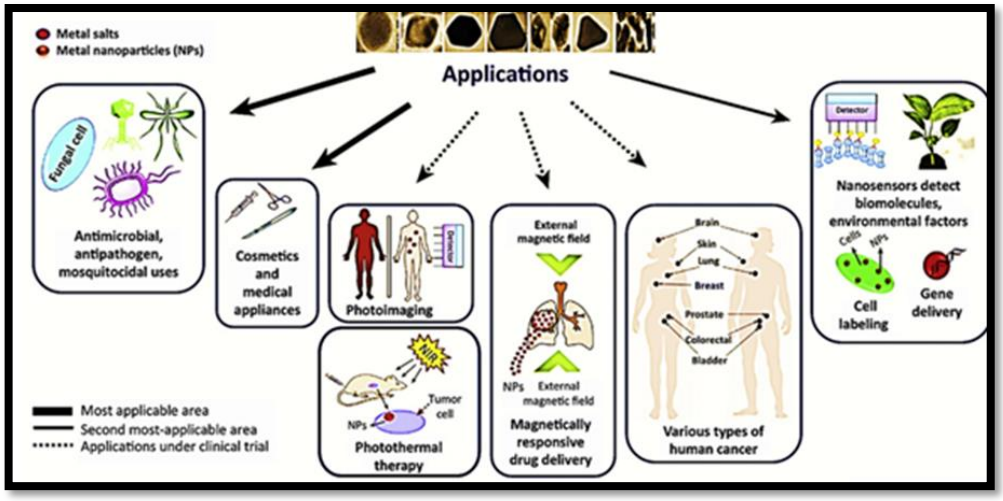


Figure 3*:** An illustration showing the biological synthesis of nanoparticles from microorganisms or plant tissue and several applications of metal nanoparticles ***(Shnoudeh et al. 2019)

I.3. Silver nanoparticles

Depending on their size, ranges from 1 to 100 nm shape, and morphology, nanoparticles exhibit novel properties that allow them to interact with plants, animals, and microorganisms Silver nanoparticles (Ag-NPs) exhibit excellent bactericidal properties against a variety of microorganisms **(Siddiqi, Husen, and Rao 2018).** They are prepared from different angles, usually to study their morphology or physical properties. Some authors have used chemical methods confusing it with green synthesis, although they did so unintentionally. The Ag NPs and their applications in electronics, catalysis, pharmaceuticals, and controlling microbial development in biological systems make them environmentally friendly Bacteria, fungi, yeast, actinomycetes, and plant extracts are involved in the biosynthesis of Ag NPs **(Siddiqi, Husen, and Rao 2018; Zhang et al. 2016).**

I.3.1 History

The employment of silver bowls to keep water and other drinks pure for long periods was a common practice in ancient civilizations, such as the Greeks, as reported by Herodotus, or the Romans who kept wine in silver containers to avoid moldering. The empiric knowledge that such practice could prevent festering and decomposition, and probably led to the custom of using silverware and cutlery by the wealthy throughout time **(Medici et al. 2019).**

The use of silver in various fields was a common practice in ancient civilizations, such as in medicine where it was used to treat wounds, infections, and as a germicide. Colloidal silver was widely used in hospitals in the early 20th century and was considered effective and safe. Although its use declined during the antibiotic era, it has recently become popular as an "alternative" medicine for certain pathologies, although most are only presumed and not scientifically verified. Silver salts have been used in the past as antibacterial agents and to treat mental illnesses and nicotine addiction.

Silver colloids have been used in the medical field for over 100 years, as shown by the use of "Collargol" in 1897. The first biocidal silver product, "Algaedyn," was registered in the U.S. in 1954 and is still used in disinfectants today. The development of nanotechnology in the past two decades has allowed for the exploration of new applications for AgNPs, which exhibit novel properties due to their nano-scale dimensions. Bacteriamediated AgNP synthesis was first reported in 1999 when AgNPs were found to accumulate inside cells of Pseudomonas stutzeri **(Yu, Yin, and Liu 2013)**.

I.3.2. Properties

The physical and chemical properties of AgNPs, such as surface chemistry, size, shape, particle morphology, composition, coating, agglomeration, dissolution rate, and particle reactivity, are important factors in determining their cytotoxicity. Smaller particles with larger surface areas can be more toxic than larger particles. The shape of AgNPs can also influence their toxicity. Biological reducing agents can be used to synthesize AgNPs in different shapes, such as spherical, rod, octagonal, and flower-sharped. The availability of chemical and biological coatings on the nanoparticle surface can also affect their toxicity. The surface charge of AgNPs can play a role in their toxicity, with positively-charged NPs being more suitable for long-term administration in the bloodstream. Overall, further research is needed to fully understand the potential risks and benefits of AgNPs **(Abbasi et al. 2014)**.

I.3.3. Synthesis of silver nanoparticle

The production process of Ag NPs can be classified into two main methods: top-down and bottom-up strategies. Top-down approach involves breaking down the bulk materials to create the desired nanostructures, while the bottom-up method involves assembling single atoms and molecules into larger nanostructures. Additionally, synthesis techniques can be categorized into physical, chemical, and biological methods. **(Poudel et al. 2022)**

I.3.3.1. Physical methods

Physical methods require high temperatures and can result in the formation of large aggregates, which can affect the properties of the nanoparticles and their applications. Therefore, physical methods are mostly suitable for industrial-scale production, where large quantities of nanoparticles are required, and the application requirements are less stringent **(Abou El-Nour et al. 2010; Zhang et al. 2016)** .

I.3.3.2. Chemical methods

This paerty describes the various chemical methods used for the synthesis of Ag nanoparticles. Chemical methods involve the use of organic solvents and typically include metal precursors, reducing agents, and stabilizing agents. The reduction of silver salts involves two stages: nucleation and subsequent growth. Chemical methods can be classified as "top-down" or "bottom-up" methods. The major advantage of chemical methods is high efficiency, although some of the materials used for AgNP synthesis, such as citrate, borohydride, thio-glycerol, and 2-mercaptoethanol, are toxic and hazardous. Chemical methods can use techniques such as cryochemical synthesis, laser ablation, lithography, electrochemical reduction, laser irradiation, sono-decomposition, thermal decomposition, and chemical reduction. **(Zhang et al. 2016)**

The advantages of chemical methods are the ease of production, low cost, reproducibility and high efficiency. However, the use of chemical reducing agents can be harmful to living organisms. To regulate the production of metal nanoparticles, small nanoparticles that have a spherical form with confined diameter dissemination are essential. Silver nanoparticles may be manufactured at a minimal expense, which is very well known. The stabilizing agents, such as dodecanethiol, play an important role in the stability of colloidal dispersion, which protects the system from crystal growth, coalescence, and agglomeration **(Bayda et al. 2019).**The size, shape, morphology, polydispersibility index, self-assembling, and zeta potential of the synthesized nanoparticles can be affected by a small change in parameters. Commonly used ingredients in the synthesis of AgNPs and AuNPs are glycol derivatives polyvinyl pyrrolidone (PVP) and polyethylene glycol (PEG) . **(Abou El-Nour et al. 2010).** Polyacrylamide can also play a dual function as a reducing and stabilizing agent in the synthesis of AuNPs. Surfactants containing functional groups such as amines, thiols, and acids play an important role in the stability of colloidal dispersion. The modified Tollens method utilizes saccharides and silver hydrosols and reducing agents to yield AgNPs

in the range of 50–200 nm and 20–50 nm, respectively, for AuNPs **(Abou El-Nour et al. 2010; Zhang et al. 2016).**

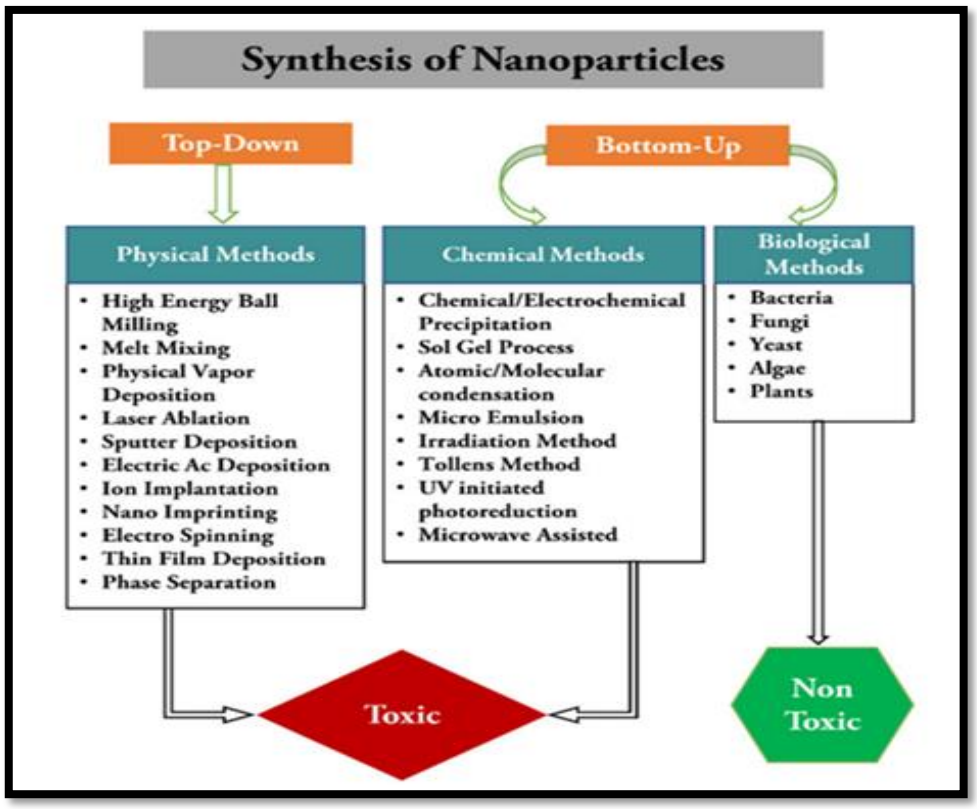


***Figure 4*:** Different methods used for synthesis of nanoparticles **(Raj, Trivedi, and Soni 2021)**

I.3.3.3. Biological methods

Biosynthesis techniques uses natural reducing agents such as polysaccharides, microorganisms (like bacteria and fungi), or plant extracts have gained significant attention recently. These methods involve using biological sources to reduce the silver ions into silver nanoparticles **(Natsuki 2015)**.

Biosynthesis is a simple and cost-effective method for synthesizing silver nanoparticles using natural reducing agents such as bacteria. Bacteria can produce several substances both inside and outside the cell, making them a suitable candidate for large-scale production of nanoparticles. Biotechnology is also used to develop the biosynthesis of AgNPs. Biological synthesis involves the reduction of metal ions by combined efforts of herbs, enzymes, proteins, micro-organisms, algae, bacteria and fungi **(Singh et al. 2015; Abou El-Nour et al. 2010)**.

A. Plant

The main advantage of using plant extracts for the synthesis of AgNPs is that they can be used as reducing and stabilising agents simultaneously. nanoparticles with a size range of 10-40 nm and good stability. The extract contained flavonoids, terpenoids, and tannins, which were found to be responsible for the reduction of silver ions and stabilisation of the nanoparticles.

Overall, biosynthesis of AgNPs using plant extracts has several advantages, including their biocompatibility, availability, low toxicity, and eco-friendliness. Moreover, the use of plant extracts allows for the synthesis of nanoparticles with a narrow size distribution and controlled shape. However, it is important to note that the choice of plant extract and the conditions of the synthesis process can have a significant impact on the size, shape, and stability of the nanoparticles produced **(Cao 2017; Arif and Uddin 2021).**

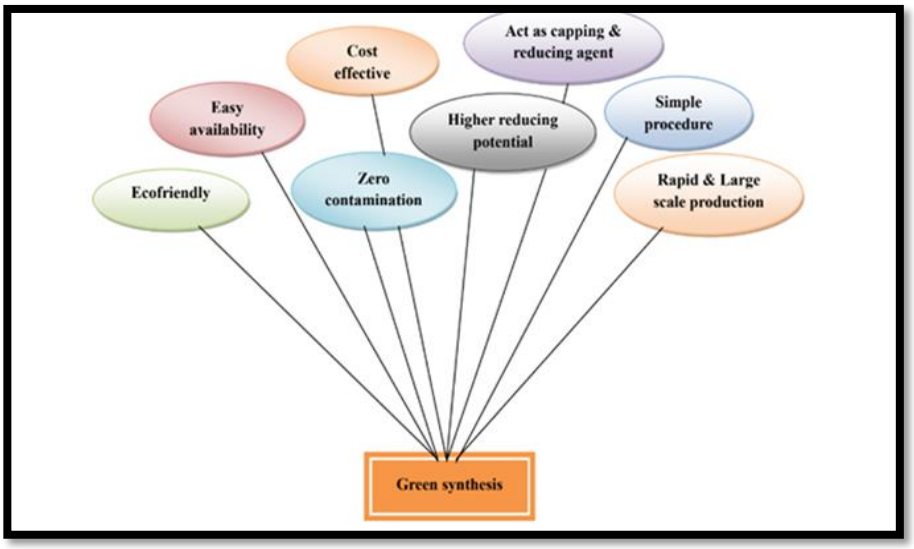


Figure 5*:** Advantages of green synthesis of AgNPs by plant extracts ***(Haneen Ali Jasim 2020).

B. Algae

Algae have been found to be useful in the synthesis of silver nanoparticles through the release of proteins or metabolites that reduce silver ions. This process leads to the nucleation and growth of silver nanoparticles that can act as powerful antibacterial agents against various pathogenic bacteria. The use of algae offers a quick and low-cost method for the synthesis of silver nanoparticles, as the nucleation and growth of crystals are accelerated due to the negative charge on the surface of algal cells. Different types of algae, such as *Chorella vulgaris*, *Pithophora oedogonia*, *Caulerpa racemosa*, *Spirogyra*, and marine macro-algae, have been utilized for the synthesis of silver nanoparticles. The silver nanoparticles

synthesized using algae have been found to have various sizes and shapes, and their stability and functionality have been confirmed using various analytical techniques such as UV-Vis spectroscopy, SEM, DLS, EDX, FTIR, XRD, and MIC/MBC assays.**(Poulose et al. 2014; Kanchi and Ahmed 2018; Raj, Trivedi, and Soni 2021; Arif and Uddin 2021)**

C. Fungi

Fungi have gained attention for their potential in the synthesis of silver nanoparticles due to their ability to secrete enzymes and proteins that can reduce and stabilize metal nanoparticles. Fungi are more efficient in this process compared to bacteria, leading to higher yields of nanoparticles. Many types of fungi, such as *Aspergillus sp.*, *Fusarium sp.*, and *Penicillium sp.*, can synthesize silver nanoparticles both intracellularly and extracellularly. Intracellularly formed nanoparticles are smaller in size compared to extracellularly formed nanoparticles. The selective accumulation and absorption of silver by certain fungi have also been reported. Extracellular synthesis of nanoparticles has many applications due to the negation of redundant adjoining cellular machineries from the cell. The synthesized nanoparticles are stabilized by secretory proteins. Different strains of fungi form different sizes and shapes of nanoparticles, including spherical, rods, hexagons, cubes, and stars **(Cao 2017; Poulose et al. 2014; Kanchi and Ahmed 2018; Raj, Trivedi, and Soni 2021).**

D. Yeast

The yeast strain MKY3 has the ability to produce silver nanoparticles extracellularly, this is observed by the formation of a characteristic black precipitate after 24 hours of contact between a supernatant of yeast and silver ions. The shape of the silver nanoparticles was found to be hexagonal, mostly twinned or multi-twinned, face-centered structures with particle sizes ranging from 3 to 8 nm. The broad peak observed in X-ray diffraction analysis suggests high polydispersity of the nanoparticles, with a relation between peak width and nanoparticle radius indicating an inverse relationship.

To recover the silver nanoparticles, an apparatus was designed in which the sample was allowed to freeze at -20 C, causing the denser silver nanoparticles to settle. The apparatus was then thawed to drive the settled silver nanoparticles through the sides of the apparatus, where they were collected **(Poulose et al. 2014).**

E. Bacteria

Klaus and his team reported the initial discovery of bacterial-mediated synthesis of silver nanoparticles (AgNP) in 1999, where they observed AgNP aggregation within cells of *Pseudomonas stutzeri* AG259, a bacterium obtained from a silver mine **(Siddiqi, Husen, and Rao 2018 ;Raj, Trivedi, and Soni 2021).** It is clear that the bacterial synthesis of nanoparticles involves both nanotechnology and biotechnology. The process originated from a biosorption study of metals with different bacterial strains, but the nanoscale production of silver was only launched in the age of nanotechnology. Bacterial cells are used as biomachinery for the synthesis of AgNPs, which can occur through either the intracellular or extracellular route. Bioreduction processes are mainly involved in the microbial production of nanoparticles, and extracellular reductase enzymes of microorganisms reduce the silver ions and form particles in the nanometer-size range. The size and shape of nanoparticles can be controlled by these bacterial strains. The effect of silver ion concentration was studied, and it was found that higher concentrations of silver ions are sensitive for the production of AgNPs, but beyond 10 mM, the organism undergoes cell death within minutes. The passage also provides a list of organisms that were reported for the synthesis of AgNPs **(Cao 2017).**

Table 1: Size and shape of silver nanoparticles using bacteria ***(Siddiqi, Husen, and Rao 2018; Chokriwal, Sharma, and Singh, n.d.)***

Bacteria	Size (nm)	Shape
Pseudomonas aeruginosa	15.1 ± 5.8	Spherical
Bacillus cereus	50–80	Spherical
Gordonia amicalis	5–25	Spherical
Streptomyces coelicolor	28–50	Irregular
Rhodopseudomonas sps.	6-10	

I.4. General Characteristics of *Staphylococcus aureus*

Staphylococci are a type of bacteria that are typically round-shaped, or cocci, and appear purple when viewed under a microscope after being stained with Gram stain. They tend to grow in clusters, resembling bunches of grapes, which is where the name derives from. They can be found in various environments, including on the skin and mucous membranes of humans and animals. *Staphylococcus aureus* is a specific type of staphylococcus that is commonly found on the skin and mucous membranes. It can produce various toxins that can cause a range of syndromes, including food poisoning. Staphylococci

are usually non-motile and do not form spores. They are facultative anaerobes, meaning they can survive in both the presence and absence of oxygen, and they are catalase-positive, which means they can break down hydrogen peroxide into water and oxygen **(Abou El-Nour et al. 2010; Abbasi et al. 2014).**

I.4.1. History

Staphylococci were first observed in the late 1800s by Robert Koch and Louis Pasteur. It was during this time that Rosenbach isolated Staphylococcus aureus in pure culture for the first time. The observation under the microscope revealed "grape-like clusters" in the pus of boils. It was first observed by Robert Koch in 1878 and recognized by Louis Pasteur two years later. In 1881, Alexander Ogston isolated the bacterium from post-operative abscesses and reproduced the infection in animals. These clusters were the origin of the name he gave it, Staphyle meaning grape cluster in Greek. OGSTON thus differentiated Staphylococcus from Streptococcus. It was in 1884 that Anton Rosenbach cultivated Staphylococcus in vitro and described the first known species: *Staphylococcus aureus*, or Golden Staph, named after the color of the colonies obtained in culture **(Rasheed and Hussein 2021; Bernier-Lachance, n.d.; Abbasi et al. 2014; Fetsch 2018).**

I.4.2. Habitat

Staphylococcus bacteria are widely distributed and can survive in various environments, including the natural outdoor environment and the skin and mucous membranes of humans and animals. Humans are the main reservoir of these bacteria, whether they are healthy or sick carriers. Staphylococci can be found in various sites of the human body, such as the nasal cavities, intestine, skin, and glandular annexes. They can also contaminate surfaces, air, and water. Transmission of *Staphylococcus* bacteria is primarily through direct human-to-human contact or by contact with contaminated objects. The most common site of colonization for *S. aureus* is the human nose, but it can also be found on the skin, hair, and mucous membranes. Although most resident bacteria do not cause disease, nasal carriage of *S. aureus* is strongly associated with infection, and a small percentage of carriers may develop *S. aureus* infections. **(Brown et al., 2013).**

I.4.3. Taxonomy

According the 9th edition of Bergey's Manual of Systematic Bacteriology, Staphylococcus are classified as follows:

- Domain : Bacteria
- Division: *Firmicutes*
- Class: Bacilli
- Order: Bacillales
- Family: *Staphylococcaceae*
- Genus: *Staphylococcus*

Through analysis of the 16S ribosomal RNA gene, the family *Staphylococcaceae* was established in 2002, which includes the genus Staphylococcus as well as *Gemella*, *Macrococcus*, *Jeotgalicoccus*, and *Salinococcus*. Within the genus Staphylococcus, there are 47 species and 24 subspecies, of which 17 are commonly found in humans.

I.5. Applications of silver nanoparticles

AgNPs are extensively utilized in different industries, ranging from household utensils and healthcare to food storage, environmental, and biomedical applications, owing to their exceptional properties. The main emphasis of the review is on the biological and biomedical applications of AgNPs, which include their potential roles as antibacterial, antifungal, antiviral, anti-inflammatory, anti-cancer, and anti-angiogenic agents.

I.5.1. Antibacterial

AgNPs, or silver nanoparticles, are being explored as a potential alternative to antibiotics for their ability to overcome bacterial resistance. Their unique properties, such as their large surface-to-volume ratio and crystallographic surface structure, contribute to their antibacterial activity, with smaller particles and specific shapes being more effective. AgNPs have been shown to effectively combat various pathogenic bacteria and yeast, and can be synthesized using different methods, including biological synthesis. They work by disrupting bacterial membranes, leading to cell death through the release of reducing sugars and proteins. AgNPs also exhibit anti-biofilm properties against Pseudomonas aeruginosa and Staphylococcus epidermidis, which are implicated in ocular-related infectious diseases such as microbial keratitis **(Abbasi et al. 2014; Richa Singh et al. 2015; Zhang et al. 2016; Wei et al. 2015).**

I.5.2. Antiviral

The antiviral activity of silver nanoparticles (AgNPs) against various viruses, including HIV-1, herpes simplex virus, influenza virus, and others. While the exact

mechanism of their antiviral activity is still unclear, studies suggest that AgNPs can inhibit viral entry and adsorption to cells. AgNPs have also been found to improve mice survival after infection with H3N2 influenza virus and reduce HSV-2 infectivity both in vitro and in vivo. Ag+ alone and in combination with carbonate ions have also been shown to have effective antiviral properties against bacteriophage MS2 phage. Treatment with AgNPs has also been found to decrease virus concentration, percentage of infection, and disease severity in Bean Yellow Mosaic Virus (BYMV). Although AgNPs show potential as antiviral agents, further research is necessary to fully understand their mechanisms of action **(Zhang et al. 2016; Khatoon, Mazumder, and Sardar 2017).**

I.5.3. Anti-inflammatory

AgNPs have anti-inflammatory properties and can reduce colonic inflammation in rats, improve cosmetic appearance in mice, and reduce wound inflammation. They are also effective against antimicrobial properties and can modulate fibrogenic cytokines. AgNPs can down-regulate the quantities of inflammatory markers and suppress inflammatory events in the early phases of wound healing. In a porcine contact dermatitis model, treatment with nanosilver increases apoptosis in inflammatory cells and decreases the levels of pro-inflammatory cytokines. Biologically-synthesized AgNPs can inhibit cytokine production induced by UV-B irradiation and reduce edema and cytokine levels in paw tissues. However, more research is needed to fully understand the mechanisms of action of AgNPs as anti-inflammatory agents **(Zhang et al. 2016).**

I.5.4. Anticancer

Several studies have investigated the use of nanomaterials, particularly silver nanoparticles (AgNPs), for anticancer therapy. These studies explored the molecular mechanisms and anticancer activities of AgNPs in various cell lines and found that they can induce apoptosis, alter cell morphology, reduce cell viability and metabolic activity, increase oxidative stress, cause mitochondrial damage, and generate reactive oxygen species (ROS) that can lead to DNA damage. Researchers also experimented with different carrier molecules and formulas to deliver AgNPs to cancer cells, and biologically synthesized AgNPs exhibited significant toxicity in different cancer cells, with the ability to target cell-specific toxicity. Although these findings suggest that nanomaterials, particularly AgNPs, hold potential for anticancer therapy, more research is needed to determine their safety and effectiveness **(Zhang et al. 2016).**

I.5.5. Antidiabetics

The prevalence of diabetes is on the rise worldwide and it is an intriguing disease. Type 1 diabetes, caused by the total lack of insulin secretion and damage to pancreatic β-cells by autoimmune response, tends to be more common among relatives of affected individuals. On the other hand, Type 2 diabetes, accounting for 90% of cases, is initiated by a combination of insulin resistance and reduced insulin secretion. Researchers have investigated the potential of silver nanoparticles produced using stem extract of Tephrosia tinctoria to regulate blood sugar levels. These nanoparticles were found to scavenge free radicals, decrease levels of enzymes that catalyse the hydrolysis of complex carbohydrates (α-glucosidase and α-amylase), and improve glucose consumption rates **(T. Galatage et al. 2021).**

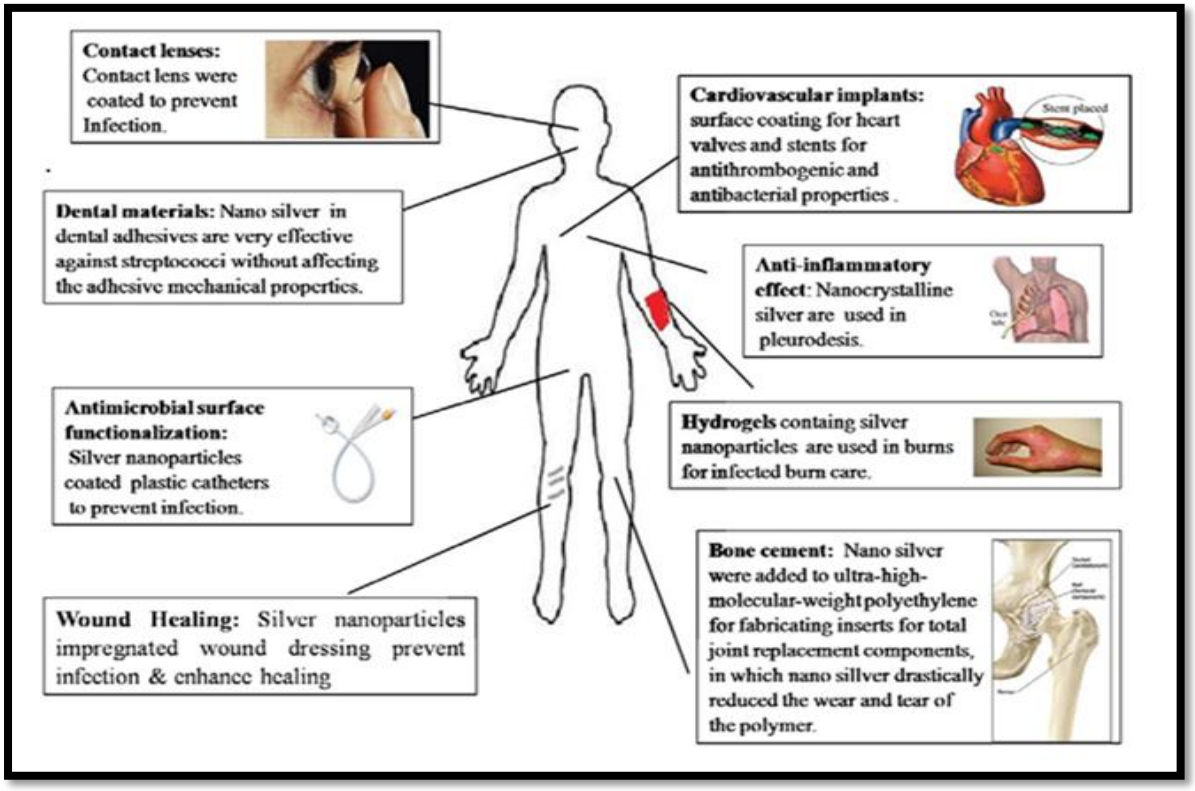


Figure 6*:** Biomedical application of silver nanoparticles ***(Khatoon, Mazumder, and Sardar 2017).

I.6.Toxicity of Ag NPs in Humans

Recent research has focused on investigating the impact of silver nanoparticles (Ag NPs) on human cells, specifically keratinocytes, lung fibroblasts, glioblastoma cells, and mesenchymal stem cells. Findings indicated that higher concentrations of Ag NPs can cause cytotoxicity and oxidative stress, which may damage proteins, lipids, and DNA, leading to

cell apoptosis. Additionally, the accumulation of Ag NPs in cells can potentially harm the mitochondrial membrane and cause DNA damage. However, in vivo acute/subacute toxicity data on mice suggested a low level of toxicity, with only a reduced number of mast cells detected on the skin. Nonetheless, further research on the safety of Ag NPs for humans is required **(Nakamura et al. 2019).**

Silver nanoparticles are widely used in various industries, including biomedical applications such as cell imaging, drug delivery, and implantation. However, some studies have reported potential adverse effects of these nanoparticles on humans and the environment. In particular, in vitro toxicity assays on rat liver cells have demonstrated that even low levels of exposure to silver nanoparticles (10-50 μg mL^{-1}) can cause oxidative stress and impair mitochondrial function. Higher doses (> 1.0 mg L^{-1}) have been found to induce significant cytotoxicity, resulting in abnormal cellular morphology and shrinkage. While these studies suggest that silver nanoparticles can have harmful effects, in vivo toxicology studies have reported comparatively less toxicity. Therefore, further research is necessary to fully understand the potential toxicity of silver nanoparticles in vivo and their impact on humans and animals **(Kanchi and Ahmed 2018).**

Chapter II. Materials and methods

II.1 Place and duration of studies

This research is being conducted at the Technical Platform for Physicochemical Analysis (PTAPC - CRAPC Ouargla). CRAPC is a specialized institution that primarily focuses on research activities. The primary goals of this study include the advancement of novel physical and chemical analytical techniques, as well as the development of innovative protocols for chemical synthesis and application. The research was carried out from February 12, 2023, to May 28, 2023. The goals of this study is to use biosynthesis techniques to elaborate silver nanoparticles (AgNPs) by bacteria, followed by their characterization. Additionally, the study includes optimization experiments and investigates the antibacterial, photocatalytic, and antioxidant activities of the synthesized AgNPs.

II.2 Biological material

For this study, three reference strains from the American Type Culture Collection (ATCC) were used. *Staphylococcus aureus* (ATCC13300) was employed for the synthesis of silver nanoparticles. Additionally, *Staphylococcus aureus* (ATCC25923) and *Pseudomonas aeruginosa* (ATCC 9027) were used to evaluate the antibacterial activities of the synthesized nanoparticles.

II.3 Methods for synthesis of silver nanoparticles

II.3.1 Synthesis of silver nanoparticles

The test was carried out using the protocol described by **(Hassan Javed Chaudhary 2012)** with modifications**.**

II.3.1.1 Revival of strains

The strains preserved in glycerol were revived as follows: an inoculum of the reference strain was seeded in 5 ml of nutrient broth followed by incubation at 37°C for 24 hours. The results were evaluated by the appearance of turbidity in the nutrient broth medium **(Annexe 2).**

II.3.1.2 Sub-culturing and purification

The revived strains were purified by alternate sub-culturing on nutrient broth medium and streaking onto nutrient Agar by making successive streaks until obtaining well-defined and identical colonies, then incubated at 37°C for 24 hours to obtain pure strains.

II.3.1.3 Biosynthesis of silver nanoparticles

In this method, a flask containing 250 ml of nutrient broth was prepared and inoculated with *Staphylococcus aureus*. The inoculated broth was then incubated at 37°C for 24 hours and agitated at 150 rpm in an orbital shaker. Once turbidity became evident in the nutrient broth medium, the culture solution was centrifuged at 4000 rpm for 20 minutes to separate the supernatant from the sediment (culot). The obtained supernatant was then transferred to a clean 250 ml flask and utilized for the synthesis of silver nanoparticles (AgNPs) **(Rajeshkumar.S et al 2013) .**

The bacterial supernatant was mixed with a 1 mM solution of silver nitrate ($AgNO_3$), which was prepared by dissolving $AgNO_3$ in distilled water. The mixture consisted of 1/4 bacterial supernatant and 3/4 metal solution. The obtained solution were kept in incubator for 24h at 37°C under string (200 rpm).Finally, the solutions were exposed to light until a noticeable change in the colour of the solution to dark brown was observed **(Hassan Javed Chaudhary 2012; Chaudhari et al. 2012).**

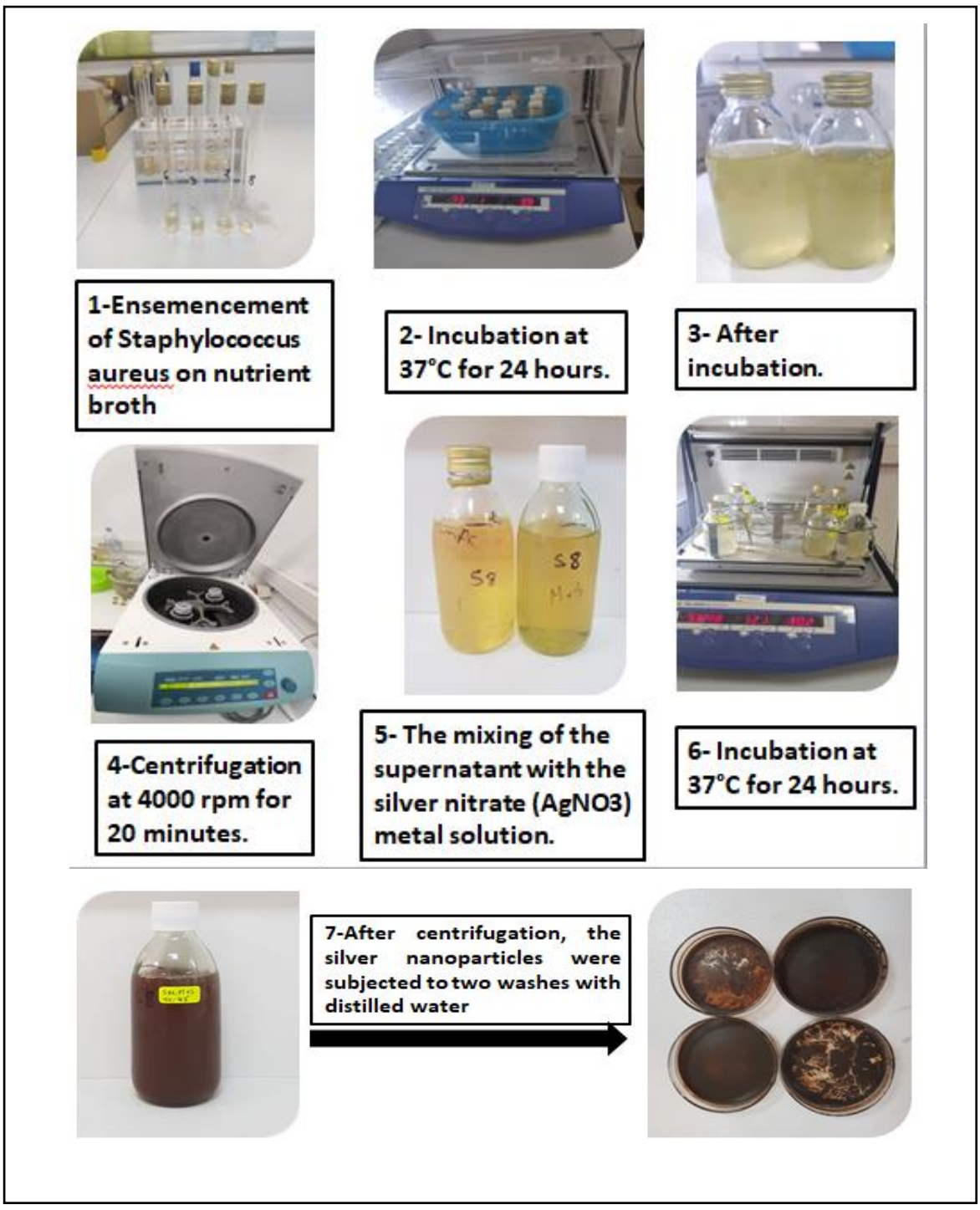


***Figure 7*:** Steps of silver nanoparticle synthesis

II.3.1.4 Silver nanoparticles purification

The AgNPs solution obtained after 24 hours was centrifuged at 4000 rpm for 20 minutes. The resulting precipitate (culot) was carefully washed with distilled water through centrifugation at 4000 rpm for 10 minutes. This washing process was repeated twice. After centrifugation, a small quantity of ethanol was added to the precipitate, and the mixture was dried in an oven. The purified AgNPs were obtained in the form of powder after drying **(Arif and Uddin 2021).**

II.3.2 Characterization of nanoparticles

Characterization is a crucial step in the study of nanoparticles (NPs) after their green synthesis, as it enables the determination of their shape, size, and surface properties **(Madkour, L. H. 2018).** The physicochemical characteristics of NPs have a significant impact on their behaviour, their distribution in biological systems, their safety and their efficacy.. Therefore, the characterization of AgNPs is essential for evaluating the functional aspects of the synthesized particles. Various analytical techniques, including UV-vis spectroscopy, X-ray diffractometry (XRD), and scanning electron microscopy (SEM) are employed for characterization. Extensive literature, including books and reviews, offers comprehensive information on the principles and applications of these techniques for AgNPs characterization. To ensure clarity, let's delve into the fundamental principles of these important techniques used in AgNPs characterization **(Poulose et al. 2014; Patil and Chougale 2021).**

II.3.2.1 Spectroscopie UV-visible

The UV-visible spectrometer utilizes two lamps, namely the deuterium lamp and the tungsten lamp, to emit light across the UV-Visible wavelength range. Following incubation, the resulting solution (supernatant mixed with the metallic solution of concentration 1 mM) was analyzed by UV-visible spectroscopy for the characterization of synthesized silver nanoparticles. This involved measuring the absorption or transmission of light in the UV-visible range, absorption was recorded at wavelengths ranging from 200 to 800 nm. **(Zhang et al. 2016; Khatoon, Mazumder, and Sardar 2017)**. The obtained UV-visible spectra were then analyzed to identify characteristic absorption peaks or shifts, which offer insights into the size, shape, and stability of the silver nanoparticles **(Cao 2017).** The absorbance characteristics of the particle suspensions were studied using a Cary 100 UV-VIS spectrophotometer (Agilent Technologies).

II.3.2.2 X-ray diffractometry (XRD)

X-ray diffractometry (XRD) is a commonly employed technique for characterizing materials, including biosynthesized silver nanoparticles**(Khatoon, Mazumder, and Sardar 2017)**. It involves the use of an X-ray diffractometer, which consists of an X-ray source, a sample holder, and a detector. The X-ray source emits X-rays that interact with the prepared sample, resulting in diffraction patterns. In the case of biosynthesized silver nanoparticles, the nanoparticles are prepared by drying and grinding them into a fine powder to ensure a uniform sample **(Panja 2021)**. This powdered sample, usually around 3 mg of AgNPs, is then analyzed using X-ray diffraction (XRD) with a D8 Advance. By examining the resulting diffraction patterns, valuable information about the crystal structure, crystallinity, and phase composition of the nanoparticles can be obtained **(Zhang et al. 2016; Pourali et al. 2013).**

This characterization technique helps researchers understand the properties and composition of the biosynthesized silver nanoparticles**.**

II.3.2.3 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is a widely used technique for the characterization of materials, including biosynthesized silver nanoparticles. SEM allows for high-resolution imaging of the surface morphology and structure of the nanoparticles**.** In the characterization process, the biosynthesized silver nanoparticles are typically collected and prepared for SEM analysis. This involves depositing a thin film of the nanoparticles onto a conductive substrate, such as a carbon-coated grid or a metal stub. The sample is then placed in the SEM chamber, where it is subjected to a beam of electrons. The scanning electron microscopy images presented in this work were obtained at the (PTAPC CRAPC) Ouargla using a ZEISS (EVO 15) microscope **(Zhang et al. 2016).**

II.3.3 Optimization of silver nanoparticles

In these experiments, and in order to reach the best synthesis conditions for the highest yield of AgNPs, serval parameters are tested; like concentration of $AgNO_3$, volume and light effect (Rose et al. 2019a). These several factors that affect the, shape and size of metal nanoparticles **(Mohamed T 2018; Peiris et al. 2018).**

II.3.3.1. Effect of AgNPs concentration

In this experiment different concentrations of $AgNO_3$ solution (0,2; 0,5 ; 0,8 ; 1 and 10mM) that used with 1:4 of supernatant and the absorbance of the solution was monitored

using the UV-Visible spectrophotometer **(Mohamed T 2018; Peiris et al. 2018; Huang et al. 2020).**

II.3.3.2. Effect of volume

In order to optimize the distilled water/supernatant ratio, a 1 mM AgNO3 solution was added to a 10 ml bottle. The rations of the experiment are given below.

V1:10 ml of solution (culture supernatant and silver nitrate solution, 100%)

V2: 08 ml of solution with 2ml of distilled water (80%)

V3: 5 ml of solution with 5ml of distilled water (50%)

V4: 2 ml of solution with 8ml of distilled water (20%)

Culture supernatant without silver nitrate was kept as control. These were incubated for 24 h in rotary shaker at 100 rpm. The silver nanoparticles formed were characterized. The absorbance of the solution was monitored using the UV-Visible spectrophotometer.

II.3.3.3. Effect of light

The influence of light on the rate of nanoparticles synthesis was investigated by preparing a mixture of 1:4 culture supernatant and 1mM silver nitrate. This mixture was then divided into two flask samples. The first flask was exposed to sunlight, while the second flask was keep in the dark.

II.3.4 Antioxidant activity

The antioxidant activity of biosynthesized AgNPs was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) based on free radical scavenging assay (RSA), following a modified method of **(Safawo et al. 2018)**. In this assay, 1500 mL of 0.1 mM DPPH solution was mixed with 50 mL of biosynthesized AgNPs at various concentrations (1, 0.5, 0.25, 0.125, 0.06 mg/mL). The mixture was incubated in the dark for 30 minutes, and the absorbance was measured at 517 nm using a UV-Visible spectroscopy. A control solution containing 1500 mL of DPPH and 50 mL of ethanol was used, as well as a blank solution containing 1500 mL of ethanol and 50 mL of NPs. Ascorbic acid was used as a standard antioxidant for comparison **(Mohd Yusof et al. 2020)**. The percentage of DPPH scavenging activity was calculated using the following formula **(Shanmugasundaram et al. 2013):**

[(Control OD – Sample OD)/ Control OD] × 100.

II.3.5 Antibacterial activity

In our research, we will explore the efficacy of AgNPs against different bacterial strains. AgNPs have shown promising biocidal properties, effectively targeting a wide range of bacteria, including both Gram-negative and Gram-positive species .**(Richa Singh et al. 2015; Rajan, Cherian, and Baskar, n.d.)**

II.3.5.1. Growth medium used

The bacterial suspension of the tested strains is diluted using a nutrient broth (NB) medium, while the action of AgNPs against the described strains is evaluated using the Muller Hinton medium, which serves as a suitable culture medium.

II.3.5.2. Tested bacterial strains

The tested strains are: *Staphylococcus aure*us (ATCC13300) and *Pseudomonas aeruginosa* (ATCC 9027).

II.3.5.3. Taxonomy and characteristics of the tested bacterial strains

II.3.5.3.1. *Pseudomonas aeruginosa*

P. aeruginosa is a ubiquitous bacterial species**.** Among the commonly encountered Gram-negative bacilli responsible for hospital-acquired infections **(Cross et al. 1983)**, also opportunistic pathogen **(Thomas et al. 1983)**.

Pseudomonas aeruginosa is a heterotrophic, motile, Gram-negative rod-shaped bacterium about 1–5 μm long and 0.5–1.0 μm wide. It is a facultative aerobe that grows via aerobic respiration and anaerobic respiration with nitrate as the terminal electron acceptor. The organism can utilize over 100 organic molecules as a source of carbon and/or energy and as a prototroph, generally has the ability to grow on a minimal salts growth medium with a single source of carbon and energy **(Diggle and Whiteley 2020).**

P. aeruginosa grows well at 37 °C, but it can survive in broad temperatures ranging from 4 to 42 °C and also resistant to many classes of antibiotics and therapeutic agents, and this makes it problematic during infection as it can be difficult to treat. It is often termed an ‘opportunistic’ pathogen because it rarely infects healthy individuals **(Alhazmi 2015).**

II.3.5.3.2. *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, aerobic and facultative anaerobic **(Bernier-Lachance, n.d.)** and causative agent of wide range of infectious diseases such as skin infections,and food poisoning **(Gnanamani, Hariharan, and Paul-Satyaseela 2017; Fetsch 2018).**

S. aureus cells are appeared in spherical shape. They are often in clusters resembling bunch of grapes when observed under light microscope after Gram staining. The diameter of the cells ranges from 0.5 to 1.0 μM. It is the leading cause of bacteremia, pneumonia, myocarditis, acute endocarditis, pericarditis, osteomyelitis, encephalitis, meningitis, chorioamnionitis, mastitis, and scalded skin syndrome. Has catalase and coagulase **(Karmakar, Dua, and Ghosh 2016).**

II.3.5.3 Diffusion method on agar medium

The antibacterial activity of biosynthesized AgNPs was assessed using the agar well diffusion method against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) pathogens **(Samundeeswari et al. 2012)**. The biosynthesized AgNPs, which were in powder form, were first suspended in sterilized deionized water. Overnight cultures of each pathogen were prepared in nutrient broth at 37 °C, and sterilized swabs were used to streak the pathogens onto nutrient agar plates. The optical densities of the overnight cultures were adjusted to 0.2 OD (0,5 Mc Farland 10^6 ufc). Wells were then created in the agar using a sterile borer, and approximately 10 □l of the biosynthesized AgNPs at a concentration of 1 mM was added to each well. The plates were incubated at 37 °C for 24 hours, and the diameter of the resulting inhibition zones was measured (Nanda and Saravanan 2009a). The experiment was performed in triplicates to ensure accuracy and reliability of the results **(Sousa-Pedrares et al. 2010; Riaz Rajoka et al. 2020; Bayda et al. 2019; Boudghene-Guerriche et al. 2020).**

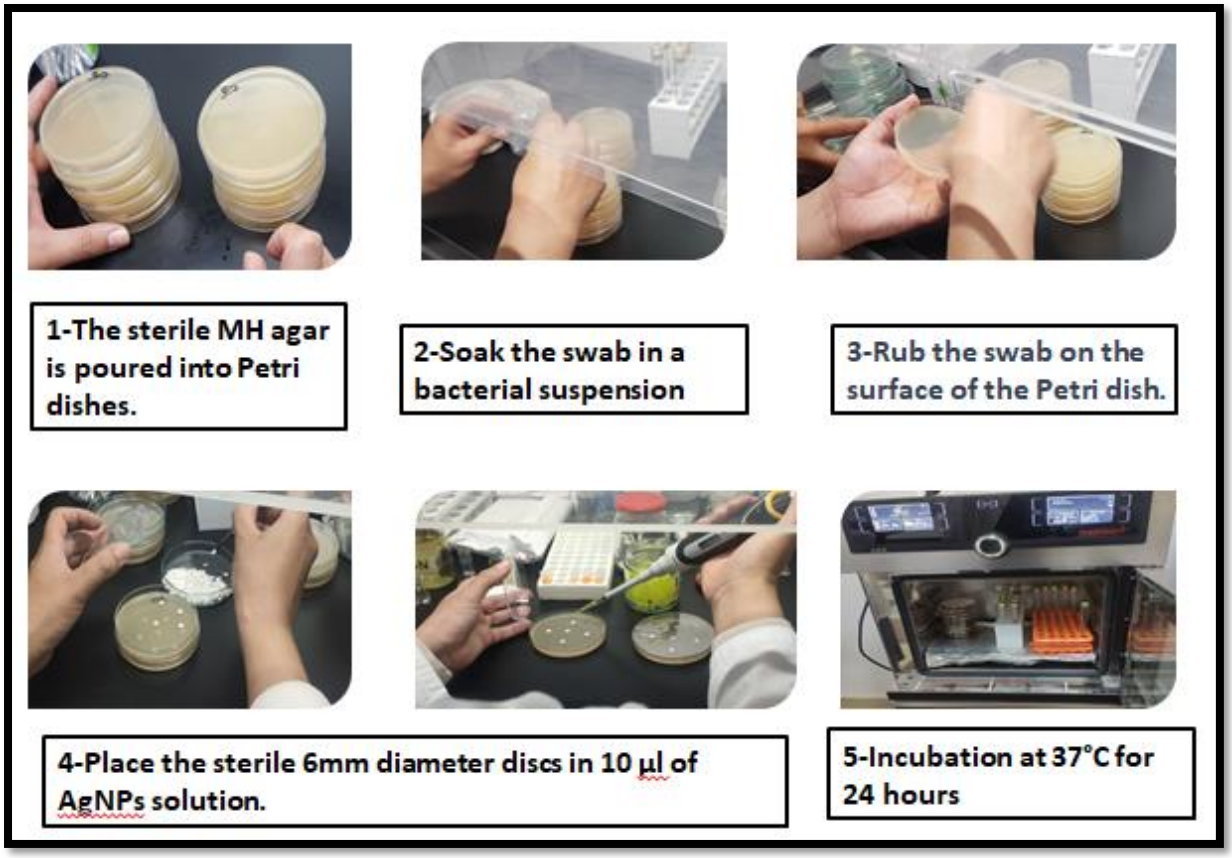


***Figure 8*:** The steps for the antibacterial activity of silver nanoparticles

Chapter III. Discussion and results

III.1. Biosynthesis of silver nanoparticles

This study focused on the extracellular biosynthesis of silver nanoparticles using the culture supernatant of *Staphylococcus aureus* by mixing the culture supernatant with a 1 mM silver nitrate solution in a 1:4 ratio. After incubation at 37°C for 24 hours, a distinct change in colour from yellow to brown have been observed. This colour transformation is commonly considered to be indicative of nanoparticle synthesis see the **(*Figure 9*).**

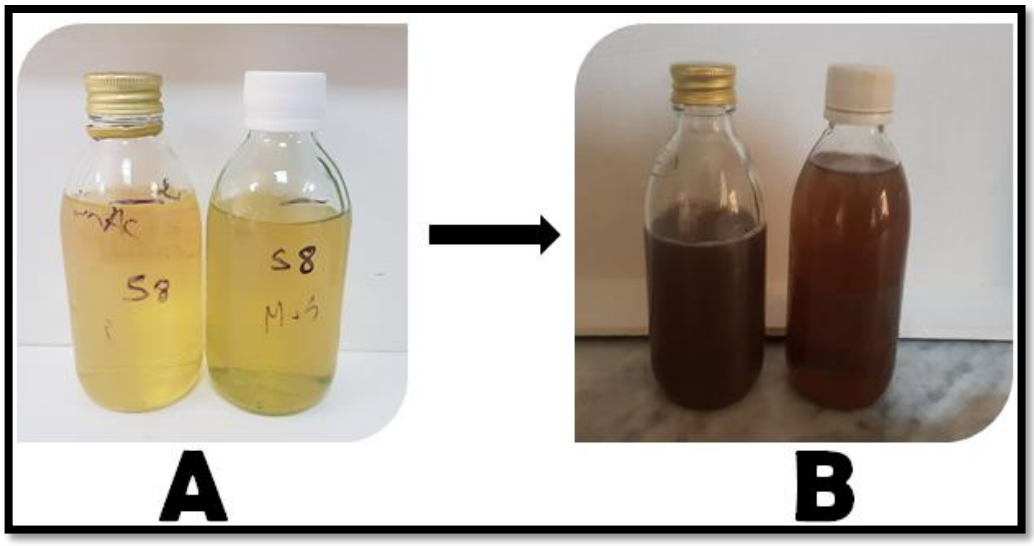


***Figure 9*: A**: culture supernatant with $AgNO_3$ solution (no color change) **B**: culture supernatant with $AgNO_3$ solution after synthesis (with color change)

The brown color observed in the silver nanoparticles is attributed to the excitation of surface plasmon vibrations **(Das et al. 2014)**. Previous studies have also reported a similar color change from pale yellow to brown in the supernatant of *Staphylococcus aureus* **(*Figure11*)**. This indicates the bacterial ability to reduce Ag^+ ions to their elemental form Ag^0, which accumulates outside the cells and promotes the formation of silver nanoparticles (AgNPs). This phenomenon was confirmed in a study conducted by **(Javaid et al. 2018a)**The presence of Ag nanoparticles was verified by analyzing the UV-visible spectra of the cell filtrate treated with $AgNO_3$ **(Javaid et al. 2018b)**. The spectra exhibited a distinctive and broad peak in 435 nm which is a characteristic feature of the surface plasmon resonance (SPR) band associated with silver nanoparticles **(Figure 10) (Rose et al. 2019b; Saifuddin, Wong, and Yasumira 2009a; Liaqat et al. 2022).**

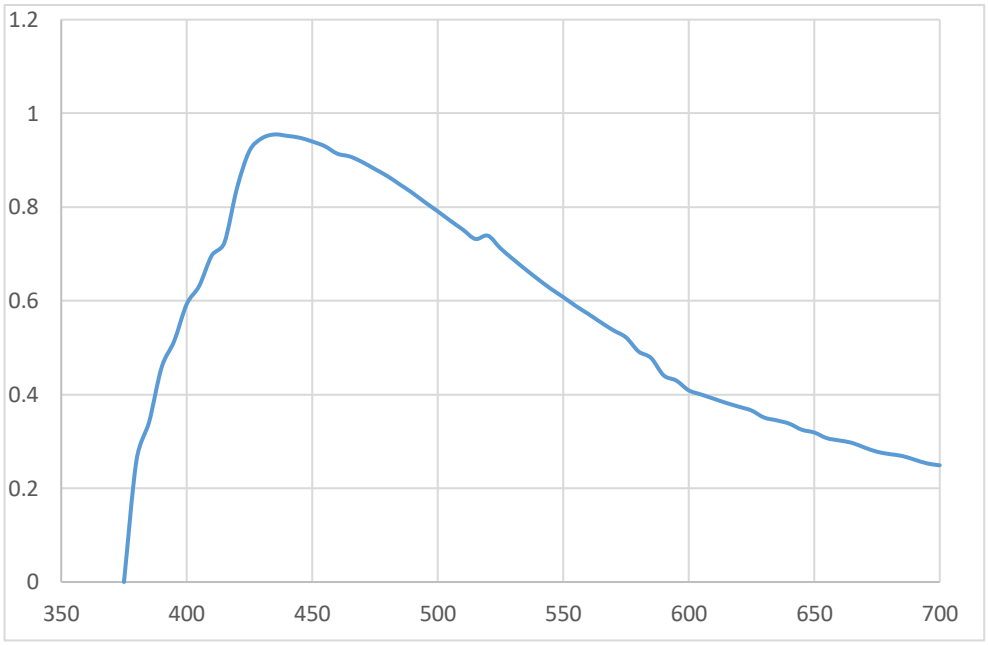


Figure 10 : *Uv-visible résulte for 1Mm concentration*

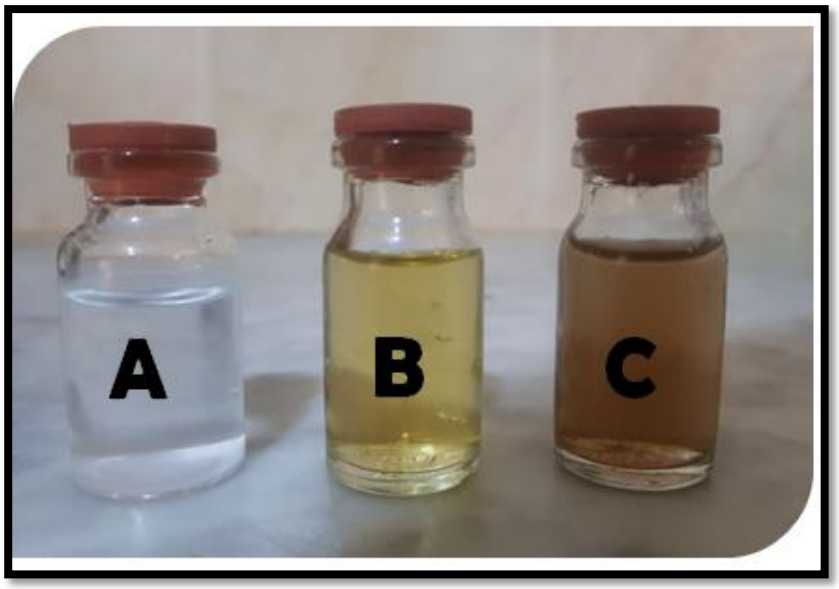


Figure 11 **:** A : solution of AgNO3 **B**: supernatant of Staphylococcus aureus **C**: AgNPs

In contrast, the reduction of silver ions was not observed in the absence of bacterial cells, indicating that the presence of bacterial cells is essential for the reduction process. This finding strongly suggests that specific reducing agents are released by the bacterial cells into the culture medium, facilitating the reduction of silver ions to silver nanoparticles **(Saifuddin, Wong, and Yasumira 2009b).** During this process, where NO^-_3 is converted into NO^-_2,

electrons are transferred to silver ions (Ag^+) resulting in their reduction to metallic silver (Ag^0). The proposed mechanism for the reduction of Ag^+ ions in these extracts involves the combination of various biomolecules such as enzymes/proteins, amino acids, polysaccharides, and vitamins. However, the precise mechanism is still not fully understood. One widely accepted mechanism, as illustrated in **(Figure 12)**, suggests the involvement of the enzyme "nitrate reductase". This enzyme is believed to play a crucial role in facilitating the reduction reaction of Ag^+ ions **(Javaid et al. 2018a)**.

In addition to nitrate reductase, the functional groups present on the bacterial cell wall also contribute to the reduction of Ag^+ to Ag^0 even in the absence of nitrate reductase. This highlights the multiple pathways involved in the reduction process. Furthermore, apart from bacteria, fungi also offer potential for the nanoparticle synthesis. Fungi have an advantage in terms of productivity due to the abundant presence of enzymes, proteins, and reducing components on their cell surfaces. This expands the scope of nanoparticle synthesis using biological methods **(Roy et al. 2019).**

Microorganisms produce extracellular reductase enzyme that reduces the silver ions to nanomaterials. The enzyme NADH-dependent reductase is involved in the reduction of silver ions into nanoparticles. While, in some cases, the enzyme nitrate-dependent reductase is also involved in the bioreduction process **(Vala, n.d.; Tashi, Gupta, and Mbuya 2016).**

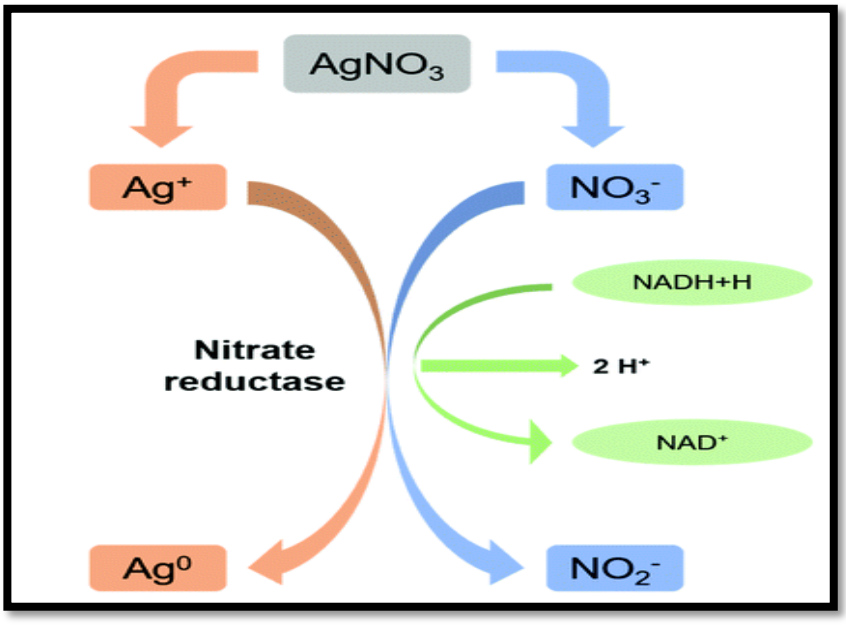


***Figure 12*:** Nitrate reductase mediated synthesis of AgNPs **(Roy et al. 2019)**

III.2. Optimisation

The optimization of physicochemical parameters is crucial in achieving a higher production rate of nanoparticles with improved physical, morphological, and biochemical characteristics. Several parameters have been optimized in the biosynthesis of silver nanoparticles (AgNPs), such as contact time, concentration of silver nitrate, light, and bacterial supernatant concentration **(Abdelmoneim et al. 2022).**

III.2.1 Effect light

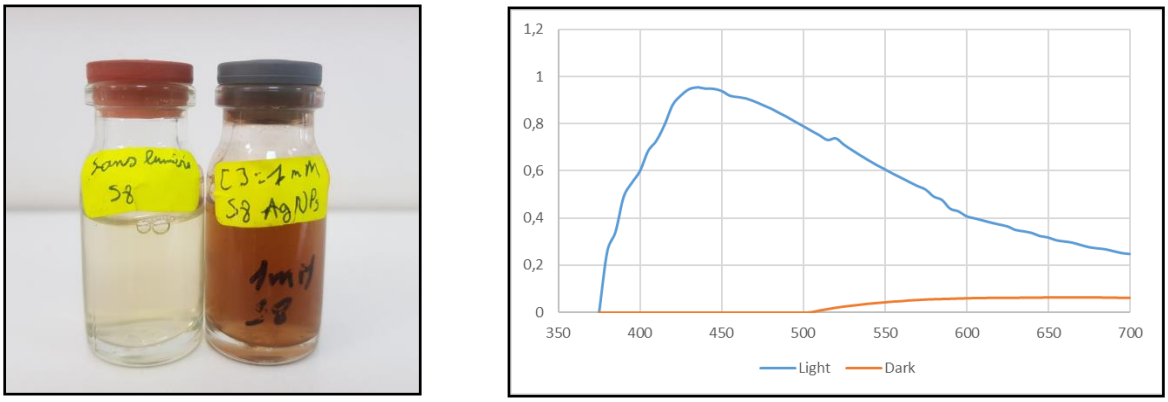
The impact of light on AgNPs production was investigated by examining the mixture under both illuminated and dark conditions **(Figure 13).** Based on the color changes of solutions, it can be deduced that the transformation from yellow to dark brown means the formation of AgNPs. This occurred exclusively in the sample exposed to sunlight. This confirms the role of sunlight in stimulating the synthesis of silver nanoparticles**.** Conversely, in the dark sample, no color change have been observed.

These color changes were attributed to the surface plasmon resonance (SPR) phenomenon, resulting from the oscillation of electrons on the metal particle surfaces in response to the incident light's electric field component. UV-vis absorption spectra analysis revealed a maximum absorbance wavelength (λ_{max}) around 435 nm, indicating the presence of spherical AgNPs. The peak intensity increased with light intensity, correlating with the observed color changes. The reduction rate of Ag^{+} to Ag^{0} was efficiently accelerated by visible light exposure. The color change and SPR effect were consistent with the synthesis of AgNPs using different plant extracts, supporting the formation of noble metal nanoparticles. The smooth spectrum without significant sharp peaks suggested homogeneous dispersion of the synthesized AgNPs without agglomeration. Light intensity played a crucial role in the synthesis, as evidenced by the gradual increase in the peak height with increasing light intensity. However, higher light intensity did not significantly affect the synthesis, indicating a saturation point in the reaction **(Rose et al. 2019b).**

Silver nanoparticles and other noble metal nanoparticles strongly interact with light due to the collective oscillation of conduction electrons at their surfaces when excited by specific wavelengths of light. This oscillation, leads to much higher light absorption and scattering for silver nanoparticles compared to non-plasmonic nanoparticles of similar size. The absorption and scattering properties of silver nanoparticles can be modulated by

controlling the particle size, shape, and the local refractive index near their surfaces **(Geddes and Lakowicz 2002).**

The experiments clearly demonstrated that the metabolic byproducts released by the cultures of *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae* exposed to silver were capable to reducing silver ions. This reduction process occurred outside the cells, indicating that extracellular reducing agents were responsible for the conversion of silver ions. Importantly, these reactions only took place in the presence of light, and no nanoparticle formation was observed in the absence of light or under dark conditions **(Minaeian and Shahverdi 2008).**



A **B**

***Figure 13*: A:** Color change depending light **B:** Effect of light on the synthesis of silver nanoparticles.

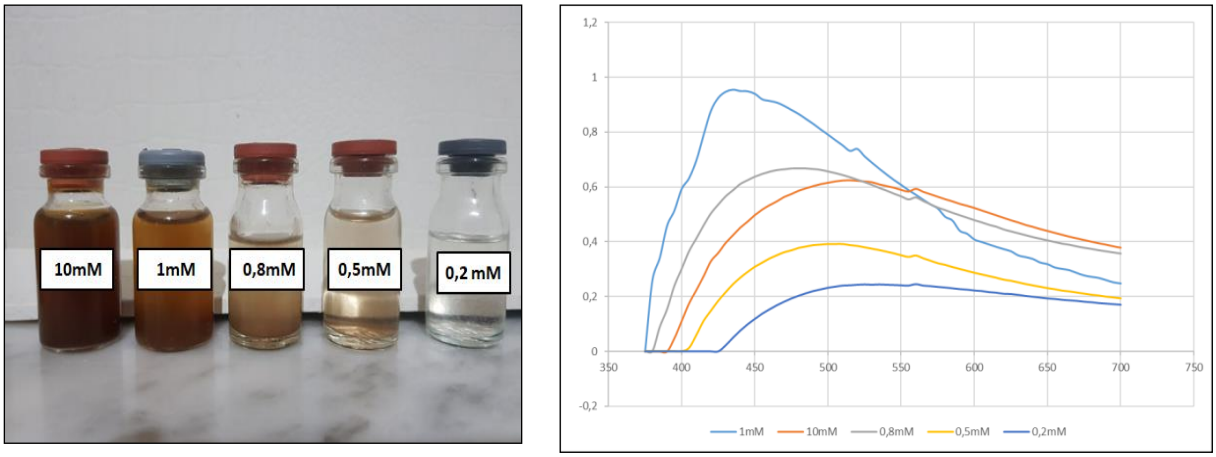
III.2.2 Effect concentration

The effect of varying $AgNO_3$ concentration on the production of AgNPs was investigated **(Figure 14)** , The results showed that as the concentration of $AgNO_3$ increased, there was a corresponding increase in the synthesis of AgNPs, which was evident from the gradual change in color from yellow to dark brown.

The optimization study showed that varying the concentration of $AgNO_3$ resulted in different concentration-dependent effects. The maximum concentration of nanoparticles was observed at 1 mM of $AgNO_3$, as indicated by the blue color observed in the UV-Vis spectrum at 435 nm. Additionally, the UV-Vis spectrum exhibited a dark blue color at low concentration of 0.2 mM, while an orange color was observed at high concentration 10 mM,

indicating site saturation. The optimized concentration of 1 mM of $AgNO_3$ demonstrated the production of AgNPs with a narrower size distribution. Therefore, 1 mM of $AgNO_3$ was identified as the commonly used optimal concentration for synthesizing AgNPs **(Bamsaoud et al. 2021)**.

Based on the study, it was found that a higher concentration of silver nitrate leads to an increased number of silver ions, which are subsequently reduced and stabilized to form nanoparticles **(Apriani 2022; Sila et al. 2019)**. This indicates a direct relationship between the substrate concentration ($AgNO_3$ concentration) and the particle sizes. The particle sizes observed ranged from 79 to 107 nm for substrate concentrations varying from 0.5 to 2.0 mM **(Panchangam and Upputuri 2019).** Notably, higher concentrations of silver nitrate resulted in the formation of larger-sized nanoparticles, suggesting that the concentration of the precursor solution plays a significant role in determining the size of the nanoparticles. Additionally, it was noted that a high concentration of silver ions requires a corresponding high concentration of the supernatant to facilitate nanoparticle production **(Nt, N, and Sua 2016).** Our result is consistent with the findings of the study **(Sharma, Guleria, and Razdan 2020; Nagar et al. 2016).**



A **B**

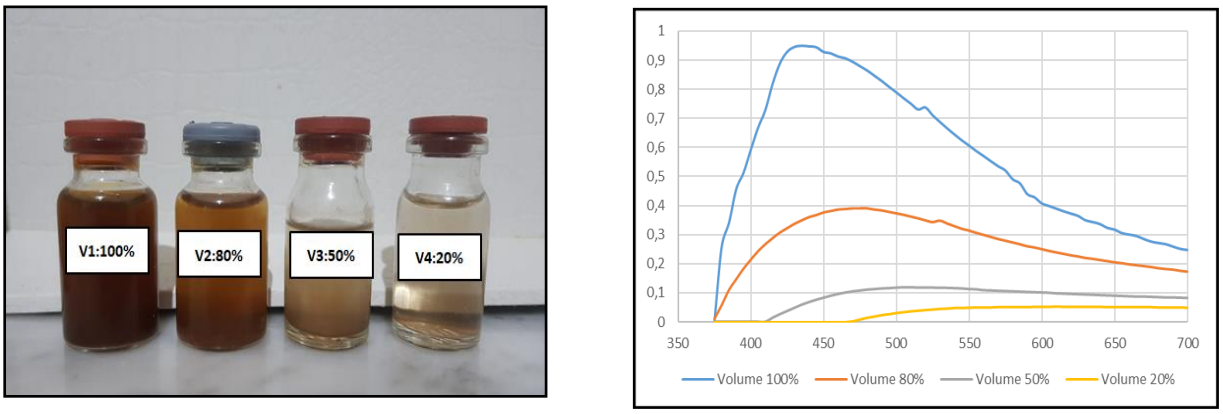
***Figure 14*: A**: Color change depending concentrations **B:** Effect of the concentration on the synthesis of silver nanoparticles.

III.2.3 Effect volume

The result shows in (**Figure 15-A),** illustrates that when the volume of the floating material and the metal solution increases, accompanied by a decrease in distilled water, a color change to brown occurs, indicating an enhanced production of AgNPs.

The analysis conducted using a spectrophotometer, as shown in (**Figure 15-B)** demonstrated a significant rise in absorbance as the volume increased. Notably, the absorbance reached its maximum value at 435 nm with 100% volume indicating the substantial impact of distilled water on the solution.

Water is essential for the biosynthesis of silver nanoparticles as it acts as a medium for the chemical reactions involved in the process. It helps in dissolving and dispersing the silver precursor, allowing it to interact with the biomolecules present in the biological system. Water also serves as a solvent for the reducing agents and stabilizers, facilitating their efficient participation in reducing silver ions and forming nanoparticles. The unique properties of water, such as its high dielectric constant and hydrogen bonding capability, contribute to the stability and dispersion of the silver nanoparticles. Additionally, water molecules can interact with the nanoparticle surface, influencing their surface charge, stability, and interactions with other molecules **(Durán et al. 2005; Rai et al. 2013)**.



A B

***Figure 15*: A**: Color change depending ration **B:** Effect of ration on the synthesis of AgNPs

III.3 Charactrization

III.3.1 Scanning Electron Microscopy (SEM)

To accurately determine the size and morphology of the AgNPs obtained from *Staphylococcus aureus* after calcination at 450 °C, Scanning Electron Microscopy (SEM) was employed.**(Figure16)** presents SEM images revealing the presence of small spherical nanoparticles. However, (**Figure17)** indicates that these particles tend to aggregate, forming larger aggregates with diameters of 400 nm and above 3m. In order to obtain precise

information about the exact size and morphology of the AgNPs, Transmission Electron Microscopy (TEM) would be necessary. The results obtained are similar to those of **(Beeler et al. 2020; Tashi, Gupta, and Mbuya 2016).**

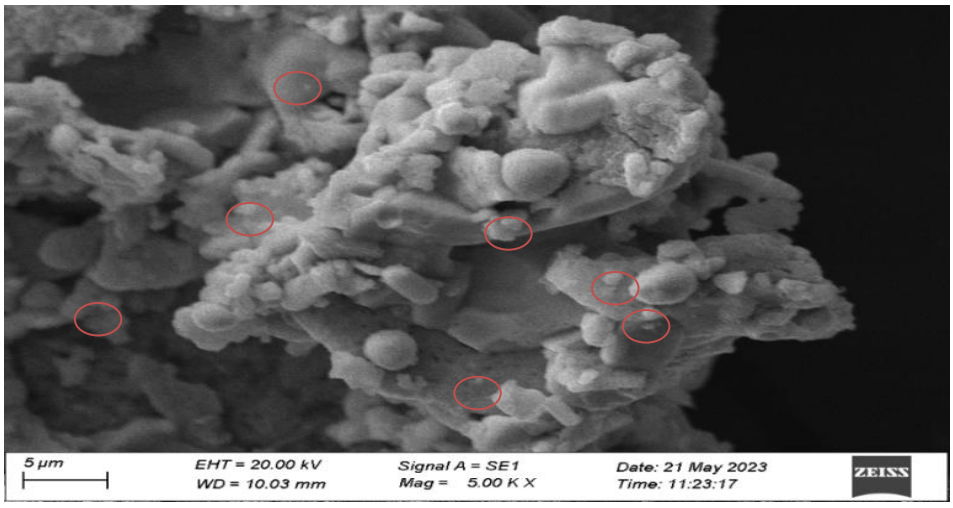


***Figure 16*:** Scanning electron microscopy image

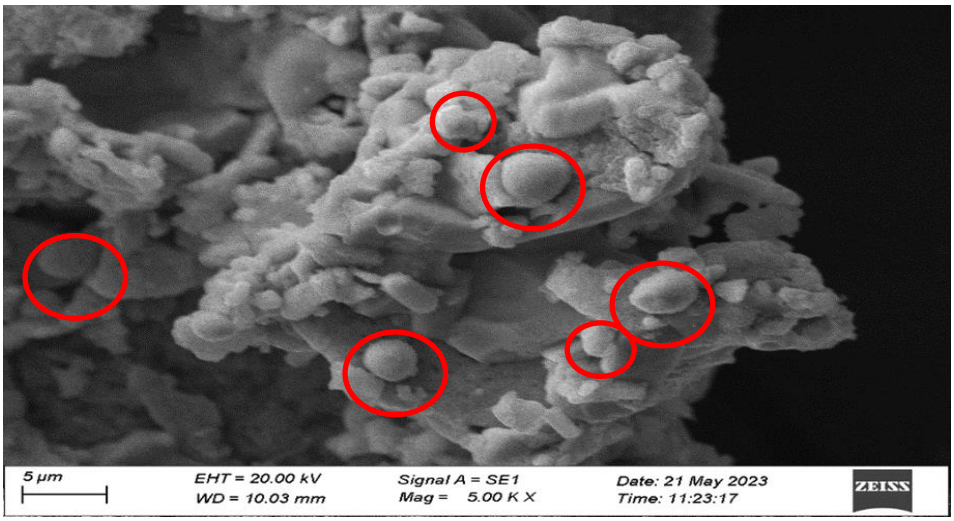


***Figure 17*:** Scanning electron microscopy images of AgNPs

The EDX analysis confirmed the presence of AgNPs, with sharp peak at 3.0 keV indicating silver as the dominant element.**(Shahverdi et al. 2007)** Furthermore, the metallic nature of the synthesized nanoparticles was confirmed by a strong signal at 3 keV in the EDX spectrum, specifically indicating the synthesis of AgNPs. The presence of other elements such as chlorine (Cl), carbon (C), silicon (Si), and oxygen (O) was also observed in the EDX

analysis, possibly originating from proteins or enzymes in the culture supernatant ***(Figure 18)*** **(Mondal et al. 2020).**

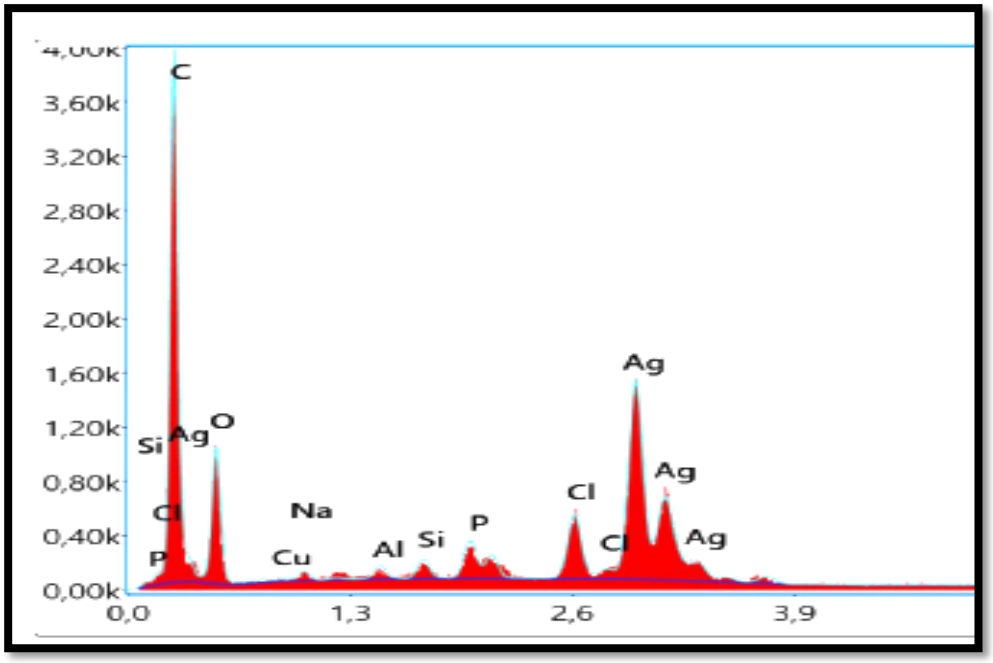


Figure 18: The EDX analysis of $AgNO_3$

III.3.2 X-ray Diffraction (DRX)

The XRD patterns of the AgNPs synthesized by *Staphylococcus aureus* are shown in **(Figure 19).** There are four peaks observed at 38.04°, 44.06°, 64.34° and 77.17° corresponds to (111), (200), (220) and (311), respectively. Crystalline nature of the formed AgNPs was confirmed by their XRD analysis facets which agree with the values reported for face cantered cubic (fcc) silver nanocrystals **(Probin Phanjom 2015; Kalishwaralal et al. 2008)**, **(Gurunathan et al. 2009)** This result of crystalline structure also supports with the XRD results and it indicates reduction of silver ions to silver nanoparticles **(Rajeshkumar et al. 2016).**

The average size (D) of crystal can be determined using Debye-Scherrer's equation, which relates the size of the crystallite domain to the X-ray wavelength, the full width at half maximum (FWHM), and the diffraction angle. The equation is given as $D = 0.94 \lambda / \beta \cos \theta$, where D represents the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the FWHM, and θ is the diffraction angle. By applying this equation to the obtained data, the calculated average size was found to be 29 nm based on the FWHM of the (111) peak. This indicates the average dimension of the crystalline regions

within the synthesized nanoparticles. **(Rajeshkumar et al. 2016; El-Shanshoury, ElSilk, and Ebeid 2011) .**

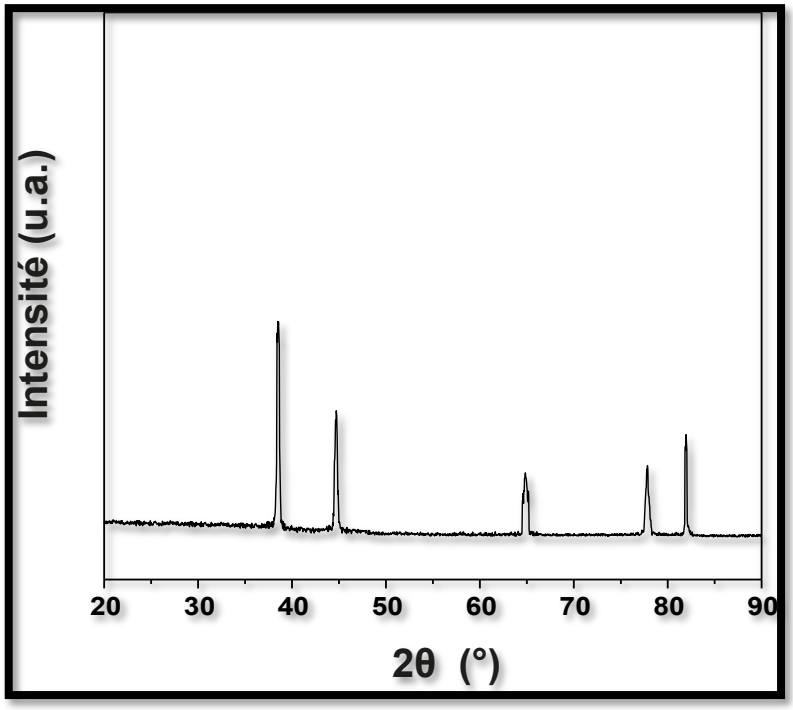


***Figure 19*:** XRD diffraction spectra of AgNPs.

III.4. Antimicrobial activity

In order to evaluate the antimicrobial activities of Ag-NPs synthesized by the strain staphylococcus aureus against Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria by the disc diffusion method after the incubation 24h at 37C°. The findings of this study demonstrated that the AgNPs synthesized at a concentration of 1 mg/mL exhibited a distinct zone of inhibition **(Figure 20).** *Staphylococcus aureus* demonstrated the greatest susceptibility, whereas *Pseudomonas aeruginosa* exhibited the least susceptibility among the tested strains. This finding implies that gram-positive bacteria tend to be more sensitive compared to gram-negative bacteria. Similar results to **Saleh and Khoman Alwan; Azarbani and Shiravand .**

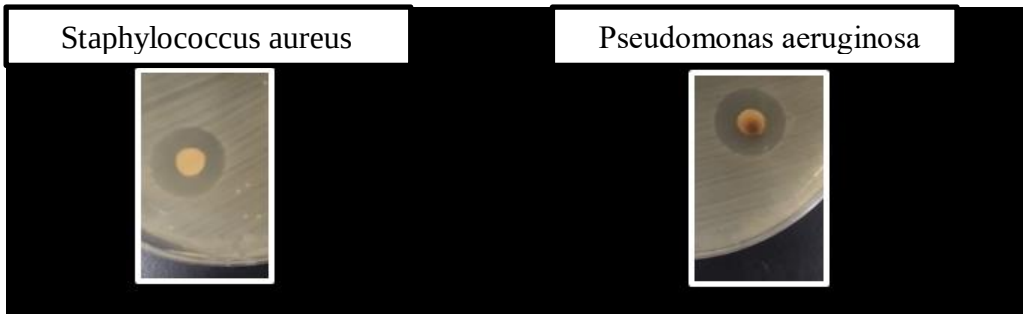


***Figure 20*:** Antibacterial activity of silver nanoparticles against various pathogenic bacterial strains**.**

Table 2: Inhibition zone of silver nanoparticles against various pathogenic bacteria.

Strain	Zone of inhibition	Sensibility
Staphylococcus aureus(ATCC25923)	12mm	Sensible (+)
Pseudomonas aeruginosa (ATCC 9027)	10 mm	Sensible (+)

Silver nanoparticles (AgNPs) have been found to exhibit greater antimicrobial efficiency against Gram-negative bacteria compared to Gram-positive bacteria. This can be attributed to the structural differences in their cell walls, where Gram-negative bacteria have a narrower cellular wall and a thick layer of lipopolysaccharides (LPS) compared to the thin peptidoglycan layer found in Gram-positive strains. The presence of a thick peptidoglycan layer in Gram-positive bacteria limits the penetration of AgNPs into cells. It is widely recognized that the uptake of AgNPs is crucial for their antibacterial effects and smaller AgNPs (less than 10 nm) have been shown to directly alter cell permeability, enter bacterial cells, and cause damage to cellular components such as DNA, proteins, and lipids. **(Yin et al. 2020)**. AgNPs can induce oxidative stress, disrupt cell membranes, and modify DNA through the generation of reactive oxygen species (ROS). Additionally, in Gram-negative bacteria, the involvement of porins in the outer membrane facilitates the uptake of AgNPs**(Noronha et al. 2017)**. Overall, the antimicrobial activity of AgNPs is a complex process involving various mechanisms, and understanding the structural and functional differences between Gram-negative and Gram-positive bacteria is important for optimizing their antibacterial effects (**Figure 21). (Kalwar and Shan 2018).**

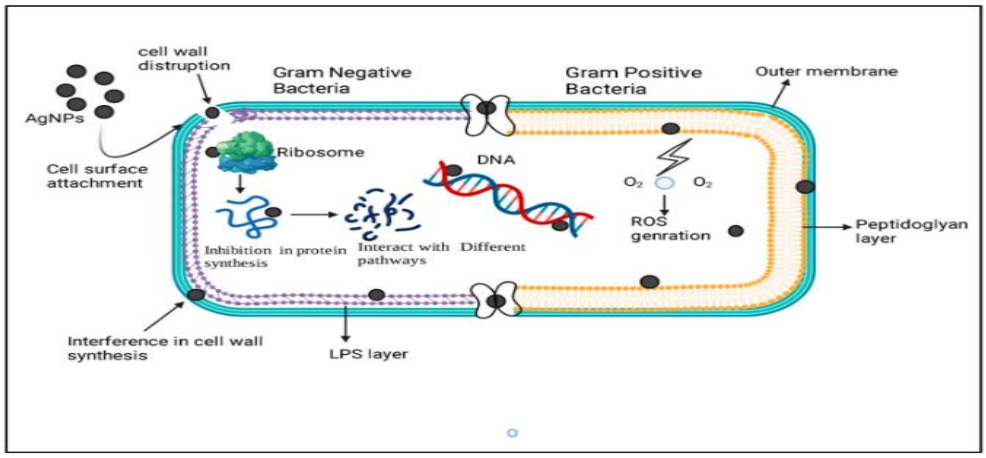


Figure 20: The comparative mechanistic approach of AgNPs on Gram-positive and Gram-negative bacteria.***(More et al. 2023)***

The antibacterial activity of the nanoparticles against MRSE, MRSA, and *S. pyogenes* was found to be the highest, with respective inhibition zone diameters of 18 mm, 17.5 mm, and 16 mm. These bacteria are gram-positive, and they exhibited greater susceptibility to the nanoparticles compared to gram-negative bacteria such as *S. typhi*, *K. pneumonia*, and *V. cholerae*. The increased sensitivity of MRSA and MRSE to AgNPs could be attributed to the plasmolysis of their cell walls or the separation of cytoplasm from the cell wall. It should be noted that the antimicrobial mechanisms of bionanosilver particles may vary depending on the bacterial species and the size of the nanoparticles **(Nanda and Saravanan 2009b) .**

Previous research supported the susceptibility of MRSA to AgNPs, attributing this susceptibility to the inhibition of bacterial cell wall synthesis. **(Nanda and Saravanan 2009b**). Based on the results of **(& Hussein 2020),** it can be concluded that the biosynthesized AgNPs using bacteria exhibit a strong inhibitory effect on the growth of pathogenic microbes compared to the bacteria supernatant. According to the protocol , **Reyna et al.** the results indicated a positive correlation between the volume of supernatant aqueous solutions and the zone of inhibition, with an increase in volume leading to a corresponding increase in the observed zone of inhibition .

Another important physico-chemical property of AgNPs is their size. In general, for nanoparticles to be effective their size typically should be no larger than 50 nm. More

precisely, silver nanoparticles with size between 10 and 15 nm have increased stability, biocompatibility and enhanced antimicrobial activity **(Dakal et al. 2016).**

Several studies have indicated that the antibacterial efficacy of AgNPs is enhanced against *S. aureus* and *K. pneumoniae* when nanoparticles with smaller diameters (<30nm) are used. **(Collins et al., 2010**).

Results indicated that biologically synthesized AgNPs exhibited greater efficacy in against *E. coli* at lower concentrations compared to *P. aeruginosa* and *S. aureus*. This observation can be attributed to the variations in the cell wall structure among these three species. The presence of peptidoglycans in the cell wall of *S. aureus*, along with its ability to produce pigments as a defense mechanism against reactive oxygen species (ROS), contributes to its higher resistance to the bactericidal effects of AgNPs**. (Khorrami et al. 2018) .**

The bactericidal activity of silver nanoparticles (AgNPs) is attributed to several mechanisms. When AgNPs come into contact with microbial cells, they interact directly and produce various effects. Smaller AgNPs with spherical or quasi-spherical shapes are more likely to release silver ions due to their larger surface areas **(Kalwar and Shan 2018)**, and they have a strong affinity for the sulfur-containing proteins in the microbial cell wall**(Yin et al. 2020)**. Here are some suggested mechanisms that explain the bactericidal action of AgNPs and shown in **(Figure 22) (Salleh et al. 2020a; Carrapiço et al. 2023):**

1. Interaction with cell membranes: AgNPs can attach to the surface of microbial cell walls or membranes through electrostatic attraction. This interaction causes irreversible morphological changes in the cell membrane, affecting its integrity and permeability. For example, the release of silver ions by the nanoparticles can alter the transport and release of potassium ions (K^+), thereby affecting cell transport activity**(Salleh et al. 2020b).** The attachment of AgNPs can depolarize and destabilize the membrane.
2. Penetration into cells: AgNPs can enter bacterial cells through water-filled channels called porins in the outer membrane of Gram-negative bacteria. Once inside the cells, AgNPs bind to cellular structures and biomolecules such as proteins, lipids, and DNA, causing damage to the internal structure of the bacteria. AgNPs interfere with the normal growth and metabolism of bacterial cells by inhibiting respiratory chain enzymes and converting various enzymes, such as glycerol-3-phosphate dehydrogenase, into inactive forms.**(Salleh et al. 2020b).**

3. Generation of reactive oxygen species (ROS): AgNPs can induce the production of ROS, including superoxide, hydrogen peroxide, and hydroxyl radicals, within microbial cells. The production of ROS leads to oxidative stress and damage to cellular components, ultimately resulting in cell death. AgNPs increase the formation of ROS, which can cause membrane damage and cell death.**(Pandian et al. 2010; Salleh et al. 2020b).**
4. Disruption of signal transduction pathways: AgNPs act as modulators of signal transduction in microbial cells. They disrupt bacterial actin cytoskeletal networks, causing morphological changes in bacterial shape and increasing membrane fluidity, leading to cell rupture and death. AgNPs can also interfere with the phosphorylation and dephosphorylation cascades of proteins or enzymes involved in cellular activity and bacterial growth.**(Reyna et al. 2015b).**

These mechanisms collectively contribute to the antibacterial effects of AgNPs. It's important to note that the exact mechanisms may vary depending on factors such as nanoparticle size, concentration, and the specific characteristics of the targeted microorganisms. Compared to other metallic nanoparticles like copper nanoparticles (CuNPs), AgNPs exhibit higher inhibition activity against bacteria but may have higher toxicity towards cells.**(Khorrami et al. 2018; Le Ouay and Stellacci 2015).**

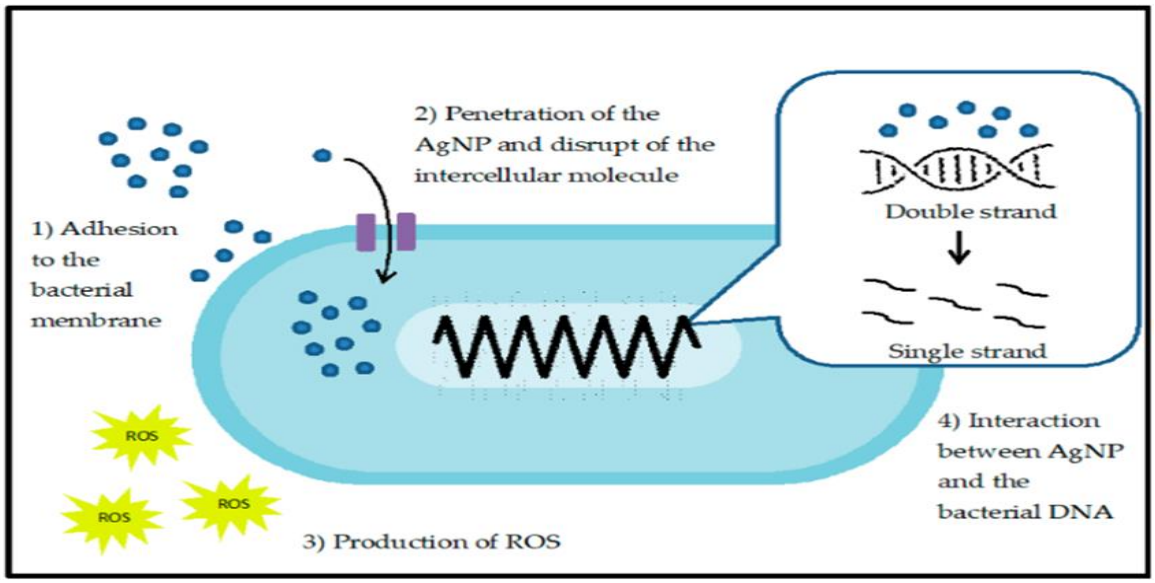


Figure 22: Mechanism of antibacterial activity of silver nanoparticles **(Salleh et al. 2020b)**

III.5. Antioxidant activity

The antioxidant activity of silver nanoparticles (AgNPs) produced in this study was assessed using the DPPH radical scavenging assay. The DPPH test is widely used to measure

antioxidant activity due to the stability of the DPPH. **(Figure 23)** depicts the relationship between different concentrations of AgNPs and their corresponding DPPH free radical antioxidant activity. The results indicate that the biosynthesized AgNPs exhibit a moderate level of antioxidant activity. However, compared to ascorbic acid; known for its strong antioxidant properties, AgNPs show relatively lower activity. While the AgNPs possess some antioxidant capability, their effectiveness is not as potent as that of ascorbic acid shown in (**Figure 24)**. Our findings align with those of **Mohd Yusof et al ,(Odeniyi et al. 2020) ,(Bedlovičová et al. 2020).**

In the DPPH assay, the antioxidant properties of AgNPs are manifested by their ability to donate electrons to the unpaired electron in the DPPH solution. This electron transfer causes a decrease in the intensity of the DPPH solution, leading to a noticeable color change from deep purple to pale yellow. This color change have been observed when the concentration of AgNPs reached 1 mM. It indicates the antioxidant activity of the biosynthesized AgNPs in neutralizing the free radicals present in the DPPH solution **(Figure 25) (Safawo et al. 2018; Riaz Rajoka et al. 2020).**

Based on the calculated IC_{50}, an IC_{50} value of 1.78 suggests that the tested antioxidant concentration was effective in reducing 50% of the DPPH present in the solution. This means that a relatively low concentration of the antioxidant was needed to achieve the desired reduction in DPPH°, indicating a strong antioxidant activity. A lower IC_{50} value indicates that the antioxidant has a greater ability to scavenge or neutralize free radicals (such as DPPH°), and protect against oxidative damage **(Figure 26). (Genc 2021; Abdellatif, Alturki, and Tawfeek 2021).**

The IC50 value of AgNPs is higher than that of ascorbic acid; it suggests that the AgNPs have a lower antioxidant activity compared to ascorbic acid, the AgNPs may have a weaker antioxidant potential than ascorbic acid ***(Shanmugasundaram et al. 2013; Reetika Singh et al. 2021).***

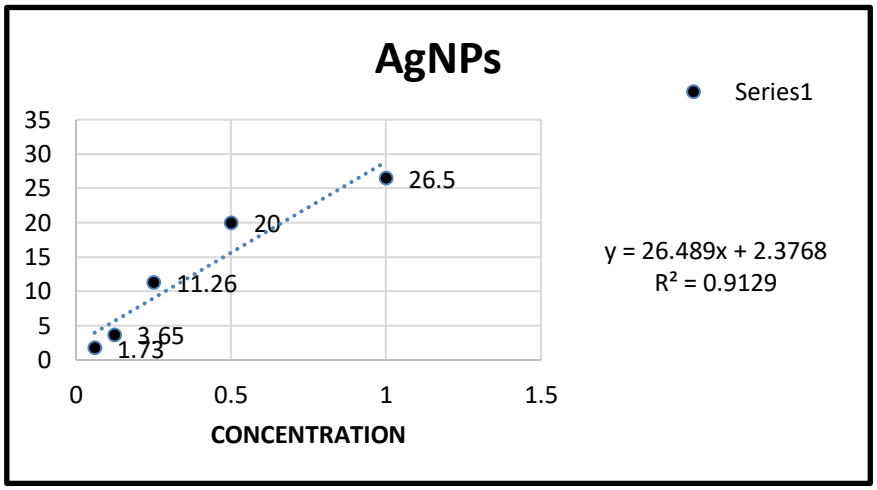


Figure 21: Antioxidant activity of biosynthesized AgNPs

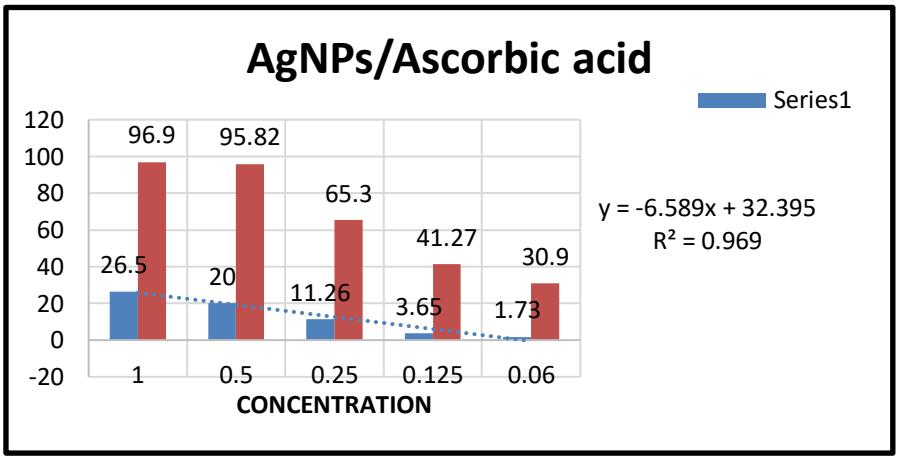


***Figure 22*:** Bar charts comparing the antioxidant activity of biosynthesized silver nanoparticles (AgNPs) with that of ascorbic acide

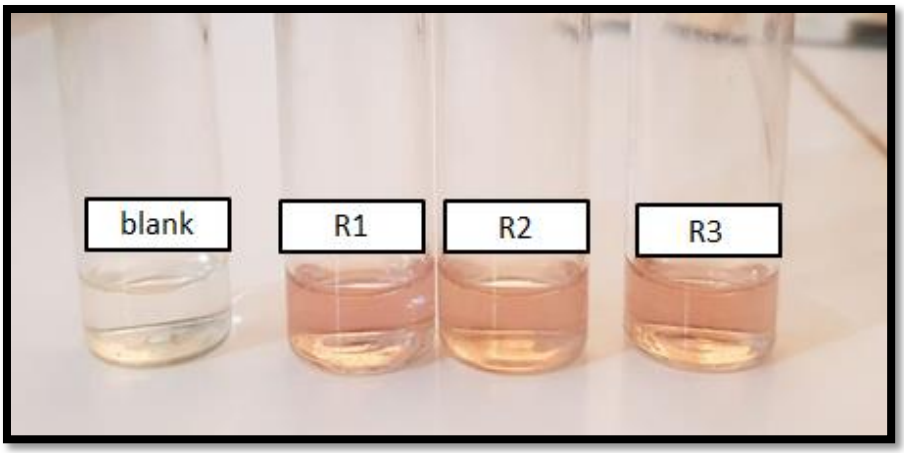


***Figure 23* :** *Antioxidant activity of silver nanoparticles at a concentration 1mM*

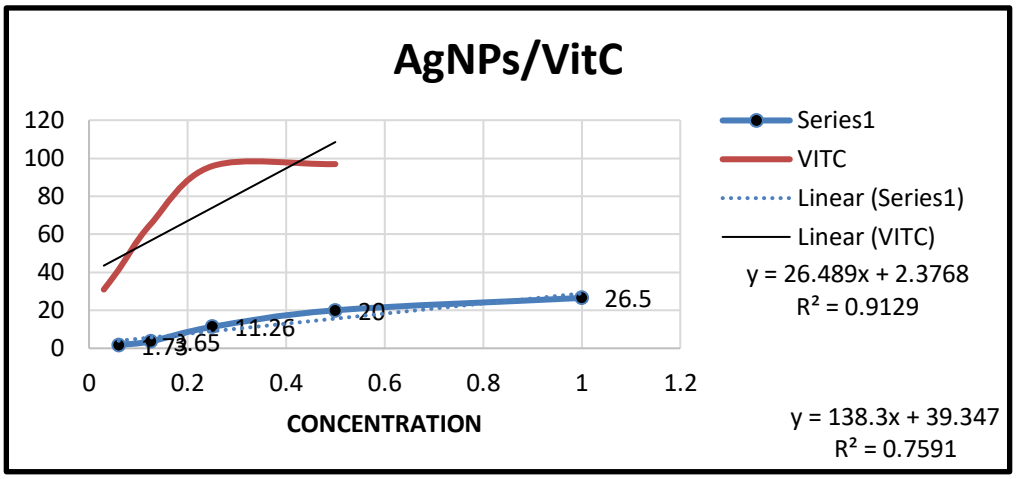


Figure 24: *The antioxidant activity of biosynthesize AgNPs with oascorbic acide*

Conclusion

The aim of this study was to perform the biosynthesis of silver nanoparticles using Staphylococcus aureus and evaluate their antioxidant activity and antibacterial efficacy. Additionally, an optimization of the operational parameters was conducted to improve the performance of the biosynthesis process.

In this study, we successfully synthesized silver nanoparticles using a simple biological method based on the culture supernatant of a reference strain of Staphylococcus aureus. This approach offers several advantages, including simplicity, low cost, and ease of implementation. The obtained silver nanoparticles demonstrated exceptional stability, making them promising for various applications.

The biosynthesis of silver nanoparticles is confirmed by a color change, shifting from yellow to dark brown, which is also verified by a UV-visible analysis showing an absorbance at 440 nm.

The EDX analysis also confirmed the presence of silver nanoparticles with a sharp peak at 3.0 keV, indicating silver as the dominant element. To study the morphology of the silver nanoparticles, we used scanning electron microscopy (SEM), which revealed a spherical shape with a size ranging from 30 to 100 nm. X-ray diffraction (XRD) was employed to confirm the crystalline structure of the silver nanoparticles and to calculate the average nanoparticle size, which was determined to be 29 nm in this study.

To enhance the performance and properties of silver nanoparticles, an optimization of operational parameters was carried out, which involved adjusting the concentration of AgNO3, the volume of the culture supernatant (supernatant + AgNO3) and distilled water, as well as exposure to light. The findings of this investigation indicated that an AgNO3 concentration of 1 mM and a volume of 100% (supernatant + AgNO3), combined with the presence of light, resulted in an optimal yield of silver nanoparticles

The results of the study suggest that the antioxidant potential of silver nanoparticles may be lower compared to that of ascorbic acid, with a difference of 1.71. The study aimed to evaluate the ability of silver nanoparticles to counteract free radicals and reduce damage caused by oxidation. To assess this, the researchers employed the DPPH assay, which is a commonly used method to measure antioxidant activity.

The biosynthesized silver nanoparticles from Staphylococcus aureus exhibited antibacterial activity against Staphylococcus aureus (Gram+) and Pseudomonas aeruginosa (Gram-) with inhibition zones measuring 12 mm and 11 mm, respectively. These findings are of great significance in the medical field, which is facing an increasing challenge of

antibiotic-resistant pathogenic bacteria. Silver nanoparticles have the potential to be a promising solution to this problem.

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Annexe

Annex 01 : Culture medium

BN Nutrient Broth

BN……...……....08 g

Glucose……..10 g

Distilled water ……..1000ml

Mueller Hinton medium

Dehydrated MH……38g

Distilled water: ……1000ml

pH 7.4

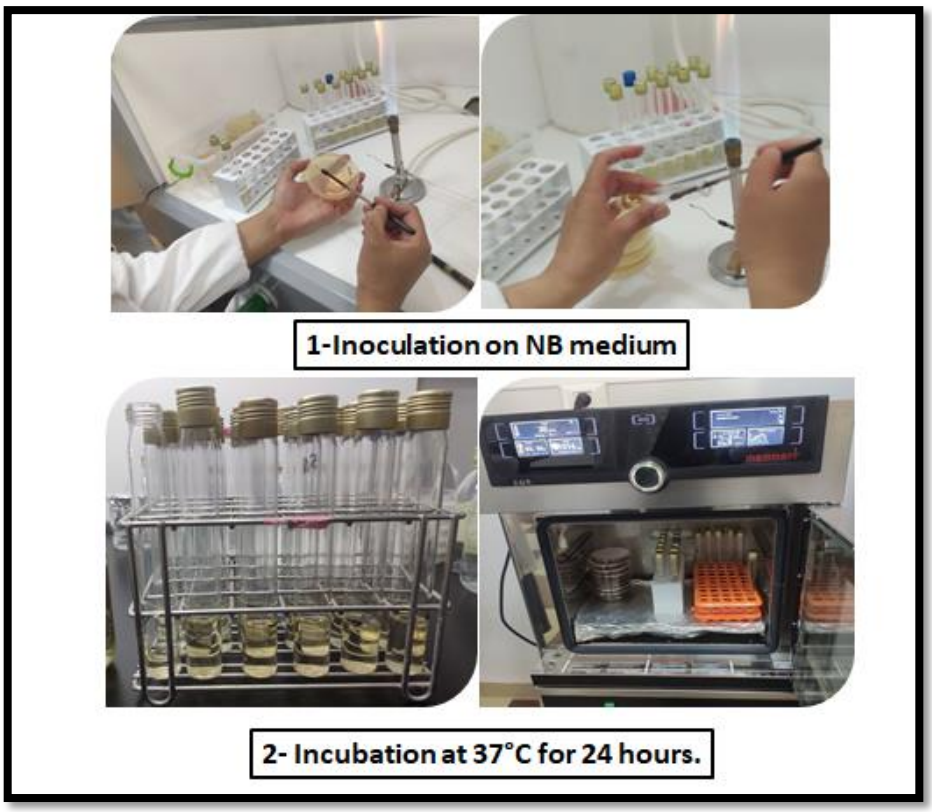
Autoclaving at 120°C for 20 minutes

$AgNO_3$ solution 1 mM

Distilled water:……...1000 ml

AgNO3..……0.08g

Annex 02



Revival step of strains

Annex 03: Materials

Glass beaker

Glass Erlenmeyer flask

Micropipette

Pipette

Benzene beaker

Spatula

Glass bottle

Glass tube

Petri dish

Graduated cylinder

Balance

Stirrer

Annex 04: Taxonomy

Phylum : Prokaryota

Division : Proteobacteria

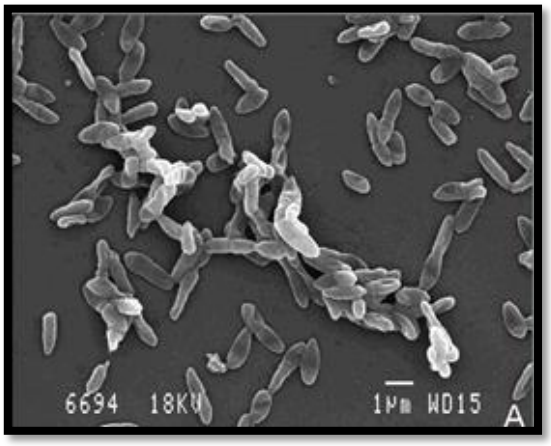
Class : Gammaproteobacteria

Order : Pseudomonadales

Family : Pseudomonadaceae

Genus :*Pseudomonas*

Species :*Pseudomonas aeruginosa*



Pseudomonas aeruginosa fixed on glass using scanning electron microscopy

Phylum : Prokaryota

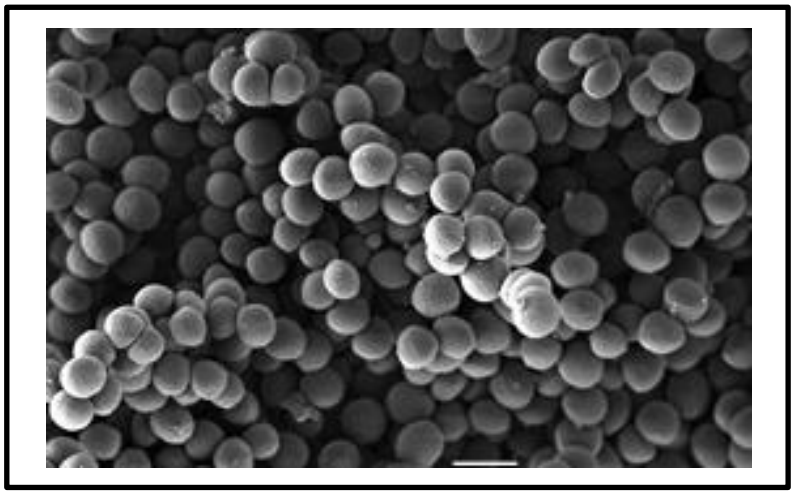
Division : Proteobacteria

Class : Bacilli

Order : Bacillales

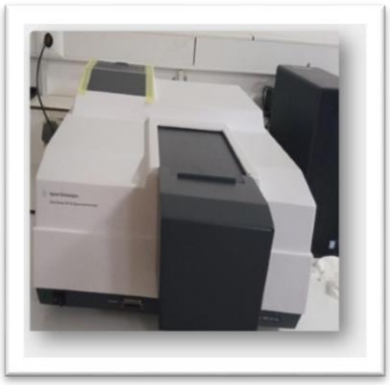
Genus : *Staphylococcus*

Species : *Staphylococcus aureus*



Staphylococcus aureus Scanning electron microscopy **(Norbert.B 2020)**

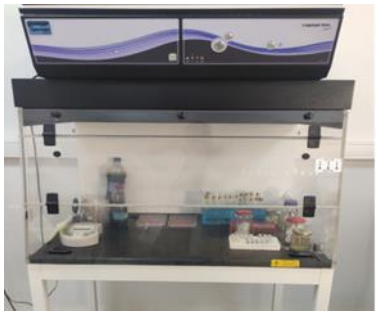
Annex 05: Equipments



Spectrophotometer UV

Centrifuge

Scanning electron microscopy



Biological safety cabinet

Ultrasound

Very high precision balance

Agnetic agitator

Shaking incubator

Bacteriological incubator

Abstract

This study utilized *Staphylococcus aureus* to biosynthesize stable silver nanoparticles with potential applications. Optimization of operational parameters, including 1mM AgNO3 concentration, 100% volume, and light exposure, resulted in optimal nanoparticle yield. Biosynthesis was confirmed through color change and UV-visible analysis. Characterization techniques (SEM, EDX, and XRD) verified nanoparticle presence, morphology, and crystalline structure. The synthesized silver nanoparticles exhibited antibacterial activity against both Gram-positive (Staphylococcus aureus) and Gram-negative (Pseudomonas aeruginosa) bacteria, with inhibition zones of 12mm and 11mm, respectively. The antioxidant potential of the silver nanoparticles was lower than that of ascorbic acid by a factor of 1.71

Key word: Staphylococcus *aureus*, Biosynthsis, Silver nanoparticle, caracterization , Activity antibacterial , Activity antioxydant ,Optimization

Résumé

Cette étude a utilisé *Staphylococcus aureus* pour la biosynthèse de nanoparticules d'argent stables avec des applications potentielles. L'optimisation des paramètres opérationnels, y compris une concentration de 1 mM d'AgNO3, un volume de 100% et une exposition à la lumière, a conduit à un rendement optimal en nanoparticules. La biosynthèse a été confirmée par un changement de couleur et une analyse UV-visible. Les techniques de caractérisation (MEB, EDX, DRX) ont vérifié la présence, la morphologie et la structure cristalline des nanoparticules. Les nanoparticules d'argent synthétisées ont présenté une activité antibactérienne contre les bactéries à Gram positif (Staphylococcus aureus) et à Gram négatif (Pseudomonas aeruginosa), avec des zones d'inhibition de 12 mm et 11 mm respectivement. Le potentiel antioxydant des nanoparticules d'argent était inférieur à celui de l'acide ascorbique d'un facteur de 1,71.

Mot clé : *Staphylococcus aureus*, Biosynthèse, Argent nanoparticule, Caractérisation, Activité antibactérienne, activité antioxydant, Optimisation

ملخص

تهدف هذه الدراسة إلى استخدام *Staphylococcus aureus* لتخليق جسيمات الفضة المستقرة ذات الاستخدامات المحتملة. تم تحسين العوامل التشغيلية، بما في ذلك تركيز AgNO3 بمقدار 1 ملي مولار، ونسبة حجم 100٪، وتعرضها للضوء، مما أسفر عن حصول أفضل عائد للجسيمات. تم تأكيد التخليق الحيوي من خلال تغير اللون وتحليل الأشعة فوق البنفسجية والمرئية. تقنيات التوصيف مثل المجهر الإلكتروني الماسح (SEMوالتحليل بالطاقة الانتشارية للأشعة السينية (EDXوالتحليل بالأشعة السينية (XRDأكدت وجود جسيمات الفضة وتوضح شكلها وهيكلها البلوري. أظهرت جسيمات الفضة المخلقة نشاطًا مضادًا للبكتيريا ضد البكتيريا الإيجابية للجرام (Staphylococcus aureusوالبكتيريا السالبة للجرام (Pseudomonas aeruginosa)، مع مناطق تثبيط تبلغ 12 ملم و 11 ملم على التوالي. تم توجيه الاهتمام إلى أن قدرة جسيمات الفضة على مكافحة التأكسد كانت أقل من قدرة حمض الأسكوربيك بنسبة تقدر بـ 1.71.

الكلمات المفتاحية : *Stphylococcus aureus* ,التخليق الحيوي، جسيمات الفضة النانوية، التوصيف، النشاط المضاد للبكتيريا، النشاط المضاد للأكسدة، تحسين