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Green synthesis, analysis and characterization of silver nanoparticles loaded on Ginkgo biloba and estimation of their antibacterial activity

Safa Salah Salman[1], Asaad T. Al-Douri[2,], Albosale Abbas Hadi[3], Saeb Jasim Mohammed Alnajm[4]*

[1]Department of Biology, College of Science, Tikrit University, Iraq
[2]Department of Biotechnology, College of Applied Science, Samarra University, Iraq
[3]Department of Immediate Ambulance/ Al-Dour institute, Northern Technical University, Mosul, Iraq
[4]Department of Biotechnology, College of Applied Science, Samarra University, Iraq
*) Email: asta7062@gmail.com

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A scientific study evaluated the effectiveness of green synthetic nanoparticles containing Ginkgo biloba extract and silver nitrate as an antibacterial agent. The scientists created the green nanoparticles by mixing the plant extract with different amounts of silver nitrate (1 mM, 1.5 mM, 1.75 mM, and 2 mM). The combination changed color from yellowish to brown in 10 minutes, and after an hour, it turned into a dark brown shade. This color change indicates the formation of silver nanoparticles. The researchers observed a distinct surface Plasmon resonance (SPR) band at around 433 nm, which suggests the presence of nanoparticles with a green color. The highest peak intensity of this band is observed at a concentration of 1.75 mM $AgNO_3$. Atomic Force Microscopy is used to analyze the form and size of the green nanoparticles. The findings showed that the nanoparticles' size depended on the silver nitrate concentration. The average diameters are 74.86, 42.70, 36.01 and 39.27 nm for $AgNO_3$ concentrations of 1 mM, 1.5 mM, 1.75 mM, and 2 mM, respectively. The height of the nanoparticles varied based on the concentration of silver nitrate. The heights measured are 46.497 nm, 18.360 nm, 19.233 nm, and 24.293 nm for silver nitrate concentrations of 1 mM, 1.5 mM, 1.75 mM, and 2 mM, respectively. The silver nanoparticles synthesized had a mostly spherical shape. The study tested the efficacy of green synthetic silver nanoparticles against two strains of bacteria: Gram-negative Pseudomonas aeruginosa and Gram-positive Staphylococcus aureus. The findings showed that nanoparticles at different doses (1, 1.5, 1.75 and 2 mM) successfully suppressed bacterial growth, resulting in inhibition zones of different sizes. The inhibitory zones for Staphylococcus aureus are measured to be 8 mm, 8 mm, 9 mm, and 7 mm at concentrations of 1 mM, 1.5 mM, 1.75 mM and 2

mM, respectively. The inhibitory zones for Pseudomonas aeruginosa are 9 mm, 10 mm, 11 mm, and 9 mm at concentrations of 1 mM, 1.5 mM, 1.75 mM and 2 mM, respectively.

Keywords: Green synthesis; Silver nanoparticle; Characterization; Antibacterial.

1. INTRODUCTION

The extensive utilization of complementary and alternative medicine (CAM) is of great significance to healthcare consumers, practitioners, researchers, and policymakers in the present era. Less than 40 percent of individuals in the United States who utilize complementary and alternative medicine (CAM) inform their physicians about their usage. According to estimates from 1997, around 15 million persons used prescription drugs at the same time as herbal remedies or high-dose vitamins. This raised concerns about the potential for negative interactions between these substances. The origins of medicine and using plants for healing may be traced back to ancient times when herbal remedies are the primary solution for all illnesses [3]. Currently, there is a renewed focus on phytotherapy on a global scale [4]. Herbal medicine uses various parts of plants, such as seeds, roots, leaves, bark, flowers, and extracts, for medicinal purposes [5]. It is a traditional form of medicine that utilizes natural products derived from plants to treat diseases and emphasizes the connection between humans and their environment [6]. The research and application of herbal medicine in treating diseases is growing steadily. Historically, medicinal plants are commonly regarded as therapeutic agents for treating typhoid, cholera, measles, and others [7,8].

Nevertheless, the understanding of herbal remedies for curing illnesses is primarily limited to herbalists or botanists who believe that the effectiveness of herbal medications will diminish if shared with the general public. While certain herbs may possess medical properties, preparing them for medicinal use can sometimes have specific adverse effects [9]. Ginkgo biloba is an ancient botanical species that is thought to provide diverse health advantages to living organisms. This plant is chemically varied due to its abundance of bioactive components. G. biloba is categorized in the plant kingdom and is a member of the Ginkgoaceae family. This plant is commonly called a "living fossil" because it is one of the earliest seed plants in evolution [10]. However, the plant Ginkgo biloba is a vital source of new herbal drugs, including numerous bioactive ingredients with therapeutic effectiveness. Ginkgo biloba has been employed as a traditional herbal remedy for more than two millennia in China and many regions across the globe. It is cultivated in Europe, Asia, Argentina, North America, and New Zealand [11].

Recently, nanotechnology has developed as a highly influential technology in the past few centuries [12]. Nanoparticle-based medicines have gained popularity due to their targeted nature, enabling the administration of lower medication doses and minimizing side effects [13]. Furthermore, drug delivery nanoparticles can increase the amount of a drug that the body can absorb at specific locations, which is essential for the effectiveness of a treatment [14].

2. EXPERIMENTAL

2.1 Ginkgo biloba collection and identification

The aboveground portion of Ginkgo biloba is gathered from Arbil city, in the northern region of Iraq. Dr. Ibrahim identified the plant at the College of Pharmacy of Al-Mustansiriya University. The plant components are rinsed with deionized water and left to dry naturally in a shady location for one week. Subsequently, the substance is pulverized into tiny particles using an electric grinder, transferred into an airtight container and kept at ambient temperature until required.

2.2 Preparation of plant extract

Ginkgo biloba is prepared according to the procedure specified by Kadhim (2016). Commence by acquiring 50 grammes of the botanical specimen and carefully cleaning it. Next, immerse it in one liter of distilled water at 40 degrees Celsius for 24 hours, ensuring the combination is continuously agitated using shaking incubators. After 24 hours, strain the suspension using cheesecloth to eliminate any solid particles that are not soluble. To make nanoparticles in the subsequent experiment, it is necessary to dry a specific portion of the extract and keep the rest of the solution in the refrigerator at a temperature of 4 °C [15].

2.3 Detection of G.biloba active compound

The Biotechnology Research Center of Al-Nahrain University detected the active compounds in the plant.

2.4 Biosynthesis of sliver nanoparticle

Silver nanoparticles are synthesized by utilizing Ginkgo biloba as a botanical source and silver nitrate ($AgNO_3$) as a source of silver. The reaction mixtures are evaluated using varying doses of $AgNO_3$ (1.0, 1.5, 1.75, and 2 mM) and Ginkgo biloba extract at 9:1, respectively. Subsequently, the combination is placed in a lightless chamber at a temperature of 30°C to avoid the activation of silver nitrate by light while maintaining a stable environment. The research is carried out by [16].

2.5 Detection of nanoparticles

The characterization of nanoparticles involves observing color changes, a crucial technique for the early identification of green synthetic nanoparticles [16]. A spectrophotometer can measure the absorbance of green synthetic nanoparticles at a wavelength of 433 nm to detect them [17].

2.6 Atomic force microscopy

The investigation is conducted using a scanning probe microscopy technique called atomic force microscopy (AFM), employing the NT-MTD instrument. The Nanoparticle solution samples are diluted using distilled water and placed onto a glass slide measuring 1×1 cm. Once the samples had dried, the slide is positioned on the AFM sample stage, and the analysis is carried out following the conventional procedure [18].

2.7 Scanning electron microscope (SEM)

The size and form of AgNPs are determined using Scanning Electron Microscopy (SEM) [19].

2.8 Determination of antibacterial activity

Muller Hinton agar is prepared by dissolving 38 grammes of media in 1000 milliliters of distilled water. Subsequently, the concoction is subjected to boiling and filtration to eliminate contaminants. The agar is placed onto a glass plate to a 3-4 mm depth and allowed to harden. The plates are stored at a temperature of 4°C until ready to be used. To assess the impact of green synthetic nanoparticles derived from Ginkgo biloba on bacterial growth, predetermined concentrations of these nanoparticles are introduced into agar plates. Subsequently, the bacteria are inoculated into the plates [20]. The present investigation employed two bacterial species: Staphylococcus aureus and Pseudomonas

aeruginosa. Individual colonies from each bacterial strain indicated in the previous text are cultivated on nutrient agar for 18-24 hours. Next, they are transferred using a reed containing 5ml of normal saline and thoroughly mixed using a vortex. The bacterial proliferation is assessed using the McFarland tube as a reference. The turbidity of the standard saline tube is equivalent to a bacterial inoculum concentration of 1.5×108 cells/ml. A cotton swab inoculated a tiny bacterial culture from the normal saline onto the Muller Hinton agar. The plate is subsequently streaked three times by rotating the swab about 60° each time to ensure uniform dispersion of the inoculums. The plates that are treated with an inoculum are left at room temperature for ten minutes to facilitate the absorption of any surplus moisture. Next, using a sterilized Pasteur pipette, we generated wells (placed strategically to avoid the formation of overlapping inhibitory zones). We filled them with 100 µl of green synthetic nanoparticles derived from Ginkgo biloba extract. Following incubation at 37°C for 18-24 hours, the individual in charge utilized a restricted area to measure the diameter of the plates in millimeters and duly documented the obtained outcomes [21].

3. RESULTS AND DISCUSSION

3.1 Chemical analysis of G.biloba

The methanolic extract of Ginkgo biloba is subjected to chemical analysis, revealing the existence of glycosides, flavonoids, polyphenols, and alkaloids. The results are presented in Table 1.

Table 1 Chemical compounds identified in the methanolic extract Ginkgo biloba.

Ginkgo extracts	Chemical compounds	Reagents	Indication	Results
Methanolic extract	Tannins	Ferric chloride	Green-blue ppt	Positive
	Glycosides	Fehling reagent	Red-ppt.	Positive
	Flavonoids	Ammonia	Yellow-ppt.	Positive
	Saponines	1-Shaking of extract	Foam	Positive
	Alkaloids	2-Mercuric chloride	White-ppt	Positive
	Terpens	Myers regent	White-ppt	Negative
		Concentrated Sulfuric acid	Brown colour	Positive
Hexane extract	Steroids	Liebermann's reagent	Green color	Positive

Scientific evidence supports the efficacy of Ginkgo biloba as an herbal medicine. It attributes its effectiveness to the presence of bioactive compounds such as flavonoids, glycosides, alkaloids, and polyphenols. These components function as antioxidants and offer a multitude of health advantages. Ginkgo biloba is rich in flavonoids and terpenoids, widely recognized for their strong antioxidant properties. Furthermore, research has demonstrated that these elements exhibit anti-inflammatory characteristics [22].

3.2 Biosynthesis and detection of silver nanoparticles

This work aimed to investigate the process of synthesizing green silver nanoparticles by utilizing colour change and UV spectroscopy absorption. The reaction mixture underwent a colour

transformation from a yellowish hue to brown in a span of 10 minutes. Eventually, it turned dark brown after an hour, signifying the production of silver nanoparticles. The presence of active molecules in the Ginko Biloba extract caused a reduction of silver metal ions Ag to form silver nanoparticles, Ag. The alteration in colour is ascribed to the stimulation of the Plasmon resonance characteristic (SPR). A distinct surface plasmon resonance (SPR) band at around 433 nm is observed for silver nanoparticles with a green colour, a typical feature of these nanoparticles. The surface plasmon resonance (SPR) peak of green silver nanoparticles grew more pronounced as the concentration of silver nitrate increased. The highest intensity of the peak is seen at a concentration of 1.75 mM of $AgNO_3$ (Figure 2). The creation of silver nanoparticles can be related to the interactions between hydrophilic and hydrophobic substances, which lead to intermolecular forces [23]. Additionally, there is extensive evidence that AgNPs display a brown hue when dissolved in methanol due to the stimulation of surface Plasmon vibrations [24]. It is understood that changes in the composition of biological material and the concentration of metal salts can impact the formation of nanoparticles. Finally, it is important to mention that noble metals have distinct optical properties because of the surface Plasmon resonance (SPR) phenomenon [25,37]. The capacity of Ginko Biloba extract to produce AgNPs may be ascribed to the presence of secondary metabolites in the plant.

Figure 1 Visual observation of synthesized nanoparticle after 24 hours (A) *Ginko Biloba* extract (B) *Ginko Biloba* extract loaded with silver nanoparticles.

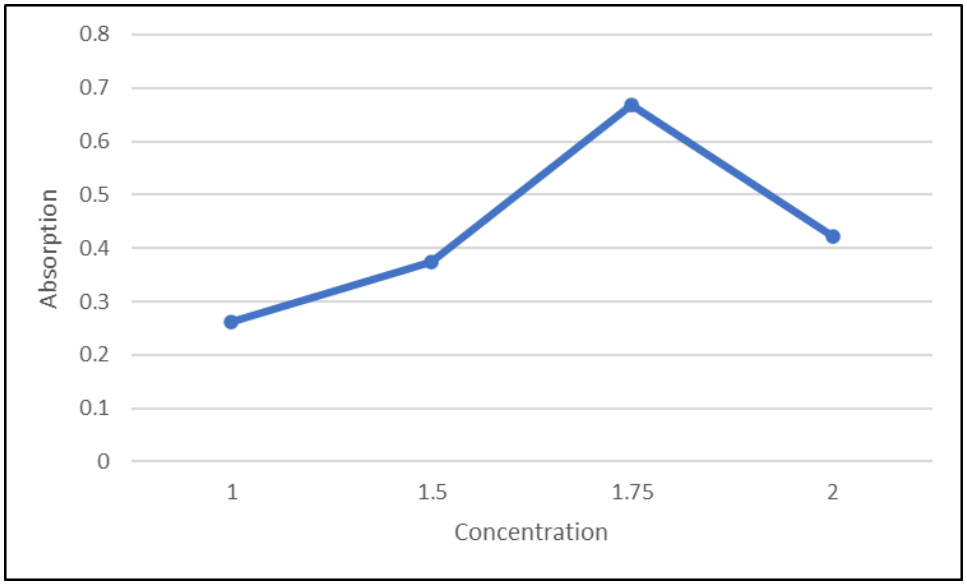


Figure 2 UV-visible absorption spectra of green synthesized silver nanoparticles at 433 nm.

3.3 Characterization of Green Synthetic Silver Nanoparticles

The form and size of the green-synthesized silver nanoparticles are analyzed using AFM. The results showed that the average sizes of the nanoparticles varied depending on the silver concentrations, as depicted in Figures 3A74.86, B 42.70, C 36.01, and D 39.27. A study conducted on silver nanoparticles [26] discovered that the organic chemicals identified in the extract are accountable for reducing silver ions and stabilizing the resulting nanoparticles. The components mainly comprise pectin, cellulose, and hemicelluloses [27]. The functional groups associated with these polymers and the proteinaceous materials may play a role in decreasing the nanoparticle. Compounds with reducing power can donate electrons and lower the oxidized intermediates of lipid peroxidation processes. This makes them effective as both primary and secondary antioxidants [28].

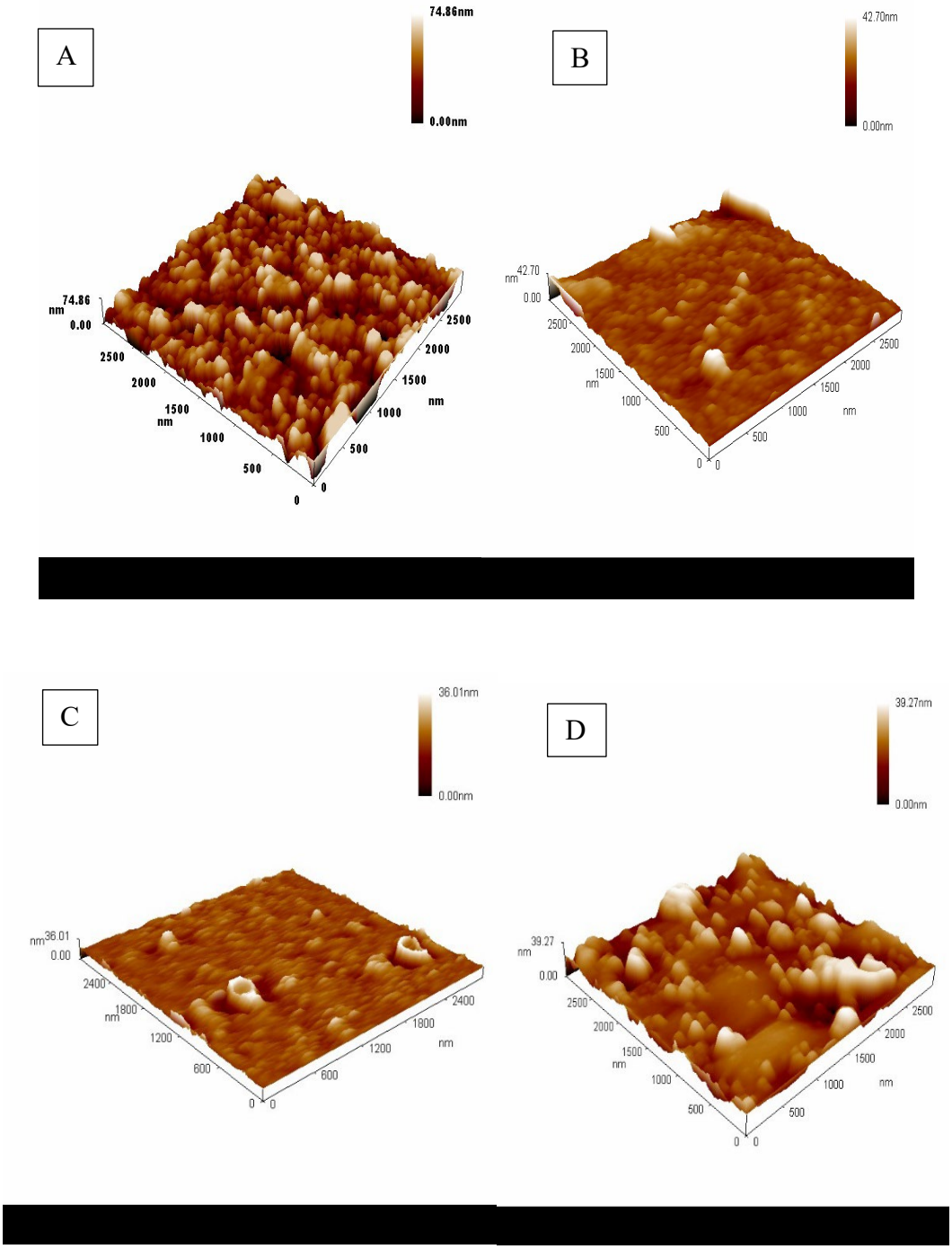
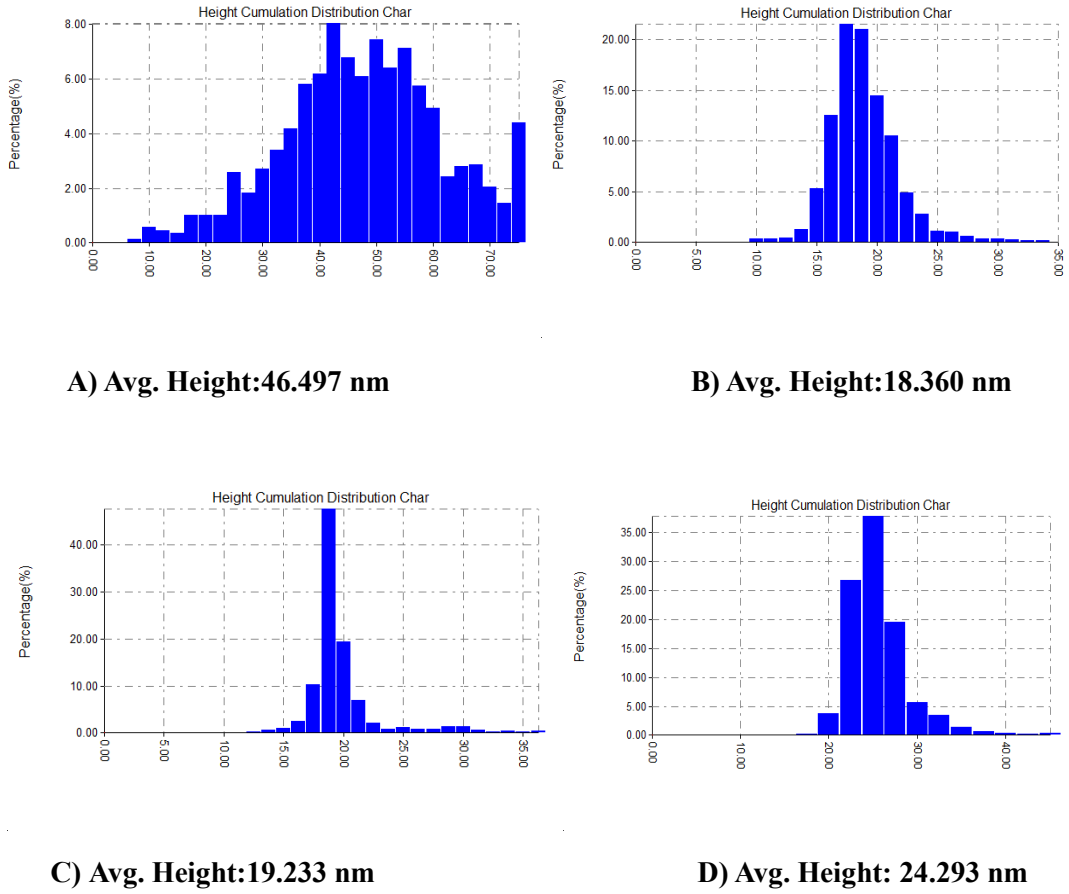


Figure 3 AFM Segments of green-produced silver nanoparticles with varying concentrations of the particles (A 1 mM 74.86, B 1.5 mM 42.70, C 1.75 mM 36.01, and D 2 mM 39.27nm).

AFM analysis showed that the size of Ginko Biloba NPs, determined by their height, is (A) 46.497 nm, (B) 18.360 nm, (C) 19.233 nm, and (D) 24.293 nm.



A) Avg. Height:46.497 nm

B) Avg. Height:18.360 nm

C) Avg. Height:19.233 nm

D) Avg. Height: 24.293 nm

Figure 4 AFM analysis explained that the height of *Ginko Biloba* NPs in which the height is (A) 46.497 nm, (B) 18.360 nm,(C) 19.233 nm and (D)24.293 nm for (1,1.5,1.75 and 2) respectively.

The nanoparticles' primarily spherical morphology is apparent from the Figure 4 scanning electron microscope (SEM) image.

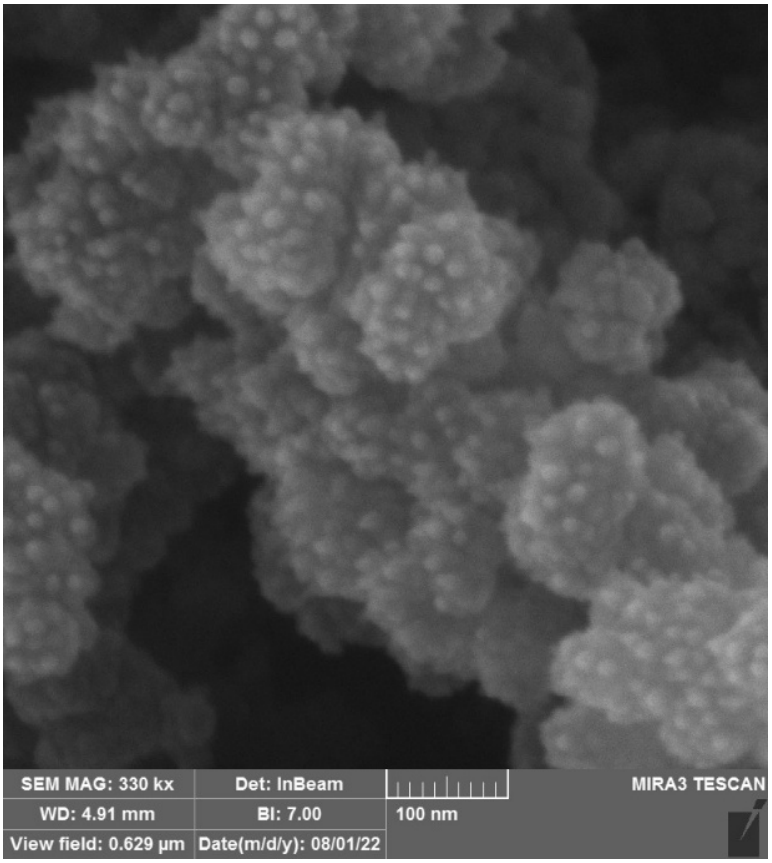


Figure 5 Scanning electron microscopy images of nanoparticles at different concentrations (1 mM, 1.5 mM, 1.75 mM, and 2.5 mM).

In a study conducted by it is discovered that silver nanoparticles may be generated by reducing silver ions using organic chemicals contained in the extract [29]. The primary constituents of these organic substances are cellulose, hemicelluloses, and pectin suggest that the presence of proteinaceous materials and functional groups on these polymers could potentially decrease the size of nanoparticles [30-32].

3.4 Antibacterial Activity of Green-Synthesized Silver Nanoparticles:

We investigated the antibacterial efficacy of green synthetic nanoparticles against Gram-negative (Pseudomonas aeruginosa) and Gram-positive (Staphylococcus aureus) bacteria by measuring the zone of inhibition. The nanoparticles are tested at 1, 1.5, 1.75 and 2 mM concentrations. The findings demonstrated a positive correlation between the concentration of green synthetic nanoparticles and their antibacterial effectiveness against G- bacteria. The diameter of the inhibitory zone varied between 9 and 11 mm. The green synthetic nanoparticles exhibited antibacterial action against Gram-positive bacteria, as demonstrated by the inhibition zone ranging from 7 to 9 mm (Table 2, Fig 6). Gram-negative bacteria exhibited greater inhibitory zones than Gram-positive bacteria, possibly due to cell wall composition differences [33]. Gram-positive bacteria possess a cell wall composed of a substantial coating of peptidoglycan. This layer consists of linear polysaccharide chains that are interconnected by short peptides. Consequently, the structure becomes stiffer, impeding the penetration of silver nanoparticles. In contrast, Gram-negative bacteria possess a narrower peptidoglycan layer [34]. Silver nanoparticles can inflict more harm on bacterial cells by entering the cell and reacting with DNA, proteins, and other cellular components that contain phosphorus and sulphur [35]. The third consequence of silver nanoparticles entails the liberation of silver ions, leading to an intensified

biocidal impact contingent on both the size and quantity [36]. Nevertheless, the study demonstrates that silver nanoparticles exhibit a greater antibacterial efficacy against Gram-negative bacteria than Gram-positive bacteria [37-41].

Table 2 The diameter of the zone of inhibition caused by green synthetic silver nanoparticles (AgNPs) at different doses (1, 1.5, 1.75, and 2 mM) is determined in mm for both Gram-negative (P. aeruginosa) and Gram-positive (S. aureus) bacteria.

Concentrations of green synthetic silver nanoparticles	***P. aeruginosa***	***S. aureus***
1	9	8
1.5	10	8
1.75	11	9
2	9	7

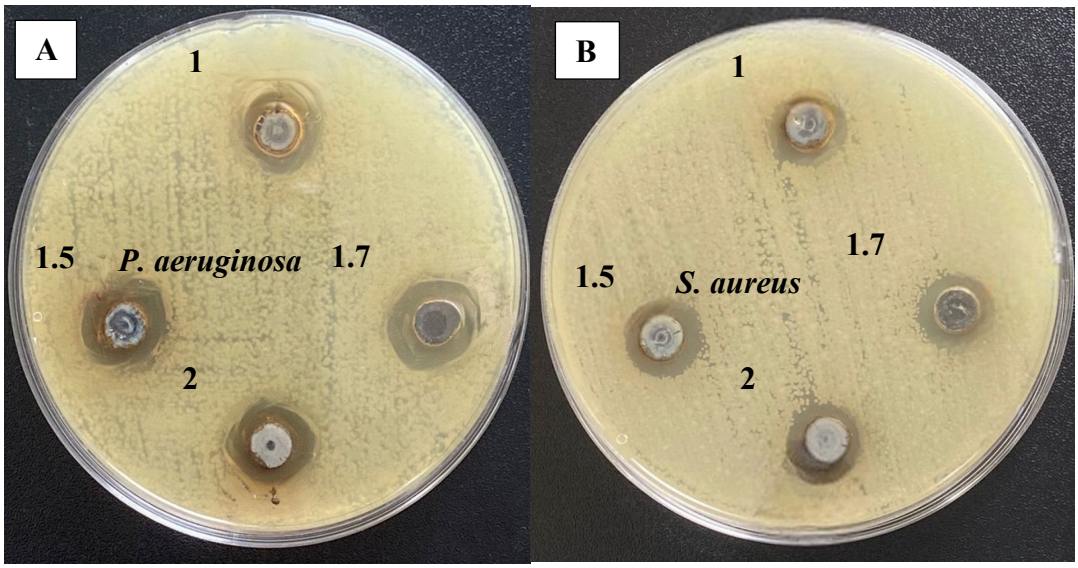


Figure 6 The antibacterial activity of green synthetic silver nanoparticles (at concentrations of 1, 1.5, 1.75, and 2mM) is assessed against two types of bacteria: Gram-negative bacteria (P. aeruginosa) and Gram-positive bacteria (Staphylococcus aureus) by measuring the zone of inhibition.

4. CONCLUSIONS

This study investigates the phytochemical characteristics of Ginkgo Biloba and produces silver nanoparticles by an environmentally friendly synthesis process. We employ UV-vis, AFM, and SEM techniques to characterize the synthesized nanoparticles. The results of our study suggest that silver nanoparticles can suppress the growth of Gram-negative bacteria more effectively than Gram-positive bacteria. This significant finding emphasizes the promise of green synthesis nanoparticles for medical purposes, given their extensive utilization across several domains.

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