

Bioactivity of silver nanoparticles synthesized using extracts of *Blepharis*

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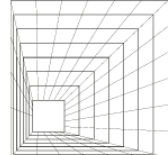
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Abstract: The data on how to quantify the antibacterial and antioxidant capabilities of silver nanoparticles is the focus of this review article. Below, we have compiled the results of many studies that have examined the antioxidant activity of silver nanoparticles. Several studies have shown that the antioxidant activity of silver nanoparticles is higher than that of the extract that is utilized in their synthesis. In this review, we primarily looked at the (DPPH) test as a way to measure antioxidant activity. The results showed that the extract, gold nanoparticles, and ascorbic acid had antioxidant activities of (53.67), (55.35), and (72.12), respectively. In vitro and in vivo studies have shown that silver nanoparticles (AgNPs) are effective against infectious microorganisms. Both Gram-negative and Gram-positive bacteria, even those that are resistant to several drugs, are susceptible to the antibacterial action of AgNPs. Here are the *E. coli*, *K. pneumoniae*, and *P. aeruginosa* antibacterial results for the negative type concentrations that were chosen: 5, 7, 8, and 8 mm; 7, 8, and 10 mm; and 8, 10, and 11 mm. The results for *S. aureus* (7,7,9,10) mm, *S. pneumoniae* (7,8,10,11) mm, and *S. sanguis* (8,9,10,11) mm, all of which were of the positive type, are as follows.

Keywords: Silver nanoparticles; *Blepharis* extract; Antibacterial activity; Antioxidant; Antibiotic substitute ; Medicine uses

1. Introduction

Natural remedies and pharmaceuticals can be found in medicinal plants. The capacity for plants to create secondary metabolites was bestowed upon them by nature [1]. Natural resources are utilized nowadays. [2]. One cutting-edge biological approach that uses therapeutic herbs is nanoparticle production [3]. Dispersion, shape, and size are three distinguishing features of nanoparticles [4]. The production of nanoparticles using *Blepharis* extract is an efficient, safe, and easily scalable process that may be scaled up to huge quantities [5]. Chemical catalysts, biolabeling, antimicrobial, antioxidant, gene therapy, optical effectors, photonic crystal coatings for solar energy absorption and solar energy batteries, green AgNPs are utilized for these and other purposes [6]. In order to develop methods for making nanoparticles that are safe for the environment and do not harm living things, scientists are looking at biological systems. Antibacterial medications utilizing silver nanoparticles, either singly or in conjunction, have an extensive history of usage. Burn treatments, ulcer dressings, food packaging to prevent contamination, home equipment (such as refrigerators and washing machines), and industrial purposes have all used this metal due to its ability to restrict bacterial growth [7]. The antibacterial activity of silver makes it a suitable candidate to investigate the antibacterial potential of silver nanoparticles (AgNPs), which is made possible by nanotechnology. With sizes ranging from 1 to 100 nanometers, AgNPs qualify as nanomaterials [8]. Both the surface area and the capacity of these are greater than those of bulk silver [9]. Researchers have explored and developed devices using this material for targeted medication administration, diagnostics, imaging, and detection because of its nanoscale electrical, optical, and catalytic characteristics [10]. Both academia and industry are interested in AgNPs because of their antibacterial properties [11]. Several harmful pathogens, including



those that are resistant to many drugs, are susceptible to the bactericidal effects of AgNPs [12]. Hundreds of products using AgNPs have found use in the medical and healthcare sectors, where the nanoscale antibacterial effect of silver has been particularly beneficial [13]. Items pertaining to surgery and food handling, apparel, cosmetics, dentistry, catheters, and bandages [14]. The antibacterial potential of AgNPs is enhanced by their multi-action nature [15]. A wide variety of bacterial species are eradicated by these procedures because they attack many germ structures simultaneously [16]. In oxygen-dependent (aerobic) settings, free radicals play a significant role [17]. They play an important role in cellular respiration and other processes. Additionally, they amplify the process of age-related diseases. Since free radicals include unpaired electrons, they are unstable [18]. The presence of unbound electrons, which seek for covalent bonds, makes these compounds reactive [19]. Radical free states deplete nearby molecules of electrons [20]. Damage to carbohydrates, lipids, proteins, and nucleic acids can disrupt cellular homeostasis [21]. Cells and the environment are both potential sources of free radicals. During aerobic metabolism, free radicals are generated [22]. Electron transport across plasma membranes and respiration by mitochondria are the principal energy sources. Superoxide can bypass the electron transport chain and induce oxidative damage primarily in mitochondria. To protect themselves from free radicals, cells have an antioxidant mechanism built right in [23]. Proteins play a role in this process by helping to stabilize free electrons. By blocking, postponing, or limiting biomolecule oxidation, antioxidants avoid oxidative reactions [24]. Enzymes that neutralize free radicals shield proteins from oxidative stress. Glutathione and vitamin C are two examples of non-enzymatic antioxidants that can also neutralize radicals [25]. Recently, nanomaterials have demonstrated efficacy in a variety of biomedical applications, including medical imaging, medication administration, disease detection, cancer therapy, infectious disease treatment, neurodegenerative disorder treatment (including Parkinson's disease), and many more [26].

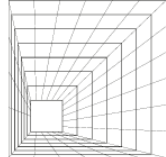
2. Experimental

2.1 preparation of *Blepharis* extract

After soaking 50 g of dried leaves in an ultrasonic shaking water bath for 48 hours, the mixture was diluted with 350 ml of ethanol. After that, it goes through a three-layer tetron cloth filter to remove plankton and other contaminants. Then, the collected extracts are placed into plates, and the solvent is allowed to evaporate at room temperature. Next, the thick liquid is filtered and kept out of direct sunlight. Using deionized water, various concentrations are created for the experiments [27].

2.2 preparation of silver nanoparticles (AgNPs)

A container with an amber hue was made by dissolving 0.042 g of AgNO₃ in 250 ml of distilled water at room temperature. This solution contained 1 mM of silver nitrate, which would prevent the silver ions from autooxidizing. the mixture was heated to 45 degrees Celsius in a water bath with a pH of 9, and 10 milliliters of silver nitrate (1 millimoles) was added to (1,2,3,4) milliliters of extract at a time while being agitated. A change to a yellowish brown hue in the solution after 30 minutes of heating was indicative of the formation of silver nanoparticles. After centrifuging the colloidal suspension at 4500 rpm for 30 minutes, the supernatant was discarded and the pellet was resuspended in deionized water. Two or three rounds of centrifugation were performed in an effort to remove impurities that had attached to the surface of the silver nanoparticles. Following drying and cooling, the nanoparticles were returned to their powdered state [28].



2.3 Oxidation activity (DPPH Radical-scavenging)

The dissolving of 0.0039 grams of DPPH in 100 milliliters of methanol yielded a DPPH solution with a concentration of 0.1 mM. Upon the addition of 1.0 mL of this solution, 2.0 mL of a solution containing silver nanoparticles was produced. The solution's concentration varied from 25 to 100 g/mL, contingent upon the volume of the solution added. Following a thirty-minute waiting time, the absorbance of the analyzed mixture was measured at 517 nanometers. Ascorbic acid was selected as a reference for comparison. The little light absorption by the reacting combination indicates that the sample exhibits optimal scavenging activity [30]. The percentage of oxidative damage from free radicals neutralized by the sample was determined using the formula:

$$\% \text{ inhibition of DPPH} = \frac{(Ac - As)}{Ac} \times 100$$

(Ac) absorbance of control, (As) absorbance of materials

2.4 Antibacterial activity method

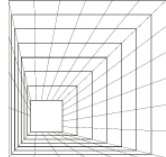
Six distinct types of bacteria were used in the experiment; three of these were Gram positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Streptococcus sanguinis*) and three of these were Gram negative (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*).

To test if the AgNPs produced by alcoholic extraction had any antibacterial action, the agar well diffusion method was employed. The experiment called for the use of a sterile cotton swab to transfer pathogenic organisms from fresh cultures onto agar plates. Next, an uncontaminated cotton swab was used to disseminate the resulting suspension on the nutritional agar. The mixture was then allowed to dry for ten minutes [29].

4. Result and discussion

4.1 Antioxidant activity

To determine the extent of their DPPH-based antioxidant activity, we compared *Blepharis* extract with AgNPs in this study. Radical scavenging activity (RSA) percentages were used to present the final figures. It is usual practice to evaluate plant extracts for their antioxidant properties using the DPPH assay. Due to their independence from the reduction of hydrogen or electron donors, free radicals focused on nitrogen are more stable than other free radicals. When testing the ability of the AgNPs to decrease DPPH in an ethanol solution, it was necessary to observe any discernible shift in color from the violet control. Diphenyl picrylhydrazine, a compound with a yellow hue, was the final product. The addition of silver nanoparticles to a purple solution containing DPPH causes the solution to become yellow. Whether the solution is reduced by donating electrons or hydrogen, this occurs. Consequently, substances that may undergo this reaction are referred to as radical scavengers and antioxidants. Because they absorb less DPPH, the extracts are better able to scavenge free radicals. The method relied on tracking variations in DPPH absorbance at 517 nm to determine antioxidant activity. Plants may have powerful free radical scavenging capabilities for some chemicals, such as phenolic compounds or flavonoids, due to the presence of hydroxyl groups. The DPPH scavenging assay shown that at pH=9, AgNPs significantly inhibited the activity of *Blepharis* extract and ascorbic acid, the gold standard in the field. It was discovered that the antioxidant capacity of *Blepharis* extract was lower than initially believed. Compared to before, our silver particles are now far more effective. Extracts of *Blepharis* resulted in AgNPs with the greatest radical scavenging activity, at 72.12%. Antioxidant activity was 53.67% for standard ascorbic



acid and 53.67% for ethanolic extract at a concentration of 100 g/mL. Its antioxidant activity might be due to components present on the metal surface and in plant extract that cap it. Figure 8 shows that plant extracts boost the DPPH radical scavenging action of AgNPs in a dose-dependent manner.

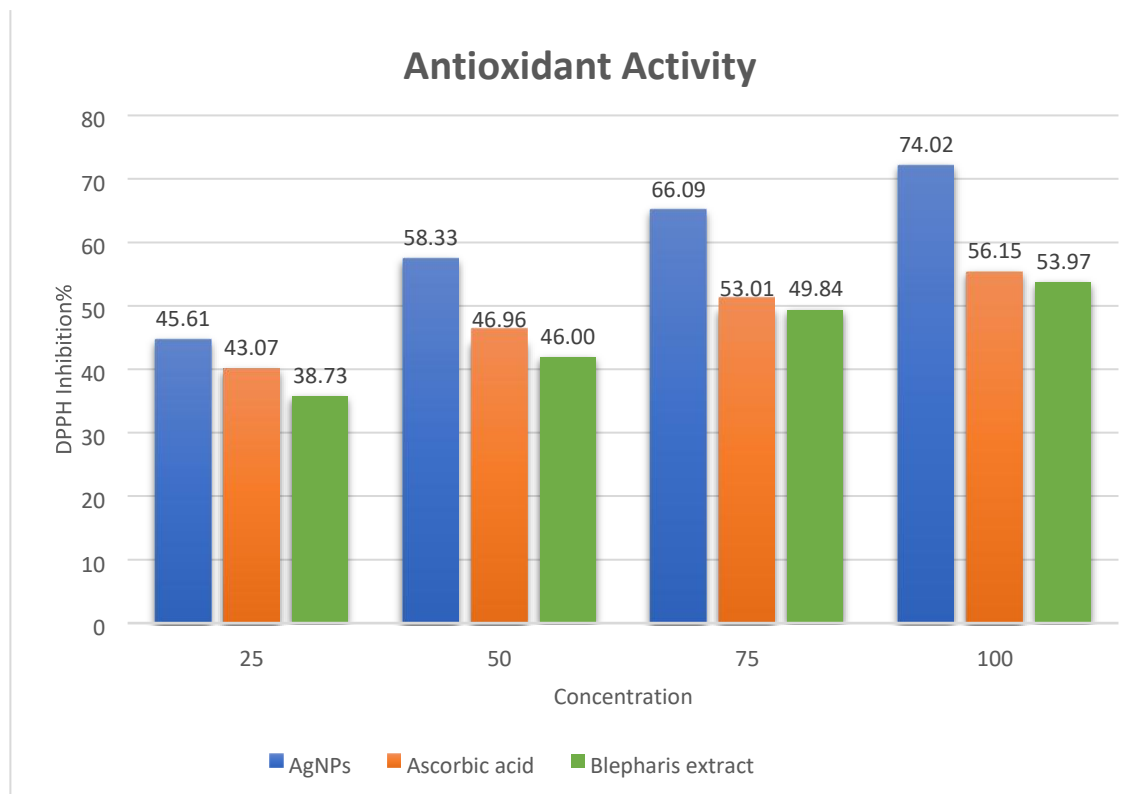


Figure 1: DPPH free radical scavenging activity of AgNPs with different concentration when compared to the standard ascorbic acid and *Blepharis* extract

4.2 Antibacterial activity

By employing the agar disk diffusion method, the antibacterial activity of six distinct microorganisms was evaluated against both Gram-negative (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*) and Gram-positive (*Staph aureus*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*) bacteria. Using disk diffusion, a commonly used technique to test for antibacterial activity, researchers have verified that the AgNPs solution kills bacteria. Any microbes in close proximity to a substance having antibacterial properties will have their growth stymied. To stop the growth of microbes surrounding a disk, place the chemical on top of the disk and place it on top of a thick agar gel containing bacteria. This zone of inhibition is directly related to the sample's efficacy against bacteria. So, an antibacterial agent's efficacy is proportionate to the size of its zone of inhibition.

The results showed that the growth of the bacterial strains was progressively affected by the concentration. The extract has varying effects on various bacterial species. The ethanolic extract was used in varying concentrations. The inhibitory zones of *K. pneumoniae*, *P. aeruginosa*, and *Escherichia coli* bacteria were 5, 6, and 7 mm in diameter, respectively. A diameter of (5,6,6,7)mm at different concentrations inhibits gram-positive bacteria such *Staph. aureus*, *Strep. pneumoniae* (6,7,8,8)mm, and *Strep. sanguinis* (5,7,7,8)mm, according to the table in figure (1).

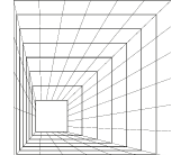


Figure 2 : Antibacterial test of *Blepharis* extract

The antimicrobial effects of AgNPs were measured against various bacteria at different concentrations. *Staph. aureus*, *Strep. pneumoniae*, *Klep. pneumoniae*, *E. coli*, *Pseudomonas aeruginosa*, and *Strep. sanguinis* were tested at 7, 7, 9, 10, and 11 mm, 7, 8, 10, and 10 mm, respectively. For *S. sanguinis*, *S. pneumoniae*, and *K. pneumoniae*, respectively, the inhibition zones of imipenem antibiotic discs at different doses were 12, 10, and 9 millimeters, as compared to antibiotic (imipenem). The antibacterial activities of the ethanolic *Blepharis* extract and the biologically generated AgNPs were more pronounced against Gram-positive bacteria than Gram-negative bacteria. The antibacterial agents have diverse modes of action due to variations in the structure of the cell walls. Gram-negative bacteria contain multiple layers of peptidoglycan in their cell walls, in contrast to cell-positive bacteria which only have one thin layer. Because of this, gram-negative bacteria become inflexible and unable to allow nanoparticles access to their cells. Lipopolysaccharides, lipoproteins, and protein lipids are common components of the cell walls of gram-negative bacteria. Bacteria, in contrast, are more aggressive than Gram-negative bacteria due to their lipid-based cell walls, which make them more permeable. Figure (2) shows that the relative adherence of particles to microorganisms is proportional to their surface area.

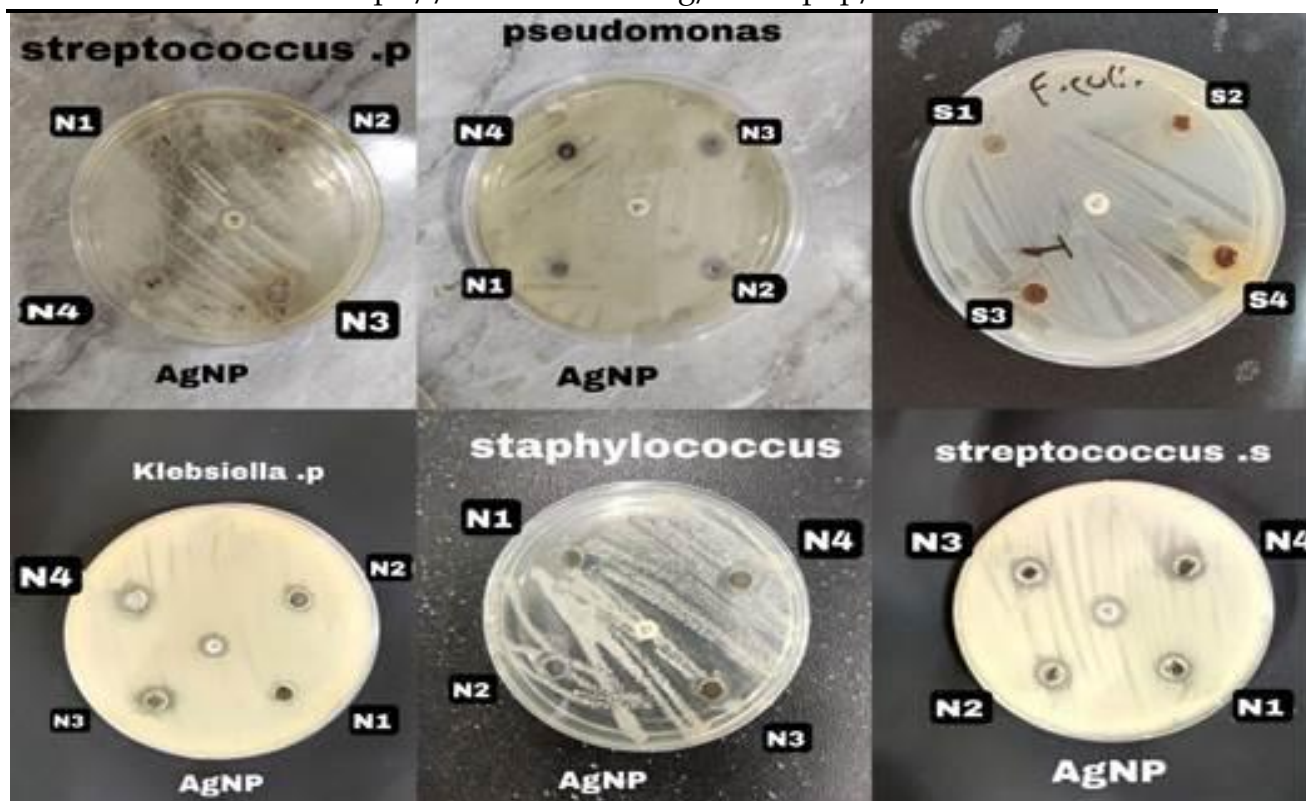
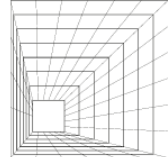


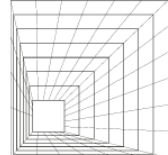
Figure 3: antibacterial test for AgNPs

5. Conclusion

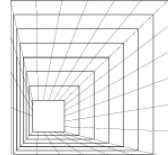
Using an ethanolic *Blepharis* extract, silver nanoparticles were produced in a "environmentally friendly" manner. As the amount of extract used grows, the rate of synthesis also increases, leading to the production of more nanoparticles of ever-tinier sizes. The ethanolic extract of *Blepharis* was found to include flavonoids, phenols, glycosides, carbs, Quinones, steroids, terpenes, tannins, and coumarins (absence of alkaloids was noted in the qualitative analysis). When compared to ascorbic acid, the industry standard, AgNPs showed a substantial improvement in peroxy radical neutralization in the DPPH assay. Although nanoparticles have some success against gram-negative bacteria like *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*, they have less success against gram-positive bacteria like *Staphylococcus aureus*, *Staphylococcus pneumoniae*, and *S. sanguinis*.

6. References

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