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Original Research Article

Antibacterial Activity of Green Synthesized Silver Nanoparticles of *Lablab purpureus* Flowers Extract against Human Pathogenic Bacteria

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ABSTRACT

There are several uses for silver nanoparticles in the field of biomedicine because of their exceptional features. The crucial use of silver nanoparticles is the development of antibiotics that are clinically effective against resistant bacteria. Due to their non-toxicity, environmental friendliness, and faster action, green syntheses of silver nanoparticles have gained some attraction. Plant extracts could be used as efficient sources of reductants and stabilizers in this process and also investigated for antibacterial action. This study mainly aimed to synthesize silver nanoparticles using *Lablab purpureus* flowers extracts and evaluate the antibacterial properties of the green synthesized sliver nanoparticles. The silver nanoparticles were synthesized and characterized by uv–vis, FT-IR spectroscopy and SEM analysis. Additionally, the disk diffusion method was used to evaluate the antibacterial activity of the silver nanoparticles against one gram positive and five gram negative bacteria. The particle size of synthesized Silver nanoparticles (AgNPs) was less than 1 µm. In bacterial susceptibility study, flower extract of *L. purpureus* showed moderate antibacterial activity but nanoparticles showed promising antibacterial potential against gram positive *S. aureus* and gram negative *E. coli*, moderate antibacterial activity against *S. typhi* bacteria, and mild activity against *P. vulgaris*, *K. pneumonia* and *P. aeruginosa*. This study indicates that AgNPs made from *L. purpureus* flower extract exhibit potent antibacterial activity, suggesting these nanoparticles may be used to develop effective anti-bacterial drugs for the treatment of bacterial infections.

Keywords: *Lablab purpureus*, Nanoparticles, green synthesis, antibacterial, disk diffusion.

Introduction

The development of pharmacopoeial, non-pharmacopoeial, or synthetic medications has placed importance on the use of medicinal plants as a rich source of phytoconstituents. In both modern and traditional medicine, medicinal plants are utilized with the intention of improving health, being administered for a particular illness, or both.[1] About one-fourth of the medications prescribed to patients in modern medicine are derived from medicinal plants and undergo thorough research. Medicinal plants may comprise the majority of what are often unofficially proposed treatments in other systems of medicine that have not undergone scientific analysis.[2]

Infectious diseases have a high global burden, particularly in low- and lower-middle-income countries (LMICs) like Bangladesh, compared to upper-middle- and high-income countries where non-communicable diseases account for the majority of deaths. Infectious illnesses led to the deaths of about 7 million people worldwide in 2019, accounting for roughly 12% of all fatalities.[3] As a result, given all the evidence of the fast worldwide spread of resistant clinical isolates, the need for novel antimicrobial medicines is essential. This has prompted a search for novel antimicrobial substances, such as plants, which generate a wide range of bioactive chemicals with recognized pharmacological potential.[4]

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The use of herbal remedies and the incorporation of these beneficial foods into the diet will help to promote health.[5] The vegetable *Lablab purpureus*, including its seeds, leaves, flowers, and pods, is popular across the South East Asia.[6] *L. purpureus* has hypoglycemic, antidote, antiviral, carminative, and anticholesterolemic properties. The flowers have carminative, alexiteric, and antiviral properties. Ear and throat inflammation is treated with the pod juice. The fully developed seeds have antispasmodic, aphrodisiac, anthelmintic, digestive, febrifuge, and stomachic properties. They are used to treat a variety of conditions, including cholera, sunstroke, nausea, vomiting, diarrhea, enteritis, and arsenic poisoning.[6-9]

Synthesis of nanoparticles using plant extracts is the most adopted method of green, eco-friendly production of nanoparticles and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites.[10, 11] Targeted medication therapy based on green synthesis nanosized formulations may be an effective way to address complications associated with infections. Green syntheses of nanoparticles from plant extracts have gained some traction due to their non-toxicity and faster onset of action.[12] Besides, it has been observed in literature review that there is no scientific evidence of antibacterial activity of green synthesis nanoparticles of flowers of *L.purpureus*. Hence the aim of this present research was to investigate the antibacterial activity of extract and green synthesized silver nanoparticles of *L. purpureus* flowers.

Material and Methods

Plant material

The flowers was collected from Chittagong, Bangladesh in August 2021 and authenticated by Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong. A voucher specimen

has been deposited at the Department of Pharmacy, University of Science and Technology Chittagong. (Voucher no. USTC/DP/050).

Preparation of Plant Extract
The flowers were properly washed before being air dried and dried in low light. The flowers were finely ground using a suitable grinder after drying. With periodic shaking and stirring, 500 g of powdered material was macerated in methanol (1:10) at room temperature for 7 days. Following that, a clean cotton filter and Whitman filter paper (No. 1) were used to filter the flowers extract. By using a Rotary evaporator (Lab Tech EV311) at 40ºC and reduced pressure, the solvent was evaporated. The extract was then kept in a refrigerator (2-8ºC) till further use.

Green synthesis of Silver Nanoparticles
Silver nanoparticles were synthesized from flower extract of *L. purpureus* according to A Rautela et al. 2019.[13] Metallic silver was obtained from a 1 mM silver nitrate solution in double-distilled water. In a 1:9 ratio, silver nitrate and flower extract were mixed. With a magnetic stirrer, the reaction mixture was continuously stirred at 800 rpm while being heated below the boiling point. Within an hour, the mixture turned reddish brown in color. The entire reaction took place in the dark. Silver nanoparticles were obtained as a suspension, and it was centrifuged for 45 minutes at 15,000 rpm. To get rid of silver ions and extract residue, the silver nanoparticle-containing pellet was washed three to four times with deionized water. In order to further characterize the nanoparticles, particles were kept in a cool, dry, and dark at 2-8ºC.

Characterization of Nanoparticles
By using a PERKIN ELMER (Lambda 35 model) spectrometer, the maximum absorption wavelength of the produced nanoparicles will be determined. FTIR spectroscopy analysis was performed using a PERKIN ELMER (Spectrum RXI) instrument at room temperature with a resolution range of 400–4000 cm^{-1} to identify the functional group of biomolecules present in the *L. purpureus* extract. SEM analysis will be used to examine at the nanoparticles' morphology.

Phytochemical screening
Qualitative phytochemical screening was conducted using standard procedures.[14] The presence of alkaloids, steroids, tannins, saponin, glycosides, phenols, flavonoids, and terphenoids were analyzed in the crude methanolic extracts of flowers.

Antibacterial activity
Microorganisms
One gram-positive bacteria, *viz. Staphylococcus aureus*, and five gram-negative bacteria *viz. Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Proteus vulgaris* were used in this study. These microbes were type cultures, obtained from the Imperial Hospital, Chattogram. All of the bacterial strains were grown and maintained on Nutrient agar (Merck, India) media at 37°C. The bacteria were subculture overnight. Test media was prepared by using Muller-Hilton agar after sterilization.

Antibacterial screening by disk diffusion technique
The antibacterial effects were tested by the disc diffusion method.[15] Briefly, the blank discs (5 mm in diameter) were individually impregnated with 50 µl of 1000 µg/mL of flower extract, silver nanoparticles and silver nitrate solution and then placed onto the agar plates which had previously been inoculated with the test microorganisms. The Petri dishes were kept at 4 °C for 3 h before incubation at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. All the tests were performed in triplicate. Blank disc impregnated with distilled water was used as negative control and disc of Kanamycin (30 µg / disc) as positive control.

Statistical analysis
Data from the experiments were analyzed using the Statistical Package for Social Science (SPSS) software for windows version 26 (SPSS Inc., Chicago, Illinois, USA). All the triplicate data were expressed as Mean ± SEM as appropriate.

Results and Discussion

Observation of Silver Nanoparticles
The initially colorless solution underwent a remarkable transformation, gradually shifting its color from yellow to brown and finally settling down into a reddish-brown shade. This striking change occurred due to the green reduction of Ag+ ions by the extract, leading to an increase in the concentration of silver nanoparticles and a distinct alteration in their particle shape.[16, 17] The reaction between the aqueous extract and silver nitrate solution was responsible for the observed shift in color, transitioning from yellow to a captivating reddish-brown (Figure 1). The synthesized nanoparticles were amorphous and had a black appearance. The nanoparticles' extracting value was 26.74%.

Characterization of nanoparticles
UV spectrometer analysis
The UV-Vis spectroscopy analysis of the prepared AgNPs revealed that the size and shape of the nanoparticles influenced the position and shape of the absorption peaks, which were attributed to plasmon resonance.[16] Table 1 provides a summary of the UV-Vis absorption spectra of the AgNPs produced at room temperature, with measurements taken at various time intervals up to 2 hours. Notably, there was a significant disparity in peak valleys between the flower extract, silver solution, and green synthesis nanoparticles, particularly evident after the 2-hour mark. This disparity suggests the formation of silver complexes with phytochemicals during the green synthesis process. A distinct absorption peak observed at 437 nm indicated the presence of AgNPs and the occurrence of surface plasmon resonance phenomena.[18] The organic compounds in the *L. purpureus* flower extract contain numerous functional groups that can interact with silver to form a complex.

FTIR spectroscopic analysis
The spectrum of Ag nanoparticles derived from flowers revealed distinct vibrational peaks indicative of various chemical functionalities. Specifically, a prominent peak observed at approximately 1477-1624 cm-1 corresponded to carbonyl stretching and N-H stretch vibrations resulting from amide linkages (Figure 2a). Another notable vibrational band centered at around 1377 cm-1 was attributed to C-N stretching vibrations found in aromatic amine compounds. Moreover, distinctive IR bands at 1103 cm-1 were associated with C-O stretching frequencies. A broad band detected at 3441 cm−1 signified the stretching of hydroxyl groups, likely stemming from alcohol or phenol groups. Additionally, smaller bands appearing at 2927 and 2852 cm-1 were attributed to C-H stretching vibrations within the -CH2 group of aliphatic chains.

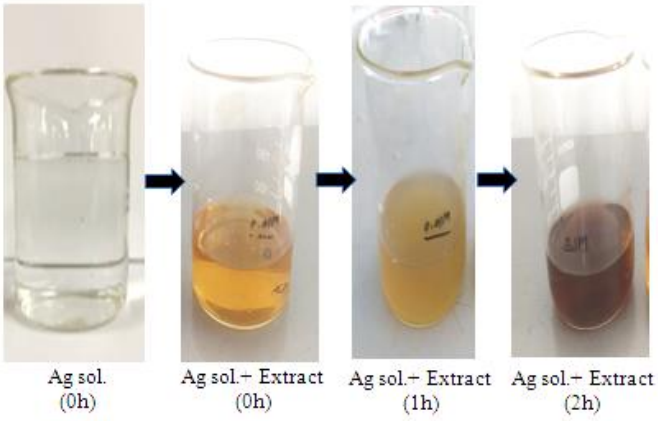


Figure 1: Synthesis of Silver nanoparticles over reaction time

A relatively strong band observed at 2065 cm−1 was identified as the CN stretching vibration. These shifts could be indicative of specific interactions and chemical bonding between silver and the phytochemical components.[19] Notably, the formation of silver-phytochemical coordination complexes led to shifts in the ligand group peaks within the IR spectrum of the silver nanoparticles (Figure 2b).

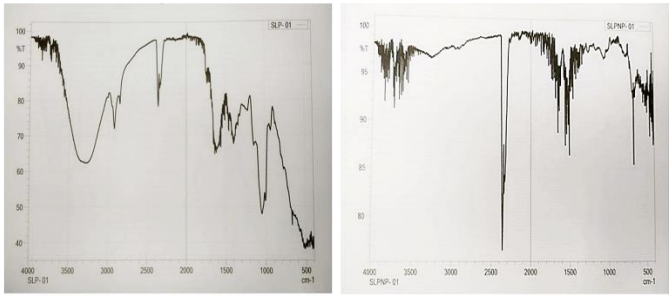
Table 1: UV spectroscopic analysis of extract and nanoparticles of *L. purpureus* flowers

Samples	Peak Valley (nm)					
	At 0 hour			At 2 hours		
Extract	225.00	290.00	345.00	225.00	290.00	345.00
Silver solution	245.00	275.00	295.00	245.00	275.00	295.00
Silver extract NPs	290.00	345.00	375.00	311.00	344.00	437.00

Table 2: Phytochemical Screening of *L. purpureus flowers* methanolic extract

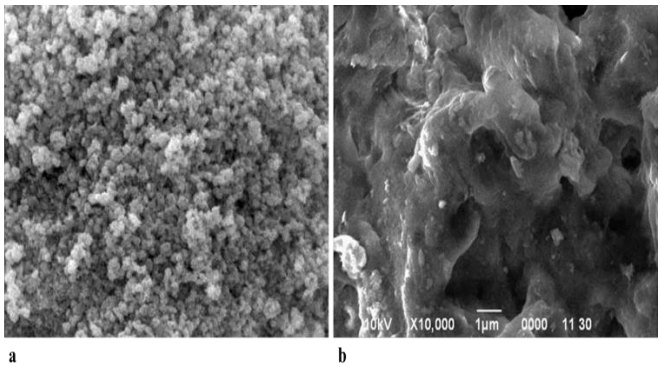
	Tested Groups	Inference
1.	Alkaloids	+
2.	Steroids/ Terpenoids	+
3.	Tannins	+
4.	Saponins	-
5.	Cardiac glycosides	-
6.	Phenols	+
7.	Flavonoids	+

(+) Indicates the presence and (-) Indicates the absence



a b

Figure 2: FTIR spectra; a: Flower extract of *L. purpureus*, b: silver nanoparticles synthesized from *L. purpureus*.



a b

Figure 3: SEM analysis; a: Flower extract of *L. purpureus*, b: silver nanoparticles of *L. purpureus*, both figures are optical images of NPs at 1000x

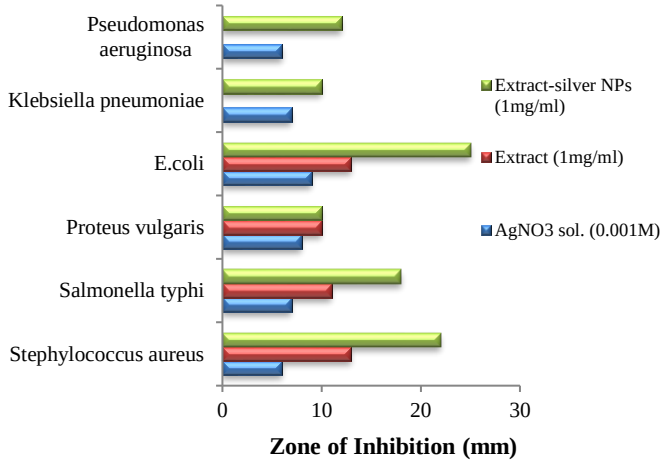


Figure 4-a: Bacterial susceptibility test of extract and nanoparticles of *L. purpureus* flowers

Morphological analysis

The Scanning Electron Microscope (SEM) image revealed that the particles possess an amorphous nature, exhibiting a closely compacted arrangement and displaying agglomeration with a semicrystalline character (Figure 3).

Qualitative Phytochemical screening

Plant secondary metabolites are abundant sources of bioactive compounds that offer numerous health benefits for both humans and animals. Plant-based foods, encompassing vegetables, fruits, grains, seeds, nuts, and legumes, can be rich in a diverse array of phytochemicals .[20] A phytochemical screening of the raw extract revealed the presence of several compounds, including alkaloids, steroids, terpenoids, tannins, flavonoids, and phenols. Among these, alkaloids, tannins, and phenols were found in higher quantities, while steroids, terpenoids, and flavonoids were moderately present. Notably, saponin and cardiac glycosides were not detected in the extract (Table 2).

Antibacterial activity

In the study of bacterial susceptibility, we observed the flower extract of *L. purpureus* to possess moderate antibacterial activity against four tested bacteria: S. aureus, S. typhi, Proteus vulgaris, and E. coli. However, there was no activity against two other tested bacteria: K. pneuminiae and P. aeruginosa. In contrast, nanoparticles displayed promising antibacterial potential, effectively combating gram-positive S. aureus and gram-negative E. coli, while also showing moderate antibacterial activity against S. typhi. Additionally, the AgNPs exhibited mild antibacterial effects against Proteus, Klebsiella, and P. aeruginosa (Figure 3 & 4-a, b). Although the precise mechanism of action of AgNPs as an antibacterial agent remains unknown, numerous studies have proposed that these silver nanoparticles attach to the bacterial cell wall, thereby disrupting its permeability and cellular respiration. Furthermore, the interaction between the nanoparticles and phosphorus- and sulfur-containing substances, such as DNA and proteins within the bacterial cell, could lead to cellular damage. The release of silver ions from the particles is what confers their antibacterial action, thus giving silver nanoparticles their bactericidal effects.[21-23]

Conclusion

The study showed efficient synthesis of green silver nanoparticles from the extract of *L. purpureus* flowers. The evaluation for antibacterial potentials revealed that these nanoparticles significantly inhibit the bacterial growth of some bacterial strains. Based on the findings, it could be depicted that green synthesis of nanoparticles from the flower extract of *L. purpureus* may be a potential source to develop an effective antibiotic.

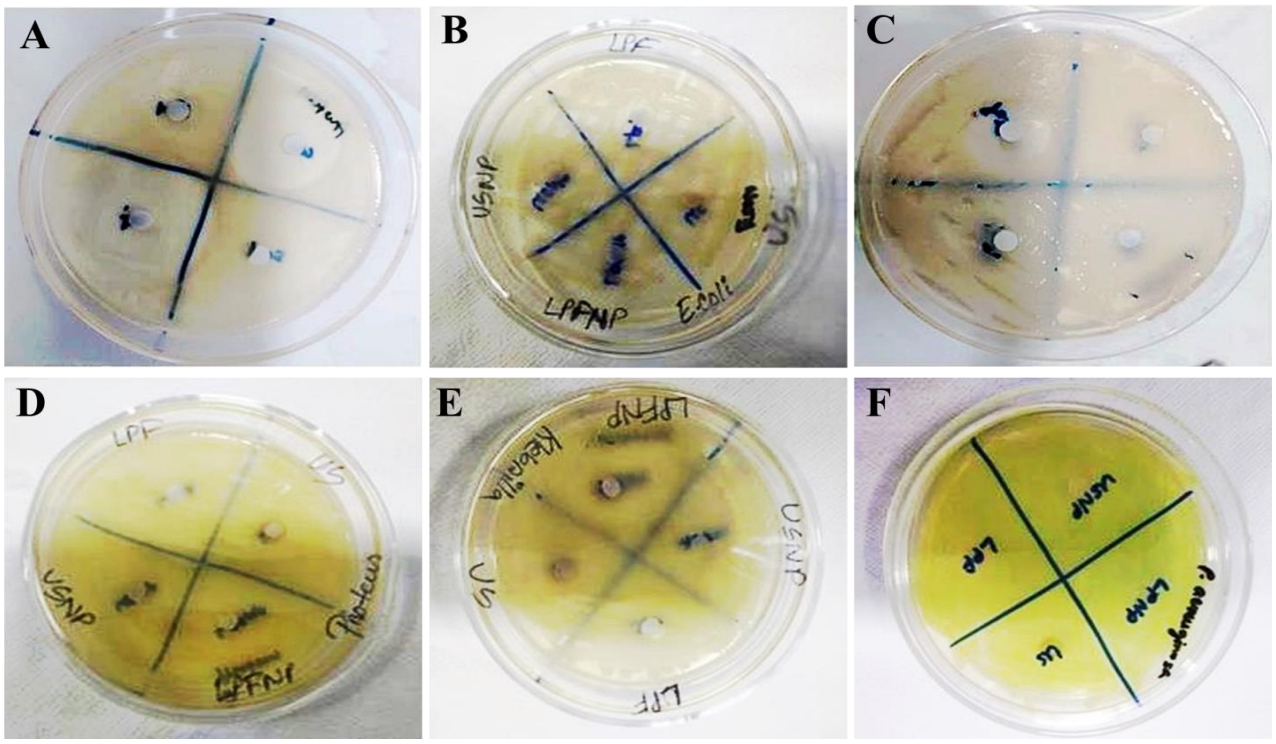


Figure 4-b: Zone of Inhibition in Disk diffusion test. Antibacterial activity of *L. purpureus* flowers, AgNPs and silver solution in the bacterial strains; A: *Staphylococcus aureus*, B: *Escherichia coli*, C: *Salmonella typhi*, D: *Proteus vulgaris*, E: *Klebsiella pneumonia*, F: *Pseudomonas aeruginosa.*

Conflict of Interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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