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Research Article

EVALUATION OF PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLE SYNTHESIZED *IPOMOEA NIL* AGAINST SELECTED PATHOGENS

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ABSTRACT

Objective: The objective of this study is to investigate a simple and feasible approach for the production of silver nanoparticles (AgNPs) by using leaf extract of *Ipmoea nil* and to evaluate its antibacterial activity.

Methods: The AgNPs synthesized was characterized by a change in color and the absorption peaks by ultraviolet-visible spectroscopy. The phyto compounds responsible for the reduction and capping of silver ions was known from Fourier transform infrared spectra and phytochemical analysis. The antibacterial effects of prepared aqueous AgNPs were detected against five types of pathogenic bacteria, including Gram-negative and Gram-positive bacteria (*Escherichia coli, Salmonella, Bacillius, Proteus,* and *Klebsilla pneumonia*) using agar well diffusion method.

Results: A peak absorption value between 400 and 450 nm and the color change of the extract from yellowish to red wine were corresponds to the plasmon absorbance of AgNPs. On the other hand, aqueous extract of *I. nil* mediated AgNPs found to be effective against tested microorganisms (*Salmonella*, *Bacillus,* and *Proteus*) with inhibition zone in the range of 10-13 mm (20 µl and 10 µl) except *E. coli* and *K. pneumonia*. Furthermore, aqueous extract of *I. nil* leaves had no ability to suppress the growth of the tested microorganisms in the concentration of 10 µl. The control also produced similar inhibition zones like AgNPs.

Conclusions: Our findings indicated that green synthesized AgNPs mediated by *I. nil* leaf extract had an efficient anti-bactericidal activity against the bacterial species tested. Hence, further studies are needed to highlight its mechanism and application as an antibacterial agent.

Keywords: *Ipomoea nil*, Silver nanoparticles, Antibacterial activity, Bacteria.

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INTRODUCTION

Advancement in nanotechnology and biotechnology has emerged into the biosynthesis of nanoparticles. A great deal of struggle has been laid into the biosynthesis of nanoparticles, especially metal nanoparticles. Metal nanoparticles were synthesized using microorganisms and plants [1-3]. Among metal nanoparticles, silver nanoparticles (AgNP) have many important applications (Jain *et al*., 2008). AgNP has attracted enormous interest because of its wide applications in food, cosmetic, clothing, and pharmaceutical industries [4-6], medical implants, textile industries, water treatment, and as antimicrobial agents [7]. Various strategies carried out for a synthesis of AgNPs [8] are reduced [9], thermal decomposition [10], microwave-assisted synthesis [11], laser-mediated synthesis [12], and biological reduction method [13]. Even though these methods effectively yield AgNPs in a useful manner, it commonly comprises the practice of toxic and hazardous chemicals, found to have numerous destructive effects on the environment and human well-being [14]. The final product, thus obtained requires more purification steps since some of the chemicals or reducing agents or by-products left behind without use during the process gets adsorbed to the nanoparticle surface and leads to adverse effects during its application. Furthermore, these methods utilize luxurious chemicals and generally require stabilizers to avoid accumulation of chemicals on the surface of nanoparticles. On the other hand, a preferred way for the synthesis of nanoparticles is green chemistry, a widely accepted alternative process for synthesizing nanoparticles.

According to Ayurveda (an ancient Indian medical treatise), Nano silver is a popular additive in several Indian health products due to its unique ability to fight infectious diseases [15-18]. On the other hand, SNPs is gaining more importance in the medical field as antimicrobials, sterilizers, and testing tools for diagnosing and detecting sensitive biomolecules.

According to a literature review, various medicinal plants have been reported for the green synthesis of AgNPs such as *Bryophyllum* sp., *Cyperus* sp., *Hydrilla* sp. [19], *Gliricidia sepium* [20], *Rosa rugosa* [21], *Cycas* [22], *Acalypha indica* [23], *Garcinia mangostana* [24], *Cocos nucifera* coir [25], *G. sepium* [20], *A. indica* [26], *Capsicum annuum* [27], *Carica papaya* [28], and *Eucalyptus hybrida* [29].

Plants offer a highly appropriate system for nanoparticle synthesis over other methods due to their ability to produce nanoparticles in a single step, cost-effective, and eco-friendly manner [30] besides the presence of wide range of secondary metabolites. Subsequently, plants are not as much of sensitive toward metal toxicity when compared to algae and bacteria. Hence, medicinal plants offer a green alternative for the AgNPs biosynthesis [31] by providing capping layers to nanoparticles. Recently, green synthesis of nanoparticles is an exciting branch evolving in the field of nanotechnology *Coriandrum sativum* [32], *A. indica* [33] and *Rhizophora mucronata* [34].

In the present work, we synthesized a stable AgNPs using aqueous *Ipomoea nil* leaf extract and investigated its antibacterial activity against selected bacterial strains.

MATERIALS AND METHODS

Materials

The chemical silver nitrate ($AgNO_3$), Mueller-Hinton agar (MHA) for antibacterial study and ampicillin antibiotic were purchased from Sigma Chemical Co.

2 mM $AgNO_3$ solution

2 mM $AgNO_3$ solution was prepared by dissolving 0.0339 g in 100 ml double distilled water. The solution is mixed thoroughly in the dark and stored in an amber colored bottle for further use.

Preparation of plant extract

The plant under study (*I. nil*) was collected from in and around the Vellore District, Tamil Nadu, authenticated, and deposited in the herbarium. 10 g of fresh leaf of *I. nil* was separated from plant sample and cut into fine pieces using a knife. To this add 100mL of double distilled water and boil for 10 minutes. The boiled solution was filtered through Whatman filter paper No.1 until a clear solution was obtained.

Synthesis of AgNPs

2 mM concentration of an aqueous solution of $AgNO_3$ prepared was used for the synthesis of AgNPs. 30 ml of *I. nil* leaves extract was added into seventy milliliter of an aqueous solution of 2 mM/L, $AgNO_3$ solution for the reduction into Ag+ ions. The solution was heated on a water bath at 80°C for 10 minutes. The change of color takes place within ten minutes from yellow to red wine. The solution was kept undisturbed for 24 hrs. The AgNPs were collected by centrifugation 10,000 rpm twice for 10 minutes to collect pellets and were used for further studies.

Characterization of AgNPs

Ultraviolet-visible (UV-VIS) spectroscopy

UV-VIS spectral analysis of synthesized AgNPs was analyzed using UV-VIS spectrophotometer from 300-500 nm range at a resolution of 1 nm.

Fourier transform infrared (FT-IR) spectral analysis

I. nil extract and pellets of *I. nil* AgNPs were subjected to FT-IR analysis. FT-IR spectrometer identifies the active biomolecules linked to extract and AgNPs. The equipment was operated with a resolution of 4 cm^{-1} and scanning range of 500-4000 cm^{-1}.

Phytochemical screening

Both the plant extract and the AgNPs were subjected to preliminary phytochemical analysis as per the standard procedure.

1. Test for carbohydrates
   a. Molisch's test
      To the test solution 1 ml of alpha naphthol was added, followed by 1 ml of concentrated sulphuric acid slowly along the sides of the test tubes. Keep the test tubes to stand in the room temperature. A violet ring at junctions of two liquids indicates the presence of carbohydrates.
2. Test for protein and amino acid
   b. Biuret test
      To the test solution1ml of biuret reagent was added. A violet or pink colouration represents the presence of protein.
   c. Ninhydrin test
      2 ml of 5% ninhydrin solution was added into the test solution and heated for 10 minutes in a boiling water bath. The appearance of purple or bluish color indicates the presence of amino acids.
3. Test for alkaloids
   d. Mayer's test
      Test solution was taken in a separate test tube and each tube 1 ml of Mayer's reagent was added, along the sides of the test tube. A white creamy or yellow coloration indicates the positive result to alkaloids.
   e. Hager's test
      1 ml of Hager's reagent was added to the test solution, and the prominent yellow precipitate indicates the presence of alkaloids.
4. Test for Saponin
   f. Foam or froth test
      1 ml of test solution was diluted with 20 ml of distilled water and shaken for 15 minutes vigorously. A permanent foam or froth stable for 10 minutes depicts the saponin presence.
5. Test for Tannin and Phenolic compounds
   g. Ferric chloride test
      To few ml of the test solution, few drops of neutral 5% ferric chloride solution were added and heated in a boiling water bath. A color change indicates the phenolic compound presence.
   h. Lead acetate test
      3 ml of 10% lead acetate solution was added to 2 ml of test solution; a bulky white precipitate confirmed the presence of both phenolic and tannin compounds.
6. Test for steroid and terpenoids
   i. Salkowski's test
      2 ml of chloroform and 2 ml of concentrated sulfuric acid was added to test solution and shaken well. Allow the test tube to stand at room temperature. Lower layer with red color indicated the presence of steroids and an upper layer with golden color confirmed the presence of triterpenoids.
7. Test for glycosides
   j. Borntrager's test
      5 ml of chloroform and test solution was mixed and shaken well. To this, 10% ammonia solution was added. Pink color indicated the presence of anthraquinone glycosides.

Screening of the antibacterial property in synthesized nanoparticles

The AgNPs synthesized using *I. n*il leaves extract, aqueous extract of *I. nil*, ampicillin antibiotic was tested for antimicrobial activity by agar well diffusion method against different pathogenic microorganisms *Escherichia coli, Salmonella* sp., *Bacillius* sp., *Proteus* sp., and *Klebsilla pneumonia*. The pure cultures of bacteria strain microorganisms were sub-cultured on MHA medium. A sterile cotton swab was used to spread the microorganisms uniformly onto the individual plates and allowed to dry for 10 minutes. Subsequently, 4 wells were made on nutrient agar plates using a sterile metal cup-borer. Using a micropipette, 20 µl and 10 µl of nanoparticle solution, 10 µl of aqueous extract of *I. nil,* and 10 µl of standard antibiotic ampicillin were poured onto respective well on all plates. The plates were allowed to incubate at 37°C for 24 hrs. The diameter of zone of inhibition was measured in millimeter, the area surrounding the hole with no growth of inoculated microorganisms was recorded as an inhibitory effect against pathogens.

RESULT

In the present work, AgNPs has been biologically synthesized using fresh leaves of *I. nil*. Synthesis of AgNPs from colourless$AgNO_3$ solution using leaves of *I. nil* was recognized by the color change of the solution (Fig. 1). Preliminary identification of nanoparticle formation was carried out by observing the color change of the reaction solution from colorless to red wine.

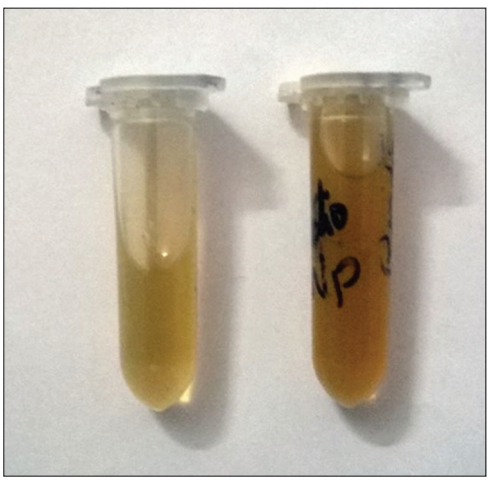

Fig. 1: Leaf extract of *Ipomoea nil* and synthesized nanoparticles

The freshly prepared *I. nil* aqueous extract was yellowish in colour. However, after adding of colourless$AgNO_3$ to aqueous extract and subsequent boiling, the suspension turned red wine. Formation of AgNPs was confirmed using UV-VIS spectroscopy. UV-VIS spectroscopy study monitors the process of reduction of silver ions to AgNPs, and the spectral analysis showed the plasmon resonance band between 400 and 450 nm. The obtained UV-VIS spectral analysis was similar to previous reports reported by other workers [35,36].

FT-IR spectroscopy analysis

It has been reported [27] in literature that plant constituents involved in the reduction and capping of nanoparticle can be identified by FT-IR technique. FT-IR measurements were taken to identify the possible biomolecules responsible for capping and efficient stabilization of the AgNPs synthesized by *I. nil* leaves. Figs. 2 and 3 shows the FT-IR spectra of leaf extract of *I. nil* and synthesized AgNPs overlay.

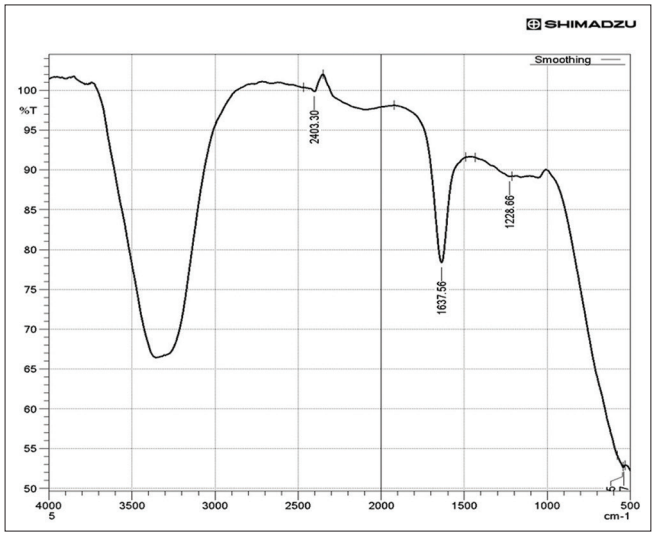

Fig. 2: Fourier Transform infrared spectrum of *Ipomoea nil* aqueous extract

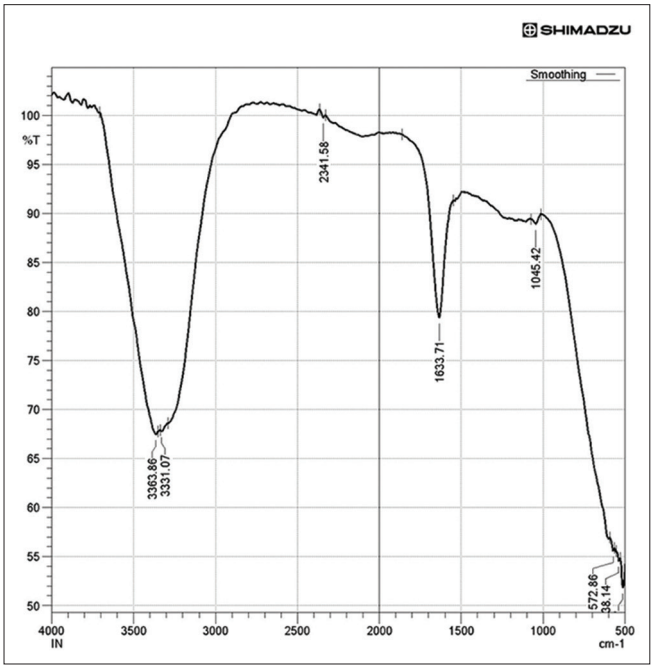

Fig. 3: Fourier Transform infrared spectrum of silver nanoparticle

FT-IR spectra of plant extract obtained showed absorption bands located at 1228.66, 1637.56, and 2403.30 cm^{-1}.The absorption peak at around 1228.66 cm^{-1} can be assigned as absorption peak of C-C,C-N,C-O. The peak at around 1637.56 cm^{-1} is assigned to the functional group C=O. The peak at 2403.30 cm^{-1} indicates the presence of O-H group in the extract.

The silver nanoparticles FT-IR spectra showed an absorption peaks at 1047.35, 1109.07, 1635.64, 2360.87, 3363.86, 3730.33, and 3801.70 cm^{-1} which corresponds to peaks in plant extracts.The chemical shift from 1637.56 cm^{-1} to 1635.64 and shift from 2403.30 to 2360.87 cm^{-1} indicates the reduction of silver ions and the capping of bioreduced silver nanoparticles synthesized by leaf extract of *I. nil*.

Phytochemical analysis

The phytochemical analysis of an aqueous extract of *I. nil* detected the presence of alkaloids, saponin, tannin, phenol, and glycosides while steroid and phenol were not found in aqueous extract. Secondary constituents such as alkaloids and flavonoids components were found in *I. nil* synthesized nanoparticles. Whereas saponin, tannin, phenol, and glycosides were absent in the synthesized AgNP (Table 1).

From the FT-IR spectrum and phytochemical analysis results, the component responsible for the reduction of silver ions and capping of AgNPs were identified as phenolic compound.

Antibacterial activity

In the present study, the antibacterial activity of AgNPs against various pathogenic organisms (*E. coli, Salmonella, Bacillius, Proteus* and *K. pneumonia*) were investigated. At 2 mM/L, concentration of AgNPs, the 12 mm inhibitory zone appeared around 20 µl against *Salmonella* sp. after incubation for 24 hrs followed by *bacillus sp*., and *proteus sp*., (11 mm and 10mm, respectively), then 13 mm for *salmonella* and *Proteus* sp. at 10 µl concentration. It is noted that there is no effect towards the growth of *E. coli* and *Klebsiella*. Control also produced a similar antibacterial activity toward bacteria as like AgNPs with a zone of inhibition of 31 mm (*Salmonella*), 17 mm (*Proteus* sp.,) and 11 mm (*Bacillus* sp.,). From the obtained zone of inhibition of *I. nil* aqueous extract, it was known at the extract at 10 µl concentration does not have any inhibitory effect on selected bacterial strains. The data suggest that the synthesized nanoparticles at 10 µl and 20 µl had pronounced a good antibacterial action against pathogens compared to aqueous extract alone. The results of the antibacterial activity of AgNPs, aqueous extract and control against tested microorganisms are shown Fig. 4.

DISCUSSION

The development of comfortable, consistent and eco-friendly methods increase the interest in the nanoparticles synthesis and application [35,36]. On exposure to plant extracts, reduction of silver ions into AgNPs could be noticed by color change. AgNPs exhibited reddish-wine color in aqueous solution due to the surface plasmon resonance phenomenon [23].

Table 1: Phyto chemical investigation of leaf extract *Ipomoea nil* and its AgNPs

Test	Plant extract	AgNPs
Test for tannins (lead acetate)	+	-
Test for saponin's (foam froth)	+	-
Test for flavonoids (sodium hydroxide)	-	+
Test for steroids (Salkowski)	-	-
Test for alkaloids (wagner and hager)	+	+
Test for phenol (lead acetate)	+	-
Test for glycoside (Bontrager)	+	-

+: Indicates presence, -: Indicates absence, *I. nil*: *Ipomoea nil*, AgNPs: Silver nano particles

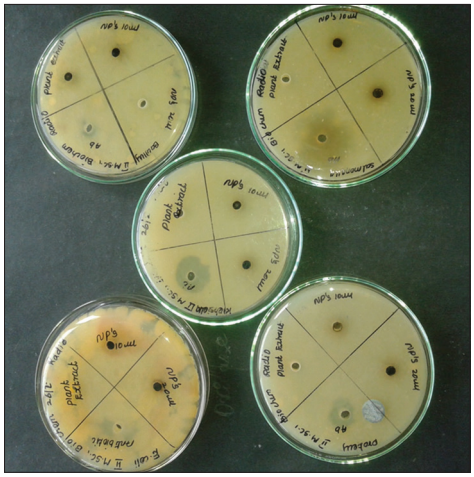

Fig. 4: Antibacterial activity

The production of the AgNPs from the leaf aqueous extract of *I. nil* was evaluated through UV-VIS spectrophotometry at a wavelength range of 300-600 nm [37]. The characteristic peak for *I. nil* AgNPs at 400 to 450 nm confirmed the formation of the AgNPs. The obtained UV-VIS spectral analysis was similar to previous reports reported by other workers [38-40]. This is similar to the surface plasmon vibrations with characteristic peaks of the AgNPs prepared by *Eucalyptus hydrida, Securinega leucopyrus, A. indica* [29,41,42], Eucalyptus chapmaniana, Eucalyptus hybrid and Eucalyptus globulus [40,43,44]. The result obtained in this investigation is very interesting in terms of the identification of potential medicinal plants for the synthesis of AgNPs.

Furthermore, the antibacterial activity of biologically synthesized AgNPs was investigated against five human pathogens and results exhibited a good antibacterial activity against tested bacteria. However, the antibacterial effect varied according to the bacterial strains [45]. In addition, the AgNPs showed good inhibition activity towards *Salmonella* (13 mm and 12 mm for 20 µl and 10 µl, respectively), *Proteus* (13 mm and 10 mm for 20 µl and 10 µl), and *Bacillus* (12 mm and 10 mm for 20 µl and 10 µl) in a dose-dependent manner. *E. coli* and *Klebsellia* sp. does not show any inhibitory effect against synthesized AgNPs. The water extract of *I. nil* when applied does not find to have effective activity against all tested bacterial strains. Thus, the ability to suppress the microbial growth was higher in AgNPs when compared to aqueous extract. Despite its action, the mechanism of action on microorganism is incompletely known. However, some previous reports suggested the inhibitory action of AgNPs may be due to its smaller size and capacity to enter into the bacterial cell. After the entry into the bacterial cell, the silver ions affect the intracellular processes such as DNA, RNA, and protein synthesis [46,47]. It was suggested that the inhibitory action of silver ions (particularly Ag^{+}) released from AgNP might be able to interact with sulfur-containing proteins and phosphorus moieties in DNA, a key element for the antimicrobial effect. This leads to the inhibition of enzyme function and inactivation of DNA replication process and eventually results in loss of cell viability and finally led to cell death [48-51].

This antibacterial ability of AgNPs might be due to the reduction of phenol, glycosides, saponin, and tannins present in the leaf extract [52]. The occurrence of tannins, saponins, alkaloids, phenol and glycosides in the leaf extract of *I. nil* was reported. This preliminary finding was similar to the presence of phytoconstituents as reported in aqueous extract of *Eucalyptus camaldulensis*, *Terminalia glaucescens* and *Rhynchotechumellipticum* [53-55]. AgNPs showed higher ability to suppress the microbial growth, which was pronounced against both Gram-positive bacteria and Gram-negative bacteria. The current investigation demonstrated that the biosynthesized AgNPs had an excellent antibacterial activity against some Gram-positive and Gram-negative bacteria.

CONCLUSION

It has been demonstrated that the aqueous extract of *I. nil* leaves is capable of producing AgNPs in a fast, green, economical method, and the AgNPs produced are relatively stable in solution. Basically, the synthesis of nanoparticle is carried out by the reduction process. In the case of phytochemical analysis result plant extracts containing tannins, saponins, phenol, and glycosides are responsible for the synthesis of nanoparticles. Based on the FT-IR spectrum reports, the phenolic compound is majorly responsible in the synthesis of nanoparticles. The biosynthesized AgNPs showed excellent antibacterial activity and the data represented in our study contribute to a novel and unexplored area of nano-materials as alternative medicine. Therefore, further studies are needed to fully characterize the mechanisms involved with the antibacterial activity of these particles.

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