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Research Article

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING THE LEAF EXTRACT OF *PUTRANJIVA ROXBURGHII* WALL. AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Objective: This study deals with the synthesis of silver nanoparticles (AgNP's) from the extract of the leaves of the plant *Putranjiva roxburghii* wall. Using biological method, i.e., green synthesis.

Methods: The extract from the leaves acts as a reducing and stabilizing agent for the AgNP's. Further characterization was done using various techniques like ultraviolet (UV)-visible spectrophotometry, which shows surface plasmon resonance, Fourier transform infra-red spectroscopy analysis shows formation of various bonds, scanning electron microscope (SEM) and transmission electron microscope (TEM) analysis depicts the distribution and average size of nanoparticles. The antimicrobial activity was also checked against various bacteria and fungi using minimum inhibitory concentration and well diffusion assay.

Result: UV analysis shows strong plasmon resonance between 420 and 480 nm SEM analysis shows the distribution of synthesized nanoparticles, whereas TEM analysis shows the average particle size to be near about 5 nm and well diffusion assay proved that these nanoparticles are effective against different microorganisms.

Conclusion: *P. roxburghii* wall. shows strong potential for the reduction of silver from Ag^+ to Ag^0 and nanoparticles so formed are strongly active against various microorganism.

Keywords: *Putranjiva roxburghii*, Fourier transform infra-red, Scanning electron microscope, Transmission electron microscope.

INTRODUCTION

The use of silver as an antibacterial agent is not new, but the application of silver as silver nanoparticles (AgNPs) against various bacteria and fungi is new. The main fact is their high reactivity due to the large surface to volume ratio. Nanoparticles play a crucial role in inhibiting bacterial and fungal growth in aqueous as well as solid media. Various strategies have been used for the biosynthesis of inorganic material, especially metal nanoparticle using microorganisms and plants [1,2]. Nanosilver has many important applications. It is used as an antimicrobial agent and also applied in textiles, home water purification systems, medical devices, electronics, refrigerators and household appliances [3]. Nanoparticles possess improved properties compared with larger particles of the bulk materials, and these extraordinary properties are derived due to the variation in specific characteristics such as size, distribution and morphology of the particle. Nanoparticles of different metals, like those of silver and gold in addition to their medical and pharmaceutical application, are also used in various other commercial products like soaps, detergents, and cosmetics. Synthesis of nanoparticles using microorganisms and plants has proved to be the valuable alternatives to various chemicals methods [4]. Various reports of the synthesis of AgNPs using extracts of plants such as *Zea mays* [5], *carob leaf extract* [6], *Calophyllum inophyllum leaves* [7], *Saraca indiaca* bark [8], *Canthium coromandelicum* leaf extract [9], *Phyllanthus amarus* [10] are present. Green synthesis of nanoparticles is an easy, efficient and ecofriendly approach, where most researchers all over the world are looking toward the ability of plants and parts of plants in synthesizing metal nanoparticles, which could be beneficial for humans. Green nanotechnology, i.e., synthesis of nanoparticles using plant or part of the plant has proved to be very effective and economical also [11]. Out of the various advantages of using plant for synthesis of nanoparticles the most important advantage over other biological method comes from the fact that in green synthesis there is no requirement of maintenance of cell culture [12]. The nanoparticles can easily penetrate inside the cell and may cause damage to DNA and protein and the release of silver ions from AgNPs makes it effective against various microorganisms [13]. Nanoparticles interacts with light more efficiently than a particle of the same dimension composed of any known organic or inorganic chromophore [14]. In current scenario nanoparticles are effective against various bacteria, including multiple drug resistant [15]. *Putranjiva roxburghii* Wall. is an evergreen tree which reaches to the height of near about 12 m with leaves being simple, alternate, dark green, shiny, elliptic-oblong. *P. roxburghii* Wall. is found wild or cultivated in almost all parts of India leaves of *P. roxburghii* Wall. are used in the treatment of various diseases like, skin disease, fever, sterility and also used in the treatment of cold, fever, and rheumatism [16]. Its leaves also possess high analgesic, antipyretic, and anti-inflammatory activity [17]. In this study, we have synthesized AgNPs using Putranjiva leaf extract for reduction of Ag^+ ions to Ag^0 nanoparticles using silver nitrate ($AgNO_3$) solution. The AgNPs were further characterized by using ultraviolet-visible (UV-Vis) spectra, scanning electron microscopy (SEM), Fourier transform infra-red (FT-IR), transmission electron microscopy (TEM) and their antibacterial and antifungal activities have been investigated using various Gram-positive and Gram-negative bacteria and fungus.

METHODS

Chemical and plant material

$AgNO_3$ was purchased from Sigma-Aldrich chemicals and fresh *P. roxburghii* leaves were collected from Aligarh, UP India. The leaves were washed several times with distilled water to remove the dust particles and then sun-dried to remove the residual moisture. The dried leaves were crushed using mortar and pestle and 10 g of dried powder was mixed with 100 ml of deionized water for 10 minutes. The aqueous extract was then separated by filtration with Whatman No. 1 filter paper (Maidstone, UK) and then centrifuged at 1200 rpm for 5 minutes to remove heavy biomaterials, the extract was then stored at 4°C for further experiments.

Synthesis of AgNPs

A volume of 10 ml of leaf extract was mixed with 90 ml of 1 mM $AgNO_3$ solution. The color change of the solution from yellow to dark green was indicative of the reduction process Ag^+ to Ag^0 nanoparticles. As the concentration of $AgNO_3$ increases in the solution the size of the particle also increases [18].

UV analysis

Synthesized AgNPs were scanned by UV-Vis spectrophotometer at the wavelength of 300-800 nm on Perkin-Elmer Lambda 25 spectrophotometer. It is basically done for monitoring the AgNPs as UV-Vis spectroscopy is used for the characterization of colloidal particles. Noble metal particles possess strong surface plasmon resonance (SPR) absorption in the visible region and are highly sensitive to the surface modification.

FT-IR spectroscopy

The FT-IR spectrum was recorded using Perkin Elmer spectrum between 4000 and 400/cm. The FT-IR spectrum obtained for *P. roxburghii* leaf extract displays a number of absorption peaks reflecting its complex nature.

SEM and TEM analysis

SEM (Jeol jsm 6510 LV) analysis shows the distribution of nanoparticles in which thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper then the film on the SEM grid was allowed to dry and the images of nanoparticles were taken. Whereas in TEM (jeol 2100) near about a drop of synthesized nanoparticles were placed on copper grid and the sample was illuminated with electronic radiation under vacuum and detection of sample was done by the beam of electron which was transmitted through the sample placed and then the photographs were taken.

Antimicrobial assay

The synthesized AgNPs were tested for their antimicrobial activity against various microorganisms, *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 10231), and Candida tropicalis (ATCC 13803), by the well diffusion method. The pure cultures of the organism were subcultured on nutrients broth and sabouraud dextrose agar (SDA) broth at 37°C and 28°C on a rotary shaker at 200 rpm for 24 hrs and 48 hrs respectively. Each strain was swabbed uniformly on the individual plates of nutrient agar and SDA using sterile cotton swabs. Wells of size 6 mm diametre were punched on each plate using gel puncture. Using a micropipette varying concentration of synthesized nanoparticles were poured into wells on all plates and left for incubation at 37° and 28°C for 24 and 48 hrs, respectively.

Minimum inhibitory concentration (MIC)

The antimicrobial properties of AgNPs were analyzed by means of MIC. Most commonly employed method for MIC includes agar dilution and broth dilution, MIC is defined as the lowest concentration of the antimicrobial agent that prevents the visible growth of a microorganism under defined conditions [19].

RESULTS AND DISCUSSION

UV analysis

The color of the solution changed from pale light yellow to dark deep green (Fig. 1) depending on the extract concentration indicating the formation of AgNP`s as the color change corresponds to the excitation of surface plasmon vibration in the AgNPs after 24 hrs. It can be seen that the SPR of AgNPs is between 420 and 480 nm (Fig. 2).

FT-IR

FT-IR spectra of green synhtesised nanoparticles from leaves of *P. roxburghii* revealed various absorption peaks (Fig. 3). Strong absorption peaks at 3,436/cm result from stretching of the -NH band of amino groups. The absorption peaks at about 2,074/cm could be assigned to stretching -CH. The peaks at 1,634.53 could be assigned to the CH_2 stretching The FT-IR spectrum also shows bands at 1402 and 676/cm which corresponds to -C-H bending (alkane) and =C-H bending (alkene).

SEM and TEM analysis

SEM (Fig. 4) shows the distribution of AgNPs and TEM (Fig. 5) reveals the fact that these nanoparticles are spherical in shape. The TEM analysis also shows the average size of the particle to be near about 5.74 nm.

Antimicrobial properties

As previously reported extracts of *P. roxburghii* contains antioxidant activities [20] besides containing anti-diabetic activity [21]. Increasing the concentration of AgNPs leads to increase in the zone size surrounding the wells starting from 0.062 mg/ml to 1 mg/ml (Figs. 6-9) both in case of bacteria as well as fungi. MIC values for different strains of bacteria and fungi are shown in Table 1 depicting that green synthesized nanoparticles from the extract of *P. roxburghii* are effective against bacteria as well as fungi. These results agreed with previous work carried out by Kim *et al*. [22], Li *et al.* [23,24].

CONCLUSION

The study reveals the biosynthesis of AgNPs from the aqueous extract of leaves of *P. roxburghii* which do not involve any of the harmful

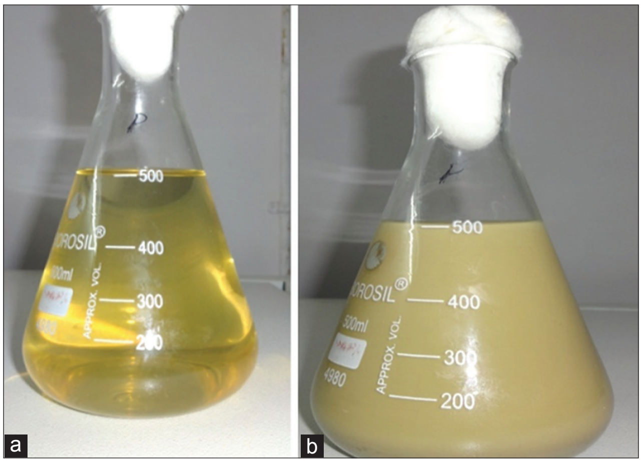

Fig. 1: (a) Extract+silver nitrate at 0 hr, (b) Extract + silver nitrate after 3 days

Table 1: MIC of green synthesized AgNPs tested against different microorganisms

Nanoparticles	Microorganisms	MIC (mg/ml)
AgNPs	*S. aureus*	0.5
AgNPs	*E. coli*	0.25
AgNPs	*C. albicans*	0.5
AgNPs	*C. tropicalis*	0.5

AgNPs: Silver nanoparticles, MIC: Minimum inhibitory concentration, *C. albicans: Candida albicans, C. tropicalis: Candida tropicalis, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus*

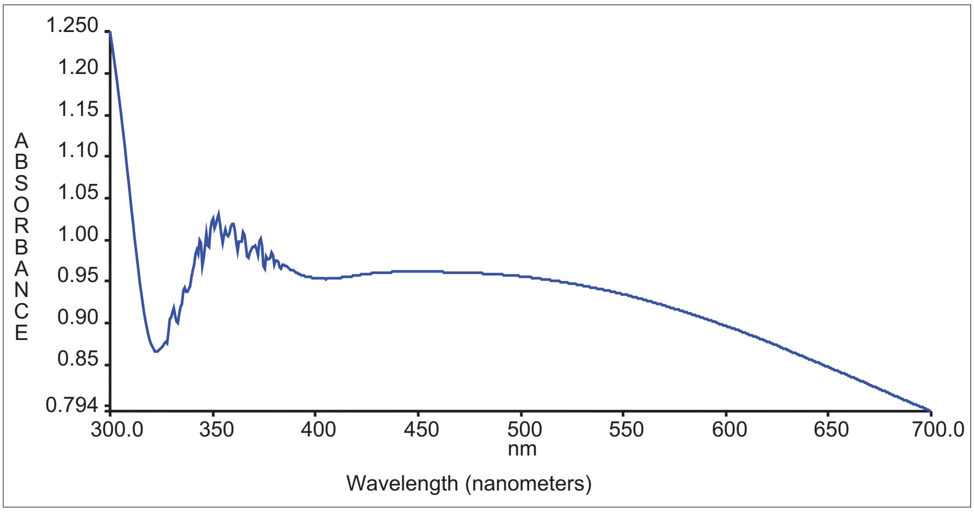

Fig. 2: Ultraviolet spectra of synthesized nanoparticles

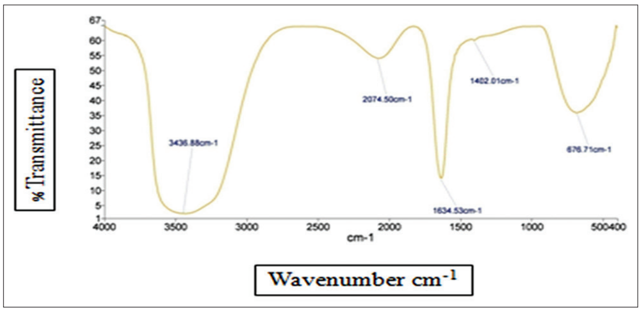

Fig. 3: FTIR spectra

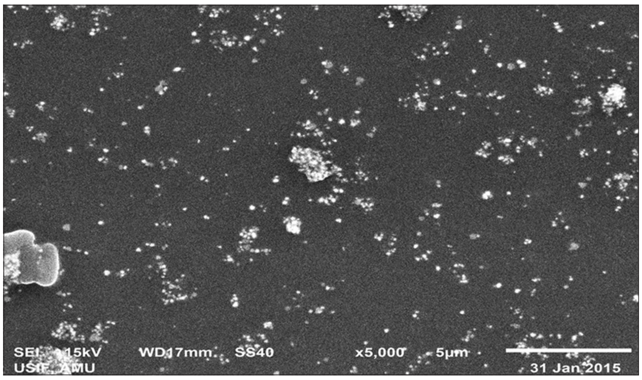

Fig. 4: Scanning electron microscope image of synthesized nanoparticles

chemicals and very simple biological and low cost approach for the preparation of stable AgNPs by the reduction method. Aqueous extract of the leaves are used for the reduction purposes and further the characterization of the nanoparticles was done using various techniques like UV-Vis, FT-IR, SEM and TEM. The results from these techniques confirmed the reduction of $AgNO_3$ to AgNPs and also their nature i.e., size and shape of the particles. The zone of inhibition in well diffusion assay confirms the efficacy of synthesized nanoparticles against various bacteria and fungi.

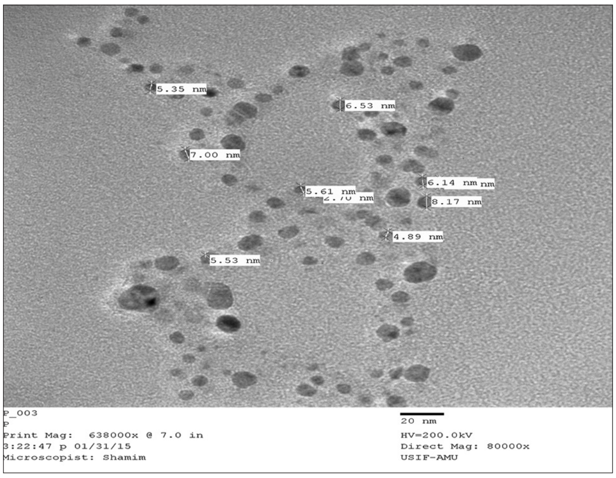

Fig. 5: Transmission electron microscope image showing size of nanoparticles

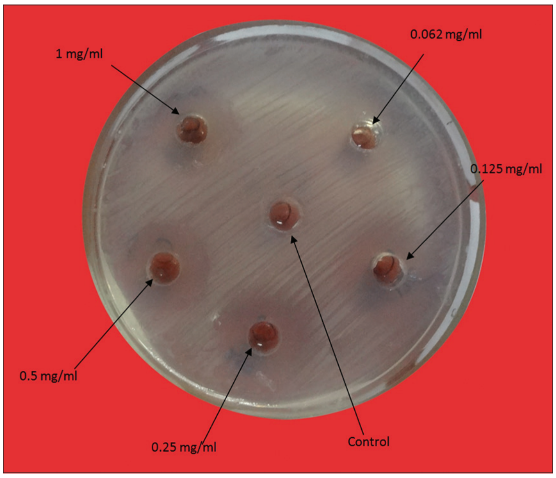

Fig. 6: *Candida albicans*

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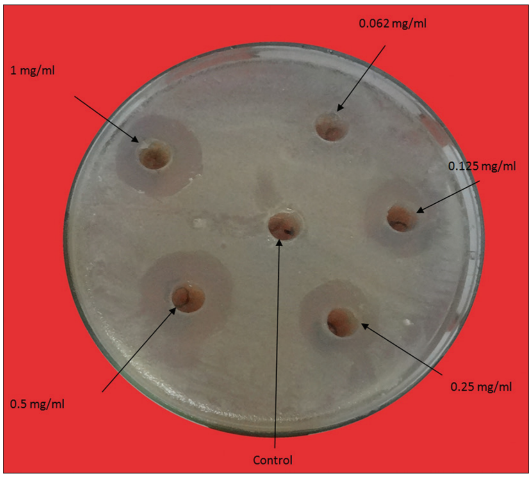

Fig. 7: *Candida tropicalis*

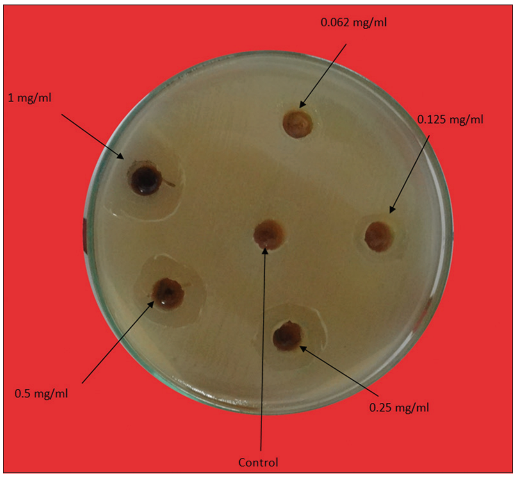

Fig. 8: *Staphylococcus aureus*

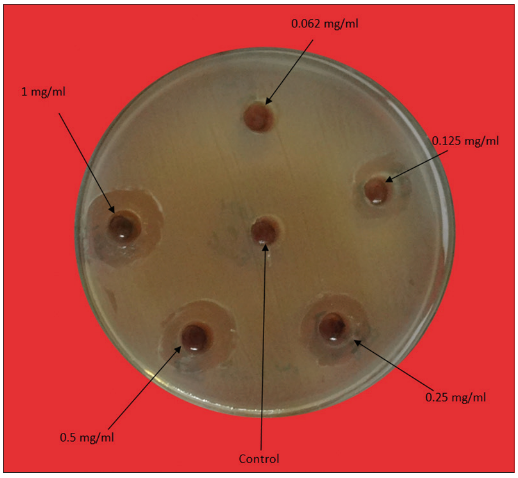

Fig. 9: *Escherichia coli*, control is taken as distil water, zones of inhibition showing antimicrobial activity

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