

BIOGENIC SYNTHESIS SILVER NANOPARTICLES USING *PELTOPHORUM PTEROCARPUM* LEAF EXTRACTS AND ITS ANTIMICROBIAL EFFICACY AGAINST SELECTIVE PATHOGENS

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ABSTRACT

Objective: The present study was aimed to screen silver nanoparticles (AgNPs) using different plant extracts and also to study their antimicrobial property against different human pathogens.

Methods: Nine different plants, namely *Parthenium hispidum*, *Vinga rose*, *Catheranthus roseus*, *Phyllanthus amarus*, *Azadirachta indica*, *Jatropha curcas*, *Tectona grandis*, *Ocimum sanctum*, and *Peltophorum pterocarpum* were screened for the synthesis of AgNPs. The AgNPs were synthesized using leaf extracts and was well characterized using a UV-Visible spectrophotometer, transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD) and zeta potential measurement. The AgNPs was tested for their antibacterial and antifungal efficacy using agar well diffusion method.

Results: Among the nine different plant extracts screened, AgNPs synthesized using *Peltophorum pterocarpum* leaf extract showed good stability even after one month with maximum absorption spectra of 425 nm. The synthesized AgNPs was found to be spherical in shape with an average size ranging from 20 to 60 nm. The EDX spectrum reveals the presence of silver peaks and the XRD spectrum confirms the crystalline nature of AgNPs. A Maximum zone of inhibition of 18.04 ± 0.74 was found when the synthesized AgNPs was tested against *B. subtilis*, and 12.34 ± 0.31 against *A. niger* when the concentration was AgNPs was maintained at 100 µg/ml.

Conclusion: The results of the present study conclude that the AgNPs synthesized using *Peltophorum pterocarpum* leaf extracts is found to be stable and possesses broad-spectrum antibacterial activity against different tested pathogens.

Keywords: Antibacterial, Antifungal, Plant extracts, Silver nanoparticles

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INTRODUCTION

The field nanoscience deals with the study of fabricating nanostructures between the diameters of 1 to 100 nm which can be used in different areas of science including medicine, biology, materials science, and chemistry etc. Recently, the field of nanotechnology has started emerging one of the most promising areas of science with diverse potential applications. Among the different nanostructures, the application of metal nanoparticles, especially silver and silver associated nanostructures has created a major impact in the field of biomedicine. The silver nanoparticles (AgNPs) are found to be very attractive due to its antimicrobial, angiogenic and anticancer properties [1, 2]. Due to a wide range of biological applications of AgNPs, there is huge interest in developing a novel protocol using various biological sources to synthesize nanoparticles with different size and shapes [3]. Traditionally nanoparticles were synthesized using the physical and chemical process where it included the utilization of various chemicals which could be hazardous to both biological systems and environment [4]. Comparing with the existing physical and chemical methods, the biologically mediated nanoparticles synthesis process was found to be less toxic, safe and eco-friendly [5].

The utilization of plant and plant-based components for the treatment and prevention of various health-related complications have been in practice for many decades. Several bioactive components were used for the treatments which found to be similar to various chemical compounds [6, 7]. In the plant-mediated synthesis of metal nanoparticles, various active molecules of plants such as flavonoids, terpenoids, amines, tannins, ketone, and proteins were used as reducing and capping agents [8, 9]. Several researchers have studied the green biosynthesis of AgNPs using different plants, including *Pulicaria glutinosa* [10], *Plumbago zeylanica* [11], *Albizia adianthifolia* [12], *Achillea biebersteinii* [1], *Origanum vulgare* [13], *Annona squamosa* [14] and *Erythrina indica* [15].

Recently, the development of drug-resistant pathogens has created a major impact globally, and demands for the production of novel

compounds with potent antimicrobial property have been increased [16]. Currently, the AgNPs have been thoroughly investigated extensively for its antimicrobial potential due to its strong bactericidal and inhibitory properties against several pathogenic strains [17-19]. Silver in the form of AgNPs tends to have increased surface area, resulting in an enhanced antimicrobial property. Though AgNPs has been extensively used in many areas, the mechanism of the antibacterial is still unclear. Studies have hypothesized that AgNPs may cause cell lysis by interacting with a thiol group, thus inactivating enzymes [20, 21].

Globally, the development of multidrug-resistant pathogens has created a serious issue which has a profound impact on public health. This is mainly due to the extreme and improper use of antibiotics, thus, leads to the development of antibiotic-resistant genes which may transfer from between the bacteria in higher level organisms. The metallic nanoparticles such as AgNPs can be replaced in future as an alternate substitute due to its broad-spectrum antimicrobial property. In this view, the present study aims to explore the different plants for the synthesis of AgNPs using plant extracts as reducing agent and to study the efficacy of synthesized silver nanoparticles as a potent source of the antimicrobial agent against several bacterial pathogens.

MATERIALS AND METHODS

Preparation of the plant extract

The mature leaves of the following plants *Parthenium hispidum*, *Vinga rose*, *Catheranthus roseus*, *Phyllanthus amarus*, *Azadirachta indica*, *Jatropha curcas*, *Tectona grandis*, *Ocimum sanctum*, and *Peltophorum pterocarpum* were locally collected from the Centre for advanced studies in Botany, University of Madras, Chennai (Latitude 13.010 ° and Longitude 80.239 °). Identification of plant materials was done at the Centre for Advanced studies in Botany and voucher specimens of all plants were serially numbered as given: *Parthenium hispidum* (KA1), *Vinga rose* (KA2), *Catheranthus*

roseus (KA3), *Phyllanthus amarus* (KA4), *Azadirachta indica* (KA5), *Jatropha curcas* (KA6), *Tectona grandis* (KA7), *Ocimum sanctum* (KA8) and *Peltophorum pterocarpum* (KA9). The leaves of the different plants were brought to the laboratory, thoroughly washed with distilled water, made into small pieces and kept in shadow drying. The dried leaves were made into powder using a mixer grinder and stored in the airtight container until further use. Ten gram of leaf powder of each plant were mixed in 100 ml of distilled water and kept in a rotary shaker incubator for 48 h. After incubation, the extracts of each plant were collected by passing it through Whatman's No.1 filter paper, and the filtrate was stored at 4 °C for further analysis.

Screening of plant extracts for AgNPs synthesis

In the present study, the leaf extracts of different plants were screened for the synthesis of AgNPs. To one ml of different leaf extracts, 9.0 ml of 1 mmol silver nitrate was mixed separately, and the tubes were incubated in dark conditions at room temperature for 24 h. The solution containing plant extracts and silver nitrate was monitored for colour change, precipitation and agglomeration of nanoparticles [22]. During the preliminary investigation, among the different plant extracts tested, the AgNPs synthesized using *Peltophorum pterocarpum* leaf extracts showed good stability and hence it was utilized for further studies.

Characterization of synthesized AgNPs

UV-Visible spectroscopy

The AgNPs synthesized using *Peltophorum pterocarpum* leaf extracts were characterized using the UV-Visible spectrophotometer. The freshly synthesized AgNPs using plant extracts was analyzed for their maximum absorption spectra using UV-Visible spectrophotometer (U 2900, Hitachi) in the range of 300 to 700 nm [22].

Stability studies of AgNPs

The AgNPs synthesized using *Peltophorum pterocarpum* was investigated for their stability by analyzing the UV-Vis spectra, precipitation and agglomeration property. The synthesized AgNPs was regularly monitored for the maximum absorption spectra from 0 h to 1 mo using UV-Vis spectrophotometer and in addition, was also monitored for precipitation and agglomeration of AgNPs.

X-ray diffraction (XRD)

The AgNPs synthesized was subjected to XRD studies in order to study the crystalline nature of AgNPs. For this analysis, the AgNPs was dried; coated on XRD grid and the XRD spectrum was recorded at a voltage of 40 kV with Cu-K α radiations using Philips X-ray diffractometer [23].

Fourier transform infrared (FTIR) spectroscopy

The FTIR study was performed to determine the different functional groups associated with the synthesized AgNPs. For FTIR measurement, the phyto-genic AgNPs was dried and mixed with potassium bromide to form a pellet. The pellet was then analyzed for FTIR spectra in the range of 4000 to 400 cm⁻¹ using FTIR spectroscopy.

Transmission electron microscopy (TEM)

The TEM analysis was performed to determine the size of the synthesized AgNPs. The AgNPs synthesized using plant extract was diluted with sterile distilled water; applied to carbon-coated copper TEM grids and allowed to dry under room temperature [24]. The grids were then analyzed using a TEM microscope (FEI Tecnai) at 100 kV coupled with energy dispersive X-ray spectroscopy (EDX) and also selected area electron diffraction (SAED).

Antibacterial property of AgNPs

Four different bacterial strains, namely two Gram-positive bacteria, *Staphylococcus aureus* (MTCC-96), *Bacillus subtilis* (MTCC-441) and two Gram-negative bacteria such as *Escherichia coli* (MTCC-443),

Pseudomonas aeruginosa (MTCC-1688), were procured from the Institute of Microbial Technology, Chandigarh, India and were used for antibacterial efficacy of synthesized AgNPs.

The antibacterial activity of phyto-genic AgNPs was evaluated using a modified agar well diffusion method. Briefly, under aseptic conditions, sterile mueller hinton agar (MHA) was prepared in sterile Petri plates. Freshly prepared overnight bacterial test strains from mueller hinton broth were inoculated on sterile mueller hinton agar plates using lawn culture method.

The media plates were then punched with five different wells of 4 mm diameter using a sterile punch borer. Each well was loaded with varying concentrations of synthesized AgNPs (25, 50, 75, 100/ml) and positive control, gentamicin (10 μ g/ml). Finally, the petri plates loaded with AgNPs were incubated at 37 °C for 24 h. At the end of incubation, the diameter of the inhibition zone was measured for each test strain and was recorded [25].

Antifungal activity of AgNPs

The AgNPs synthesized using *Peltophorum pterocarpum* leaf extract was tested for their antifungal activity against human fungal pathogens. Modified agar well diffusion method was used to determine the antifungal activity of AgNPs against *Aspergillus niger* [26]. Briefly, fungal spores of 1 \times 10⁶ spores/ml were inoculated on sterile potato dextrose agar plate using the spread plate technique. Five wells of 4 mm diameter were made using sterile punch borer and varying concentration of synthesized AgNPs (25, 50, 75, 100/ml) and positive control, amphotericin B (10 μ g/ml). The plates were then incubated at 27 °C for 3 to 4 d, and the zone of inhibition was measured.

Statistical analysis

In the present study, the experiments were performed independently for three times. The obtained values were processed and calculated using GraphPad Prism 6.0 (GraphPad Software, Inc.; USA) software.

RESULTS AND DISCUSSION

In the present study, nine different plant leaves extracts namely, *Parthenium hispidum*, *Vinga rose*, *Catheranthus roseus*, *Phyllanthus amarus*, *Azadirachta indica*, *Jatropha curcas*, *Tectona grandis*, *Ocimum sanctum*, and *Peltophorum pterocarpum* were tested for the biogenic synthesis of AgNPs. All the five different plant extracts supported the synthesis of AgNPs within 24 h by a visible change in the colouration from pale yellow to brown.

However, AgNPs synthesized using *Peltophorum pterocarpum* leaves extract showed much more stable when compared with AgNPs synthesized using other plant extracts (fig. 1). Okafor and co-workers investigated the synthesis of AgNPs using reducing agents from aqueous plant extracts from seven different plant leaves [27]. The colour change was due to the presence of various plant components such as alkaloids, saponins, flavonoids, steroids which may play a role as reducing agent. Similar results were observed by various researchers who also synthesized AgNPs using different plant extracts [28-30].

UV-Visible spectrophotometric analysis of AgNPs

The silver ion reduction to a silver atom in the test solution was observed using the UV-Visible spectrophotometer. The change in colour was observed due to the reduction of Ag⁺ leading to the formation of AgNPs. The colour change is primarily due to excitation of surface Plasmon vibrations which is a characteristic observation during the formation of AgNPs. In the present study, maximum absorption peaks were recorded and found to be 425 nm after 24 h which further confirmed the formation of AgNPs (fig. 2). The synthesized AgNPs absorbs radiation strongly at characteristic wavelengths due to its electrons transition. The exact mechanism of metal nanoparticles synthesis is not well understood. However reports suggest that the reduction was due to the role of NADH coenzyme in the medium [31, 32].

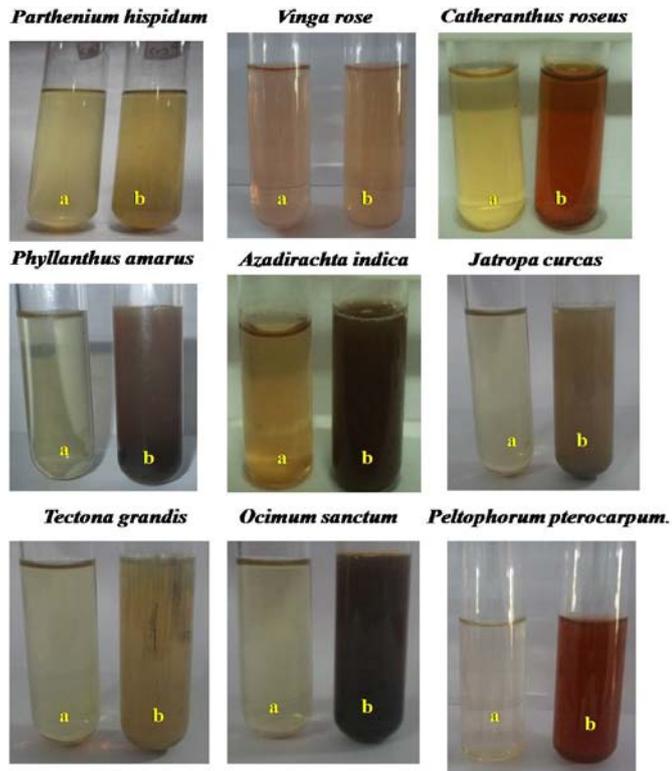


Fig. 1: Screening of AgNPs synthesis using different plant extracts

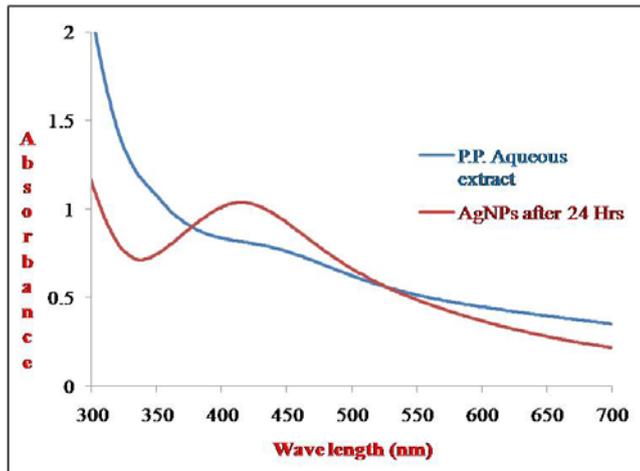


Fig. 2: UV-Visible spectrophotometric analysis of synthesized AgNPs

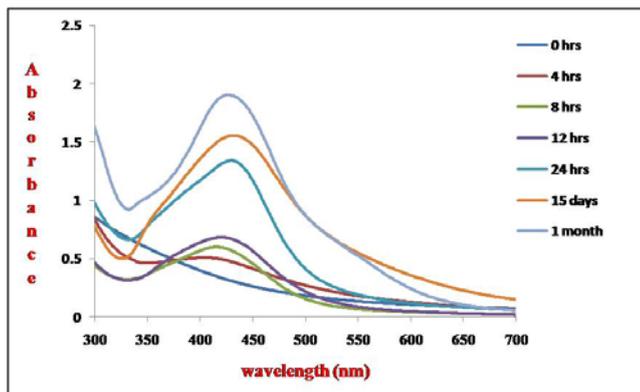


Fig. 3: Stability studies of synthesized AgNPs

Stability studies of phytosynthesized AgNPs

The *Peltophorum pterocarpum* leaves extract mediated AgNPs was analyzed for their stability studies using UV-Visible spectroscopy. The maximum spectra of synthesized AgNPs were monitored using UV-Visible spectrophotometer from 0 h to 1 mo. Initially, at 4 h, a maximum spectrum was recorded at 380 nm which confirmed the formation of AgNPs, further there is an increase in peak from 380 nm to 420 nm at the end of 12 h. The results reveal the maximum absorption spectra of synthesized AgNPs was found to be 425 nm even after 1 mo, which confirms the potential stability of AgNPs synthesized using *P. pterocarpum* (fig. 3). The above results conclude that the silver nanoparticle activity was started at 8 h and it was ended at 24 h and after that its activity was stable up to a month.

XRD analysis of AgNPs

The XRD spectra of the synthesized AgNPs showed three distinct peaks representing Bragg reflections of 1 1 1, 2 0 0, and 2 2 0 which corresponds to the face-centered cubic (fcc) of the silver confirming the crystalline nature of the phytosynthesized AgNPs. The spectral

data obtained were well matched with previously reported literature which also shows similar results [33, 34].

FTIR analysis of leaf extract and AgNPs

Results of the FTIR spectrum of both the leaf extract and AgNPs synthesized were shown in fig. 4. The spectrum of *Peltophorum pterocarpum* leaf extracts and synthesized AgNPs shows several absorption peaks at 3250, 1614, 1535, 1333, 963 and 883 cm^{-1} . The intense absorption peaks of 3250 cm^{-1} represent the hydroxyl groups of plant components such as alcohols and phenolic compounds. An absorption peak of 1614 indicates C=O amide stretch due to the presence of proteins which were similar to the observation reported by Ahmad and co-workers [35]. Less intense peak at 1333 cm^{-1} denotes the C-N stretching vibrations due to the aromatic amines present in both the spectra of synthesized AgNPs and leaf extracts [2, 36, 37]. The results of both FTIR spectra clearly reveals that the dual role of leaf components which acted as both reducing and capping agents during the synthesis of AgNPs which was observed well in the presence of various corresponding peaks.

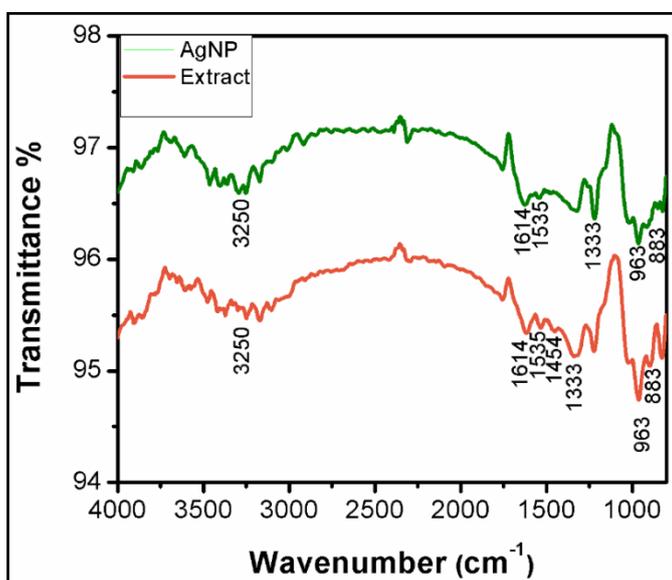


Fig. 4: FTIR analyses of leaf extract and synthesized AgNPs

TEM analysis of AgNPs

The TEM analysis was used to determine the size and shape of the AgNPs. The TEM results of the present study revealed that the nanoparticles synthesized were of spherical shape with an average size ranging from 20 to 60 nm distributed evenly (fig. 5a) and the inset (fig. 5b) shows SAED pattern. The EDX spectrum also revealed the presence of silver peaks which confirms the synthesis of AgNPs

(fig. 6). Gavade and co-workers developed a single step biogenic method for the synthesis of AgNPs using leaf extract of *Ziziphus jujube*. They also measured the size of AgNPs using TEM analysis, averaging about 25 nm [38]. Several researchers also studied the morphology and size of AgNPs synthesized from different plant extracts using TEM analysis also reveals the presence of monodisperse spherical nanoparticles ranging between 20 60 nm [39, 40].

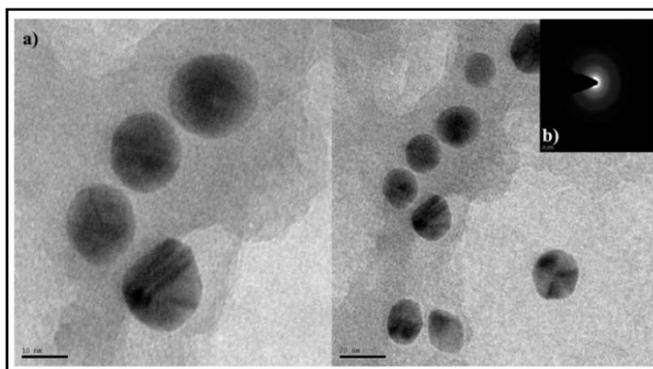


Fig. 5a and b: TEM and SAED analysis of synthesized AgNPs

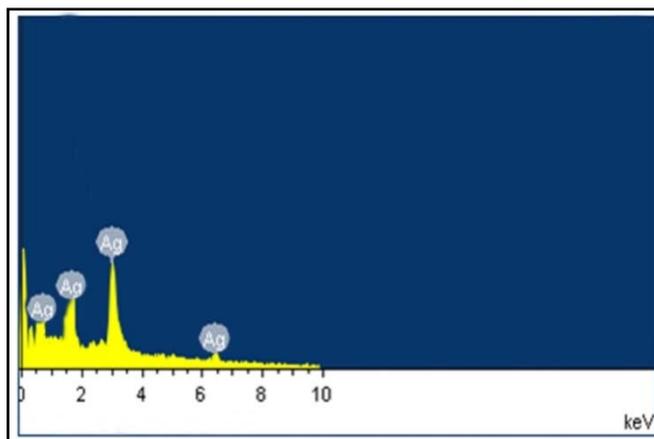


Fig. 6: EDX analysis of synthesized AgNPs

Antibacterial studies of phytosynthesized AgNPs

In the present study, AgNPs synthesized using *Peltophorum pterocarpum* leaves extract was tested for their efficacy against four different bacterial pathogens, namely *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* at four different concentrations. Among the tested strains, in general, AgNPs shows good inhibition activity against Gram-negative bacteria compared to

Gram-positive bacteria. Maximum inhibition activity was found when AgNPs (100 $\mu\text{g/ml}$) was treated against *P. aeruginosa* which recorded a zone of inhibition of 16 mm (table 1). This followed by AgNPs treated *B. subtilis* showed a zone of inhibition of 18 mm when tested at 100 $\mu\text{g/ml}$ (fig. 7). This present study also shows that the antibacterial activity was concentration dependent as the concentration of AgNPs increases the antibacterial activity. Standard positive control was tested for antibacterial activity against all the tested strains.

Table 1: Antibacterial Activity of AgNPs using well diffusion method

Test bacterial pathogens	Zone of inhibition (mm)*				
	AgNPs 25 $\mu\text{g/ml}$	AgNPs 50 $\mu\text{g/ml}$	AgNPs 75 $\mu\text{g/ml}$	AgNPs 100 $\mu\text{g/ml}$	Gentamicin 10 $\mu\text{g/ml}$
<i>Staphylococcus aureus</i>	12.12 \pm 0.14	12.07 \pm 0.52	14.07 \pm 0.22	15.18 \pm 0.43	18.07 \pm 0.24
<i>Bacillus subtilis</i>	14.59 \pm 0.23	15.67 \pm 0.75	16.04 \pm 0.38	18.04 \pm 0.74	20.04 \pm 0.40
<i>Escherichia coli</i>	8.16 \pm 0.37	8.35 \pm 0.98	10.25 \pm 0.55	12.08 \pm 1.09	15.51 \pm 0.59
<i>Pseudomonas aeruginosa</i>	12.15 \pm 0.52	12.80 \pm 1.21	14.25 \pm 0.73	16.49 \pm 1.47	14.27 \pm 0.78

*All the experiments were repeated independently three times. Values were represented as an average means \pm standard deviation.

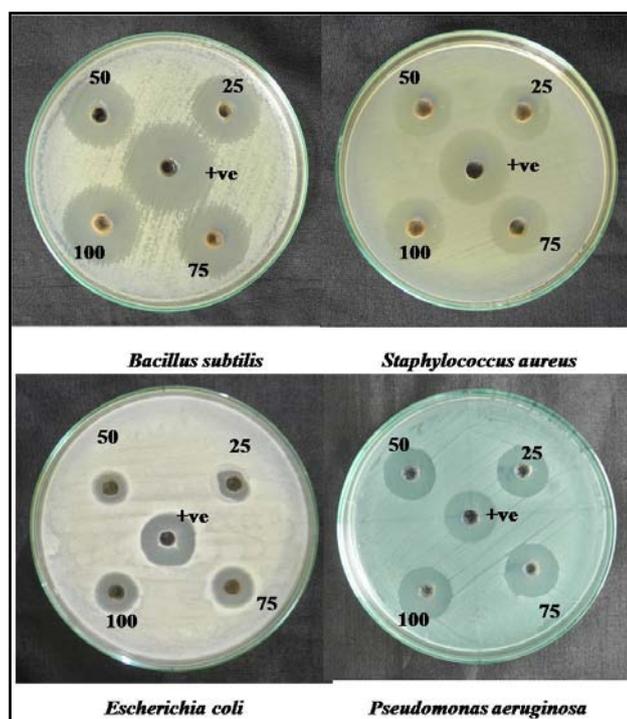


Fig. 7: Antibacterial activity of AgNPs using well diffusion method

Though the antibacterial effect of AgNPs was well studied, the mechanism of antimicrobial action was still unclear. Few reports suggested that the size of nanoparticles plays a major role in the antibacterial property against pathogens. Earlier studies also show that the antibacterial property may be due to electrostatic interaction, increased permeability and influence on membrane transport [41-44]. Several researchers have investigated the antibacterial property of AgNPs against human pathogens using the well diffusion method. The findings of our study were well supported by researchers who have tested the antibacterial activity of AgNPs against human pathogens [25, 45, 46].

Antifungal studies of phytosynthesized AgNPs

The antifungal activity of AgNPs against *A. niger* was studied using the agar well diffusion method. The maximum zone of inhibition (12 mm) was found when AgNPs was tested with 100 µg/ml of concentration against *A. niger*. The positive control amphotericin B was tested against *A. niger*, and the zone of inhibition was found to be 18 mm (fig. 8). The mechanism of antifungal action of AgNPs was still not clear. Similar studies were performed by Bahrami-Teimoori and their co-workers (2017) who have reported the antifungal property of AgNPs against *Macrophomina phaseolina*, *Fusarium oxysporum* and *Alternaria alternata* [47]. Similar studies were also reported by earlier researchers who investigated the antifungal activity against human fungal pathogens [23, 48].

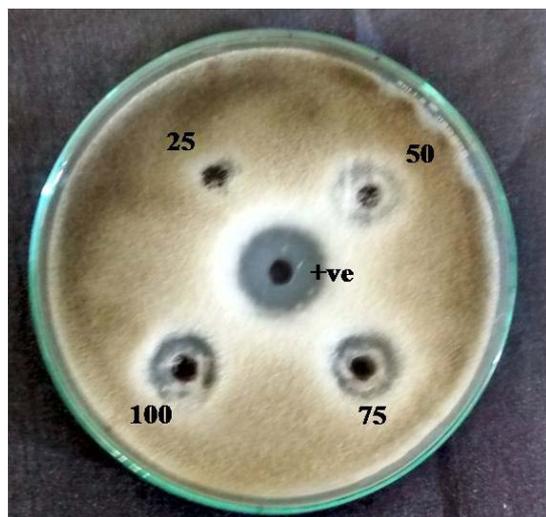


Fig. 8: Antifungal activity of AgNPs using well diffusion method

CONCLUSION

In the present study, five different plants were studied for the synthesis of AgNPs. Among the five plants, the AgNPs synthesized using *Peltophorum pterocarpum* leaves extract shows good stability, which was further characterized and tested for their antimicrobial property. The synthesized AgNPs was found to be spherical and had potent antimicrobial activity against both bacterial and fungal pathogens. The synthesized AgNPs showed a broad spectrum of antibacterial activity against both the Gram-positive and Gram-negative bacteria. Maximum activity AgNPs was found against *P. aeruginosa* followed by *B. subtilis*. The AgNPs also showed potent activity against filamentous fungi, *A. niger* which clearly confirms that the synthesized AgNPs could be a potent antimicrobial agent. However, a further detailed study is required to reveal the exact mechanism and role of AgNPs against the human pathogens.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICTS OF INTERESTS

Authors declare no conflicts of interest

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