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Research Article

POTENTIAL ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES BIOSYNTHESIZED BY LEAF EXTRACT OF *ANDROGRAPHIS PANICULATA*

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ABSTRACT

Use of biological system for the nanoparticle synthesis is the current trend in life science research. Use of these biosynthesized nanoparticles for various biological activities is area of interest for many scientists. In this research a well-known medicinal plant, *Andrographis paniculata*, which is having an antimicrobial activity is used for green synthesis of silver nanoparticles. Water extract of leaves of this medicinal plant was used for the reduction of Silver Nitrate for silver nanoparticle synthesis. It was observed that plant extract was quite effective in reduction of silver nitrate and nanoparticle particle synthesis. Nanoparticles synthesized were primarily characterized by UV-Visible spectroscopy and FTIR analysis. These nanoparticles then screened for antibacterial and antifungal activity. These particles have shown considerable antimicrobial activity against two most common human bacterial pathogens, *Staphylococcus aureus and Escherichia* coli, and also against two most common plant fungal pathogens, *Penicillium notatum* and *Aspergillus niger*.

Keywords: Biosynthesis, Green synthesis, Silver Nanoparticles, *Andrographis paniculata*, UV-Spectroscopy, FTIR, Antimicrobial activity.

1. INTRODUCTION

The field of nanotechnology is acquiring significance step by step in all parts of life. In practically every one of the fields like food industry, horticulture, medication, aviation, defense area and space science nanoparticles are acquiring their traction. Physical and chemical methods of particle synthesis due to use of toxic chemicals do leftover some inappropriate or non-desirable effects in the nano-particles. Consequently, it is need of an hour to create eco-accommodating and safe nanoparticle creation strategies which won't utilize poisonous synthetic compounds in the conventions to dodge hostile impacts in biomedical applications [1]. Consequently, bio-synthesis of nanoparticles (NPs) is acquiring significance angle in nanoparticle union.

Nanoparticles especially metal nanoparticles, received special attention from research community [2]. This is because their application in development of new technologies. They have their application in medical field, new generation antimicrobials, environment, electronics, chemical sciences, solar energy harvesting, batteries and many more [3-8].

When we do physical or chemical synthesis of nanoparticles it leads to absorption of some toxic or unwanted chemical substances on the surface of nanoparticles which limits the use of these nanoparticles. Also, these physical or chemical methods of nanoparticle synthesis are harmful to the environment and also very expensive. Remedy for this problem is biological synthesis of nanoparticles, also known as green synthesis of nanoparticles. We find many references on biogenesis of nanoparticles, especially silver nanoparticles (AgNPs) by using plant extracts. Reducing properties of variable phyto-components have variable role in reduction of silver metal to silver metal nanoparticles [9]. We use plant extracts [10] or microorganisms like bacteria [11], fungi [12], and enzymes [13] for the synthesis of nanoparticles. Biosynthesis of nanoparticles has edge over chemical and physical synthesis. Green synthesis is environment friendly, do not use any toxic or expensive chemical in synthesis or do not need any expensive instrument for synthesis. Green synthesis does not need high energy, high temperature, high pressure for particle synthesis. Green synthesis of nanoparticles is environment friendly, very cost effective and we can easily scale up these processes for large scale production of nanoparticles [14-15].

Silver nanoparticle are the first choice of researchers as

they have variable applications in biological sciences, chemical and physical sciences. AgNPs does not show any ill effect on viable cells and microbes cannot develop resistance to AgNPs. Thus, products having silver material found their application in agriculture, medical industry, pharma and antimicrobials, food industry, fiber industry, packing and plastic industry [16-24].

One of the best multipurpose medicinal plants in Ayurveda is *Andrographis paniculata* (Acanthaceae). This medicinal plant is native to India and very popular in southeast Asia. Most important traditional applications of this plant is in the treatment of malaria, skin diseases, blood pressure, diabetes, cancer, leprosy, influenza and ulcer in central Asia, America and African continents [25-28].

It is a source for various bioactive molecule like andrographolide and andrographin. These compounds have board-spectrum of biological actions [27]. Many researchers have reported antimicrobial [29-31], anticancer [32] and hepatoprotective [33] properties for Silver Nanoparticles synthesized by *Andrographis paniculata* extract (AP-AgNPs) [34].

2. MATERIAL AND METHODS

2.1. Plant collection

Healthy and fresh mature leaves of *A. paniculata* were collected from Local fields outside periphery of Nagpur City, Maharashtra, India.

2.2. Preparation of plant extract

The collected healthy fresh matured leaves were washed with tap water thoroughly for 5 min and then immediately rinsed with sterile distilled water for 2 min to remove the soil and dust particles and possible microbes from the surface of leaves. Washed leaves then properly dried in shade for three to five days at room temperature. These shade dried leaves then grinded to fine powder. Plant extract for biosynthesis was freshly prepared by adding 2 g of leaf powder in 20 mL of autoclaved distilled water and vertexing them for at least 5 min. The resulted infusion was filtered thoroughly Whatman filter paper and the filtrate extract was collected in a 15 mL sterile tube.

2.3. Chemicals and media used

All the chemicals used are of analytical grade. Ethylene-diaminetetraacetic acid (EDTA), sodium hydroxide (NaOH) (HiMedia), silver nitrate ($AgNO_3$) (HiMedia), ascorbic acid ($C_6H_8O_6$) (HiMedia), dextrose (HiMedia), LB (Luria-Bertani) broth (M1245, HiMedia) and bacto-agar (RM026, HiMedia), PDA (potato dextrose agar) (M096, HiMedia). autoclaved distilled water (ADW) was used for all the reaction setups.

2.4. Microbes used for screening

For the antimicrobial screening, bacterial cultures of *Staphylococcus aureus* and *Escherichia coli* and fungal cultures of *Aspergillus niger* and *Penicillium notatum* were isolated and characterized in the department itself.

2.5. Biological synthesis of AgNPs

Green synthesis of silver nanoparticles was done using freshly prepared plant extract and the final volume of reaction mix was 100mL. Green synthesis of nano-particles was done as described follows,

i. Seventy milliliter sterile distilled water (SDW) was taken in 250mL beaker (beaker was completely covered with silver foil) on magnetic stirrer.
ii. From the stock of 0.1 M silver nitrate, 10 mL of silver nitrate solution was added in the above 70 mL SDW (with constant stirring) to make the concentration of silver nitrate 0.01 M in final 100mL of reaction.
iii. pH of the reaction was adjusted to 6 with 1 M NaOH solution.
iv. 20 mL of fresh plant extract was added to the above reaction, drop by drop with constant stirring for next one hour a room temperature. After complete addition of plant extract, keep the reaction for 12 hours at room temperature with constant stirring.
v. You will observe the color change from colorless to colored, which indicates the synthesis of nanoparticles. This reaction mix was centrifuged at 15,000 rpm for complete hour at 10°C.
vi. AgNPs pellets collected at the bottom of the tube were washed with 10 mL sterile distilled water and again the washed AgNPs were collected by centrifugation at 15,000 rpm for complete hour at 10°C.
vii. Pelleted AgNPs were redistributed in 10 mL sterile distilled water and used for further analysis.

2.6. Chemical synthesis of AgNPs

Chemical synthesis of AgNPs was done as described above for green synthesis, only difference is that the pH of reaction is set to be at 8 and 0.1 M solution of ascorbic acid solution was added for reduction of silver ions instead of plant extract. Collection of AgNPs was done in the same way as done for AP-AgNPs.

2.7. Characterization of synthesized AgNPs

In both the methods biological synthesis and chemical synthesis, first confirmation of nanoparticle synthesis was the color change and precipitate formations. Further characterization was done by spectral analysis of the reaction mixture. The spectral scanned between 200 and 800 nm was done to find the absorbance peak on the UV-VIS spectrophotometer (UV-1800; Shimadzu). Further characterization was done by Fourier Transform Infrared Spectroscopy (FTIR) measurements by taking liquid suspension of washed AgNPs pellets. The samples were analyzed on a Nicolet IR 200 (Thermo electron corp.) model in Department of Chemistry, RTM Nagpur University, Nagpur.

2.8. Antimicrobial activity bioassay of AP-AgNPs

2.8.1. Antibacterial activity bioassay of AP-AgNPs

In this antimicrobial bioassay of AP-AgNPs, antibacterial activity was tested against common human bacterial pathogens, *Staphylococcus aureus and Escherichia* and antifungal activity was also established against common plant fungal pathogens, *Aspergillus niger* and *Penicillium notatum*. For this bioassay well diffusion method was implemented [35]. We also took chemically synthesized AgNPs and fresh plant leaf extract in the bioassay for comparison and as a positive control we took antibacterial drug Cefixime dispersible 200 mg, a combination of Fluconazole and Cefixime. Here, a combination of an antifungal (Fluconazole) and antibiotic (Cefixime) medicines work by killing the fungi and bacteria by attacking their outer protective covering [36]. The stock was made by dissolving 200 mg tab in 2 mL ADW.

Bacterial culture of *E. coli* and *S. aureus* was grown overnight in 2 mL of Luria Bertoni (LB) broth (LB broth was prepared as per the manufacturer's instructions) at 37 ^{O}C. This 2 mL culture was diluted to 20 mL by adding 18 mL ADW and from this diluted bacterial culture 100 µL of *S. aureus* and 200 µL of *E. coli* culture was spread on their respective LB agar plates (LB agar was prepared as per the manufacturer's instructions with Bacto agar).

Spores of the fungal pathogens were collected in 50 mL ADW from well grown fungal culture in potato dextrose agar (PDA) (PDA is synthesized as per the manufacturer's instructions) plates. From this spore suspension, 100 µL of spore suspension was spread on their respective PDA agar plates.

The petri plate was divided in four quadrants and four wells were made in center of each quadrant with the help of sterile steel borer (0.8 diameter) (Fig. 4). 100 µL of respective samples are added in each of the well as follows,

In the 1st well we added 1st sample which is 100 µL fresh plant extract, in the 2nd well, 2nd sample which is 100 µL chemically synthesized AgNPs, In the 3rd well, 3rd sample, 100 µL Cefixime (20 mg/mL) and in the 4th well, 100 µL biologically synthesized AP-AgNPs.

Bacterial LB agar plates were incubated at overnight at 37 ^{O}C and next day the zone of inhibition measured with the measuring scale. Similarly, fungal PDA plates were incubated at 27°C for two days and then zone of inhibition is also measured in these plates. This experiment was carried out in the triplicate. The results were read by the presence or absence of zone of inhibition in cm.

3. RESULTS AND DISCUSSION

3.1. Biological and chemical synthesis of AgNPs

Synthesis of AgNPs was visually confirmed by color change of reaction mix from colorless to brown colored. This color change is due to the formation of AgNPs by reduction of silver ions due to reduction action of plant extract and ascorbic acid. It is notable that AgNPs show yellowish earthy colored tone in aqueous solution because of excitation of surface plasma vibrations in AgNPs [37]. As the water extract of *A. paniculata* leaf was added in the solution of silver ion complex, it gradually changes the colour from green to yellowish brown, and this is due to the reduction of silver ion which is considered first visual indicator of AgNPs formations (Fig.1). Many researchers have observed this effect of colour change in the formation of AgNPs by plant extract [34, 38-45].

3.2. UV-spectral and FTIR analysis of AgNPs

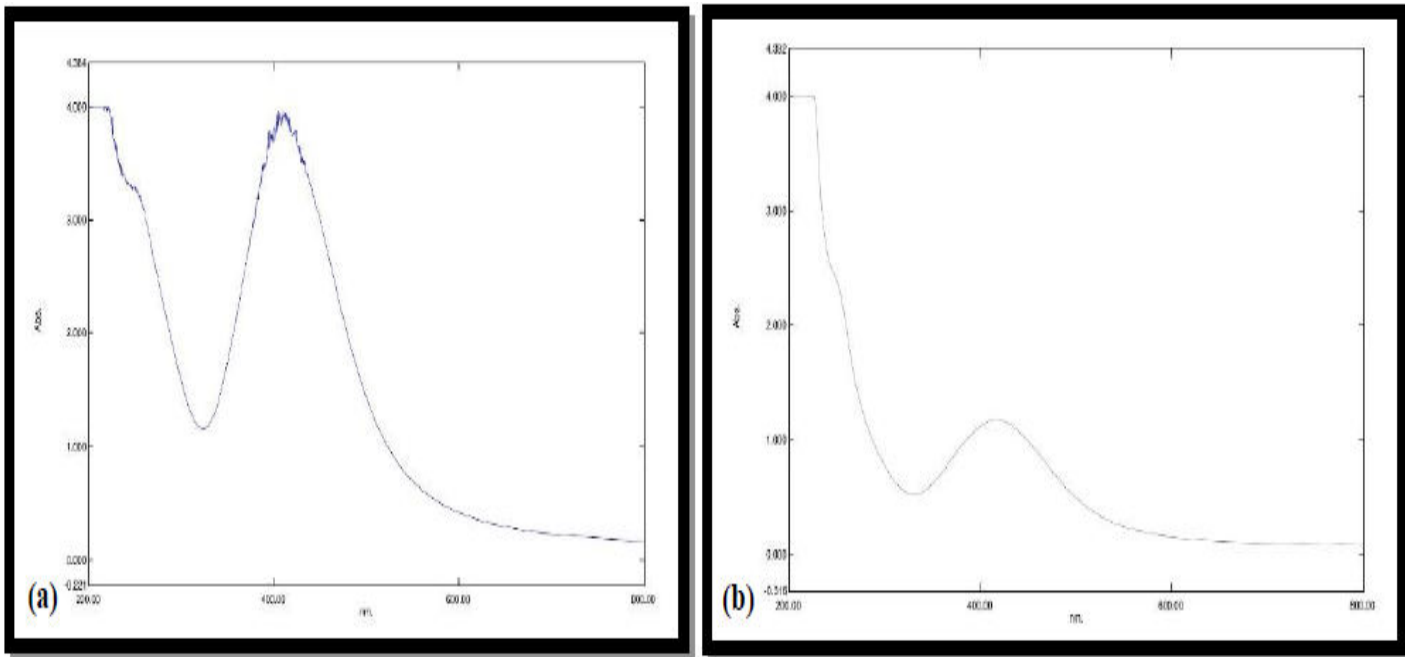
After visual confirmation, analytical confirmation was done by UV-spectral and FTIR analysis. Spectral analysis of AP-AgNPs was done in-between 200 nm to 800 nm. A distinct peak near 420 nm was recorded in the screening of AP-AgNPs (Fig.2.a) and chemical AgNPs (Fig.2.b). This peak, near 420 nm indicates the reduction of Ag^{+} which also confirms the synthesis of AgNPs in both reactions. This result was also coinciding with the results obtained by various researchers who studied AgNPs synthesis using *A. paniculata* extract [25-28, 30, 43-45]. Similar kind of results were also obtained in previous studies with other medicinal plants [46], *Citrullus colocynthis* [47] and from *Allium cepa* [48].

The FTIR measurements of biosynthesized silver nanoparticles were carried out to identify the possible

interaction between protein and silver nanoparticles. Results of FTIR study showed sharp absorbance peaks located at about 1635 and 3430 cm^{-1} (Fig.3). Absorption peak at 1635 cm^{-1} may be assigned to the amide I bond of protein arising due to carbonyl stretch in proteins, and peaks at 3430 cm^{-1} are assigned to OH stretching in alcohol and phenolic compounds [49]. The absorption peak at 1635 cm^{-1} is close to that reported for native proteins [50] which suggest that proteins are interacting with biosynthesized nanoparticles and also their secondary structure were not affected during reaction with Ag^{+} ions or after binding with silver nanoparticles (Fig.3) [51].

(a) Colorless solution of 0.1 M silver nitrate ($AgNO_3$); (b) Light brown colored fresh plant extract; (c) Dark brown colored silver nanoparticle solution (AP-AgNPs) after addition of plant extract. Color change from colorless to colored is an indication of synthesis of silver nanoparticles.

Fig. 1: Green synthesis of silver nanoparticles by plant extracts



(a) biologically synthesized AP-AgNPs and (b) chemically synthesized AgNPs UV-absorbance graph shows typical absorbance near 420 nm which indicates successful production of silver nanoparticles.

Fig. 2: UV-spectral analysis of AP-AgNPs and chemically synthesized AgNPs

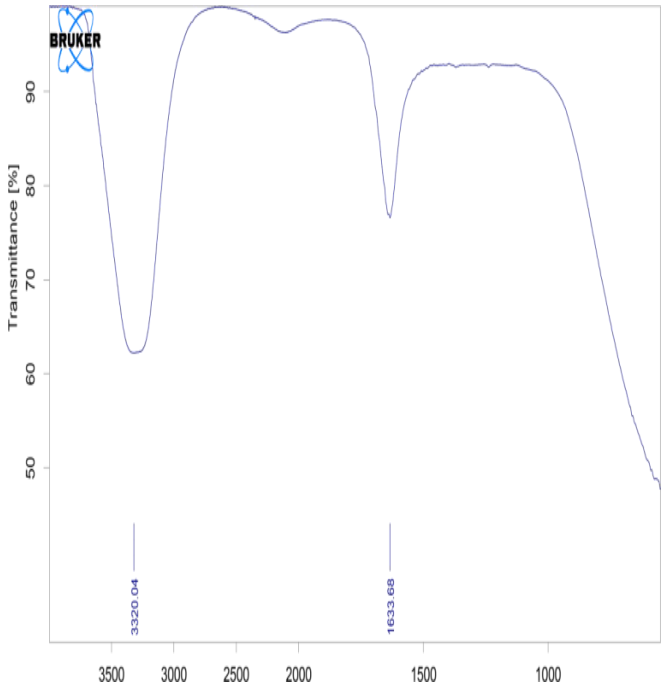


Fig. 3: FTIR spectral analysis of biologically synthesized silver nanoparticles (AP-AgNPs) using leaf extract of *A. paniculata*

3.3. Antibacterial and antifungal activity of AP-AgNPs

The results of well diffusion assay for antimicrobial and antifungal activity of AP-AgNPs has shown promising results. We took chemically synthesized AgNPs, fresh plant extract and Cefixime as a positive control to compare the effectiveness of antimicrobial potential of AP-AgNPs. AP-AgNPs have shown higher antifungal and antibacterial activity in comparison to all the other samples taken (Table-1). In case of human pathogens, AP-AgNPs have shown good antimicrobial activity against *S. aureus* (Fig. 4a) as compared to *E. coli* (Fig. 4b).

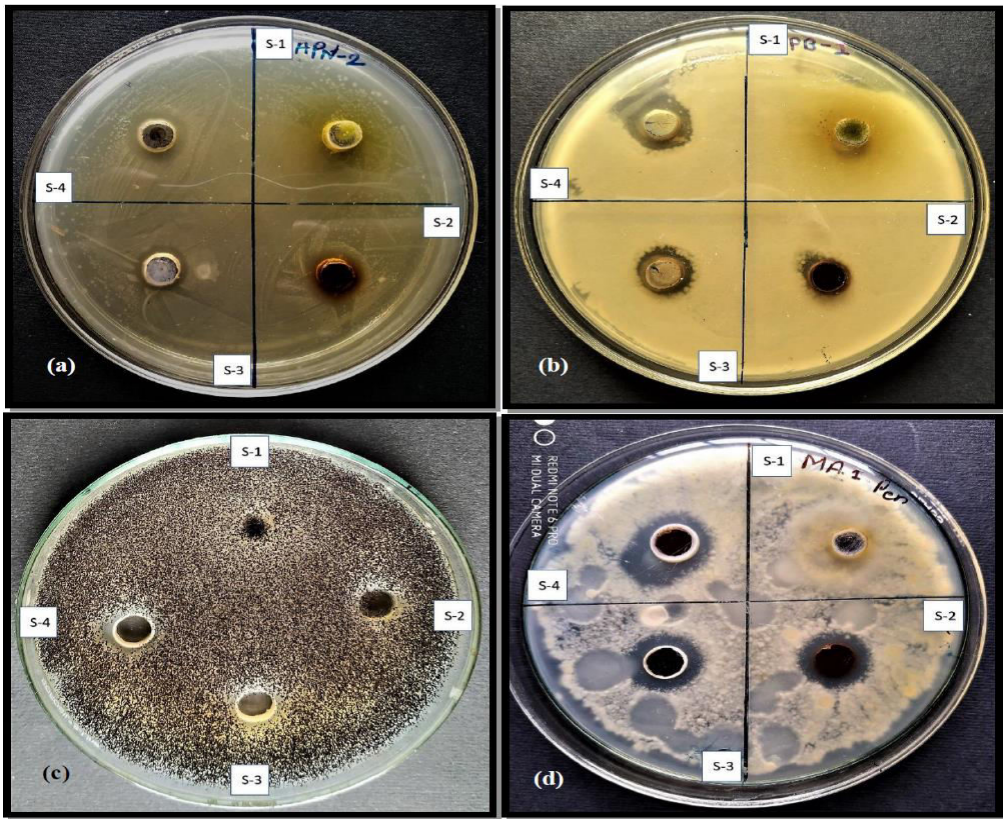
Similarly, in case of plant pathogens, antifungal activity of AP-AgNPs was more against *P. notatum* (Fig. 4d) than *A. niger* (Fig. 4c). These results also indicates that, chemically synthesized silver nanoparticles and plant extract of *A. paniculata* were less effective against pathogens as compared to the combination of both i.e., silver nanoparticles synthesized by plant extract of *A. paniculata*. This proves that during green synthesis of silver nanoparticles some functional groups must be attached with AgNPs, which have enhanced the antimicrobial activity of AP-AgNPs. If we compare positive control i.e. Cefixime results with AP-AgNPs, AP-AgNPs have equal or slightly higher antimicrobial activity than Cefixime.

These results showing antimicrobial activity of biologically synthesized silver nanoparticles against bacterial and fungal pathogens have also been reported by other researchers. Recently, S. Anantharaman and coworkers (2020) have reported enhanced antimicrobial activity of AP-AgNPs against bacterial pathogens *Staphylococcus aureus, Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhimurium,* and fungal pathogens *Candida albican* and *Botrytis cinerea* [41]. Similarly, antimicrobial activity of AP-AgNPs have been reported against many bacterial and fungal pathogens [34,43-45]. Biologically synthesized silver nanoparticles synthesized by other plant extracts also showed antimicrobial activity, such as, Cocoa bean extract AgNPs [52], miracle fruit plant AgNPs [53], Cocoa pod AgNPs [54], *Buchholzia coriacea* AgNPs [55], leaves extract of *Berberis vulgaris*, *B. nigra, C. bursa-pastoris*, *L. angustifolia, O. vulgare* [56], leaf extract of *Salvia spinosa* [57], Waste grass mediated green AgNPs [58], *Acacia lignin* AgNPs [59], green synthesis with chitosan incorporated AgNPs [60] and many more.

Table 1: Antimicrobial activity of synthesized AP-AgNPs

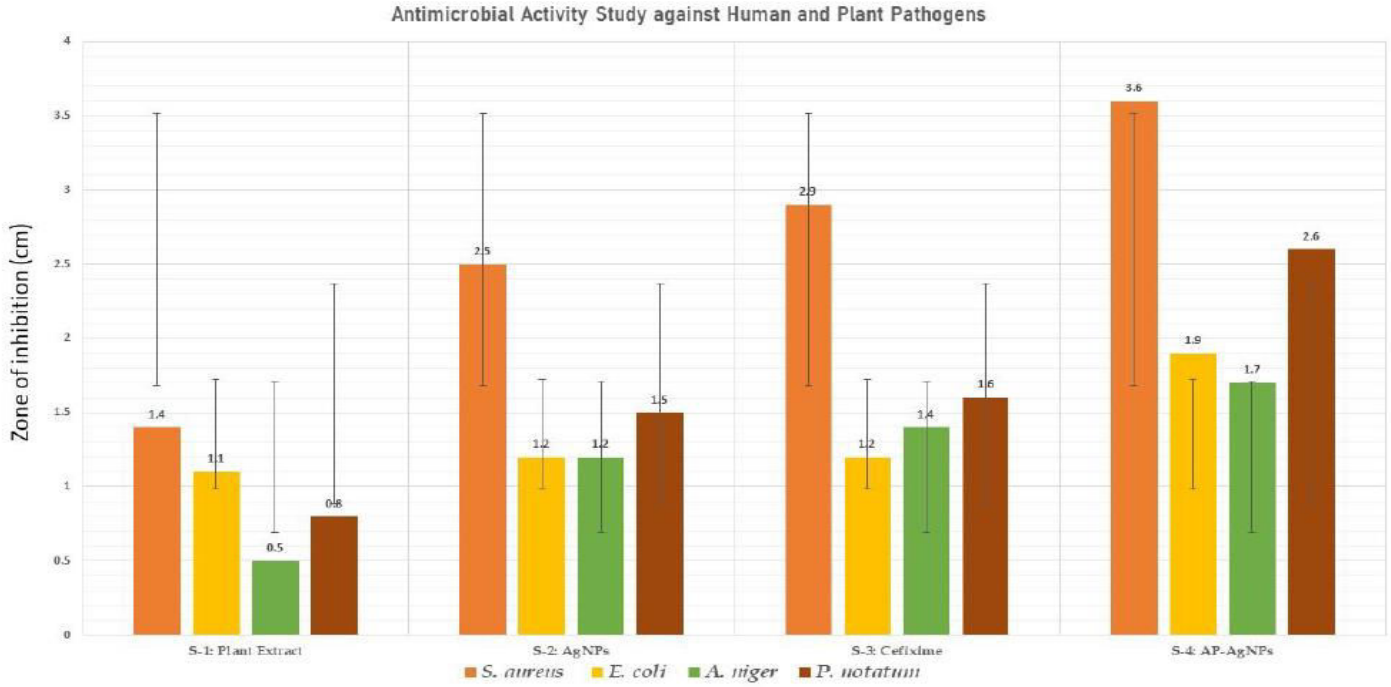
Pathogen	Zone of inhibition (cm)							
	Sample-1		Sample-2		Sample-3		Sample-4	
	Average	St. Deviation	Average	St. Deviation	Average	St. Deviation	Average	St. Deviation
S. aureus	**1.4**	0.15	**2.5**	0.31	**2.9**	0.26	**3.6**	0.15
E. coli	**1.1**	0.06	**1.2**	0.06	**1.2**	0.06	**1.9**	0.15
A. niger	**0.5**	0.25	**1.2**	0.15	**1.4**	0.12	**1.7**	0.12
P. notatum	**0.8**	0.12	**1.5**	0.21	**1.6**	0.12	**2.6**	0.15

Readings of zone of inhibition against common human bacterial pathogens S. aureus and E. coli and against common plant pathogens, Penicillium notatum and Aspergillus niger in triplicate. Sample-1: Plant extract; Sample-2: Chemically synthesized AgNPs; Sample-3: Positive control Cefixime; Sample-4: AP-AgNPs.



Antibacterial activity of AP-AgNPs against (a) S. aureus and (b) E. coli. Antifungal activity of AP-AgNPs against (c) A. niger and (d) P. notatum. Here, S-1: Plant extract; S-2: Chemically synthesized AgNPs; S-3: Positive control Cefixime; S-4: biologically synthesized AP-AgNPs.

Fig. 4: Antimicrobial bioassay of AP-AgNPs



Comparative antimicrobial activity of plant extract, chemically synthesized AgNPs, positive control Cefixime and biosynthesized AP-AgNPs against common human bacterial pathogens S. aureus and E. coli and against common plant pathogens, Penicillium notatum and Aspergillus niger

Fig. 5: Antimicrobial activity study against Human and Plant pathogens

4. CONCLUSION

In the given study it is clear that biological synthesis of silver nanoparticles is better option than chemical and physical synthesis. These bio-nanoparticles have better and enhanced antimicrobial activity and can solve the problem of development of multiple drug resistance developed in pathogens and can be a good candidate for next generation antibiotics. These bio-nanoparticles synthesized by *A. paniculata* leaf extract can also be used in agriculture industry for effective control of plant pathogens.

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Conflict of interest

There is no conflict of interest.

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