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Green Synthesis of Silver Nanoparticles Justicia Gendarussa Leaves Extract for Biological Activities and Antimicrobial Activity

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Abstract

The fast-paced development of nanomaterial production has enhanced the application of silver (Ag) in a wide variety field. We prepared silver nanoparticles using *Justicia gendarussa* leaf extract. The green synthesis process reports ecofriendly, nontoxic, cost effective and simple approach to the synthesis of metal and metal oxide nanoparticles. Leaf extract of *Justicia gendarussa* acts as capping as well as stabilizing agent. The average crystalline size of the XRD pattern is estimated to be 20.34nm. FT-IR (Fourier Transform Infrared) spectra confirm the existence of Ag NPs. The UV-Visible spectrum exhibits absorption peak range of between 420nm. SEM and TEM technique. Structural analysis for silver nanofibers reveals. The antimicrobial activity of silver NPs was tested for bacteria and fungi by disc diffusion method.

***Key words*:** Silver NPs, Biosynthesis, Characterization, Antimicrobial activity

Nanotechnology is a modern disciplinary domain for research that is more likely to anticipate in numerous technologies. It is the driving force of the innovations in the current century. Owing the excellent properties, the interest in nanoscience application is growing significantly through developing new material [1-2]. With the advancements of the science and technology, the progress in almost every sphere of life is now gaining a key interest. Towards this, the multifunctional nanostructured materials have garnered a plethora of potential application especially in day-to-day life. Further, the green nanotechnology has gained more recognition for the synthesis of nanoparticles due to its unique property of not affecting environment, renewable feedstock, high production rare, low cost and low energy requirements [3].

Nanotechnology exposure in scientific world covers all area of science [4]. Nanotechnology deals with small dimensional materials under the range of 1-100nm [5]. Nanomaterials are widely used in electronics, biomedical, biotechnology and therapeutic applications, etc. Silver nanoparticles were approving clinically for vaccine infections, inflammations and renal diseases [6]. Metal oxide base NPs shows promising interest in scientist mind for their extensive application in field of biomedical and material science. The strong absorption in the visible region due to free electron excitation of noble metal nanopaticles also gain high interest in scientist to work on it [7]. In the recent studies, AgNPs importance from various biomedical applications such as antibacterial, anti-fungal, antioxidant, anticancer and anti-inflammatory activity [8-9].

Justicia gendarussa belongs to the family of Acanthaeceae. Every part of the plant has medicinal properties. Notably, the leaves are a valuable source of drugs for many infectious diseases. In the light of the other differences in the biomedical applications of AgNPs based on the various biological synthesis, this study hypothesized to synthesize AgNPs using *Justicia gendarussa* leaf extract concerning physical, chemical and biomedical properties. Thus, herein the medicinally important *Justicia gendarussa* leaf extract has been used as a potential bio-reductant agent for the fabrication of AgNPs through a novel green-chemistry, in an economical and ecofriendly approach. The present study was carried out biosynthesized AgNPs were characterized by UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FT-IR), X-Ray diffraction (XRD), scanning electron microscope (SEM), energy dispersive X-ray spectroscopy (EDS), transform electron microscopy (TEM) and antimicrobial activity of silver nanoparticles in *Justicia gendarussa*.

MATERIALS AND METHODS

Justicia gendarussa leaves were collected from Kadukaval village in Thanjavur, Tamil Nadu, India. Silver nitrate purchased from Hi Media laboratories Pvt. Ltd. All purchased chemicals are analytically used without any further purification process. Antimicrobial pathogens obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

Preparation of leaves extract

The dried leaves of *Justicia gendarussa* were pulverized well with pestle and mortar to make a fine powder. Twenty grams of powder sample was mixed into one hundred mille liter of deionized water and the mixture was boiled for 10 min. After

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cooling the leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4°C for further use.

Synthesis of silver nanoparticles

Silver nanoparticle was synthesized by the method 5 ml of *Justicia gendarussa* leaves extract was added to 45 ml of 1 mM aqueous $AgNO_3$ solution in a 250 ml Erlenmeyer flask. The flask was then incubated in the dark for five hours (to minimize the photo activation of silver nitrate), at room temperature. Silver NPs formation was confirmed by change of color in the solution [10].

Characterization

Silver NPs were successfully synthesized by *Justicia gendarussa* leaves extract as capping and reducing agents. Silver nanoparticles were studied by optical, morphological and biological properties. Silver nanoparticles were characterized by using X-ray Diffractometer, Fourier Transform Infrared Spectroscopy, UV-Visible Spectroscopy and Scanning Electron Microscopy and antimicrobial activity of the silver nanoparticles.

X-ray diffractometer

The phase evolution of calcined powder as well as that of sintered samples was studied by X-ray diffraction technique (Philips PAN analytical, The Netherland) using Cu Ká radiation. The generator voltage and current were set at 35 KV and 25 mA respectively. Ag samples were scanned in the 2è ranges 15 to 700 °C range in continuous scan mode. The scan rate was 0.04/sec.

UV-Visible spectroscopy

The silver nanoparticles were examined under UV and visible spectrophotometer analysis. The silver nanoparticles were scanned within the wavelength starting from 200-1000 nm using Perkin Elmer photometer and also the characteristic peaks were identified. The peak values of the UV were recorded. Each and every analysis was repeated thrice for the spectrum confirmation.

Fourier transform infrared spectroscopy

FTIR analysis was performed using Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm-1 for the functional group identification. The peak values of the FTIR were recorded. Each and every analysis was repeated thrice for the spectrum confirmation.

Scanning electron microscope with EDS

The particle size and morphology of nanoparticles were analyzed by ZEEISS-SEM machine. The dried form of silver nanoparticles were sonicated with distilled water, small droplet of silver nanoparticles were placed on glass slide and allow to dry. The ZEEISS-SEM machine was worked at a vacuum of the order of 10-5 torr. The accelerating voltage is 10 kV. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached with the SEM. The EDX analysis of Ag sample was done by the SEM machine.

Transmission electron microscope

Shape, selected area diffraction and average of particle size analyses of synthesized silver NPs were carried out with the help of TEM. The TEM study was done by CM30–Philips at functioning voltage of 80 kV.

Microorganisms

The microbial strains employed in the biological assays were *Escherichia coli* (MTCC 732), *Pseudomonas aeruginosa* (MTCC 1035) and *Staphylococcus aureus* (MTCC 3160) for bacteria while fungal strain *Candida albicans* (MTCC 183), *Aspergillus niger* (MTCC 1783) and *Aspergillus flavus* (MTCC 10180), obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

Antimicrobial activity

Antibiogram was done by disc diffusion method [11-12] using AgNPs samples. Petri plates were prepared by pouring 30 ml of NA/PDA medium. The test organism was inoculated on solidified agar plate with the help of micropipette into spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized microbes test suspension and used to evenly inoculate the entire surface of the Nutrient agar /PDA plates. Briefly, inoculums containing of microbial strains were spread on Nutrient agar /PDA plates. Using sterile forceps, the sterile filter papers (6 mm diameter) containing 50µl of samples of leaf extract, 1mMAgNO_3, AgNPs(50µl) and Chloramphenicol and Fluconazole 30µl standard solution were laid down on the surface of inoculated agar plate. The plates were incubated at 37 ºC for 24 h for the bacteria and 48 hr. for fungal strains. Each sample was tested in triplicate.

RESULTS AND DISCUSSION

Visual confirmation of silver nanoparticles

Visual confirmation of silver NPs was confirmed using color changes (Fig 1). Color changes from pale green to brown in color. The formation of nanoparticles takes time from minutes to 5 hours incubation.

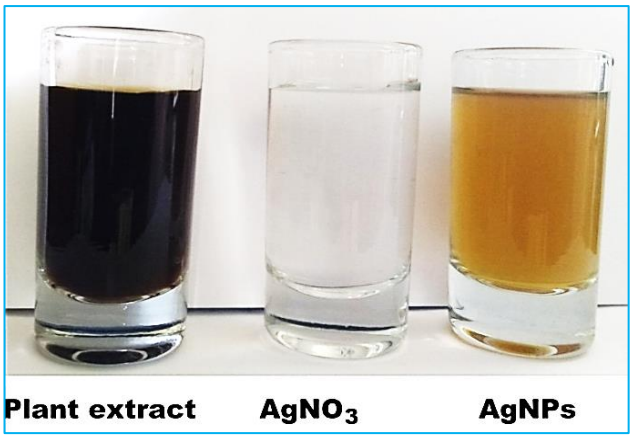


Fig 1 Colour changes before (Plant extract) and after (AgNPs) the process of reduction of Ag^+ to Ag nanoparticles and control ($AgNO_3$) {$AgNO_3$: 1 mM $AgNO_3$ (White colour) and AgNPs: 1mM $AgNO_3$ in the presence of *Justicia gendarussa* leaves extract after 5 hours of incubation (Brown colour)}

XRD analysis

The (Fig 2) shows the XRD (X-Ray Diffraction pattern silver NPs XRD pattern reveals the 2ɵ peaks obtained at 38º, 43º, 55º, 68º and 83º obtained pattern 2ɵ correspond to Bragg's reflection planes 111, 200, 211, 310 and 320 respectively [13]. XRD pattern synthesized silver nanoparticles results crystalline nature and having a face-centered cubic crystalline size values were calculated using Debye Scherer formulae $D=0.9\lambda/\beta \cos\theta$. Where, 0.9-constant, λ-wavelength of X-Ray, D is the average crystalline size and β full width half maximum [14].

Average crystalline size values of silver nanoparticles reveal 20.34nm peak intensity and no more impurity peak correspond to crystalline nature. Observed XRD pattern well matched with JCPDS: Silver file No.04.0783. Here, the constant is 35.5(=144.4108.9). Hence XRD pattern thus clearly illustrated that the silver nanoparticles formed in the present synthesis are crystalline in nature. In addition to the Bragg peaks representative of fcc silver nanocrystals, additional as yet unassigned peaks are also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles. The line broadening of the peaks is primarily due to small particle size. Indexing has been done and data are in (Table 3).

X-Ray Diffraction pattern exhibit a pure and cubic crystalline face-centered cubic structure of pure silver metal that was exhibited by all reflectance [15].

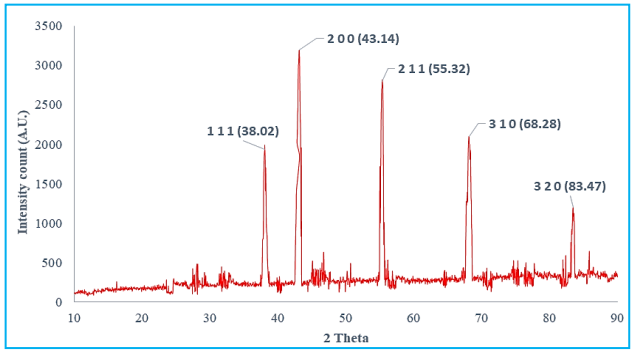


Fig 2: XRD patterns of silver nanoparticles synthesized by *Justicia gendarussa* leaves

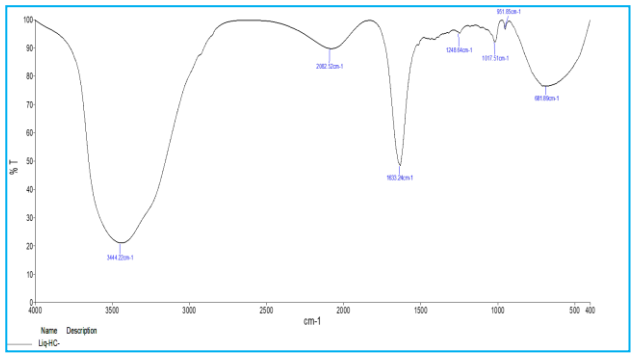


Fig 3 Fourier transform infra-red spectral analysis of AgNPs

FTIR analysis

The FTIR spectrum was used to recognize the active biomolecules present in the leaf extract and to determine functional group interaction of synthesized AgNPs by using *J. gendarussa* leaf extract. The FTIR pattern of biosynthesized AgNPs was shows Fig.3 and revealed different absorption peaks. The broad bands at 3444.22cm^{-1} were corresponding to the O-H stretch and H-bonded of Alcohol and Phenolic. The sharp and strong peaks observed 1633.2422cm^{-1} were contributed to the N-H bend of Primary amines. The absorption bands at 1248.64 and 1017.5122cm^{-1} were assigned C-O stretch bonded of Alcohols, Carboxylic Acids, Esters and Ethers. The absorbance at 681.89cm^{-1} assigned to aromatic bond C-H "oop" bending. *Justicia gendarussa* leaves extract that acts as a strong reducing and stabilizing agent polyphenol act like excellent stabilizing to reduce Ag^+ to Ag nanoparticles. FTIR spectrum results in the biomolecules present in leaves extract acting as a dual function which is responsible for stabilization and reduction of nanoparticles [16-17]. Hydrogen bonds are derived from the protein molecules and capped the AgNPs through the electrostatic attraction process prevents the agglomeration of nanoparticles and enhances the stability and biological activity [18-19].

UV-Visible spectroscopy

UV-Visible spectra of AgNPs as shown in Fig. 4 due to surface plasmon resonance (SPR) 420nm [20]. AgNPs shows SPR at around 350-460nm. Color changes from pale green to dark reddish-brown result in the formation of AgNPs [21]. Biological molecules present in leaves extract results in reduction of Ag^+ ions to AgNPs. The endothermic is commendatory resulting spontaneous increase in AgNPs due to increasing in OH – ions concentration [22]. The broad absorption peak indicates the different particle size values broad peaks are due to polydisperse nanoparticles in the reaction mixture [23].

Color variation from pale green to dark reddish brown due to the reduction of Ag^o absorbance peak was observed at 420nm which shows the excitation of SPR [24]. According to the similarly research agreement with the color change was observed during the reaction mixture and further it was conformed UV-Visible spectrum absorption peak at 380nm characteristic band [25]. Particles size values vary due to changes in wavelength. Free electrons present in AgNPs result form an increase in absorption band due to combined vibration of e^- from metal nanopaticles in resonance with light wave. In AgNPs free electrons present in which excited by absorbing UV light and transmitted to higher energy levels. When electrons are unstable at an excited state and emit photons simultaneously.

The replications of the work have been carried out and measured by UV-Visible spectrometer technique as shown in (Fig 4). The unique property of SPR has confirmed the presence and the zero valences of AgNPs the results showed the efficiency and uncertainly of the synthesis method in this experiment. The replication results are compared with original work which is depicted below.

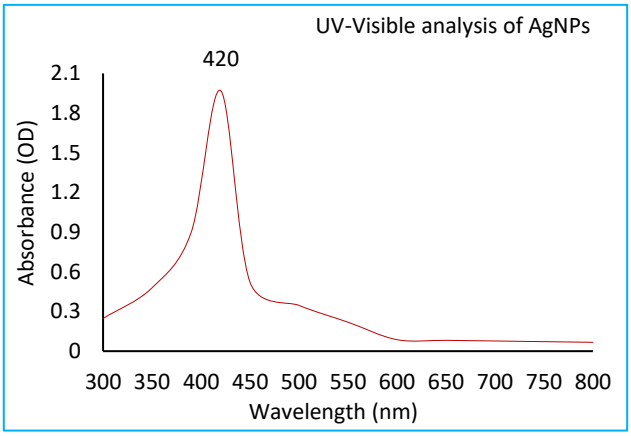


Fig 4 UV-Vis. analysis of AgNPs using *Justicia gendarussa* (Peak was observed at 420nm indicating the presence of silver nanoparticles)

Scanning electron microscope

SEM analysis was carried out to understand the topology and the size of the AgNPs, which showed (Fig 5a) the synthesis of higher density polydispersed spherical AgNPs of various sizes that ranged from 35.60 to 65.88 nm respectively as well cubic and crystalline nature of the nanoparticles. Most of the nanoparticles gathered and only a little of them were dispersed, when observed under SEM. To find out the particle size of the nanoparticles the dynamic light scattering measurement was performed. Laser diffraction had shown that particle size was found in the range of 30 to 100 nm.

The histograms plotted on the obtained data to study the particle size distribution reveals that the size of the nanoparticles ranged from 33.33 to 91.38 nm and the average particle size was found to be 57.40±14.17 nm (Fig 5b). Overall particle size of AgNPs were highly distributed between 50nm to 60nm range which is the evidence that the NPs synthesized less than 100nm (NPs < 100nm). According to the similarly research agreement with the scanning electron microscope the morphology, distribution, elemental analysis and electron diffraction of the silver nanofibers in Jasmine flower extract. The electron microscopic results revealed the information of fiber shape and evenly distribute in all surface. The higher magnification of the SEM denotes the distribution as well as the fiber shape of the silver material [26].

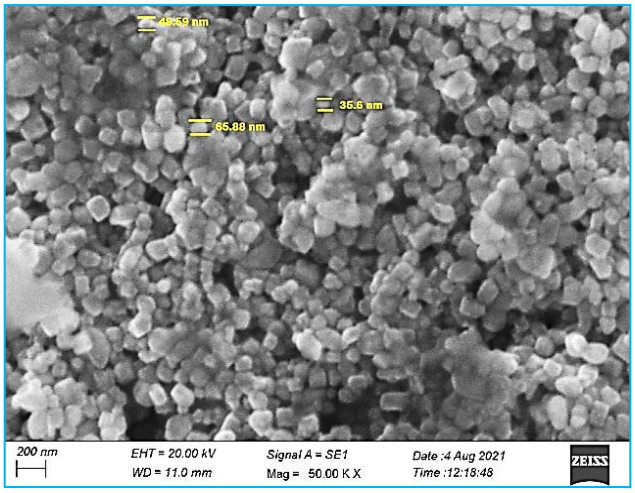


Fig 5a Scanning electron microscopical (SEM) analysis of AgNPs

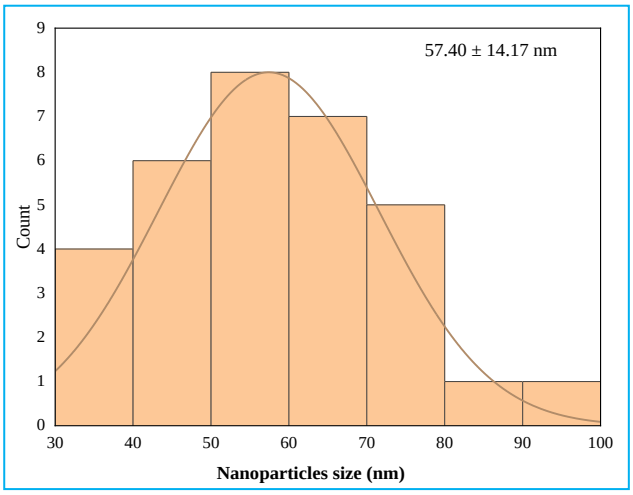


Fig 5b Histogram showing particle size distribution of AgNPs

Energy dispersive spectrum

Fig.6 exhibits the energy dispersive spectra of AgNPs. EDS spectra of AgNPs reveal the presence of Silver (Ag) and Chloride (Cl). Higher intensity peaks of Ag results in major element present in the investigated material. The Ag peak observed are 20.0KeV prove the formation of the pure silver nanoparticles (67.05%). According to the previous similar report the plant extract mediated green chemistry synthesis of NPs elemental compositions of metals were determined by using EDS analysis [27].

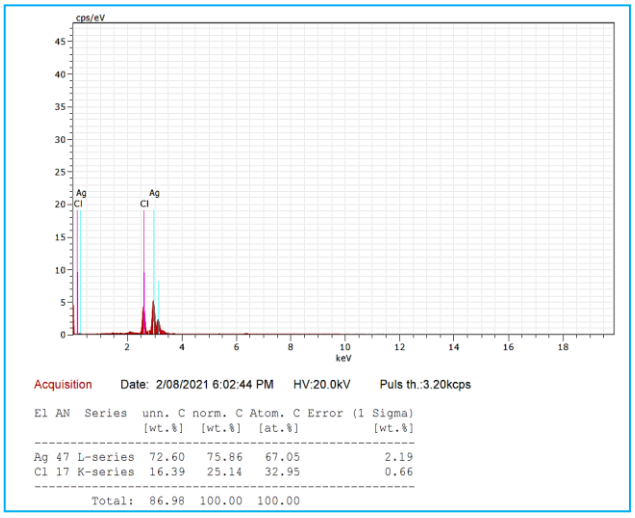


Fig 6 EDX analysis of silver nanoparticles

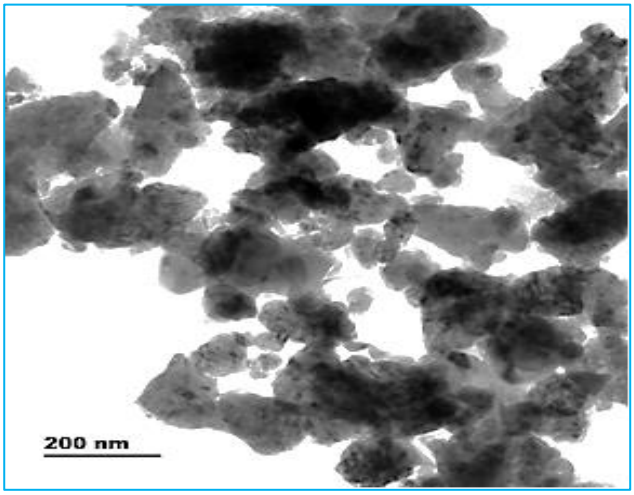


Fig 7 Transmission electron microscopic (TEM) image of AgNPs nanoparticles with cluster

Transmission electron microscope

As transmission electron microscope images represent the electron transmission is to the surface of the AgNPs as shown in (Fig 7) indicating the higher magnification of the AgNPs represent the 200nm scale of the silver nanoparticles. The observed result coincides with an earlier report a few agglomerated Ag-NPs were also perceive in some places, which shows variation in average crystalline size as well as particle size estimated was 22nm and the particle size ranged from 10nm to 40nm [28].

Antibacterial activity

Silver nanoparticles biosynthesized from *Justicia gendarussa* leaves extract was tested individually against test organisms for antibacterial activity by agar disc diffusion method. For this study both Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) organisms were used. This was performed by determining ZoI (zone of inhibition) which is rapid and inexpensive to determine the susceptibility of a particular test organism as antimicrobial agent. This was executed by measuring the zone of inhibition using a vernier caliper. After 24 hours of incubation, the inhibitory effect of AgNPs from *Justicia gendarussa* leaves extract was significant were compared to *Justicia gendarussa* leaves extract and standard chloramphenicol. Zone of inhibition (ZoI) was used as a measure for comparing bactericidal activity of these $AgNO_3$. AgNPs from *Justicia gendarussa* leaves extract showed about

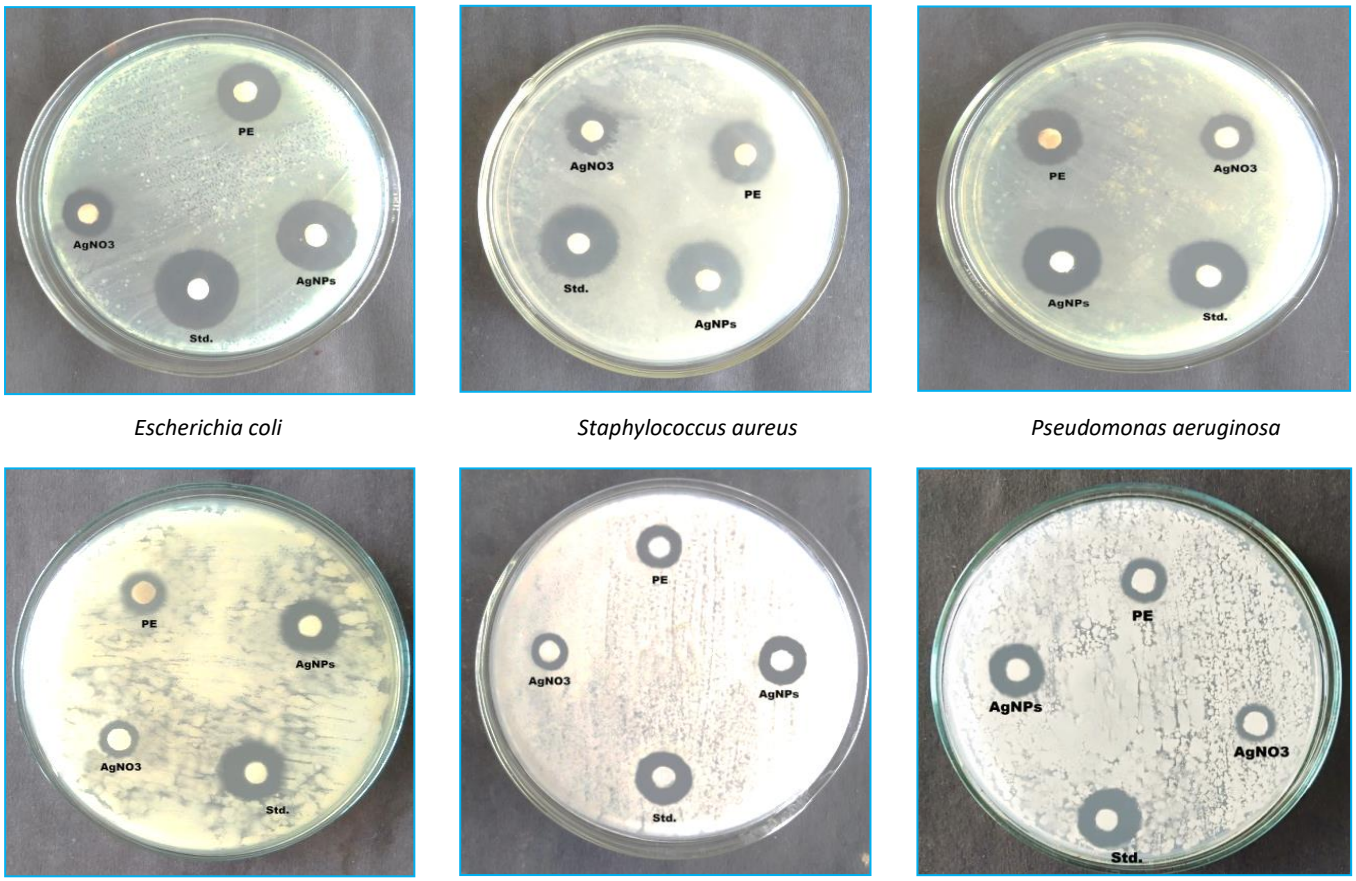
12.05mm zone against the test organisms *E. coli* maximum zone of inhibition. In previous research work carried out the maximum zone of inhibition up to 23mm has been observed for *Bacillus* Sp. followed by the *E. coli* and *S. aureus* [29]. Similarly, the AgNPs from *Justicia gendarussa* leaves extract showed 12.00mm and 11.50mm ZoI against test organisms: *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively (Table 1, Plate 1). In the previous research reported Ag nanoparticles have drawn attention as a potent antibacterial agent to outcome lacking sources of antibiotics. Silver nanoparticles (AgNPs) and AgCl nanoparticles were assessed as novel antibacterial agents [30-32].

Antifungal activity

Silver nanoparticles biosynthesized from *Justicia gendarussa* leaves extract was tested individually against test organisms for antibacterial activity by agar disc diffusion method. For this study *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger* were used. This was performed by determining ZoI (zone of inhibition) which is rapid and inexpensive to determine the susceptibility of a particular test organism as antimicrobial agent. This was executed by measuring the zone of inhibition using a vernier caliper. After 48hrs hours of incubation, the inhibitory effect of AgNPs from *Justicia gendarussa* leaves extract was significant were compared to *Justicia gendarussa* leaves extract and standard fluconazole. Zone of inhibition (ZoI) was used as measure for comparing fungal activity of these $AgNO_3$. AgNPs from *Justicia gendarussa* leaves extract showed about 8.00mm zone against the test organisms: *Candida albicans* maximum zone of inhibition. As mentioned previously, the antifungal activity of CuNPs against the *P. capsici* was stronger than that of the *F. oxysporum*. Moreover, the results of negative control test using control solution (only 3% after 2 days) also suggest that control solution would not enhance the antifungal activity, and $CuCl_2$ solution (40 ppm) partly inhibited the growth of *P. capsici* [33]. Similarly, the AgNPs from *Justicia gendarussa* leaves extract showed 6.00mm and 7.00mm ZoI against test organisms: *Aspergillus flavus* and *Aspergillus niger* respectively (Table 1, Plate 1).

Table 1 Anti-microbial activity (Values expressed as mm) Bacterial standard: Chloramphenicol and fungal standard: Fluconazole

Microbial strains	Zone of inhibition (mm) of 50µl			
	Plant extract	1mM $AgNO_3$	AgNPs	Std. (30 µl)
		Bacterial strains		
Escherichia coli	9.00	7.00	12.05	12.65
Staphylococcus aureus	8.00	6.00	12.00	12.40
Pseudomonas aeruginosa	9.00	6.80	11.50	12.00
		Fungal strains		
Candida albicans	5.00	4.00	8.00	11.00
Aspergillus flavus	4.00	2.00	6.00	9.50
Aspergillus niger	4.50	3.00	7.00	9.70



Escherichia coli *Staphylococcus aureus* *Pseudomonas aeruginosa*

Candida albicans *Aspergillus flavus* *Aspergillus niger*

Plate 1 Anti-microbial activity

CONCLUSION

In the current investigation, successfully green route biosynthesized AgNPs from the leaf extract *Justicia gendarussa*, which is first reported in their plant. The AgNPs have less toxic effect due to the presence of phytochemicals. AgNPs was characterized by FTIR and XRD spectrums, have to be confirmed biological molecules mediated development of AgNPs and their cubic crystalline in structure. SEM and TEM images revealed surface topography of spherical shapes, were suggested range between 30 to 100nm. The energy dispersive X-ray spectroscopy (EDS) pattern was suggested various elements in the prepared AgNPs and DLS also performed to confirmation of average particles sizes. The synthesized AgNPs was initially characterized and then subjected to determine the antimicrobial activity. The research could of great entity for scientist to work on and make it more reliable in various applications such as biomedical and energy source. This study provides and informational insight into the polyphenol mediated synthesis of AgNPs, their antimicrobial activity and biosynthesized AgNPs indicating a new application in the domain of drug metabolism. This investigation demonstrates a novel and environmentally friendly method for synthesizing AgNPs using plant extract, and it explores their potential applications, especially in the biomedical field and as an energy source.

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