



Biological synthesis of silver nanoparticles from *Adansonia digitata* L. fruit pulp extract, characterization, and its antimicrobial properties

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ABSTRACT

Aim: In the present study, we report a cost-effective, eco-friendly, and an efficient alternative method for large scale production of silver nanoparticles (AgNPs) from *Adansonia digitata* fruit pulp extract. The study mainly focused on the synthesis, characterization, and antimicrobial properties of AgNPs.

Materials and Methods: Synthesis of AgNPs with the help of standard protocol and characterized by ultraviolet (UV)-vis spectrophotometry, Fourier transform infra-red (FTIR), X-ray diffractometer (XRD), atomic force microscopy (AFM), scanning electron microscopy (SEM) with EDAX, transmission electron microscopy (TEM) and explore their potential growth inhibitory effect on 07 bacterial and 05 fungal pathogens. **Results:** The synthesized AgNPs are characterized by UV-vis spectrophotometry shows a broad peak at 434 nm. The FTIR spectroscopic analysis clearly reveals phenols and proteins are main responsible for reduction and stabilization of nanoparticles. XRD studies show the nanoparticles are crystalline in nature owing 44 nm in size. EDAX spectrum shows a 33.28 weight percentage of Ag metal in the reaction medium confirms the purity of AgNPs. High resolution and magnification studies with AFM, SEM, and TEM reveal the nanoparticles are polydispersed, spherical in shape, having the size range from 3 to 57 nm without any agglomeration between the particles. Further, the antimicrobial studies reveal the potentiality of nanoparticles against different microbial pathogens.

Conclusion: The present study is mainly focused on the synthesis of AgNPs from *A. digitata* fruit pulp extract. Here, we succeed to synthesize a narrow range of particles and validate its potential antimicrobial activity on different microorganisms. Based on this, we conclude that *A. digitata* pulp extract is a good source toward the reduction of AgNPs and acts as environment benign antimicrobial agents.

KEY WORDS: *Adansonia digitata*, antimicrobial activity, characterization, fruit pulp, silver nanoparticles

INTRODUCTION

Synthesis of metal nanoparticles with the help of plant extracts is an emerging field of nanotechnology, due to their novel properties, terrific applications in biomedicine and its eco-friendly nature [1]. In recent past great efforts were made for synthesis of environment benign and eco-friendly nanoparticle from plants such as iron oxide nanoparticles from *Medicago sativa* [2], copper nanoparticles from *Magnolia kobus* [3], calcium nanoparticles from *Boswellia ovalifoliolata* [4], gold nanoparticles from *Avena sativa* [5], zinc oxide nanoparticles from *Catharanthus roseus* [6], and silver nanoparticles (AgNPs) from *Syzygium alternifolium* [7]. Silver is one among the metal nanoparticles focused much interest due to its wide variety of applications [8]. It has different biological

activities such as antimicrobial [9], anthelmintic [10], antilarvicidal [11], antioxidant [12], anticancer [13], anti-inflammatory [14], hepatoprotective [15], and wound healing activity [10]. Conventional methods for synthesizing AgNPs are mainly by chemical, physical, and microbe-mediated synthesis. In these chemical and physical methods, usage of hazardous chemicals, high energy requirements, difficult and wasteful materials generate potential and biological hazards to the environment [16]. Whereas in the case of microbe-mediated synthesis is not feasible industrially due its lab maintenance. Therefore biological synthesis of AgNPs by using plant materials is easy, efficient, and eco-friendliness in comparison to chemical mediated or microbe-mediated synthesis [17] and they possess secondary metabolites having the redox capacity to reduce metal nanoparticles in an easiest way [18].

Adansonia digitata L. belongs to the family Malvaceae is a large tree indigenous to Africa and found in many countries also. Traditionally, the fruit pulp dissolved in water or milk is used as a drink or sauce for food in Africa. Fruit pulp and powdered seeds are used for the treatment of diarrhea and dysentery in India [19]. The high content of Vitamin C in fruit pulp is recommended to pregnant women's for daily intake [20]. In recent times, different scientists prove different activities of pulp such as hepatoprotective [21], antimicrobial [22], antiviral [23], antioxidant [24], antidiarrheal [25], hypoglycemic [26], anti-inflammatory, and antioxidant [27] activities. Due to high medicinal values and mythological significance of this plant is known as "Kalpavriksha" (a tree which fulfill all desires) in India [28]. In our previous studies, synthesis of AgNPs from stem bark and leaf extract of *A. digitata* acts as excellent reducing agents and show potential antimicrobial activity against different microorganisms [29,30]. However, the potentiality of fruit pulp mediated synthesis of AgNPs is not carried out so far. Hence, the present study is aimed to synthesize AgNPs from *A. digitata* fruit pulp extract, characterize and to know the potentiality of AgNPs against different microbial pathogens.

MATERIALS AND METHODS

Collection of Plant Material

2 to 4 kg weight mature fruits possess a great amount of pulp is collected from Acharya Ranga Agricultural University, Tirupati and cross checked by herbarium (Voucher no: SVU362) deposited in Department of Botany, Sri Venkateswara University, Tirupati. Cut open the fruit and separate the seeds adhesive to pulp. Grounded the collected pulp with the help of mortar and pestle, sieved the content for the synthesis of nanoparticles.

Extract Preparation and Synthesis of AgNPs

For synthesis, 25 g of fruit pulp is extracted with 100 ml of distilled water on boiling water bath for 30 min, filter the content with Whatman No. 1 filter paper and stored at room temperature. From this, 5 ml of the extract is taken into 250 ml of Erlenmeyer conical flask and titrated against with the solution of 1 mM AgNO₃ at a 60-80°C temperature. The contents are cooled and centrifuged at 10,000 rpm for 20 min to avoid the presence of any biological impurities, and it is used for further characterization and antimicrobial studies.

Characterization of AgNPs

Confirmation of synthesized nanoparticles is AgNPs by ultraviolet (UV)-vis spectrophotometry absorption spectra using a spectro UV 2080 double beam, between the wavelength scan range of 190-700 nm, 1200 L/mm spectrophotometer, Analytical technologies, India. To know the possible bio-molecules responsible for reduction and stabilization of AgNPs by Fourier transform infra-red (FTIR) spectra in the scan range of 4,000 to 500 cm⁻¹ transmittance with an ALPHA interferometer, Bruker, Ettlingen, Karlsruhe, Germany by KBr pellet method. X-ray diffraction of synthesized nanoparticles is examined by an

X-ray diffractometer (XRD) (Shimadzu, XRD-6000) equipped with Cu K α radiation source using Ni as a filter at a setting of 30 kV/30 mA to know the crystalline nature of AgNPs. Purity of AgNPs was analyzed by FEI Quanta 200 FEG high resolution (HR)-scanning electron microscopy (SEM) machine equipped with EDAX instrument. To know the size, shape, agglomeration pattern, and dispersed nature of the nanoparticles are done by atomic force microscopy (AFM) by NOVA NT-MDT SOLVER NEXT, Russia. SEM by FEI Quanta 200 FEG HR-SEM machine. Transmission electron microscopy (TEM) using HF-3300 advanced 300 kV TEM/STEM from Hitachi.

Antimicrobial Studies of AgNPs

The antimicrobial activity of biologically synthesized nanoparticles are analyzed on seven pathogenic bacteria such as *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538 (Gram-positive), *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 43816, *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 15442, *Salmonella typhimurium* ATCC 14028 (Gram-negative), and five fungal pathogens such as *Alternaria solani* ATCC 32904, *Aspergillus flavus* ATCC 9643, *Aspergillus niger* ATCC 16404, *Penicillium chrysogenum* ATCC 11709, and *Trichoderma harzianum* ATCC 20476 procured from Department of Microbiology, Sri Venkateswara University, Tirupati. Disc diffusion method [31] was followed for testing antimicrobial activity against biologically synthesized AgNPs, and comparative studies were made with plant pulp extract, 1 mM AgNO₃ as negative controls and streptomycin or fluconazole as a standard for bacteria and fungi, respectively. 7 mm sterile discs are prepared from Whatman no. 1 filter paper and 20 μ l of plant extract, 1 mM AgNO₃ solutions, and 10 μ g/disc streptomycin/fluconazole are loaded on separate discs and allowed to air dry for 1 h at sterile conditions. Apart from these, a 5, 10, 20, 40, 60, 80 μ g/ml concentrations of synthesized AgNPs are tested separately to know the minimum inhibitory concentration. 20 and 40 μ g/ml concentration of AgNPs show minimum inhibitory effect and 80 μ g/ml concentrations of AgNPs show maximum inhibitory concentration. Due to this, we prefer 80 μ g/ml concentrations of AgNPs to check the antimicrobial activity on different microbial organisms. Freshly prepared nutrient agar media for bacteria and potato dextrose agar media for fungi are poured into sterile petri plates, allowed to 30 min for solidification. The plates are swabbed with 100 μ l of microbial cultures and placed the previously prepared discs; the experiment is carried out in triplicates. The plates are incubated at 37°C for 24-48 h, and then the zone of inhibition is measured with the help of a scale and tabulated the results.

RESULTS

Reduction of Ag⁺ into Ag⁰ nanoparticles was observed visually by means of a color change pattern of the reaction medium. *A. digitata* fruit pulp having thick cream color, upon synthesis the color change from creamy to light yellow indicates the formation of nanoparticles. This color changed nanoparticles solution was analyzed by UV-vis spectrophotometry shows a broad absorption peak at 434 nm further confirms the formation of nanoparticles

are AgNPs [Figure 1]. FTIR spectroscopic studies of these synthesized nanoparticles show broad transmittance peaks at 3322 cm^{-1} assigned for an O-H bond of phenols and 1636 cm^{-1} assigned for an N-H bond of primary amines [Figure 2]. XRD spectrum of synthesized AgNPs shows the crystalline nature of AgNPs and gives four intensive peaks at 38.1° , 46.2° , 64.5° , and 77.3° of 2θ degrees of X-axis corresponds to 111, 200, 220, and 311 Bragg reflections of Y-axis [Figure 3]. These peaks are indexing the face-centered cubic nature of AgNPs. The mean particle diameter of synthesized AgNPs is 44 nm, calculated according to Debye-Scherrer equation ($D = k\lambda/\beta \cos \theta$) and it coincides with powder diffraction file of Joint Committee on Powder Diffraction Standards file No. 04-0783 [32]. The full width at half maximum values, i.e. $k = 0.44$ was derived from 38, 46, 64, and 77 Bragg reflections of the X-axis. $2\text{ }\mu\text{m}$ resolution studies of biologically synthesized AgNPs with AFM reveal the particles are polydispersed, spherical in shape, having the size range from 25 to 57 nm, and there is no agglomeration observed between the particles [Figure 4a]. Raw data obtained from this AFM microscope is treated with a specially designed image

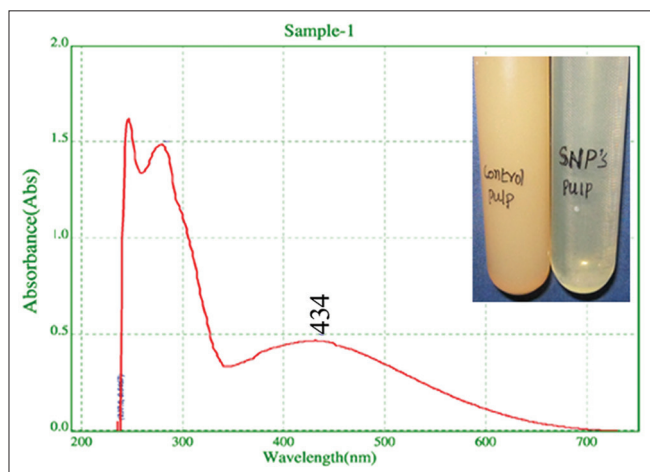


Figure 1: Ultraviolet-vis spectrum of synthesized silver nanoparticles shows broad peak at 434, 280, and 247 nm of narrow peaks is due interference of phytoconstituents in the medium. Inset figure shows color change pattern from cream to light yellow

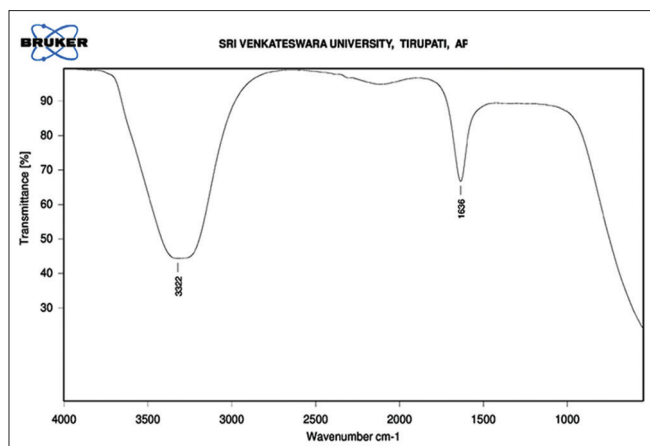


Figure 2: Fourier transform infra-red spectrum of synthesized silver nanoparticles shows broad peaks at 3322 cm^{-1} and 1636 cm^{-1}

processing software (NOVA-TX) to further exploit the three-dimensional (3D) image of nanoparticles [Figure 4b]. 500 nm resolution studies with 20 kV electron energy passing through the nanoparticles coated thin films on a clean glass slide reveals the nanoparticles are spherical in shape, polydispersed and having the size range from 18 to 32 nm [Figure 5a]. The same sample was analyzed with the help of EDAX instrument shows 33.28% presence of Ag^0 in the sample [Figure 5b] along with 05.29% of carbon, 03.28% of nitrogen, 31.80% of oxygen, 08.37% of sodium, 02.57% of magnesium, 01.01% of aluminum, 00.78% of silicon, 07.87% of aurum, and 05.74% of calcium [Table 1]. 33.28% of Ag metal in the sample indicates the high purity of

Table 1: Metal analysis by EDAX spectrum shows different weight percentages of sample

Element	Weight (%)
C	05.29
N	03.28
O	31.80
Na	08.37
Mg	02.57
Al	01.01
Si	00.78
Au	07.87
Ag	33.28
Ca	05.74

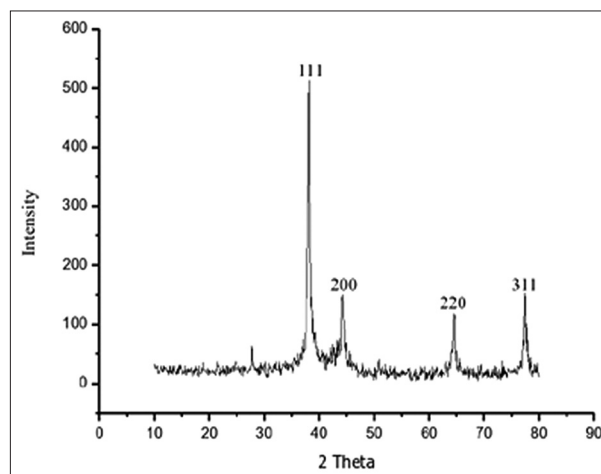


Figure 3: X-ray diffractometer pattern of synthesized silver nanoparticles shows four intensive peaks

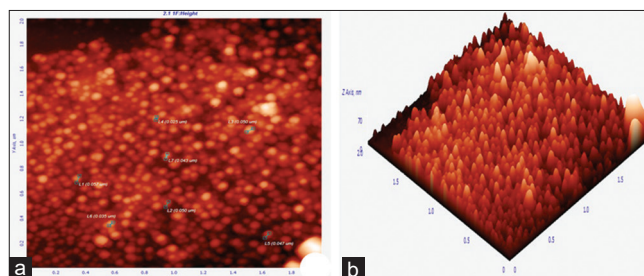


Figure 4: Atomic force microscopy micrograph of synthesized silver nanoparticles (AgNPs), (a) $2\text{ }\mu\text{m}$ resolution studies 25-57 nm size, spherical shaped, polydispersed particles, (b) three-dimensional image of AgNPs analyzed by NOVA-TX software

synthesized nanoparticles. Crystalline nature of synthesized AgNPs was analyzed using selected area electron diffraction (SAED) by directing the electron beam perpendicular to nanoparticles. The SAED pattern of synthesized AgNPs shows the spots had been corresponding to 111, 200, 220, and 311 of the crystallographic nature of face-centered cubic structures [Figure 6a]. 20 nm resolution studies with 300 kV electron energy passing through nanoparticles coated thin films on copper grid show the nanoparticles are polydispersed, spherical in shape and size range from 3 to 7 nm [Figure 6b]. To know the antimicrobial potency of biologically synthesized AgNPs from fruit pulp of *A. digitata* was analyzed against two Gram-positive, five Gram-negative, and five fungal pathogens by disc diffusion method. Among the bacterial pathogens, the highest inhibition zones were observed in *P. vulgaris* followed by *K. pneumoniae*, *P. aeruginosa*, *S. typhimurium*, *E. coli*, *B. subtilis*, and *S. aureus*. Whereas in the case of fungi, the highest inhibition zones were observed in *T. harzianum*, followed by *A. niger*, *A. flavus*, *P. chrysogenum*, and *A. solani*. [Figure 7 and Table 2].

DISCUSSION

When the addition of AgNO_3 solution to the plant extract the color of the plant extract is changed gradually according to the quantitative addition of AgNO_3 solution. This color change is due to the reduction of nanoparticles with the help of electrons present in the fruit pulp extract coming from NAD and ascorbic acid as electron donors present in the plant extracts [33]. Significantly higher amount of ascorbic acid, i.e., >300 mg/100 g was reported in fruit pulp of *A. digitata* [34]. This solution was subjected to UV-vis spectrophotometry between the scan ranges of 190-750 nm, a broad peak is obtained at 434 nm. The same type of results was observed in leaf extract mediated synthesis of AgNPs from *Couroupita guianensis* [35]. The color change pattern and broad peak obtained in UV-vis spectrophotometry are due to Surface Plasmon Resonance nature of AgNPs present in the medium. These nanoparticles absorb light at different wavelengths and excited due to charge density at the interface between conductor and insulator to give a respective peak on UV-vis spectrophotometry. FT-IR is a sensitive tool to analyze functional groups present in the biological samples. It relies

on the light absorbance between 4000 cm^{-1} to 500 cm^{-1} of the electromagnetic, infrared region. In our case, the synthesized nanoparticles show broad peaks at 3322 cm^{-1} and 1636 cm^{-1} . The same type of results was observed in *Myristica fragrans* seed extract mediated synthesis of AgNPs [36]. From this FTIR study, clearly reveals hydroxyl groups of phenols and amide groups of proteins from plant extract forming a layer to the nanoparticles and acting as a capping agent to prevent agglomeration and providing stability in the medium. Based on these FTIR studies, we suggest that the bio-molecules present in plant extracts play dual role in formation and stabilization to AgNPs.

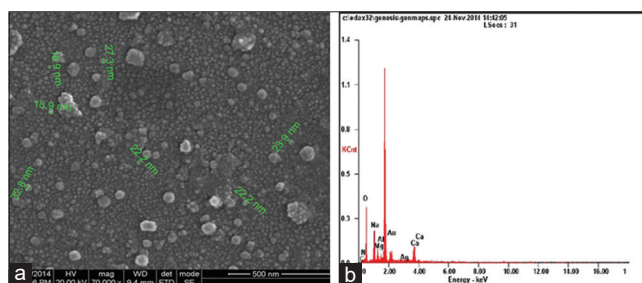


Figure 5: Scanning electron microscopy micrograph of synthesized silver nanoparticles (AgNPs) (a) 500 nm resolution studies shows 18-32 nm size, spherical shaped particles, (b) EDAX spectrum of synthesized AgNPs shows 33.28 weight percent of Ag metal in the sample

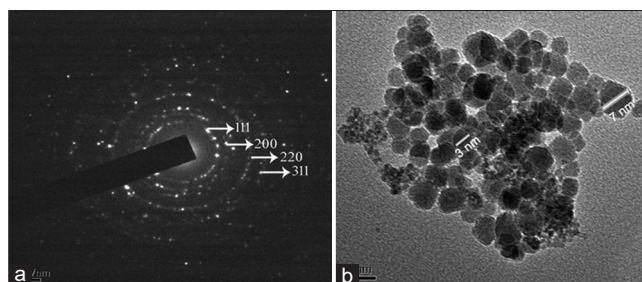


Figure 6: Transmission electron microscopy micrograph of synthesized silver nanoparticles (AgNPs), (a) selected area electron diffraction pattern shows characteristic crystal spots of elemental silver, (b) 20 nm resolution studies of AgNPs shows 3-7 nm size, spherical shaped nanoparticles

Table 2: Zone of inhibition (mm) of AgNPs on microbial pathogens and comparative studies with different controls

Name of the organism	Plant extract (mm)	1 mM AgNO_3 solution (mm)	AgNPs		Streptomycin/fluconazole (mm)
			Disk diffusion assay (mm)	Minimum inhibitory concentration ($\mu\text{g/ml}$)	
<i>B. subtilis</i>	8.9±0.25	9.3±0.69	19.3±0.96	20±0.00	26.6±1.06
<i>S. aureus</i>	7.3±0.73	9.9±0.86	15.9±1.09	40±0.00	23.2±0.85
<i>E. coli</i>	7.2±0.48	8.1±0.38	22.0±0.38	20±0.00	35.9±0.69
<i>K. pneumoniae</i>	8.9±0.38	14.1±0.53	25.8±1.22	20±0.00	36.3±0.78
<i>P. vulgaris</i>	10.3±0.18	11.3±0.81	27.3±0.72	20±0.00	37.1±0.67
<i>P. aeruginosa</i>	7.5±0.38	9.3±0.20	24.5±0.91	20±0.00	33.2±0.70
<i>S. typhimurium</i>	9.1±0.43	9.4±0.25	24.4±0.60	20±0.00	34.6±0.32
<i>A. solani</i>	0	8.2±0.43	10.1±0.39	40±0.00	17.5±0.62
<i>A. flavus</i>	8.1±0.39	8.0±0.10	12.2±0.44	40±0.00	18.1±0.42
<i>A. niger</i>	0	0	12.4±0.21	40±0.00	20.3±0.32
<i>P. chrysogenum</i>	0	8.2±0.25	11.4±0.17	40±0.00	17.3±0.36
<i>T. harzianum</i>	0	9.0±0.40	14.4±0.17	40±0.00	24.4±0.32

AgNPs: Silver nanoparticles, *B. subtilis*: *Bacillus subtilis*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. vulgaris*: *Proteus vulgaris*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *S. typhimurium*: *Salmonella typhimurium*, *A. solani*: *Alternaria solani*, *A. flavus*: *Aspergillus flavus*, *A. niger*: *Aspergillus niger*, *P. chrysogenum*: *Penicillium chrysogenum*, *T. harzianum*: *Trichoderma harzianum*

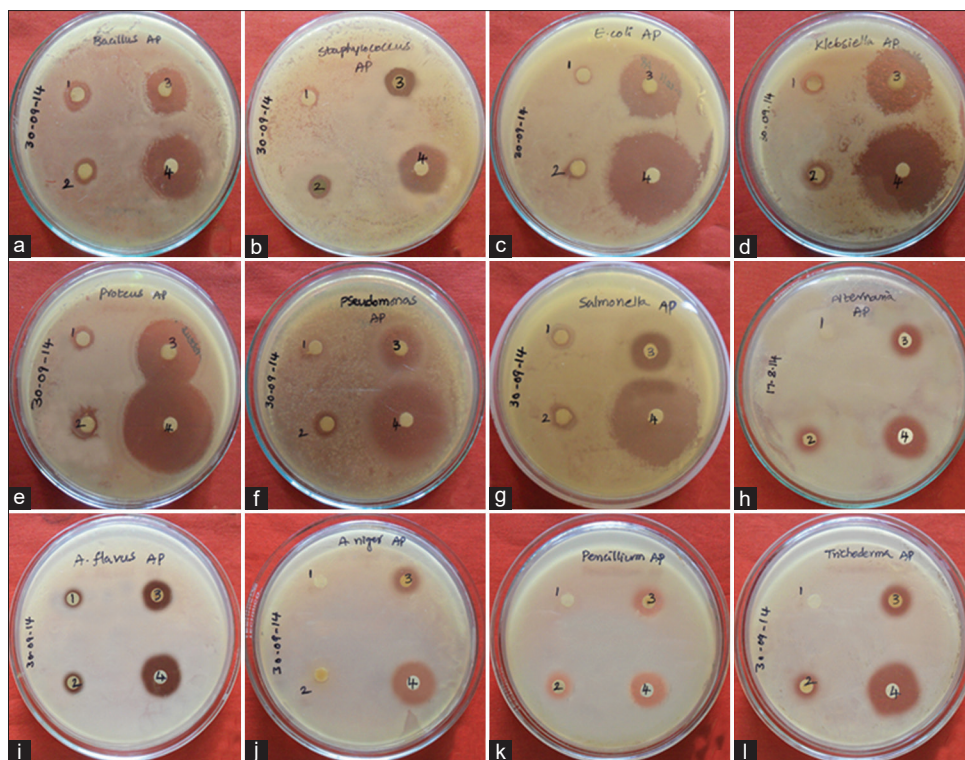


Figure 7: Antimicrobial studies of biologically synthesized silver nanoparticles (AgNPs) from *Adansonia digitata* pulp extract, (a) *Bacillus subtilis*, (b) *Staphylococcus aureus*, (c) *Escherichia coli*, (d) *Klebsiella pneumoniae*, (e) *Proteus vulgaris*, (f) *Pseudomonas aeruginosa*, (g) *Salmonella typhimurium*, (h) *Alternaria solani*, (i) *Aspergillus flavus*, (j) *Aspergillus niger*, (k) *Penicillium chrysogenum*, (l) *Trichoderma harzianum*, (1) Plant extract, (2) AgNO₃, (3) AgNPs, (4) streptomycin/fluconazole

AFM is a primary tool for analyzing size, shape, agglomeration pattern and also offers visualizations of 3D views of the nanoparticles, unlike the electron microscopes. It has an advantage over combination of HR, samples do not have to be conductive and does not require the high-pressure vacuum conditions. Better resolution and percentage presence of nanoparticles were demonstrated by SEM with EDAX instrument provides reliable characterization and morphology of the particles when compare to AFM. The four intensive Bragg reflections are appear in XRD pattern [Figure 3] is correlated with SAED pattern [Figure 6a] of AgNPs, clearly indicates the nanoparticles are crystalline in nature. Higher resolution studies with TEM analysis shows size, shape, and agglomeration pattern of nanoparticles. It achieves better resolution than SEM due to electron energies higher than 20 kV used in SEM. Finally, all the microscopic studies reveal the biologically synthesized AgNPs are spherical in shape, having the size range from 3 to 57 nm polydispersed and no agglomerations were found between the particles.

To test the inhibitory effect of biologically synthesized nanoparticles on different microorganisms shows potential antimicrobial activity. Bacterial species show the highest inhibitory activity when compare to fungi because the cell walls of fungi are made up of chitin is more rigid when compare to bacterial cell walls containing peptidoglycans. Among the bacteria, Gram-negative species show the highest inhibitory zones when to compare to Gram-positive species, because the Gram-negative species containing a less amount of

peptidoglycans. Silver is a precious metal used as an effective antimicrobial agent before the advent of AgNPs. Due to overuse of silver products, decreased the efficiency of silver agents as antibiotic. In recent times with the advancement of nanotechnology, the interest in the use of AgNPs as antibacterial agents had been rekindled [37]. Antimicrobial activities of AgNPs are dependent on the size and shape of the particles. Small sized nanoparticles have higher antimicrobial activity than larger particles because they have large surface area to interact bacteria efficiently [38]. In recent times, the scientists produce 30 to 40 nm size, spherical shaped AgNPs from fruit extract of *Vitis vinifera* shows excellent antimicrobial activity against *B. Subtilis* and *K. planticola* [39]. 10 to 70 nm size, spherical shaped AgNPs synthesized from *Emblia officinalis* fruit extract shows potential antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli*, and *K. Pneumoniae* [40]. *Euphorbia hirta* leaf mediated synthesis of AgNPs having a spherical shape with 40-45 nm size shows good antifungal efficacy [41]. In our studies also 3-57 nm size, spherical shaped AgNPs produced from *A. digitata* pulp extract confirmed by HR microscopic studies with AFM, SEM, and TEM proved these AgNPs are eco-friendly antimicrobial agents against different microorganisms.

CONCLUSION

In the present study, we report an eco-friendly, non-toxic, cost-effective method for synthesis of AgNPs from *A. digitata* pulp extract as reducing agent. In this method, naturally

occurring materials are acts as reducing agents such as biomolecules such as phenols and proteins present in plant extract as a simple and alternative to complex physical or chemical synthetic procedures. 3 to 57 nm size, spherical shaped, polydispersed nanoparticles are produced from *A. digitata* pulp extract confirmed by AFM, SEM, and TEM prove this plant extract as an effective reducing agent for the production of narrow range of nanoparticles by industrial scale. Further antimicrobial studies reveal that small size, spherical shaped particles have immense activity against different microbial pathogens and acts as eco-friendly antimicrobial agents.

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