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Research Article

Synthesis of silver nanoparticles using the medicinal plant *Allmania nadiflora* and evaluation of its anti microbial activities

Karunakar Rao Kudle[1], Manisha R Donda[1], Prashanthi Y[2], Ramchander Merugu[2], Pratap Rudra M.P*[1]

[1]Department of Biochemistry, Osmania University, Hyderabad-500007, India
[2]University College of Science and Informatics, Mahatma Gandhi University, Nalgonda-508254, India

ABSTRACT

In the present study, we report an eco-friendly and economical way for the synthesis of silver nanoparticles using the weed *Allmania nadiflora*. The plant popularly known as "Erra baddiaaku" belongs to the family Amaranthaceae. Characterization of the silver nanoparticles was done using UV Visible Spectroscopy (Uv-Vis), Fourier Transform Infra Red Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Transmission electron Microscopy (TEM). All the synthesized nanoparticles exhibited significant antimicrobial activity. Significance of the above in the light of existing literature is discussed in the present communication.

Keywords: *Allmania nadiflora;* nanoparticles; UV-Vis-spectra; FTIR, SEM-EDX; TEM; antimicrobial activity

1. INTRODUCTION

Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts (Raveendran, P et al., 2003).These silver nanoparticles have emerged as novel antimicrobial agents due to the growing microbial resistances against metal ions, antibiotics and the development of resistant strains (Juliet and John W.2012). Nanotechnology has diversified into various branches, encompassing new approaches based upon molecular self assembly for developing new materials of less than 100 nm range (Kannan and Vasanthi, 2012). Since physical and chemical methods for the synthesis of nanomaterials are posing a concern for environmental contaminations in terms of the hazardous by-products, there is an important need for green synthesis of nanoparticles (Mukherjee et al., 2001).

Silver nanoparticles have been applied in catalysis (Jana et al., 1991) to photonics (Velikov et al., 2003) bio sensing, diagnostics (Songping and Shuyuan, 2005), antimicrobial (Pal et al., 2007) and DNA sequencing (Thompson et al., 2008). Silver nanoparticles were also shown to promote wound healing (Govindaraju K et al., 2010), antitumor activity (Rani et al., 2009) Synthesis of silver nanoparticles have been reported using different biological materials like microorganisms (Nair and Pradeep T., 2002), fungus (Vigneshwaran et al., 2007), enzymes (Willner et al., 2006) and plant extracts. Synthesis of silver nanoparticles have also been reported from different plant extracts such as *Andrographis paniculata* (S ulochana, et al., 2012), *Catharanthus rosesus* (Mukunthan et al., 2011), *Coleus aromaticus* (Mahendran et al., 2012) *Nicotiana tobaccum* (Prasad., 2011), *Nerium indicum* (Mano priya., 2011) Ocimum sanctum (Ramteke., 2013), fruit seeds of Pomegranate fruit extracts of *Lantana camara* (Pour., 2011) root extracts of *Glycyrrhiza glabra* (Dinesh et al., 2012).The plant *Allmania nadiflora* popularly known as "Erra baddiaaku" belongs to the family Amaranthaceae which is a straggling, branched herb that grows about 10-50cm in height. The leaves are edible and locally consumed in the diet. The leaves have the highest content of crude protein 62.7% (Dewanji et al., 1997). The flowers are usually sessile, brown in colour, globose, bisexual. The fruit is a pyxis. The plant is used to cure snake bite ripe fruits for constipation and dysentery. The plant also gives good organic manure to soil. In the present study synthesis of silver nanoparticles using the weed *Allmania nadiflora* are described along with their antimicrobial activities.

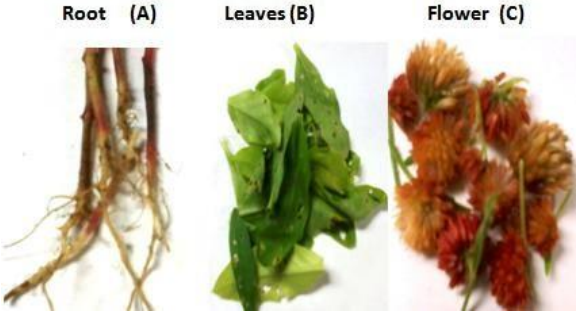

Figure 1: *Allmania nadiflora* (A) Root (B) Leaves (C) Flowers

* Corresponding Author
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2. MATERIALS AND METHODS

Materials used for the synthesis of Silver nanoparticles (AgNPs) are AR grade Silver nitrate ($AgNO_3$), purchased from Merck, India. The test strains, *Escherichia coli, Bacillus subtilus, Pseudomonas putida, Staphylococcus aureus, Micrococcus luteus and Klebsiella pneumonia* were procured from IMTECH, Chandigarh. Yeast extract, Tryptophan and Bacterial grade Agar-Agar purchased from Himedia Laboratories, Mumbai, India.

2.1. BIOSYNTHESIS OF SILVER NANOPARTICLES

Fresh plants of *Allmania nadiflora* were collected with their roots, leaves and flowers from botanical garden Osmania University campus, Hyderabad, India. The leaves, roots and flowers are separated were shown in Fig.1 (A-C). The plant material was washed individually and placed on a blotting paper to remove the water that is adhering to the plant material. Then 5g of the leaves are taken in a 250 ml Erlenmeyer flask and to it 100ml of milliQ water is added. Then the flask is kept on a sand bath for 30mins at 60^0C. This facilitates the formation of aqueous leaves extract. The plant material is then filtered through Whattmann No. 1 filter paper and the broth is used for further analysis. Similarly aqueous extracts of roots and flowers were also prepared. These extracts can be stored at 4^0C for a period of seven days. The plant extracts (root, leaf and flower extracts) were taken individually in different beakers and to it 1mM silver nitrate solution is added in the ratio 1:10. The reaction mixtures are kept for incubation on a sand bath at 60^0C for 10mins. The colours of the reaction mixtures have changed from pale yellow to reddish brown.

3. CHARACTERIZATION STUDIES

UV-Visible spectroscopy is an important technique to confirm the formation of sliver nanoparticles in aqueous solution. The synthesized silver nanoparticles from the different extracts of the plant of *Allmania nadiflora* were characterized with the help of Elico SL-159 UV Spectrophotometer by continuous scanning from 300nm to 700nm and the biological extract was used as the reference for the baseline correction. The binding properties behind the formation of silver nanoparticles using extracts of the plant were analysed by FT-IR. The spectra were recorded in the wave number range of 500-4000cm-$^{-1}$. The analysis was carried using Paragon 500, Perkin Elmer-RX1 spectrophotometer in the diffuse reflectance mode at a resolution of 4cm^{-1} in KBr pellets. Further, the formed silver nanoparticles were centrifuged at 12, 000rpm for 15min, followed by redispersion of the pellet of silver nanoparticles in 5ml of double distilled water. The solution is then kept in an oven to obtain the powdered form of the silver nanoparticles. The morphology of the synthesized silver nanoparticles was characterized by Scanning Electron Microscope (SEM). EDX analysis gives qualitative as well as the quantitative status of the elements that may be involved in the formation of nanoparticles. The size of the synthesized AgNPs were determined by using a Transmission Electron Microscope (TEM). The powdered form of the AgNPs was ultrasonically dissolved in 10µl of double distilled water. A drop of the solution was subsequently deposited onto a lacey carbon film supported on a Cu grid and allowed to evaporate under ambience conditions.

The structure and composition of the characterized

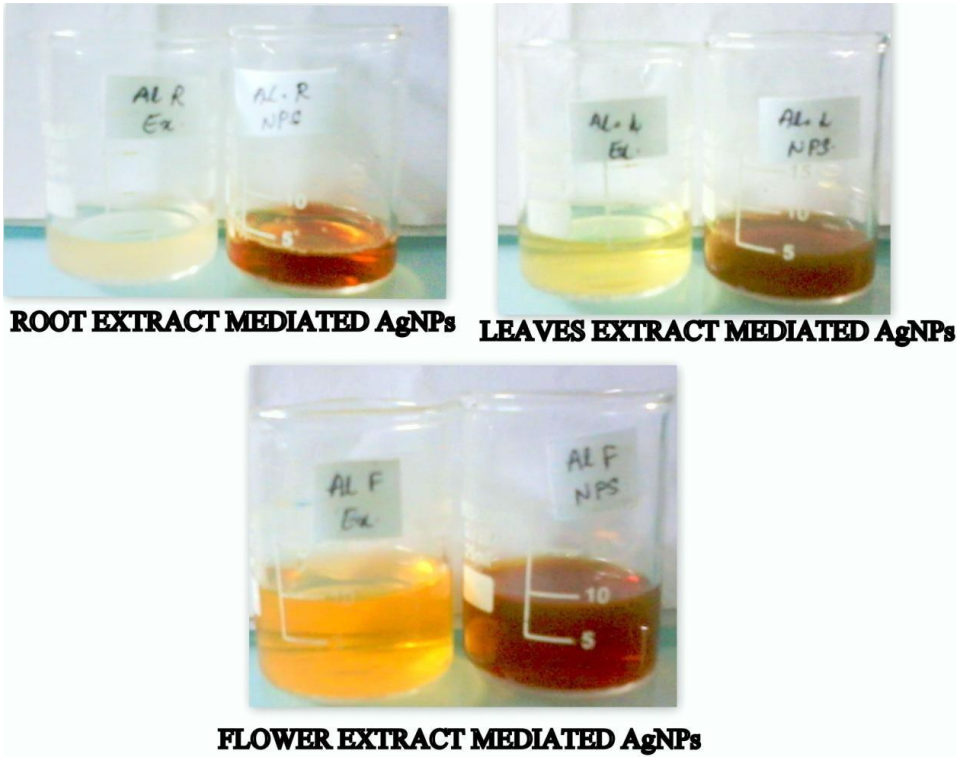

Figure 2: Colour change indicating the formation of Ag nanoparticles

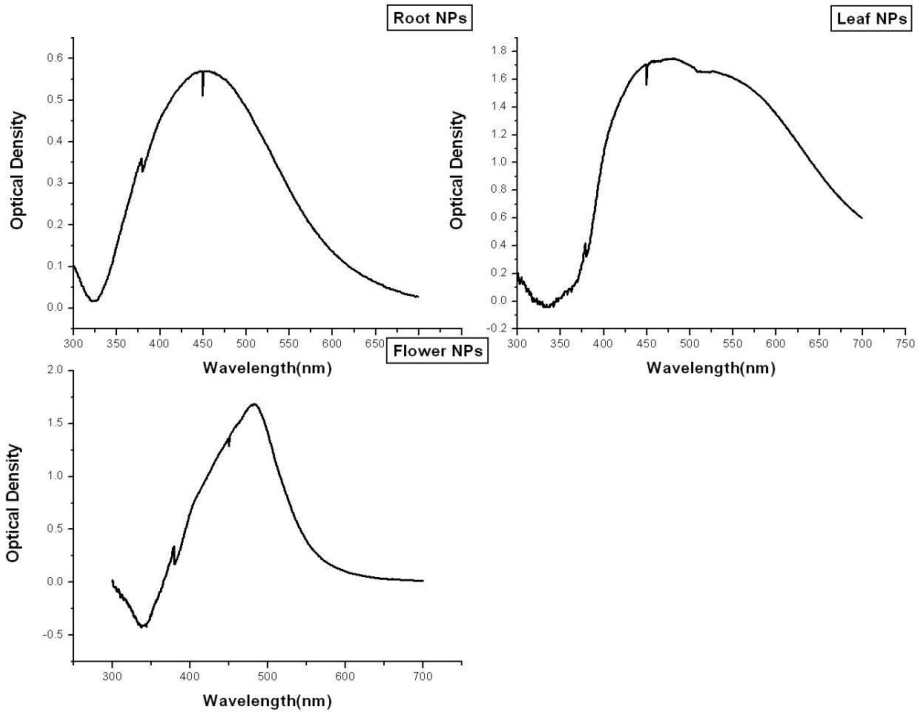

Figure 3: UV-Vis spectrum of root, leaf and flower extracts synthesized silver nanoparticles

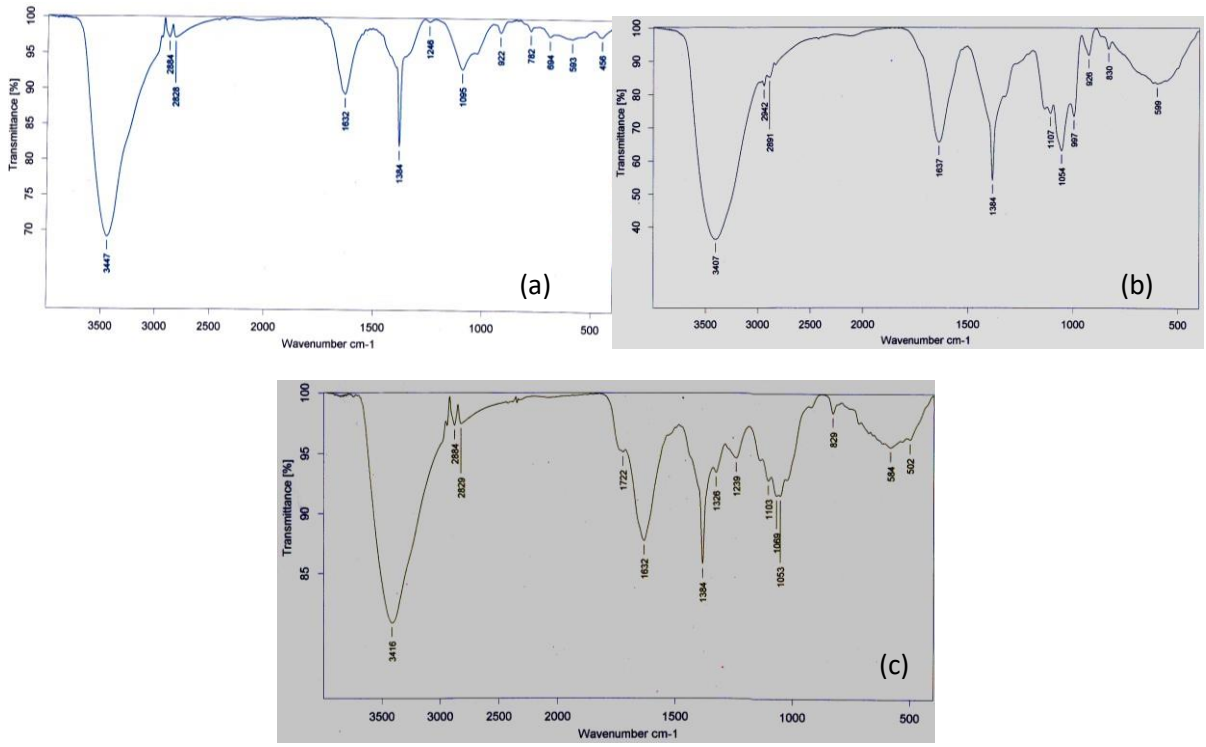

Figure 4: FTIR spectra of AgNPs synthesized by *Allmania nadiflora* (a) Leaves (b) Root extract (c) Flower extract

silver nanoparticles were obtained by X-ray diffraction (XRD) analysis. The AgNPs solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of AgNPs into 10 µL of deionised water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD. The instrument operated at a voltage of 45 kV and a current of 40 mA with Cu Kα radiation in a θ- 2 θ configuration.

3.1 Antimicrobial Assay

The antimicrobial assay of the synthesized AgNPs was done on different pathogenic bacteria and fungi by disc diffusion method. For anti bacterial studies, 10 µl of each of the strains of *Micrococcus luteus, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas putida*, and *Bacillus subtilus* were spread onto individual plates nourished with Luria Bertani (LB) media. Four sterile discs of 4mm diameter were placed

Figure 5: SEM image of silver nanoparticles synthesized using *Allmania nadiflora* extract AgNPs

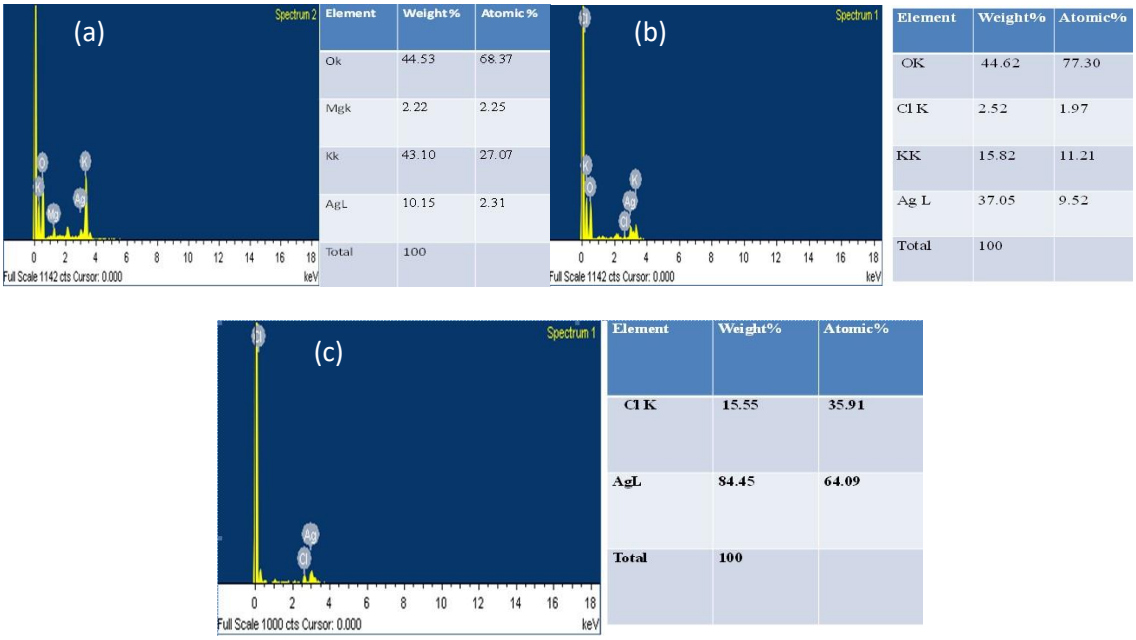

Figure 6: EDX Representative spot energy-dispersive spectrometer profile confirming the presence of silver nanoparticles. (a) *Allmania nadiflora* leaves extract (b) root extract and (c) flower extract AgNPs

equidistantly on to the plates. Then the discs were impregnated with Ampicillin (positive control, 2 µl), leaf extract mediated AgNPs (5 µl), flower extract mediated AgNPs (5 µl) and root extract mediated AgNPs (5 µl). Then the plates were sealed and incubated overnight at 37°C.

A similar method was used to assay the antifungal activity of the synthesized silver nanoparticles. Potato Dextrose Media was used for the nourishment on the plates and the discs were impregnated with each of 20µl AgNPs. The fungal strains used were Aspergillus n*iger, Aspergillus flavus, Fusarium, Trichoderma harzianum.* Fluconazole (20 µl) was used as the positive control. The plates were kept at room temperature for 78 hours.

3. RESULTS AND DISCUSSION

The roots, leaves and flower extracts of the plant *Allmania nadiflora* were used for the synthesize of silver nanoparticles in a greener approach. Upon incubation of the reaction mixture on the sand bath for ten minutes, the colour of the solutions gradually changed to reddish brown. The change in colour of the solutions is shown in Fig.2. Colour of silver colloid is attributed to surface plasmon resonance (SPR) arising due to the collective oscillation of the free conduction electrons induced by an interacting electromagnetic field. UV absorption spectra of the silver nanoparticles formed in the reaction media has high absorbance peaks at 460nm (Root mediated AgNPs), 450nm (Leaf mediated AgNPs) and at 480nm (Flower mediated AgNPs). The spectra are as shown in Fig. 3. The FT-IR measurements were carried out to identify the possible biomolecules responsible for the reduction of Ag^{+} ions.The spectra are as shown in Fig. 4. These spectra reveal major peaks at 3047cm^{-1} (root mediated AgNPs), 3347cm^{-1} (leaves mediated AgNPs) and 3416 cm^{-1} (flower mediated AgNPs) which corresponds to O-H group stretch and for the presence of alcoholic groups. Other minor peaks were also formed which corresponds to the different biomolecules present in the synthesized silver nanoparticles. The minor peaks formed and their corresponding functional groups are detailed in Table.1. The shape of nanoparticles plays a key role in its optical and electrical properties. It is evident from the SEM images Fig. 5.that the synthesized silver nanoparticles using the plant extracts of the plant *Allmania nadiflora* are predominantly spherical to oval in shape. Fig.6. shows the elemental profile of the synthesized silver nanoparticles. With this analysis, the presence of

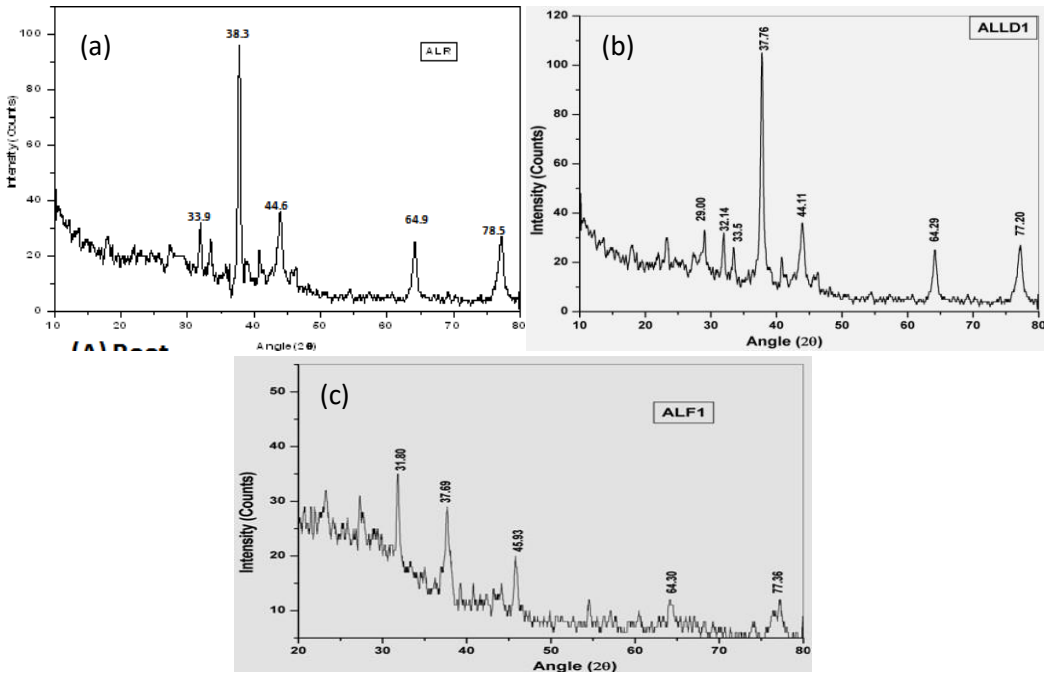

Figure 7: XRD patterns of silver Nanoparticles synthesized using *Allmania nadiflora* (A) Root, (B) Leaves and (C) Flower Extract AgNPs

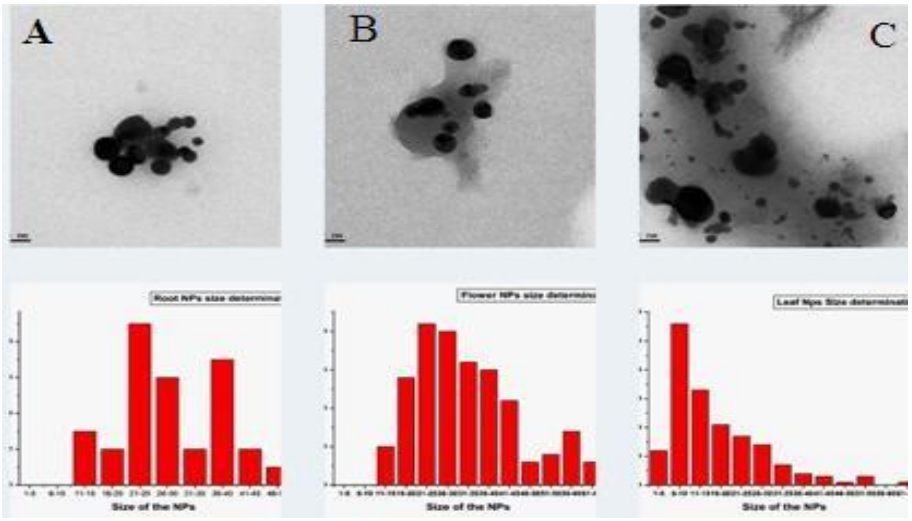

Figure 8: TEM micrograph of silver nanoparticles. (A) Root AgNPs (B) Flower AgNPs (C) Leaf AgNPs with individual Graphs

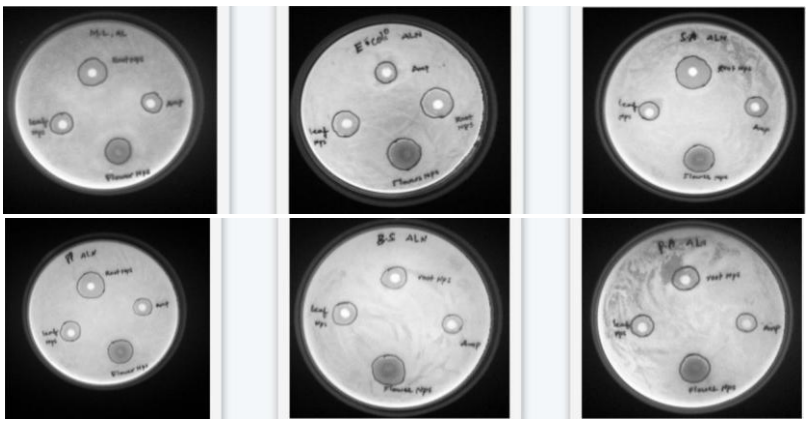

Figure 9: Zone of the inhibition in response silver nano Particle synthesized used by *Allmania nadiflora* Leaves NPs, Root NPs and Flower NPs on with antibiotics Ampicillin control

Table 1: Interpretation of FTIR spectra of synthesized silver nanoparticles with Functional Group assigned

S.No	Leaf extract FTIR peaks cm^{-1}	Functional Group assignated	Flower FTIR peaks cm^{-1}	Functional Group assignated	Root FTIR peaks cm^{-1}	Functional Group assignated
1	3447	Phenol &alcohol, OH group stretch	3416	OH group stretch	3407	OH group stretch
2	2828	Carboxylic acid and OH group stretch	2828 2829	Carboxylic acid and OH group stretch	2942,2891	Carboxylic acid and OH group stretch
3	1632	Assignated with amide in I-raised C-N protein strength	1722, 1632	Assignated C=O aldehyde in I-raised C-N protein strength	1637	Assignated I-raised C-N protein strength
4	1384	Nitrogen (N=O)bend	1384,1326	Nitrogen (N=O)bend (nitro methane)	1384	Nitrogen (N=O)bend
5	1246,1095	Methyl formate group	1239, 1069, 1059	Amine with methyl formate group, diethyl ether	1107,1054	C-N stretching vibration of amines
6	922	Assigned to CH=CH2	829	Assigned to CH=CH2	997,926,850	Assigned to CH=CH2and C-O-C
7	782,694	CH=CH (cis)	584,502	-CH=CH-	830,599	-CH=CH-

Table 2: Zone of inhibition against different bacterial pathogens

Micro Organism against which NPs activity was measured	Zone of Inhibition in (mm) using AgNPs of different extracts of the plant *Allmania nadiflora* with standard as Ampicillin			
	Root (5µl)	Leaf (5µl)	Flower (5µl)	Ampicillin (2µl)
Bacillus subtilus	11	11	11	10
Staphylococcus aureus	18	10	14	11
Pseudomonas putida	17	12	14	11
Pseudomonas aurogenosa	11	11	14	10
Micrococcus luteus	14	12	13	10
Escherichia coli	15	12	15	10

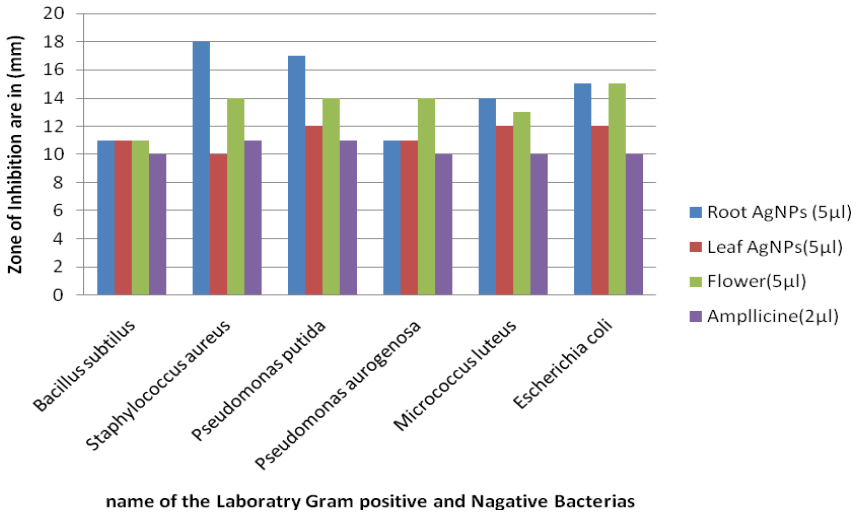

Figure 10: Zone of inhibition are in (mm) against different bacterial pathogens and Observed against Reference drug (Ampllicine)

Table 3: Antifungal activity of whole plant extracts of *Allmania nadiflora*

S.No	Name of Fungal species	Diameter of the inhibition zone (mm) using AgNPs of different extracts of the plant *Allmania nadiflora* with standard as Fluconazole (150mg/2ml)			
		Fluconazole (20 µl)	AL.Root Ag NPs (20 µl)	AL.Flower AgNPs (20 µl)	AL.Leaf AgNPs (20 µl)
1	*Aspergillus niger*	15	13	13	12
2	*Aspergillus flavus*	10	11	13	12
3	*Fusarium* sps	14	10	10	12
4	*T.harzianum*	9mm	12mm	12mm	7mm

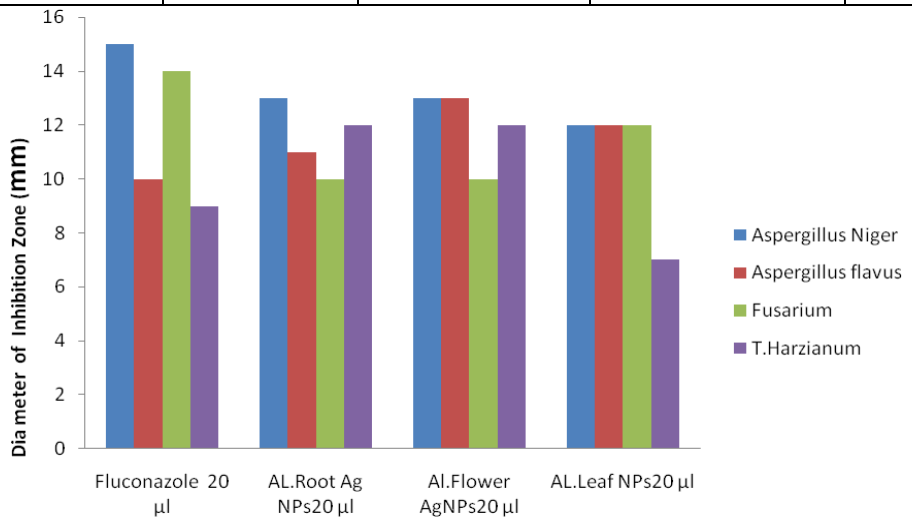

Figure 11: Comparative antibacterial activity of AgNPs against different fungi

elemental silver signal was confirmed in the sample. XRD analysis indicated strong diffraction peaks Fig.7 at 38.33°, 37.76°, and 37.69° for root, leaves and flower mediated AgNPs respectively. These peaks are characteristic to Face Centered Cubic (FCC) silver lines. The size of the synthesized silver nanoparticles was determined using a TEM. These images revealed the presence of spherical particles with smooth surface. The TEM images and the histograms of the synthesized silver nanoparticles are shown in Fig.8. There were maximum number of particles in the range 21nm-25nm (root mediated AgNPs), 6nm-10nm (Leaves mediated AgNPs) and 21nm-25nm (Flower mediated AgNPs). The mean of the diameter of the formed nanoparticles was found to be 25nm. The synthesized Ag nanoparticles from root, leaf and flower have shown good antibacterial activity Table 2and Fig:9. Highest activity of the nanoparticles against all the bacteria were seen in nanoparticles synthesized from the root Fig.10. Antifungal activity against *Aspergillus niger* was comparatively high when compared to other bacteria (Table3 and Fig11.). Compared to flucanozole the antifungal activity of the nanoparticles against *A.flavus* and *T.harzianum* were relatively high.

4. CONCLUSION

In the present study, an eco-friendly method of sliver nanoparticles synthesis was investigated using the plant *Allmania nadiflora* and significant antimicrobial activity was observed. Further studies on other biological activities are required to exploit their full potential.

5. ACKNOWLEDGEMENTS

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