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CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF BIOGENIC SILVER NANO-PARTICLES USING LEAF EXTRACT OF *THUNBERGIA ALATA* BOJER EX SIMS

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Keywords:

Biogenic, *Thunbergia alata* Bojer ex sims, Silver nanoparticle, Antibacterial, Antifungal, HR-TEM.

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ABSTRACT: The multidrug-resistant strains are a major problem in the control of infections in hospitals. The smaller size of nanoparticles is gaining importance in research for the treatment of various diseases. The main objective of this study is synthesis of silver nanoparticle in eco-friendly manner without using any hazardous chemicals. Stable and spherical shaped nanoparticles Synthesized by using aqueous leaf extract of *T. alata*. This method offers a viable and an eco-friendly way for fabrication of benign nanoparticles as it is a simple and carried out at room temperature without any huge inputs in terms of energy and waste. It is advantageous over the microbial synthesis as it is carried out using in aqueous solutions at ambient temperature, without any toxic chemicals in lesser time and could be exploited for developing cost effective biosynthesis of Ag nanoparticles at a large scale. One more aim of this study is that analysis of antimicrobial activity of biogenic nanoparticles against disease causing human pathogens. Characterizations of nanoparticles were done by UV-Vis spectroscopy, FTIR, AFM, HR-TEM, XRD and with EDS instruments. Antimicrobial test was done by agar well diffusion method. The nanoparticles appeared to be spherical in shape and the size of the particles varied from 10 to 50 nm. *T. alata* leaf extract mediated silver nanoparticles have great promise as antimicrobial agent against human pathogenic multidrug resistant microbes.

INTRODUCTION: Nanotechnology is one of the most promising areas of research in modern medical science. Nanomaterials display exclusive, superior and fundamental properties; due to small size they can be accommodated in various [1]. Nanoparticles (NPs) of noble metals, such as gold, silver, platinum, and zinc oxide are widely used in medical and pharmaceutical applications [2].

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A rapid step in synthesis and applications of nanomaterials, in recent years has been invented in almost every domain of life including health care, cosmetics, biomedical, food and feed, drug - gene delivery, environment, electronics, mechanics, catalysis, energy science, optics, chemical and space industries [3]. Nanoparticles (NPs) of noble metals, such as gold, silver, platinum, and zinc oxide are widely used in medical and pharmaceutical applications, and in an array of consumer products [4]. Synthesis of NPs has been reported using various chemical and physical methods, such as sol-gel process, chemical precipitation, chemical vapour deposition, hydrothermal and microwave methods [5].

Although chemical and physical methods are very successful to produce well- defined nanoparticles, they have certain limitations such as increase cost of production, release of hazardous by-products, long time for synthesis and difficulty in purification. Global warming a climate change has induced a worldwide awareness to reduce the toxic and hazardous waste materials, thus, the green synthesis route have raised actively the progress in the fields of science and industry [6]. Biosynthesis of nanoparticles as the name indicates help in the synthesis of very complex reaction within a fraction of minutes have now taken up the attention towards synthesis protest the need of environmentally benign technologies in material science. Use of biological organisms such as microorganism, plant extracts and biomass could be a best alternative method of physical and chemical method for synthesis of nanoparticles because the biological or green synthesis route is very spontaneous, economic, environmental friendly and non-toxic. The major biological systems involved in this are bacteria; fungi [7] and plant extract [8]. In recent years, the biosynthesis of nanoparticles using plant extracts has gained more significance. The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, practical, scalable, nontoxic and avoidance of maintaining the microbial culture [9].

In most cases, they provide broad variety of metabolites which can aid in the reduction of silver ions and are quicker than microbes in the synthesis method. Different plants have been successfully used for the synthesis of biogenic metal nanoparticles [10]. Synthesis of silver nanoparticles is of much interest to the scientific community because of their wide range of applications. These silver nanoparticles are being successfully used in the cancer diagnosis and treatment as well [11, 12]. For biomedical applications; being added to wound dressings, topical creams, antiseptic sprays and fabrics, silver functions' as an antiseptic and displays a broad biocidal effect against microorganisms through the disruption of their unicellular membrane thus disturbing their enzymatic activities. They are even being projected as future generation antimicrobial agents.

In the present investigation, we report the easy synthesis of silver nanoparticles by an environmental friendly method by using *T. alata* leaf extract and the evaluation of their antimicrobial activity against various human pathogenic microorganisms.

MATERIAL AND METHODS:

Chemicals and Microorganism: Analytical grade chemicals were used - Silver nitrate nitrate and sodium hydroxide. All glass wares were washed with sterile water and dried in an oven before use. Experimental plant *Thunbergia alata* Bojer ex Sims (Black-eye Susan vine) leaves were collected from the Karnatak University campus Dharwad, Karnataka, India. It is a flowering evergreen vine of the Acanthaceae family Native to tropical and southern Africa **(Fig. 1)**. Saponins, steroids, tannins and phenolic compounds present in *T. alata.*

FIG. 1: EXPERIMENTAL PLANT *THUNBERGIA ALATA* BOJER EX SIMS

Preparation of leaf extract: Leaves of *Thunbergia alata* Bojer ex Sims were washed 2-3 times with tap water followed by double distilled water to remove dust and impurities. Leaves were shade dried to remove the residual moisture and about 25gm. were cut into small pieces and boiled in glass beaker containing 250ml of sterile distilled water for 20 minutes. The aqueous extract was separated by filtration with whatman no. 1 filter paper and stored in refrigerator at 4^0C for further use.

Phytosynthesis of Silver nanoparticles: For reduction of silver ions, 10ml of leaf extract was added to 90ml of 1mM aqueous $AgNO_3$ solution taken in Erleyenmeyer flask (250ml). Simultaneously, the reaction mixture was adjusted to pH 8 by using 1 N. NaOH. Then the flask containing reaction mixture was incubated at 40-60^0C, resulting in the formation of pale yellow to dark brown solution indicating the synthesis of silver nanoparticles.

Detection of silver nanoparticles: A number of different measurement techniques were used for detection of Ag-NPS., including UV-Vis spectroscopy, Fourier Transform Infrared (FTIR), Atomic Force Microscopy (AFM), High Resolution Transmission Electron Microscopy (HR-TEM), X–Ray Diffraction (XRD) and Energy dispersive spectroscopy (EDS).

Characterization of nanoparticles: The reduction of metal ions was monitored by measuring the UV-Vis spectroscopy of the solution according to the method of Mie (1908), by the sampling of aliquots (3ml) of the aqueous component. The silver nanoparticles were measured in a wavelength ranging from 200-800nm. The UV-Vis spectroscopy measurement of silver nanoparticle was recorded on UV- Vis spectroscopy (Jasco V-670 UV-Vis NIR spectrophotometer) operated at resolution of 1nm. The solution containing reduced silver ions was centrifuged at 3000 rpm for 40 min to remove the unwanted biomass residue; the resulting suspension was then dispersed in 10ml of double distilled water and centrifuged again at the same condition. Re-dispersion and centrifugation process was repeated for 2-3 times to obtain silver nanoparticles free from any biomass residue. A sample taken from pellet was dispersed on a slide and dried slide was observed on contact mode of AFM. The pellet thus obtained was re-dispersed in double distilled water and oven dried at 60^0C to obtain the powder. The powder was used for FTIR and HRTEM (TECNAI 20 G2-electron microscope), X-ray diffraction (XRD) analysis and SEM with EDX analysis (Fei Quanta 200 SEM EDAX Genius Xm 4).

Antibacterial activities: The silver nanoparticles synthesized using *Thunbergia alata* Bojer ex Sims leaf extract were tested for antimicrobial activity by agar well diffusion method against human pathogenic *Staphylococcus aureus, Bacillus subtilis, Bacillus polymyxa, Escherichia coli, Salmolella typhi, Vibrio cholera, Aspergillus niger* and *Candida albicans*.This method depends on the radial diffusion of an antibiotic from the well through semisolid agar layer in Petri plate, which prevents the growth of bacteria in a circular area or the zone around the well. The pure cultures of bacteria were sub-cultured on nutrient broth at 35°C. The hot sterile medium was poured into the sterile Petri plates to form 2-3 mm thick uniform layer and allowed to solidify. Each strain was swabbed uniformly on the individual plates using sterile cotton swab. Wells of size 6 mm were made on nutrient agar plates using gel puncture. Different concentrations of silver nanoparticles (25, 50, 100, 200, 400 µl) solution were poured on to four wells and in one well 400 µl of plant extract poured as control on all plates using micropipette. After incubation at 37 °C for 24h for bacterial strains and 96h for fungal strains, the diameter of zone of inhibition was measured in millimetres and tabulated.

RESULTS:

Characterization of silver nanoparticles: Addition of leaf extract to $AgNO_3$ the colour of the reaction mixture changes from pale yellow to dark brown **(Fig. 2)** within few seconds and after incubation time (24 hours) the walls of the Erlenmeyer flask (which contains reaction mixture) showed mirror like illumination, it clearly indicates the formation of silver nanoparticles in the reaction mixture.

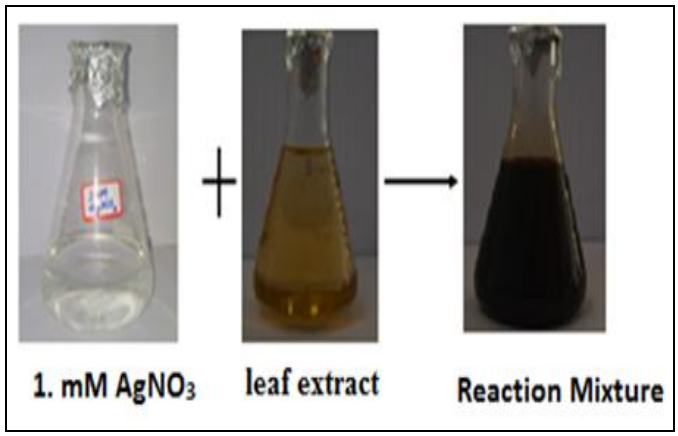

FIG. 2: VISUAL OBSERVATION OF THE FORMATION OF SILVER NANOPARTICLE SYNTHESIS

The UV-visible spectroscopic studies on the synthesis of silver nanoparticles **(Fig. 3)** have shown an absorbance at 432 nm due to surface plasmon resonance (SPR).

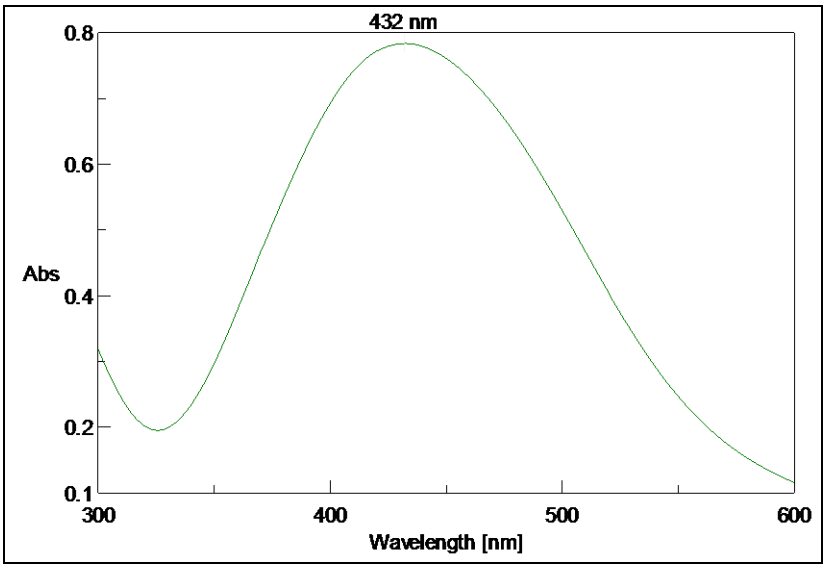

FIG. 3: UV-VIS SPECTRUM OF AGNPS IN AN AQUEOUS SOLUTION

Study of effect of physicochemical parameters on the nanoparticles synthesis: Based on UV–Vis spectroscopy the effect and interaction of various physico-chemical parameters were optimized which would increase the yield of nanoparticle synthesis. Various parameters such as concentration of the leaf extract and $AgNO_3$, pH, temperature and incubation time were optimized for the reduction of Ag^+ ions to AgNPs using *Thunbergia alata* Bojer ex sims extract. The maximum yield of AgNPs is with 1 mM, this concentration was selected for further studies. Among the various parameters, pH is one of the fundamental factors in nanoparticle synthesis. Among 8, 9, 10 pH, the reaction started rapidly at pH 8 of the reaction mixture (as observed by the change in colour). The optimal pH for nanoparticle synthesis was preferred to be pH 8.

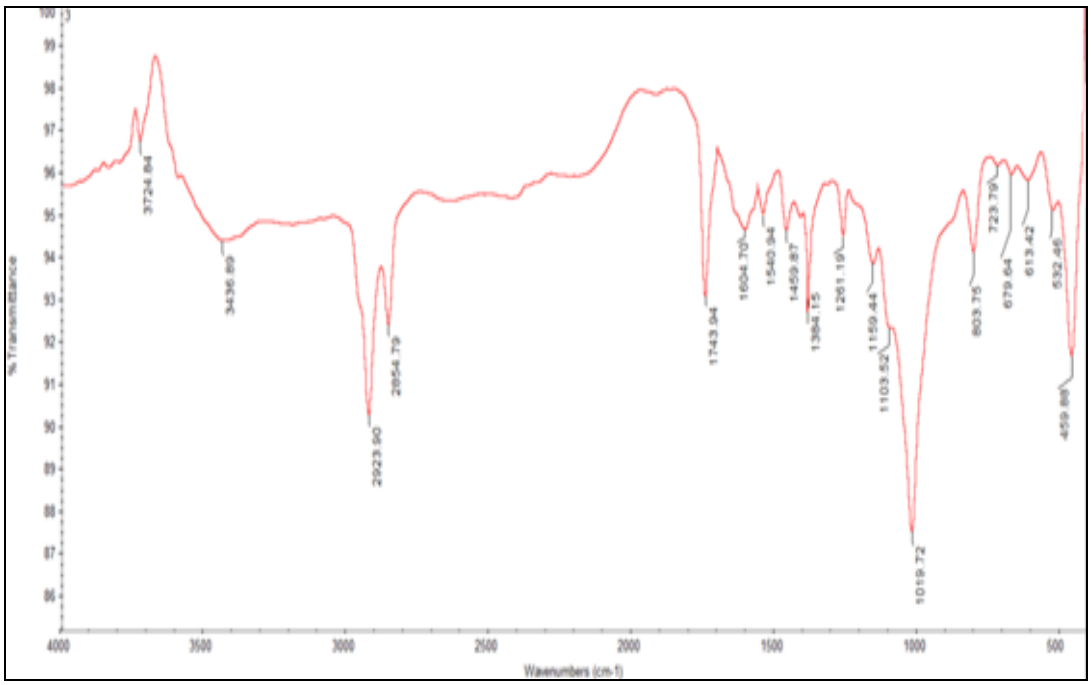

FIG. 4: FTIR SPECTRUM OF AG-NPS SYNTHESIZED FROM LEAVES OF *THUNBERGIA ALATA* BOJER EX SIMS

TABLE 1: FTIR ABSORPTION PEAKS AND THEIR FUNCTIONAL GROUPS OF SILVER NANO PARTICLES SYNTHESIZED FROM LEAVES OF *THUNBERGIA ALATA* BOJER EX SIMS

Sl. No.	Absorption peaks (cm^{-1})	Functional groups.
1	3724	The free O-H bond stretches.
2	3436	Indicative of OH stretching H-bonded alcohols and phenols
3	2923	C-H stretches, aldehyde group strongly stretches
4	2854	C-H stretch region for the aldehyde, stretch, aldehyde hydrogen (-CHO) bond
5	1743	C=O aldehyde saturated aliphatic
6	1604	C=C stretches; N-H 1^0 & 2^0 amines & amides are stretches and bends
7	1540	Nitro group show strong bands & overlaps the aromatic ring region. Stretching and bending of 1^0 & 2^0 amines & amides takes place.
8	1459	C-F stretches strongly; $-CH_3$ bends between these two regions
9	1384	C–C and C–N stretching
10	1261	C-O 1^0, 2^0, or 3^0 structures to an alcohol
11	1159	Aromatic amines
12	1103	Aromatic amines
13	1019	C-C Ring stretching

Identification of the bio-molecules involved in the formation of silver nanoparticles was done using FTIR. **Fig. 4** and **Table 1** show the FTIR spectrum of silver nanoparticles.

The FTIR study showed sharp absorption peaks located at 3724 cm^{-1}, 3436 cm^{-1} , 2923cm^{-1}, 2854cm^{-1}, 1743 cm^{-1}, 1604 cm^{-1}, 1540 cm^{-1}, 1459cm^{-1}, 1384cm^{-1}, 1261cm^{-1}, 1159 cm^{-1} etc. The absorption peaks at 3724 $cm^{-1,}$ 3436 cm^{-1} were assigned to strong stretching vibrations of O-H Stretching of phenol and alcoholic bond, the absorption peaks at 2923 cm^{-1}, 2854 cm^{-1} arose from the C-H aldehyde group, 1743 cm^{-1} is represents C=O aldehyde saturated aliphatic group, 1604 cm^{-1} peak arose due to C=C stretches; N-H 1^0 & 2^0 amines & amides are stretches and bends, The peak around 1540 cm^{-1} region represents the Nitro group, it show strong bands & overlaps the aromatic ring region, stretching & bending of 1^0 & 2^0 amines & amides takes place, 1459 cm^{-1} peak formed by C-F stretches, $-CH_3$ bends between these two regions, 1384 cm^{-1} peak arose from C–C and C–N stretching, 1261 cm^{-1} represents C-O stretch 1^0, 2^0, or 3^0 structures to an alcohol, 1159 cm^{-1} and 1103 cm^{-1} peaks formed by aromatic amines. The above mentioned functional groups of bio-molecules suggest that they are involved in the reduction as well as the capping of nanoparticles.

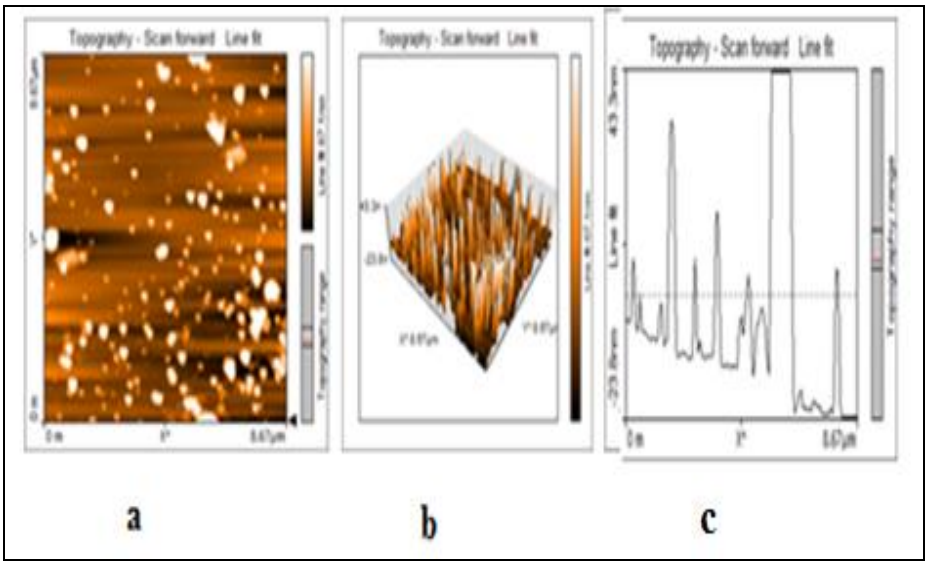

FIG. 5: AFM IMAGES OF SILVER NANOPARTICLES SYNTHESIZED FROM *THUNBERGIA ALATA* BOJER EX SIMS

AFM data reveals that the particles are mono-dispersed and spherical in shape and that the size ranges from 10 nm 80nm **(Fig. 5a, b)** in 2D and 3D structures of the nanoparticles with a distance of 25 to 30 nm from each other **(Fig. 5c)**.

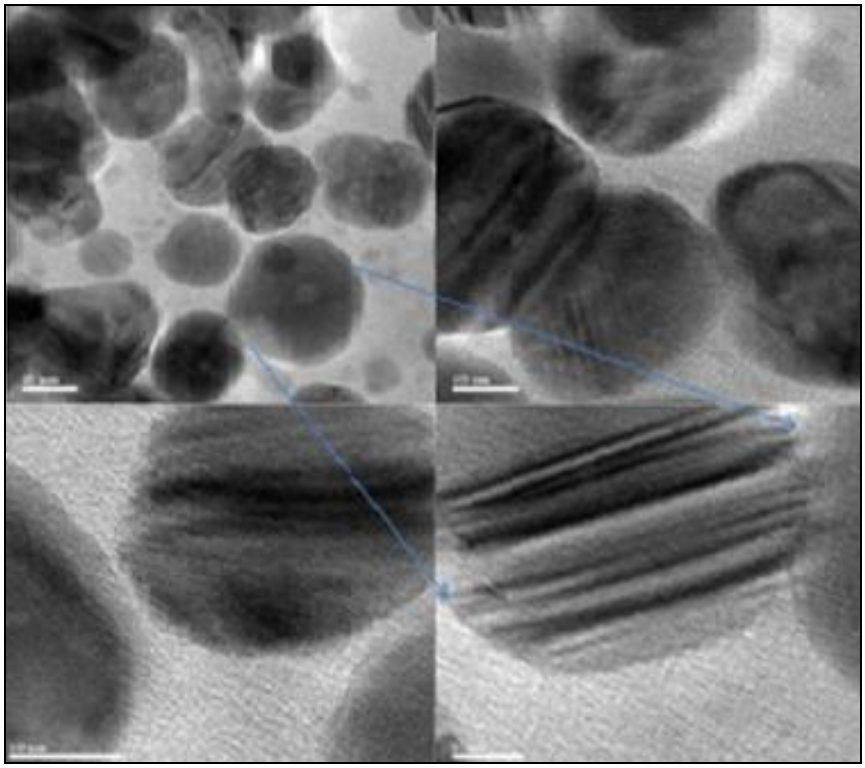

FIG. 6: HR-TEM IMAGE OF SILVER NANOPARTICLE SYNTHESIZED FROM *THUNBERGIA ALATA* BOJER EX SIMS

The silver nanoparticles were further characterized by HR-TEM micrograph, these Silver nanoparticles showed spherical shape with the size range from 10 to 20 nm **(Fig. 6)**. Further, it also shows that the biomolecules of leaf extract bound the nanoparticles as capping agents to hinder further oxidation of nanoparticles.

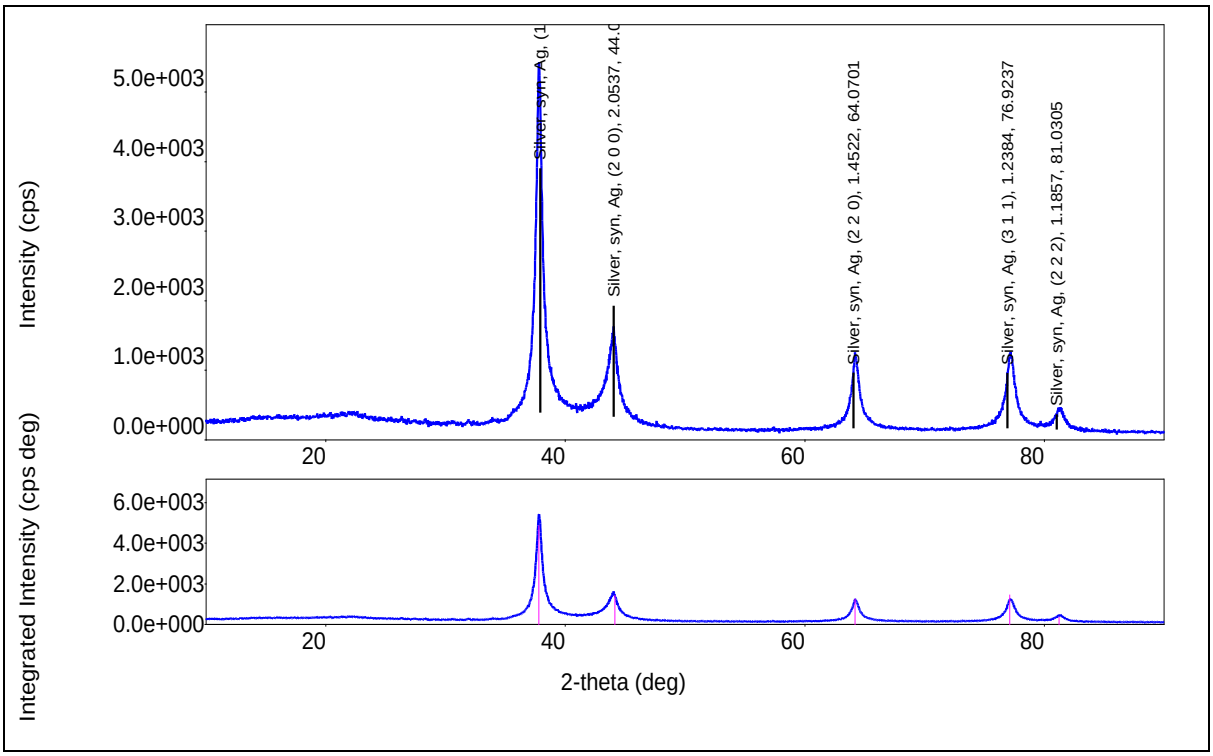

FIG. 7: X-RAY DIFFRACTION SPECTRUM OF SILVER NANOPARTICLES FROM *THUNBERGIA ALATA* BOJER EX SIMS

The X-ray Diffraction patterns of silver nanoparticle were recorded according to the description of Wang (2000). The X-ray diffraction pattern of the biosynthesized silver nanostructure produced by the leaf extract of *T.alata* represented in the figure 7 and was further confirmed by the characteristic peaks observed in the XRD image. The XRD data showed intensive diffraction peaks at a 2θ value of 38.0º from the (111) lattice plane of face centred cubic (fcc) silver unequivocally indicates that the particles are made of pure silver. The additional broad bands are observed at 44.14º (2θ), 64.18º (2θ), 77.11º (2θ) and 81.22 º (2θ) they Correspond to the (200), (220), (311) and (222) planes of silver respectively **(Fig. 7)**. Other spurious diffractions are due to crystallographic impurities. In the spectrum obtained the Bragg peak position and their intensities were compared with the standard JCPDS files 89-3722. The software gave the information about the face centred cubic (fcc) structure of silver nanoparticles. The average size of the nanoparticles is 20 nm. It can be estimated using the Debye–Scherrer equation

EDX analysis was conducted to confirm the elemental composition of the sample. The EDS images **(Fig. 8)** confirmed the presence of significant amounts of elemental silver along with other elements, which may be originate from the bio-molecules that are bound to the surface of nanosilver.

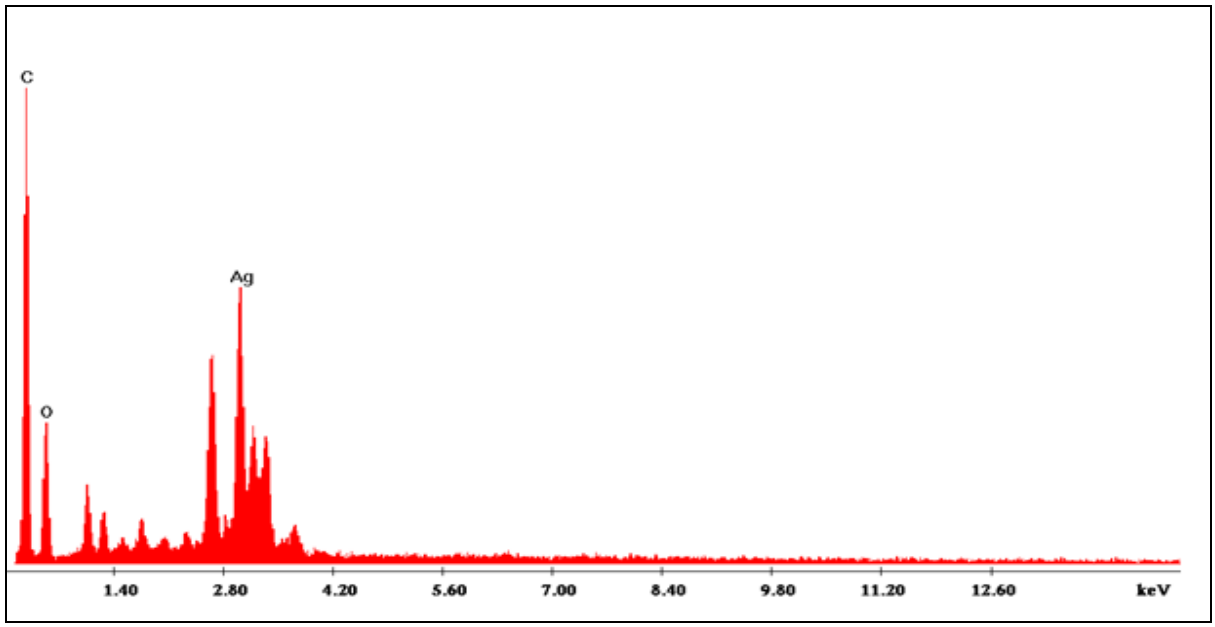

FIG. 8: EDS SPECTRA OF SILVER NANOPARTICLES SYNTHESIZED BY LEAF EXTRACT OF *THUNBERGIA ALATA* BOJER EX SIMS

Antimicrobial activity of silver nanoparticles: Synthesized silver nanoparticles exhibited antibacterial activity against gram positive *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus polymyxa* and *gram* negative *Escherichia coli*, *Salmolella typhi*, *Vibrio cholera* and antifungal activity against *Aspergillus niger and Candida albicans* **(Fig. 9 and 10)**, while controle (leaf extract) didn't show any antimicrobial activity. The antibacterial and antifungal effect of silver nanoparticles at different concentrations (25-400 µl) was quantitatively assessed on the basis of the zone of inhibition **(Table 2 and 3)**. The antibacterial activity of silver nanoparticles against *S. aureus* showed an inhibition zone of 3, 7,9,12 and 16 mm for concentration of 25, 50, 100, 200 and 400 µl respectively. 2, 6, 10 and 12 mm for concentration of 50, 100, 200 and 400 µl respectively against *B. Subtilis.* 5, 9 and 12mm for concentration of 100, 200 and 400 µl respectively against *B. Polymyxa.*9, 10, 13 and 15mm for concentration of 50, 100, 200 and 400 µl respectively against *E. Coli.* 6, 8 and 12mm for concentration of 100, 200 and 400 µl respectively against *S. typhi*. It was notice that the zone of inhibition increased with increased concentration of Ag NPs, while *V. Cholera* didn't show any antimicrobial activity **(Fig. 9)**. The antifungal

activity of silver nanoparticles also tested against *A. Niger* and *C. albicans. A. Niger* showed 3mm zone of inhibition only for 400 µl concentrations of silver nanoparticles while *C. Albicans* showed 4, 9 mm for concentration of 200 and 400 µl. The antifungal activity of Fluconazole antibiotic (positive control) also tested against *A. Niger* and *C. albicans*, but comparatively no zone of inhibition was noticed.

The minimum inhibitory concentration (MIC) studies showed varied concentrations of AgNPs against selected microbes. The gram positive *S. aureus* showed a MIC of 25 µl, *E. Coli* and *B.subtilis* showed a MIC of 50 µl, *S. typhi* and *B. Polymyxa* showed a MIC of 100 µl. The fungal strains like *C. albicans* and *A. niger* showed a MIC of 200 and 400 µl respectively.

TABLE 2: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED FROM LEAVES OF *THUNBERGIA ALATA* BOJER EX SIMS

Bacterial species	Zone of inhibition (mm)						
	Control (plant extract)	Silver nanoparticle solution					
	400 µl	25 µl	50 µl	100 µl	200 µl	400 µl	MIC µl
S. aures	0	3	7	9	12	16	25
B. subtilis	0	0	2	6	10	12	50
B. polymyxa	0	0	0	5	9	12	100
E. coli	0	0	9	10	13	15	50
S. typhi	0	0	0	6	8	12	100
V. cholerae	0	0	0	0	0	0	NF

TABLE 3: ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED FROM LEAVES OF *THUNBERGIA ALATAB* BOJER EX SIMS

Fungal species	Zone of inhibition (mm)						
	Control (plant extract)	Silver nanoparticle solution					
	400 µl	25 µl	50 µl	100 µl	200 µl	400 µl	MIC µl
A. niger	0	0	0	0	0	3	400
C. albicans	0	0	0	0	4	9	200

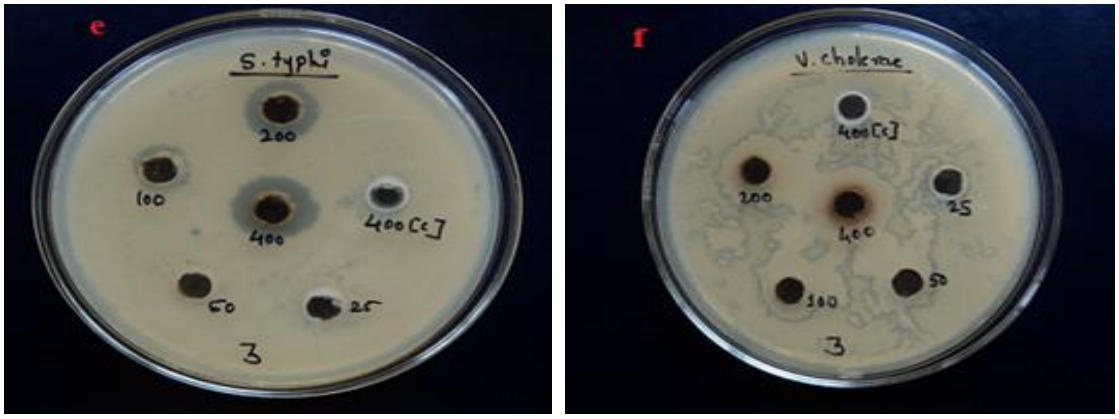

FIG. 9: ANTIBACTERIAL OF SILVER NANOPARTICLES SYNTHESIZED FROM LEAVES OF *T. ALATA* AGAINST GRAM POSITIVE *S. AUREUS, B. SUBTILIS, B. POLYMYXA* AND *GRAM* NEGATIVE *E. COLI, S. TYPHI, V.CHOLERA*.

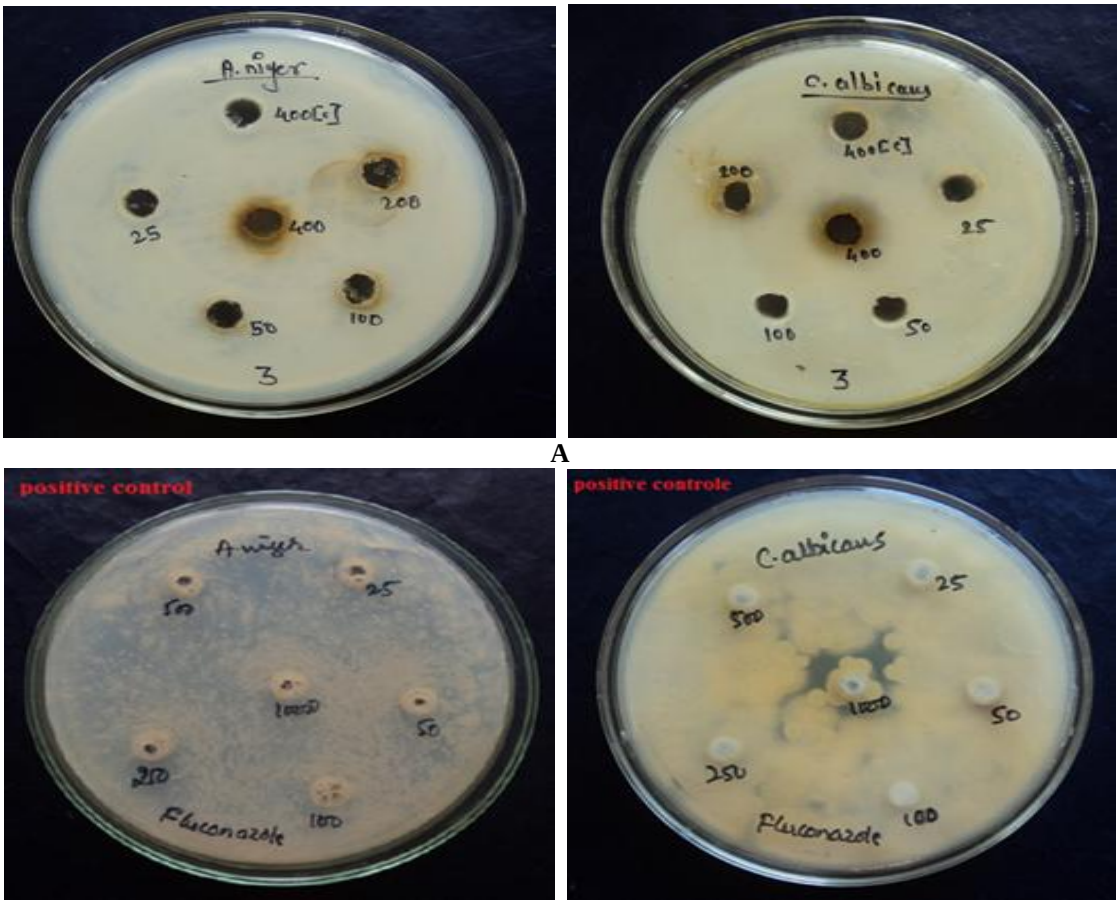

FIG. 10: COMPARISON OF ANTIFUNGAL ACTIVITY OF [A] SILVER NANOPARTICLES SYNTHESIZED FROM LEAVES OF *T. ALATA* AND [B] FLUCONAZOLE ANTIBIOTIC – A POSITIVE CONTROL AGAINST *A.NIGER* AND *C. ALBICANS*

DISCUSSION: The inhibitory action of silver compounds and silver ions had been historically recognized and applied as a useful therapeutic agent for preventing wound infections. The inhibitory action of silver on bacterial cells is related to the strong interaction of silver with thiol groups present in key respiratory enzymes in bacteria [13] Whereas, Nano crystalline silver shows the most effective inhibitory action with a rapid inhibition rate [14]. The aim of this study is based upon exploring the potential of AgNPs from *T. alata* to provide the eco-friendly, cost effective antimicrobial nanoparticles against multidrug-resistant human pathogenic microbes. The important findings of this study is that the bio-synthesized silver nanoparticles from *T. alata* Ag

Nps showed comparatively good antifungal activity than the Fluconazole antibiotic (positive control) against A.niger and *C. Albicans* **(Fig. 10)** and the antibacterial effects of were also successfully investigated *against Staphylococcus aureus, Bacillus subtilis, Bacillus polymyxa, Escherichia coli, Salmolella typhi, Vibrio cholera, Aspergillus niger and Candida albicans*. Also there are various reports which have been providing the evidences that silver nanoparticles were used as powerful tool against multidrug-resistant bacteria [15, 16].

The characterization of nanoparticles was done using various techniques. The results obtained from the techniques and antibacterial studies have coincided with the literature in the field of nanoparticle research [17, 18] and certainly gives proof about the nanoparticle synthesis and its efficiency as antibacterial agent [19]. The reduction of metal ions was primarily monitored by visual inspection of the reaction mixture [20]. The change in colour has been attributed to excitation of surface Plasmon resonance of the metal nanoparticles [21]. UV-Vis absorption spectroscopy is one of the main tools to analyze the formation of metal nanoparticles in aqueous solution [22]. The FTIR analysis was carried out to identify the possible interfacial groups between the capping agents and silver nanoparticles. Different functional groups indicate that the silver nanoparticles synthesized from the extract are surrounded by some proteins and metabolites such as terpenoids that have amine, alcohol, ketone, aldehyde and carboxylic acid functional groups [23]. This result suggests that the biological molecules could probably perform a function involving the formation and stabilization of Ag NPs through free amine groups in the proteins [24]. The HR-TEM studies have given further inputs on the morphology and size of biosynthesized silver nanoparticles ranging between 10 to 50nm with a scale of 100 nm and histogram **(Fig. 6)** showing sizes of particles with spherical morphology [25, 26]. EDX analysis shows the presence of pure silver and other elements confirming the biosynthesis of silver nanoparticles. EDX peak in the range of 3–4 keV is typical for the absorption of metallic silver nanoparticles [27].

The antimicrobial analysis of synthesized AgNPs showed profound antibacterial effect against both Gram positive and Gram negative strains. The sensitivity of microbial strains to AgNPs is increased by affecting the cell morphology and membrane permeability. It was notice that the zone of inhibition increased with increased concentration of Ag NPs. Similarly, Dipankar and Murugan have reported dose-dependent inhibition by Ag NPs synthesized from *Iresine herbstii* leaf aqueous extract [28]. This might be due to the denaturation of bacterial cell wall, blocking bacterial respiration, destabilization of outer membrane, and depletion of intracellular ATP [29]. The high bactericidal activity is certainly due to the silver cations released from Ag nanoparticles that act as reservoirs for the Ag+ bactericidal agent. Changes in the bacterial membrane structure bacteria as a result of the interaction with silver cations leads to the increased membrane permeability [30, 31]. Lin explained that in general, silver ions from silver nanoparticles are believed to become attached to the negatively charged bacterial cell wall and rupture it, which leads in to denaturation of protein and finally cell death [32]. Silver has a greater affinity to react with sulphur or phosphorus-containing bio-molecules of the cell. Thus, sulphur-containing proteins the membrane or inside the cells and phosphorus-containing elements like DNA are likely to be the preferential sites for silver nanoparticle binding [33, 34]. The vatiation in MIC values of AgNPs could be due to the existence of different modes of action on individual microorganisms [35].

This Phytosynthesis approach appears to be a cost-effective, non-toxic, eco-friendly alternative to the conventional microbiological, physical and chemical methods, and would be suitable for developing a biological process for large-scale production. These silver nanoparticles are powerful tool against multidrug-resistant bacteria and also may be used in effluent treatment process for reducing the microbial load

CONCLUSIONS: The green synthesis and characterization of -AgNPs was done and confirmed by UV–visible spectrophotometer, FTIR, AFM, HR-TEM, XRD and EDX techniques. The nanoparticles appeared to be spherical in shape and the sizes of the particles varied from 10 to 50 nm, but amongst them most of the particles obtained were sized in between 10 and 20 nm. Growth studies of different microbial cultures were performed in the presence of nanoparticles to

observe their effect on the growth profile. This study shows that *T.alata* leaf extract mediated silver nanoparticles have great promise as antimicrobial agent against human pathogenic multidrug resistant microbes. Hence, our results are promising and prove to be an important step in this direction as it decreases the burden of multidrug resistance in patients and might act as long searched alternative and could be the answer to antibiotic resistance.

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