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Use of Aerial Roots of *Ficus benghalensis* for Green Synthesis of Silver Nanoparticles with Enhanced Antibacterial Activity

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Authors' contributions

This work was carried out in collaboration between both authors. Author TRP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author ARS managed the analyses of the study, managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Basically, nanosubstances are developed by a variety of chemical methods which are not environmentally providential.
Aim: The present research work deals with the synthesis of silver nanoparticles using the aerial root of *Ficus benghalensis* extract. The absolute reduction of silver ions was observed after 48 h of reaction when extact combine with aqueous solution of Silver nitrate. The visual colour changes were observed during the reduction of silver ion into the silver nanoparticles in the reaction mixture allows producing dark brown colour. The formed silver nanoparticles was purified by high speed centrifugation, collected and stored for further characterization.
Methodology: The formation of silver nanoparticles was confirmed by UV-Visible spectroscopy, and characterised by X-Ray Diffraction (XRD) pattern, FTIR, High Resolution Transmission Electron Microscopy (HRTEM), Zeta potentiometry, ICP-AES.

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Results: The results showed that UV peak at 437.5 nm, the silver content estimation by ICP-AES was found to be 413.06 µg/mL and images were recorded by using High resolution TEM. Synthesized AgNPs were found to be effective against micro-organisms responsible for bacterial infections like Staphylococcus aureus, *Pseudomonas aeruginosa*, *E. coli* and Methicillin resistant Staphylococcus Aureus (MRSA). Further In-vitro cytocompatibility studies showed lack of toxicity at even higher concentration.
Conclusion: Still, these Silver Nanoparticles are cytotoxic in nature and could serve as a good green method for synthesis of silver nanoparticle by using plant extract.

Keywords: Antibacterial activity; silver nanoparticles; ficus benghalensis; green synthesis.

1. INTRODUCTION

The area of nano-science is one of the most effective area of research in modern days because of multidrug resistance. Nanoparticles reveales wholely new or advanced properties based on specific characteristics such as size, shape, dispersity, micro and macroscopy. Novel therapeutic uses of nanoparticles and nanomaterials are coming out rapidly [1-2]. Silver nanoparticles have found enormous applications in the field of high reactivity biomolecular sensing and diagnostics [3], antimicrobials and therapeutics [4-5] , and micro-electronics [6]. However, there is still a demand for fruitful, commercially sound as well environmental friendly way to synthesize silver nanoparticles. A number of attitudes are available for the synthesis of silver nanoparticles for example, by using microorgamisms, reduction in solutions [7], chemical and photochemical reactions in reverse micelles [8], thermal decomposition of silver compounds [9], radiation assisted [10], electrochemical [11] ,and not long ago by green method means by using plant extract [12]. Regrettably, number of methods for the synthesis of nanoparticle requires use of insecure chemicals, costly materials, skilled personnel handling, high energy requirements, hard and wasteful refining methods. Production of chemical compound by living organism or by plant extract have appeared as a simple, environment friendly and cost effective method and alternative to chemical and physical methods. Most of the methods are however in the evolutionary stage and various difficulties are experienced with the stability of prepared metallic nanoparticles.

Green method that is by the use of plant material supplies improvement above the methods which uses chemicals and physical vapour deposition as green method is economical, environmentally benign, smoothly synthesized in large-scale and aditionally there is no need to use excessive force, vibrancy, heat and hazardous chemicals. By the use of plants for synthesis of nanoparticle can be preffered over the use of microbes because it removes the complicated process of nourishing cell cultures, cmplicated sterile process and can also be appropriety scaled up for large-scale synthesis of nanoparticles. Due to interesting microstructural properties of Silver nanoparticles play a keen role in the field of nanoscience and medicine.

Silver itself have strong inhibitory and cidal effects, as well as antimicrobial activities, which has been used for recent years to prevent and treat various diseases, most notably infections [13]. Silver nanoparticles are reported to possess anti-fungal [14-15] , anti-inflammatory [16] , anti-viral [17] . Also the use of plant extract for synthesis of nanoparticle posseses a novel approach. There are some examples which have demonstrated that synthesis of AgNPs by using plants like Alfalfa Sprouts [18], *Jatropha curcas* [19] , Aloe vera [20], *Cymbopogon flexuos* [21], green tea [22] , neem leaf broth [23] , natural rubber [24], starch [25], aloe vera plant extract [26], lemongrass leaves extract [27,28] , leguminous shrub [29] , and *Emblica officinalis* [30].

Here in this research work, we reported for the synthesis of silver nanoparticles, by reduction of silver ions present in the solution of silver nitrate by the use of aqueous extract of Ficus benghalensis aerial root. In addition these synthesized nanoparticles were found to produce a high bacteriostatic as well as bactericidal action.

2. MATERIALS AND METHODS

2.1 Plant Material & Preparation of Extract

Fresh aerial root of Ficus benghalensis were collected, break it into small pieces and washed thoroughly with distilled water and air dried in

shade. About 15 gm of crushed powder weighed and mixed with 100 ml of Deionised water and boiled for 15 min. The extract obtained was filtered through Whattman filter paper and filtrate was stored properly for further use.

2.2 Silver Nanoparticle Synthesis

For synthesis of Silver Nanoparticle, (1Mm) aqueous solution of Silver Nitrate was prepared. To this aqueous solution of Silver Nitrate, the plant extract was added in different volume ratios (10:1, 10:2, 10:3, 10:4, and 10:5) for reduction into Silver ion. The formation of Silver nanoparticle was confirmed by opyical colour change from yellowish to dark brown. The formation of silver nanoparticles was also confirmed by UV-visible spectra. Synthesized Silver Nanoparticles were purified by high speed centrifuge by repeated centrifugation at 17000 rpm for 20 min.

2.3 Characterization of Synthesized Silver Nanoparticles

2.3.1 Surface Plasmon Resonance (SPR) studies

UV–Vis spectrophotometer was used to confirm the presence of SPR peak after and before synthesis of silver nanoparticles. Synthesized silver nanoparticles were diluted with deionised water and spectrum was taken in wavelength 200-800nm by using UV-visible spectrophotometer (Hitachi-Inkarp, Inkarp Instruments Pvt. Ltd., Japan) to confirm the formation of silver nanoparticles.

2.3.2 Dynamic Light Scattering (DLS) studies

The silver nanoparticles were accordingly diluted using deionised water and analysed for Zeta potential, particle size and particle size distribution of colloidal matrix of silver nanoparticles by using Malvern Zetasizer Instrument.

2.3.3 Fourier Transform Infra Red Technique

Presence of functional groups were identified by using Schimadzu FTIR Spectrophotometer, the samples were anlysed by KBr pellete technique in the range 450-4000cm-1.

2.3.4 X-Ray diffraction

Philips XPERT-PRO diffractometer was used for X-ray Diffraction Data at room temperature using nickel filtered Cu Ka radiations which was operated at 40Kv Voltage, 30mA current.

2.3.5 High Resolution Transmission Electron Microscopy (HRTEM)

Samples were spotted on a carbon coated Cupper-grid and scanned through CCD camera for clear images by using Instrument Hitachi (H-7500, Japan).

2.3.6 Quantitative Silver Content Estimation: Inductive Coupled plasma-Atomic Emission Spectroscopy (ICP-AES)

For the qualitative and quantitative finding of Silver ion content, ICP-AES (Spectro ARCOSE, Germany) was used. The synthesized silver nanoparticles were treated with nitric acid and analysed for Silver content by taking the standard silver calibetration curve and experiments were performed in triplicates.

2.4 Antibacterial Activity

Antibacterial activity was carried out by serial dilution method. In this Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and Methicillin resistant *Staphylococcus aureus* (MRSA) were determined. Before experimentation, the culture media were standerdized. Appropriately, the lowest concentration of extract loaded silver nanoparticles showing inhibition of growth was considered as the MIC while the lowest concentration of extract loaded silver nanoparticles that showed zero growth was considered as MBC. All the experiments were performed in triplicates [31].

2.5 Cytocompatibility Studies

The cytocompatibility study of prepared silver nanoparticles was performed by using standerd MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Human liver cell lines (HepG2) were incubated with prepared silver nanoparticles of different concentrations (413.06, 206.53, 103.3, 51.63, 25.81 µg/ml) equivalent to free extract for 24 and 48 hr. After completion of the incubation period, percent cell viability was calculated by standerd MTT assay [32].

3. RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nanoparticles

Plant extract was added to the Silver nitrate solution in different concentration, which showed color change from yellowish to dark brown confirmed synthesis of Silver Nanoparticles (Fig. 1). Due to Excitation of surface plasmon resonance in silver nanoparticles shows colour change from yellowish to dark brown colour. Even as the aqueous solution of Silver nitrate was mixed with aerial root of *Ficus benghalensis* extract, after few hours it started to change the colour from yellowish to dark brown because of reduction mechanism, which indicates silver nanoparicles were formed. Generally it is granted that UV-Visible spectroscopy was used to record size and shape of nano-particle in aqueous medium [33]. Purity was confirmed by taking UV spectra before and after Centrifugation, Fig. 2 depicted UV-Visible spectroscopy analysis of diluted solution of Silver nanoparticles peak at 437.5 nm confirms the synthesis of Silver Nanoparticles. In different concentrations (10:1, 10:2) plant extract to silver nitrate solution was added and showed the color change after 1hr. Besides that, 10:1 concentration was selected for sythesis of nanoparticles because we got maximum yield with this based on number of trials and optimum yield.

3.2 Characterization of Synthesized Silver Nanoparticles

The size and shape of Synthesized Silver Nanoparticles determined by UV-Visible spectrophotometer which accordingly affects the biomedicine applications. In this present research work it was confirmed that change in color from yellowish to dark brown seen due to surface plasmon resonance in nanoparticles and which was monitored by UV-visible peak at 437.5nm. Broad peak indicated that synthesized Silver nanoparticles were polydisperse in character. At an interval of one week for 60 days, shift in wavelength was measured for the confirmation of stability of silver nanoparticles.It was recorded that silver nanoparticles were stable during this period. The reason behind stable nanoparticles were alloted to capping of silver nanoparticles with phytochemical used for synthesis. Soluble phytochemicals like polyphenols, flavonoids, and tannins encapsulate silver nanoparticles which generates negatively charged Brownian moments leads to formation of stable Silver nanoparticles [34].

FTIR measurements were carried out to identify the major functional groups in the extract and their possible involvement in the synthesis and stabilization of silver nanoparticles. The spectrum of synthesized silver nanoparticles are presented in Fig. 5. The promiment bands appearing at 3802, 3725, 3664, 3625, 3567, 3529, 3482, 3444, 3332, 3019 suggest presence of O-H, C-H, N-H stretching present in polyphenols and amine, sharp peak at 2356 represents C≡C, and 1538 cm−1(C=O) as shown in Fig. 5. It was suggest that, there were a shift in the peaks in synthesized nanoparticles which showed that functional groups of aerial root extract participate in the formation of AgNPs. From the FTIR spectra this was clearly suggested that flavonoids, polyphenols and proteins present in extract *of Ficus benghalensis* and were responsible for reduction of silver into silver ion and leads to stabilization.

Particle size, Zeta potential and Polydispersity Index of Synthesized silver nanoparticles were given in Table 1. Particle size was measured by Zeta sizer which was corresponds to hydrodynamic diameter of particles which is not a actual diameter. But actual diameter was measured by TEM. Negative zeta potential value indicated the negative charge onto the synthesized silver nanoparticles coated by polyphenols and fllavonoids. Stability of siover nanoparticles is directly proportional to magnitude of zeta potential. Polydispersity Index of silver nanoparticles was below 0.4 which indicates the synthesized silver nanoparticles were monodisperse in character.

X-ray Diffraction study revealed that the crystalline nature of nanoparticles [35]. In the present research work, XRD study were showing the peaks (Fig. 3) as per Bragg's reflection from (111) and (200) planes of Face Center Cubic (FCC) crystal structure corresponding to the 2θ value of 38.03, and 44.19 which was in line with the standard values of JCPDS No. 04-0783 for silver. Evaluation of XRD study of synthesized silver nanoparticles with standard values confirmed that Silver nanoparticles were nanocrystals in nature. In addition to the typical Bragg's peak representative of FCC silver nanoparticles, additional peaks were also observed suggesting that the crystallization of bioorganic phase (phytoconstituents) occurred on the surface of the silver nanoparticles.

Furthermore a High Resolution Transmission Electron Microscopy (HRTEM) provides the size details and morphology of synthesized silver

nanoparticles were found to be polymorphic structure like triangular, spherical, hexagonal and rod shaped (Fig. 4). From HRTEM study, it could be inferred that plant material which are used as green source for synthesis of silver nanoparticles have control on particle size and morphology. Coated silver nanoparticles were found to have narrow distribution of particle size.The synthesized silver nanoparticles were in the range of 10–30 nm. The selected area diffraction pattern (Fig. 4, inset) confirms the face-centered cubic (fcc) crystalline structure of metallic silver. HRTEM images revealed the presence of both the hydrodynamic diameter and the actual diameter of silver nanoparticles which were found to be in line with zeta sizer analysis. At the same time, images show that the small percentage of particles was far from the range which could be due to incomplete or weaker reaction of the phytochemicals (polyphenols, flavonoids, proteins) with silver leading to formation of some larger particles [36].

3.4 Quantitative Estimation of Silver Content by Inductive Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES)

In this study, the synthesized silver nanoparticles were evaluated for quantitative determination of silver by using Inductive Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES).By using standard concentrations of silver in the range of 0.1-100 mg/mL, the calibration curve was plotted and which showed linearity at ppm level with correlation coeffficient of 0.999. The reason behind treating the silver nanoparticles with concentrated nitric acid was to eliminate organic compound. The concentration of synthesized silver nanoparticles measured by using ICP-AES was found to be 413.06 µg/mL.

3.5 Antibacterial Activity

By using serial dilution method MIC and MBC were observed as shown in Fig. 6. Minimum inhibitory concentration (MIC) was estimated as lowest concentration of silver nanoparticles shows zero growth when compared with control. All concentrations which were equal to or higher than MIC were inoculated onto Muller Hinton agar plates and kept for incubation for 24hrs.The concentration of silver nanoparticle which showed zero colony forming units (CFU) was recorded as Minimum Bactericidal concentration (MBC). The MIC and MBC values (Table 2) indicated that the synthesized silver nanoparticles by using extract exhibited antimicrobialactivity against MRSA, SA, E-coli and PA. The effect of silver nanoparticles against these strains confirms its potential antimicrobial activity.

3.6 Cytocompatibility Study

Cytocompatibility is the key indicator to be assessed for any biomatrix containing nanoparticle for invitro cytotoxicity study. İn the present research work, the invitro cytocompatibility study was evaluated against primary mouse fibroblast culture (L929) using colorimetric MTT assay. Cytotoxicity of samples was compared with control cells (untreated) that were considered as 100% viable (Fig. 7). Fibroblast cells were treated with synthesized silver nanoparticles with aerial root extract of Ficus benghalensis at varying concentrations of 413.06, 206.53, 103.3, 51.63 and 25.81 µg/mL and were evaluated for cell viability after 24hr. İn the present research work, phytochemicals coated silver nanoparticles do not show any toxicity against normal mouse fibroblast thereby confirming biocompatible nature of silver nanoparticles. This expressed that phytoconstituents present in aerial root of Ficus benghalensis not only effectively reduced silver nitrate to silver nanoparticles but also provides non-toxicity coating to the surface of synthesized nanoparticles.

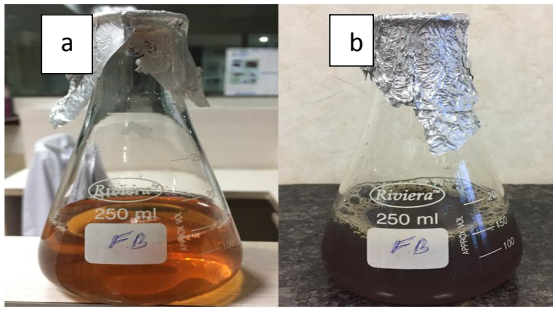


Fig. 1. Change in color from yellowish to dark brown a) Plant extract added to Silver Nitrate, b) Change in color after 1hr

Table 1. Nanoparticle size, Zeta potential, and PDI of Synthesized nanoparticles

Sr.no.	Nanoparticle size (nm)	Zeta potential (mV)	PDI
1.	130.8	-41.7	0.340
2.	134.9	-37.8	0.289
3.	132.0	-40.9	0.269
Mean	132.5667	-40.1333	0.299333
SD	2.107922	2.059935	0.036611

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of synthesized silver nanoparticle from aerial root extract

Organisms							
Methicillin-resistant Staphylococcus aureus (MRSA)		Staphylococcus aureus (SA)		E-Coli		Pseudomonas aeruginosa (PA)	
MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
51.63 µg/ml	103.2 µg/ml	103.2 µg/ml	206.53 µg/ml	25.81 µg/ml	51.63 µg/ml	51.63 µg/ml	103.2 µg/ml

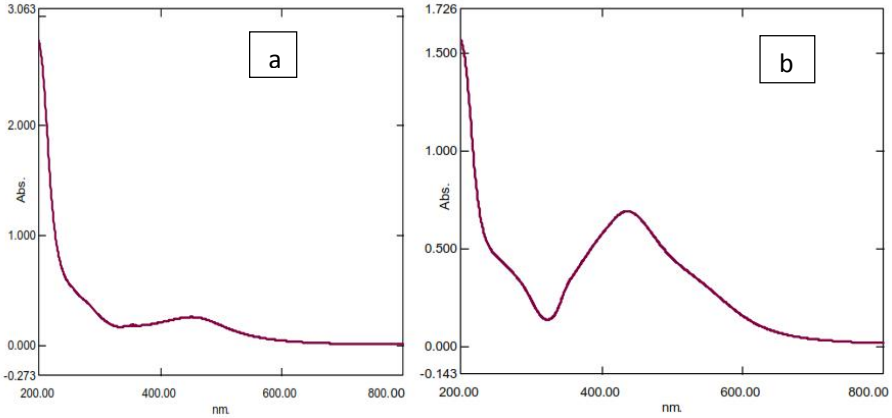


Fig. 2. UV-visible analysis (a) Before centrifugation (b) after centrifugation

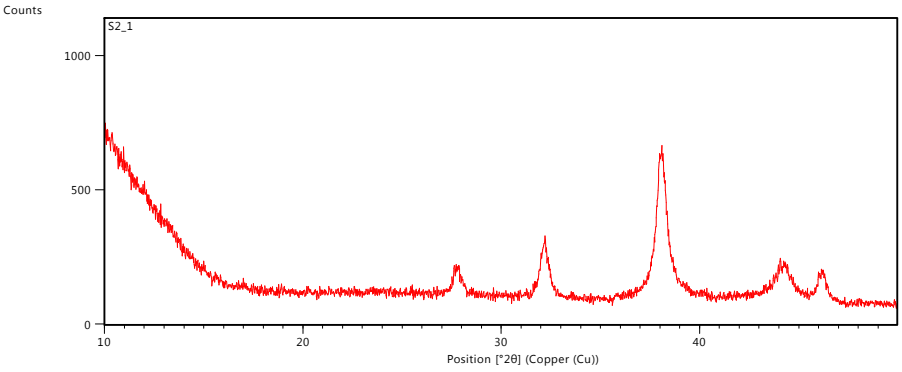
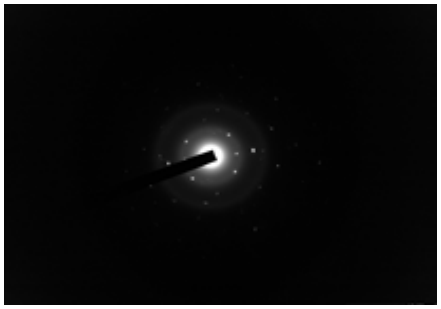


Fig. 3. XRD-spectrum of synthesized silver nanoparticles with aerial root of ficus benghalensis



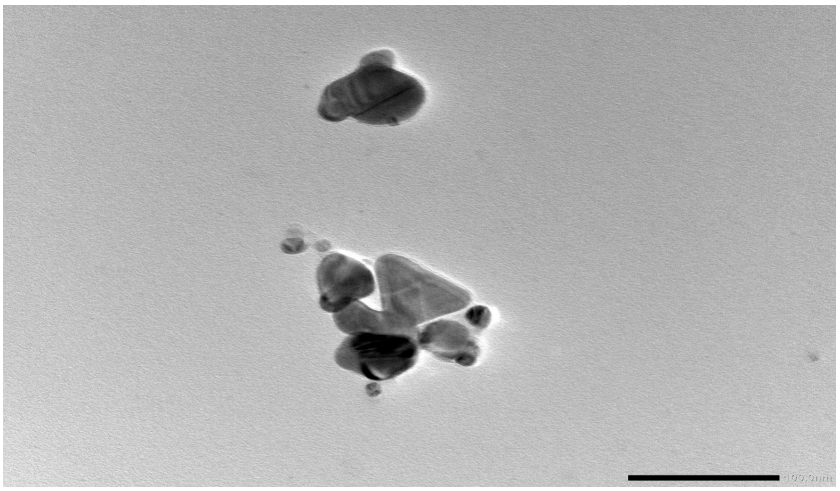


Fig. 4. TEM image of Silver nanoparticles with inset SAED pattern.

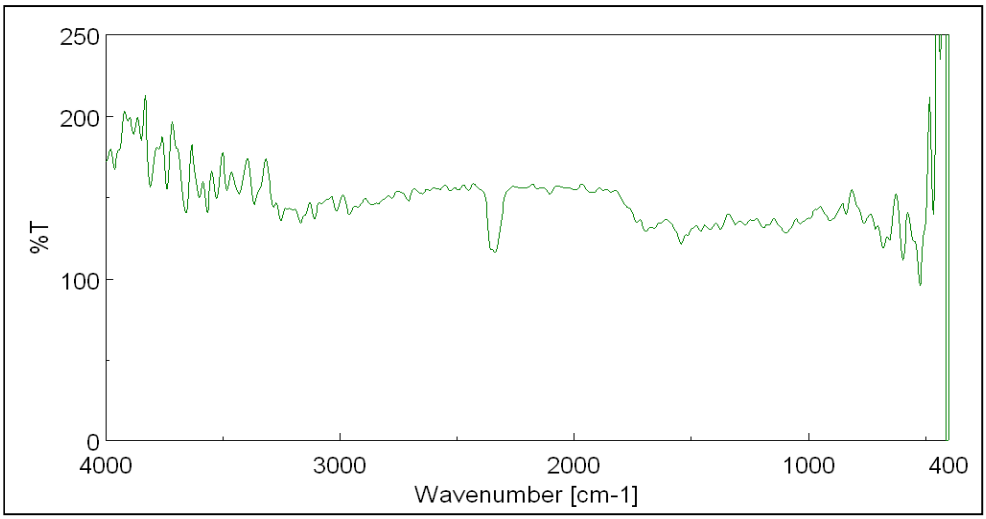
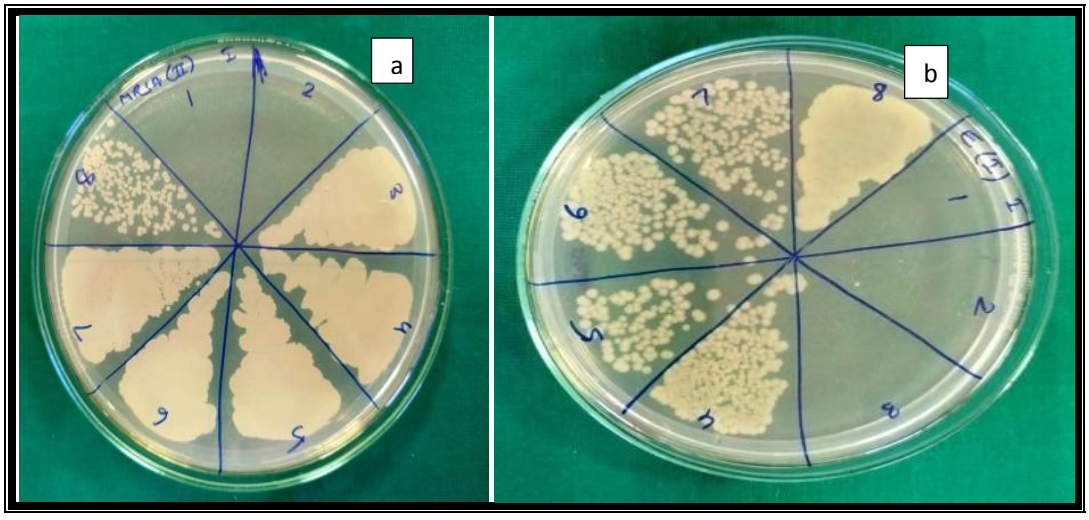


Fig. 5. FTIR spectra of synthesized silver nanoparticles



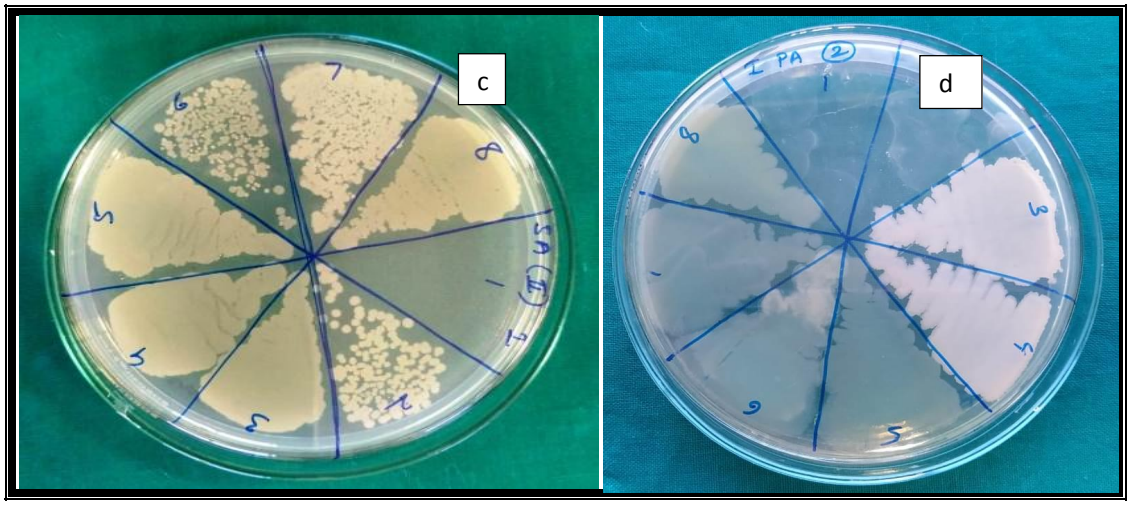


Fig. 6. Antimicrobial activity of F. beghalensis mediated silver nanoparticles effect on a) MRSA, b) E-coli, c) SA, d) PA

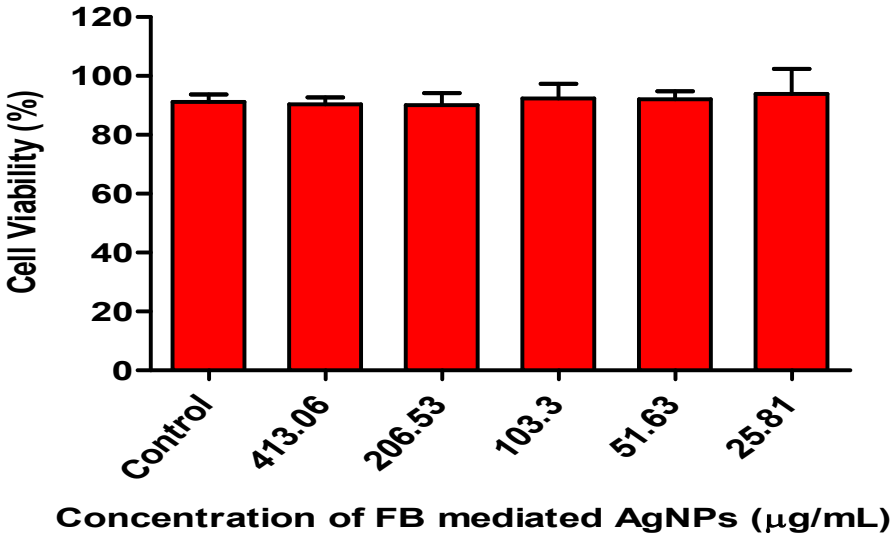


Fig. 7. Cytocompatibility study of Ficus benghalensis mediated silver nanoparticles determined by evaluating the cytotoxicity against normal mouse fibroblast cell lines (L929) using colorimetric MTT assay

4. CONCLUSION

This research work exhaustively conveyed synthesis, characterization, and biomedical application of silver nanoparticles, with special emphasis on antibacterial activity. Silver nanoparticles were successfully synthesized by using extract of F. Benghalensis, characterized and evaluated for its antibacterial activity. Plant extract of F. Benghalensis served as green source for reduction of silver nitrate to silver nanoparticles. Also silver nanoparticles were found to be nontoxic when evaluated for its cytocompatibility study. Here we concluded that the biocompatible and environment friendly silver nanoparticles can be used as an antibacterial agent against different strains.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our

area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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