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Research Article

Synthesis of Biogenic silver nanoparticles by using aqueous leaf extracts of *Ichnocarpus frutescens* (L.) W.T.Aiton and their antimicrobial activity

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Abstract
Biosynthesis of nanoparticles using angiospermic plants is a trending area of research in nanoscience and nanotechnology. The present investigation reports a simple eco-friendly method for synthesis of silver nanoparticles using leaf extract of *Ichnocarpus frutescens* (L.) W. T. Aiton. Leaf extract serve as a source of reducing and capping agents. 1 mM solution of silver nitrate was treated with the aqueous extract of leaf leading to the formation of Ag-NPs was observed visually by change in color from colorless to brown color reaction mixture. Biogenic nanoparticles were characterized by Fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM) and high resolution transmission electron microscopy. UV-Vis absorption spectroscopy is one of the main tools to analyze the formation of metal nanoparticles in aqueous solution. The FTIR analysis was carried out to identify the possible functional groups between the capping agents and silver nanoparticles. The AFM was used to analyze topography of nanoparticles and also to measure their size. The test plant mediated biogenic silver nanoparticles shows spherical Ag-NPs which range from 5 to 50 nm. This was taken to investigate their potential for antimicrobial activity Antimicrobial analysis data reveals that *S. aureus*, *E. coli*, *B. subtilis*, *B. polymyxa* and *S. typhi* microbes showed sensitive nature with suppressed growth rate; it is due to toxic effect of biogenic AgNPs synthesized by selected test plant species.

Keywords: Biogenic nanoparticles, Characterization, HR-TEM, FTIR, and Antimicrobial activity.

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Introduction

Nano size particles are quite unique in nature because nano size increase surface to volume ratio and also its physical, chemical and biological properties are different from bulk material. Nanotechnology is a fast growing area, which is a interdisciplinary field of both science and technology that rises the scope of finding and regulating at cell level between synthetic material and biological system [1, 2]. 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 [3]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 [4]. 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. Diverse plants have been successfully used for the synthesis of biogenic metal nanoparticles [5, 6].

Synthesis of silver nanoparticles is of much interest to the scientific area because of their wide range of applications. These silver nanoparticles are being successfully used in the cancer identification and treatment as well. For biomedical applications; being additional to wound dressings, topical creams, antiseptic sprays and fabrics, silver functions' as an antiseptic and displays a broad biocidal effect against microorganisms through the interference of their unicellular membrane thus disturbing their enzymatic activities. They are even being projected as future generation antimicrobial agents. The lesser size of nanoparticles is gaining importance in research for the therapy of various diseases. Therefore, we tried to establish eco-friendly, cost efficient small sized silver nanoparticles (AgNPs) by using leaf extract of *Ichnocarpus frutescens* (L.) W.T.Aiton These AgNPs are powerful tool against multidrug-resistant bacteria. Now a day the disease causes microbes that have become resistant to various antibiotics and cause increasing public health problem. Therefore there is an urgent need to develop new bactericides. Biogenic nanoparticles can act as antibacterial and antifungal agents, due to their ability to interact with microorganisms

Materials and methods



Fig. 1 Experimental plant

Ichnocarpus frutescens (L.) W. T. Aiton is belongs to apocynaceae. It is a large, much branched twining shrub, containing white latex in its all parts. Leaves of this plant contain flavonoids and phenolic acids. Flowers are small, greenish white and numerous. The fruit is a follicle type.

Methodology

Preparation of leaf extract:

Experimental plant species were collected from Botanical garden of Karnataka University campus. Leaves were washed 2-3 tunes with tap water followed by double distilled water to remove dust; next shade dried to remove the residual moisture and weighed about 50gm. The weighed leaves were cut into small pieces and boiled (60^{0} C) in glass beaker containing 500 ml of sterile distilled water for 30 min. The aqueous extract was separated by filtration with Whatman No. 1 filter paper .

Preparation of Silver nitrate solution: The ImM Silver nitrate solution *was* prepared by dissolving 0.0169 grams Silver nitrate (AR grade) in 100ml double distilled water.

Biosynthesis of Silver nanoparticles: For reduction of Silver ions, 15 ml of aqueous leaf extract was added to 95 ml of Silver nitrate aqueous solution (ImM AgNCb). Simultaneously, the reaction mixture *was* adjusted to pH 8.5 and then heated at 40- 80^{0} C resulting change in colour from yellowish to dark brown indicating the synthesis of Silver nanoparticles (7, 8). Silver nanoparticles were isolated or purified by repeated (3 times) centrifugation of the reaction mixture at 3000rpm for 50 minutes. Finally silver nanoparicles powder was collected, dried and further used for the characterization process.

Detection and characterization of Silver nanoparticles

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

Antibacterial activities silver nanoparticles

The silver nanoparticles synthesized by 'using leaf extract of *Ichnocarpus frutescens* (L.) W.T.Aiton were tested for antimicrobial activity by agar well diffusion method against human pathogenic *Staphylococcus aureus , Bacillus subtilis, Bacillus polvmyxa, Escherichia coli, Sahnolella typlii, , 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 nun thick uniform layer and allowed to solidify. Each strain was swabbed uniformly on the individual plates using sterile cotton swab. Wells of size 6 nun were made on nutrient agar plates using gel puncture. Different concentrations of silver nanoparticles (25, 50, 100, 200, 400 pi) solution were poured on to four wells and in one well 400 pi of plant extract poured as controle on all plates using micropipette. After incubation at 37 °C for 24h for bacterial strains and 96 h for fungal strains, the diameter of zone of inhibition was measured in millimetres and tabulated.

Results aud discussion

Characterization of silver nanoparticles:

Addition of leaf extract to $AgNO_3$ the color 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 Erlemneyer flask (which contains reaction mixture) showed mirror like illumination, it clearly indicates the formation of silver nanoparticles in the reaction mixture.

UV-Vis absorption spectroscopy is one of the main tools to analyze the formation of metal nanopartcles in aqueous solution. The UV-visible spectroscopic studies on the synthesis of silver nanoparticles (Fig. 3) have shown an absorbance at 415 mil due to surface plasmon resonance (SPR).

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 experimental plant leaf extract. 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 color). The optimal pH for nanoparticle synthesis was preferred to be pH 8.

Characterization of silver uauoparticles-

Visual observation-



Leaf extract **1 mM. silver nitrate** **Reaction mixture**

Fig. 2 Change in colour of reaction mixture due to formation of silver uauoparticles.

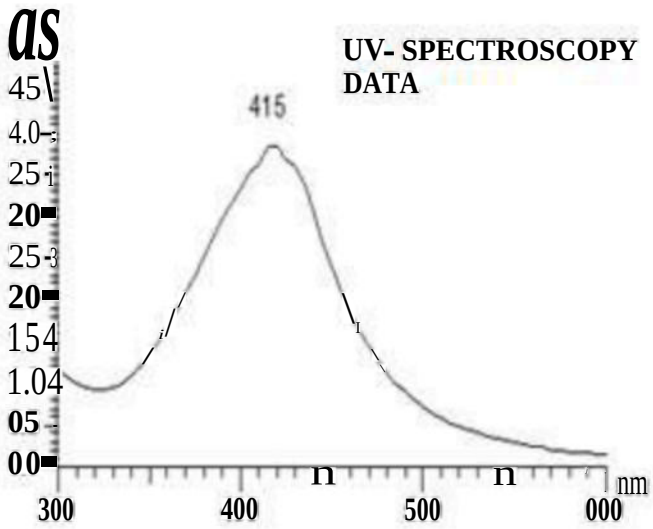


Fig. 3 UV-vis absorption spectra of Ag-NPs synthesized from leaf extract of *I. frutescens* showing SPR peak at 415 nm.

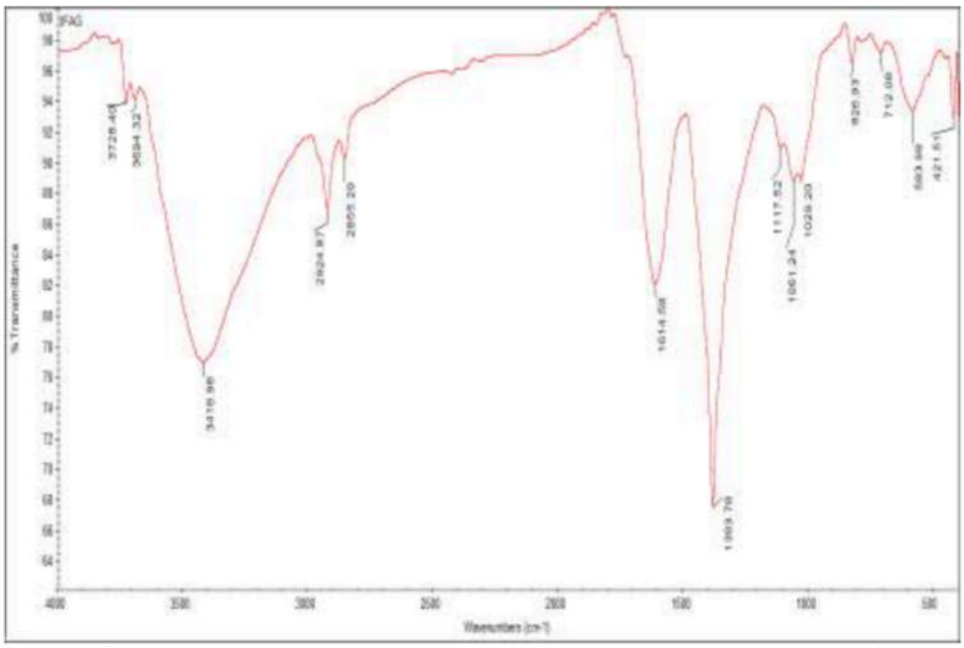


Fig.4 FTIR spectrum of Ag-NPs synthesized from Leaves of *Ichnocarpus frutescens* (L.) W.T.Aiton

Identification of the biomolecuies involved in the formation of silver nanoparticles was done using FTIR. The FTIR spectrum of silver nanoparticles synthesized from leaves of *Ichnocarpus frutescens* (L.) W.T.Aiton shows the possible interfacial groups between the capping agents and silver nanoparticles (Fig. 4) and absorption peaks value and their functional groups are represented in Table no. 1

Table 1 FTIR absorption peaks and their functional groups of Silver nano particles synthesized from leaves of *Ichnocarpus frutescens* (L.) W.T.Aiton

SI. No.	Absorption peaks (cm^{-1})	Functional groups.
1	3728	The free O-H bond stretches.
2	3694	O-H Amide N-H Stretch
3	3418	O-H Stretch alcohol & phenols N-H
4	2924	O-H stretch Corboxylic acid, Alkyl C-H Stretch
5	2855	C-H stretch region for the aldehyde, stretch, aldehyde hydrogen bond (-CHO).
6	1614	N-H, C=C Group or aromatic rings
7	1383	-C-H, C-N alkyl halide and aliphatic amine
8	1117	Aromatic amines
9	1061	C-N stretching vibrations of aliphatic amines
10	1029	C-N stretching vibrations of aliphatic amines
11	826	C-C and C-H phenyl ring substitute

The X-ray Diffraction patterns of silver nanoparticle were recorded according to the description of Wang (2000). The XRD pattern of the biosynthesized silver nanostructure produced by the leaf extract represented in the figure 5 and was further confirmed by the characteristic peaks observed in the XRD image. The XRD data showed intensive diffraction peaks at a 20 value of 37.88° from the (111) lattice plane of face centered cubic (fee) silver unequivocally indicates that the particles are made of pure silver. (7,8).

EDX analysis was conducted to confirm the elemental composition of the sample. The EDS images (Fig.6.) confirmed the presence of significant amounts of elemental silver along with other elements, which may be originate from the biomolecules that are bound to the surface of nanosilver.

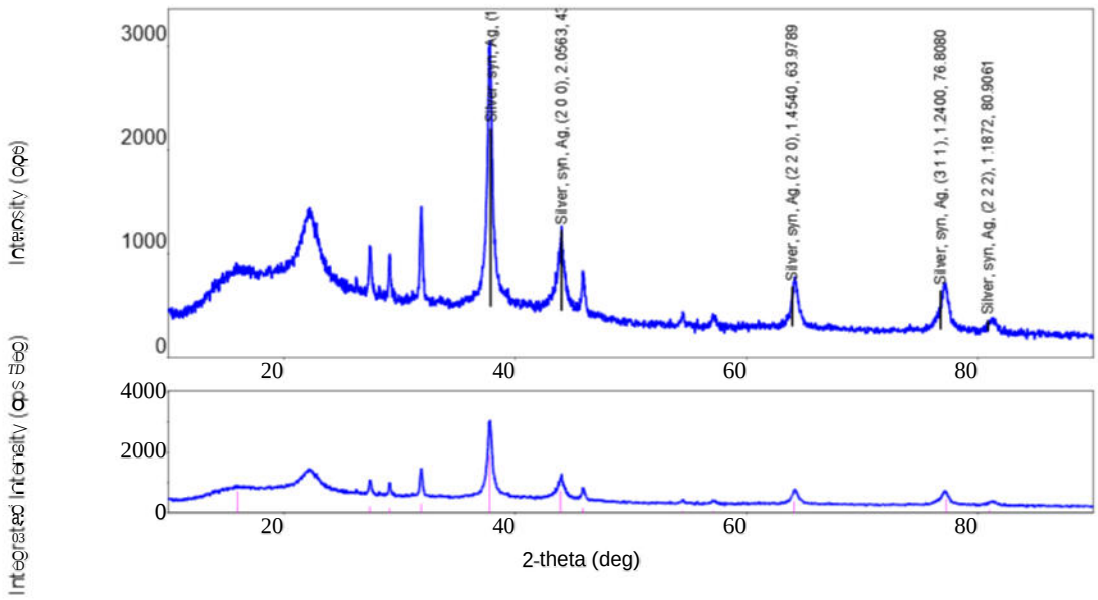


Fig. 5 X-ray Diffraction Spectrum of Silver Nanoparticles synthesized from leaves of *Ichnocarpus frutescens* (L.) W.T.Aiton

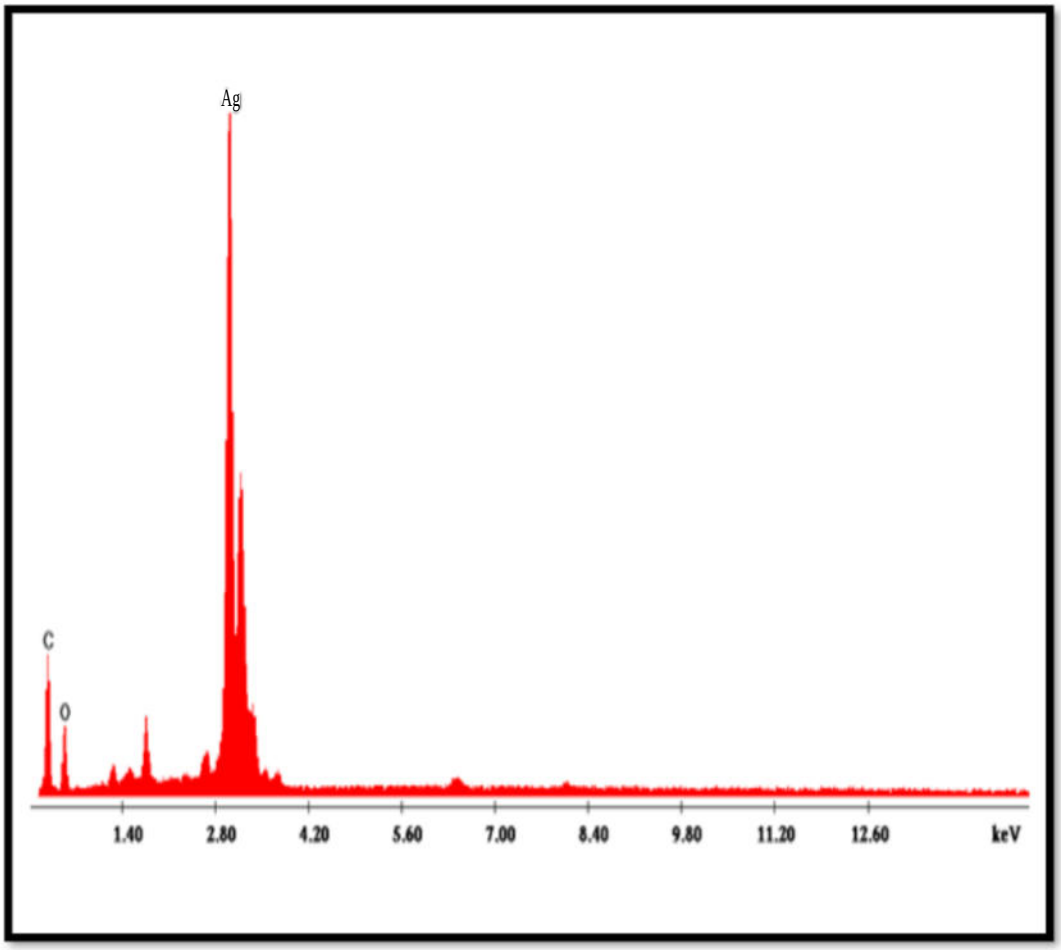


Fig. 6 EDS spectra of silver nanoparticles synthesized by leaf extract of *I. frutescens*

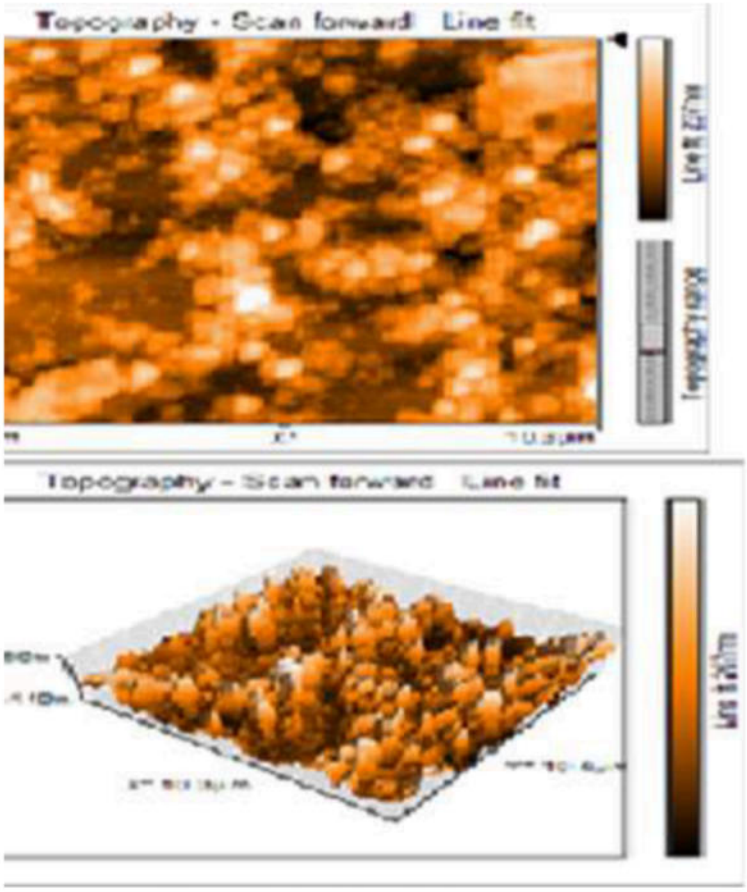


Fig.7 AFM images of Silver nanoparticles synthesized from leaf extract of *Ichnocarpus frutescens* **(L.) W.T.Aiton**

The AFM was used to analyze topography of nanoparticles and also to measure their size (9, 10 ,14). The test plant mediated biogenic silver nanoparticles shows spherical Ag-NPs which range from 5 to 50 nm. The silver nanoparticles were further characterized by HR-TEM micrograph, these Silver nanoparticles showed spherical shape with the majority size range from 10 to 20 nm (Fig. 8). Further, it also shows that the biomolecules of leaf extract bound the nanoparticles as capping agents to hinder further oxidation of nanoparticles.

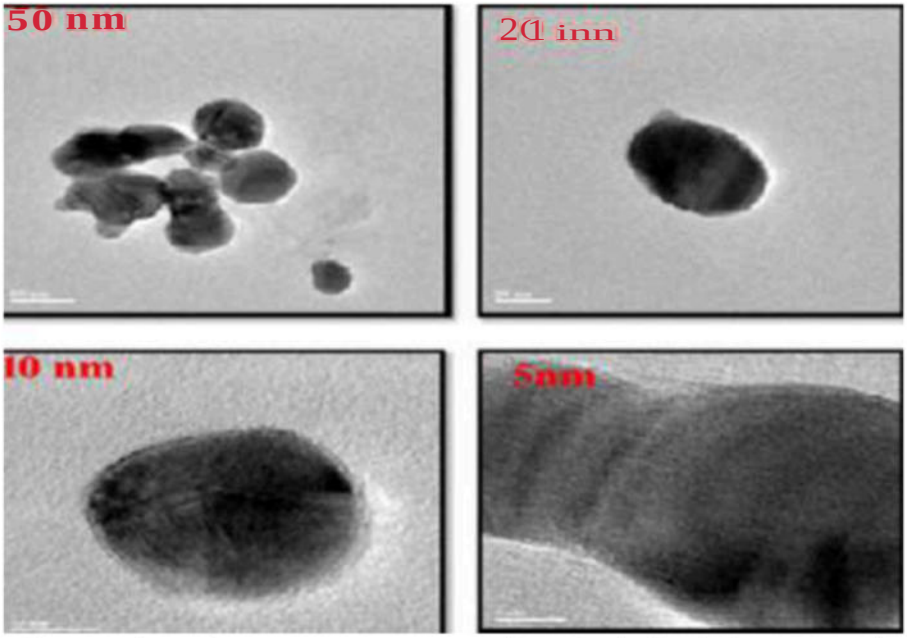


Fig.8 HR-TEM images of silver nanoparticles synthesized from leaf extract *of Ichnocarpus frutescens* **(L.) W.T.Aiton**

The Antimicrobial Activity

The antimicrobial activity of *I. frutescens* leaf extract mediated biogenic silver nanoparticles exhibited antibacterial activity against gram positive *Staphylococcus aureus* , *Bacillus subtilis, Bacillus polymyxa* and *gram* negative *Escherichia coli, Salmolella tvphi, , Vibrio cholera* and antifungal activity against *Aspergillus niger and Candida albicans* (fig 9), while control (leaf extract) do not show any antimicrobial activity. The antibacterial and antifungal effect of silver nanoparticles at different concentrations (25, 50, 100, 200 & 400 pi) was quantitatively assessed on the basis of the zone of inhibition (Table 02).

It was notice that the zone of inhibition increased with increased concentration of Ag NPs. In presence of 400 pi concentration of AgNPs the highest zone of inhibition (ZOI) was recorded (17 mm) in case of *S.* aureus and lowest ZOI was recorded in *S. tvphi* (1limn) while *V. cholera* didn't show any antimicrobial activity. The antifungal activity of silver nanoparticles also tested against *A. niger* and C. *albicans.* C. *albicans* showed 3mm zone of inhibition at 400 pi concentrations of silver nanoparticles while no ZOI was recorded in case of *A.niger*. The antifungal activity of Fluconazole antibiotic (positive control) also tested against *A. niger* and *C. albicans*, but comparatively no zone of inhibition was noticed with antibiotic.

The minimum inhibitory concentration (MIC) studies showed varied concentrations of AgNPs against selected microbes. In summary, the present investigation revealed that the MIC was lowest in *S. aureus* (25 pi). In case of fungi C. *albicans* only showed MIC of 400pi. Therefore *I. frutescens* leaf extract mediated biogenic AgNPs showed good antibacterial activity and are more potential against all selected microbes except *V. cholera* and A .*niger* (table **02)**

The antimicrobial activity of silver nanoparticles synthesized from leaves of *Ichnocarpus frutescens* (L.) W.T.Aiton against –

a) *Staphylococcus aureus*
b) *Salmolella tvphi*
c) *Bacillus subtilis*
d) *Escherichia coli,*
e) *Bacillus polymyxa*
j) *Vibrio cholera*
g) *Aspergillus niger*
h) *Candida albicans*

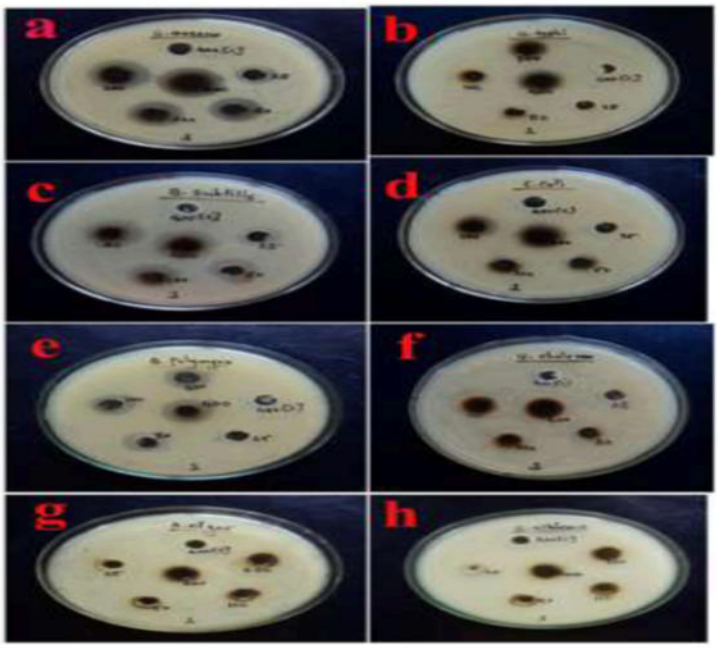


Fig. 9 The antimicrobial activity of silver nanoparticles synthesized from Leaves of *Ichnocarpus frutescens* (L.) W.T.Aiton against – a) *Staphylococcus aureus b)Salmolella typhi c)Bacillus subtilis* d) *Escherichia coli e) Bacillus polymyxa* f) *Vibrio cholera* g) *Aspergillus niger* h) *Candida albicans*

Table 2 Antimicrobial activity of silver nanoparticles synthesized from leaves of *Ichnocarpus frutescens* (L.) W.T.Aiton

Bacterial species	Zone of inhibition (mm)						
	Control (leaf extract)	Silver nanoparticle solution					
	400 pi	25 pi	50 pi	100 pi	200 pi	400 pi	MIC pi
S. typhi	0	0	0	0	8	11	200
B. polymyxa	0	0	0	4	9	12	100
E. coli	0	0	3	6	8	11	50

V. cholerae	0	0	0	0	0	0	**NF**
B. subtilis	0	0	4	7	11	15	50
S. aureus	0	2	7	10	14	17	25

The antimicrobial activity may be depends on the size of nanoparticles, smaller nanoparticles have larger surface area for interaction between silver nanoparticle and bacterial cell than the larger nanoparticles (11). It is also possible that silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the bacteria. The small size and high surface area of these nanoparticles produces electronic effects and these effects enhance the reactivity of nanoparticles surface (12,13).Similarly, in the present investigation small sized biogenic silver nanoparticles synthesized by leaf extract of *I. frutescens* showed good antimicrobial activity. Our investigation confirms the antimicrobial activity of silver nanoparticles. The nanoparticles get attached to the cell membrane and also penetrated inside the bacteria. The nanoparticles rather attack the respiratory chain and finally leading to cell death due to cell lyses.

Conclusion

It is concluded from our studies that the ecofriendly biogenic silver nanoparticles possess a very good antimicrobial activity. Therefore, applications of silver nanoparticles can cover a large area of medical and industrial. At present, clothing, respirators, household water filters, contraceptives, antibacterial sprays, cosmetics, detergent, dietary supplements, cell phones, laptop keyboards, and children's toys are among the products being marketed that purportedly develop the antimicrobial properties of silver nanoparticles.

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