

NOVEL SYNTHESIS OF SILVER NANOPARTICLES BY USING AQUEOUS FRUIT EXTRACT OF *Ichnocarpus frutescens* (L.) W.T. AITON AND THEIR ANTIMICROBIAL ACTIVITY.

¹Basavarajeshwari Hedaginal* and ²T. C. Taranath

¹Post doctoral fellow, ²Professor

^{1,2}Department of Botany, Karnatak University, Dharwad –
580 003, Karnataka, India.

Abstract

Nanotechnology is the study and application of small object which can be used across all fields such as chemistry, biology, physics, material science and engineering. In the present work, the experiments were conducted for the biosynthesis of Silver nanoparticles by using fruit extract of *Ichnocarpus frutescens* (L.) W. T. Aiton . This was taken to investigate their potential for antimicrobial activity. 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 interfacial 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. Antimicrobial analysis data reveals that *S. aureus*, *E. coli*, *B. subtilis*, *B. polymyxa* and *S. typhi* microbes showed maximum zone of Inhibition. These are sensitive with suppressed growth rate; it is due to toxic effect of biogenic AgNPs synthesized by selected test plant species.

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

1. 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]. A quick step in synthesis and applications of nanomaterials, in recent years has been invented in almost every domain of science including health care, cosmetics, biomedical, drug, environment, electronics, mechanics, catalysis, energy science, optics, chemical and space industries [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. Different plants have been successfully used for the synthesis of biogenic metal nanoparticles [5, 6]. The smaller size of

nanoparticles is gaining importance in research for the treatment of various diseases. Therefore, we tried to establish eco-friendly, cost efficient small sized silver nanoparticles (AgNPs) by using fruit 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.

II MATERIALS AND METHODS

Ichnocarpus frutescens (L.) W.T.Aiton



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.

2.1. Preparation of fruit extract:

Experimental plant species were collected from Karnataka University campus. Fruits were washed 2-3 times with tap water followed by double distilled water to remove dust, next shade dried to remove the residual moisture and weighed about 25gm. The weighed fruits were cut into small pieces and boiled (80⁰C) in glass beaker containing 250 ml of sterile distilled water for 20 min. The aqueous extract was separated by filtration with Whatman No. 1 filter paper and stored in refrigerator at 4⁰C for further use.

2.2. Preparation of Silver nitrate solution: The 1mM Silver nitrate solution was prepared by dissolving 0.0169 grams Silver nitrate (AR grade) in 100ml double distilled water.

2.3. Biosynthesis of Silver nanoparticles: For reduction of Silver ions, 10 ml of aqueous fruit extract was added to 90 ml of Silver nitrate aqueous solution (1mM AgNO₃). Simultaneously, the reaction mixture was adjusted to pH 8 and then heated at 30- 60⁰ 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 (4-5 times) centrifugation of the reaction mixture at 3000rpm for 40 minutes. Finally silver nanoparticles powder was collected, dried and further used for the characterization process.

2.4. 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 Transmission Electron Microscopy (HR-TEM), X-Ray Diffraction (XRD) and Energy dispersive spectroscopy (EDS).

2.5. Antibacterial activities silver nanoparticles

The silver nanoparticles synthesized by using fruit 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 polymyxa*, *Escherichia coli*, *Salmonella 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.

III. RESULTS AND DISCUSSION

Addition of fruit extract to AgNO₃ 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 Erlenmeyer flask (which contains reaction mixture) showed mirror like illumination, it clearly indicates the formation of silver nanoparticles in the reaction mixture.

3.1 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 fruit extract and AgNO₃, pH, temperature and incubation time were optimized for the reduction of Ag⁺ ions to AgNPs using experimental plant fruit 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.

3.2 Characterization of silver nanoparticles-

Visual observation-



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

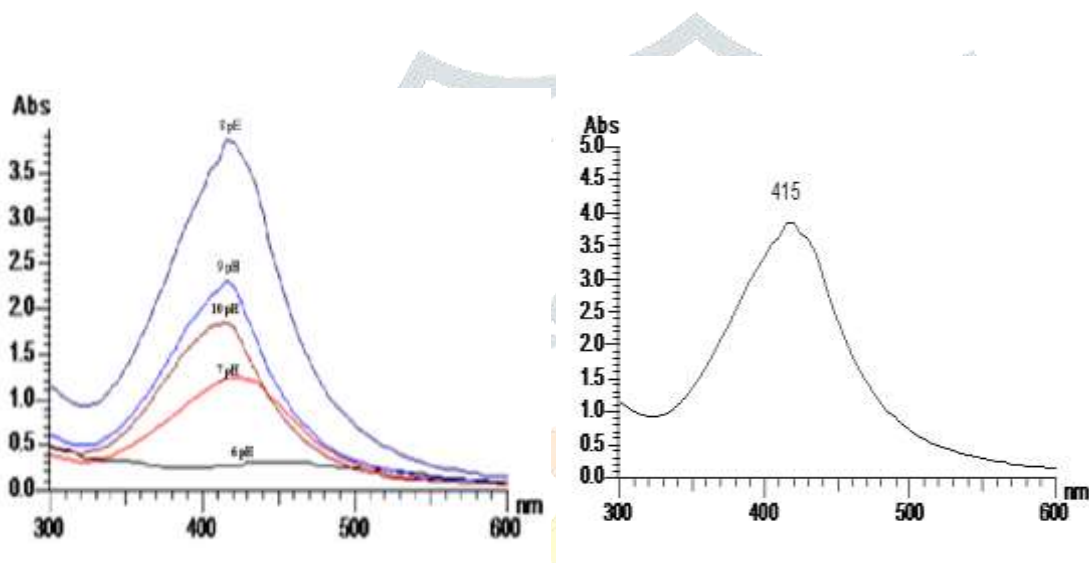


Fig. 3 UV-vis absorption spectra of Ag-NPs synthesized from fruit extract of *I. frutescens* showing SPR peak at 415 nm.

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

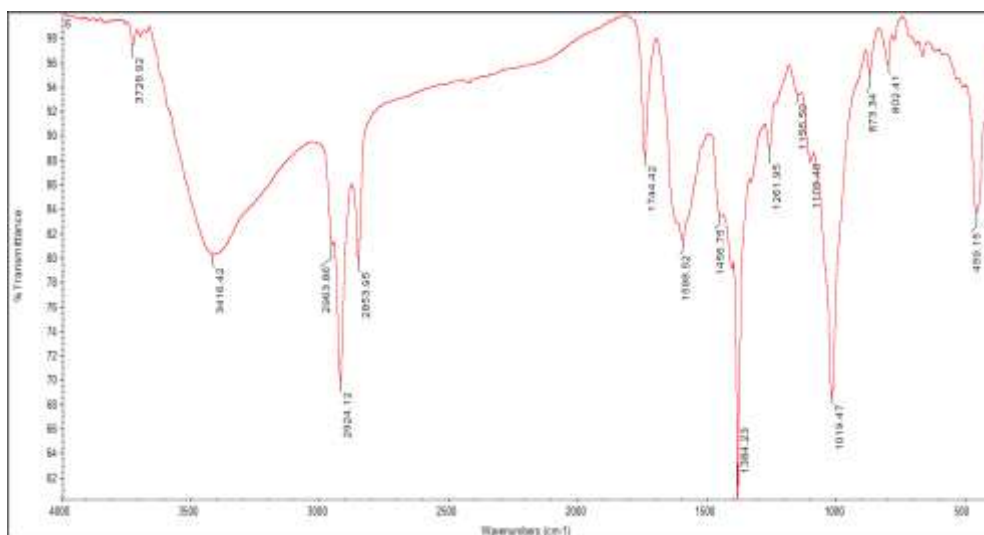
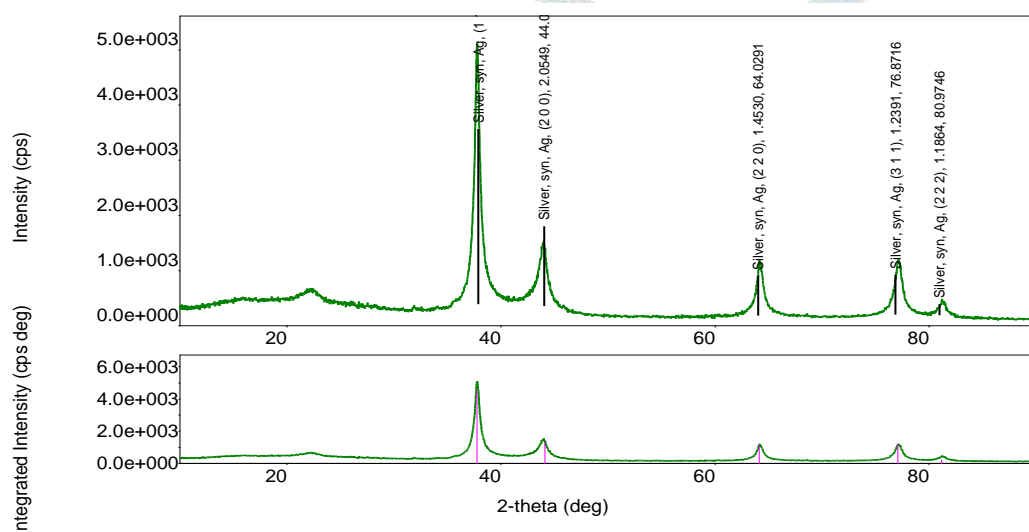


Fig.4 FTIR spectrum of Ag-NPs synthesized from Fruits of *Ichnocarpus frutescens* (L.) W.T.Aiton

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

Sl. No.	Absorption peaks (cm ⁻¹)	Functional groups.
1	3728	O-H Amide N-H Stretch
2	3416	O-H Stretch alcohol & phenols N-H
3	2924	O-H stretch Corboxylic acid, Alkyl C-H Stretch
4	2853	C-H stretch region for the aldehyde, stretch,aldehyde hydrogen bond(- CHO).
5	1744	C=O Aldehydes
6	1598	Nitro group show strong bands & overlaps the aromatic ring region. Stretching & bending of 1 ^o & 2 ^o amines & amides takes place.
7	1384	C-C , C-N stretching
8	1261	C-Cl strongly stretches in aliphatic chlorides, S-O stretch strongly

Identification of the biomolecules involved in the formation of silver nanoparticles was done using FTIR. The FTIR spectrum of silver nanoparticles synthesized from fruits 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

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

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 fruit 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 2θ value of 37.88° from the (111) lattice plane of face centered cubic (fcc) 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.

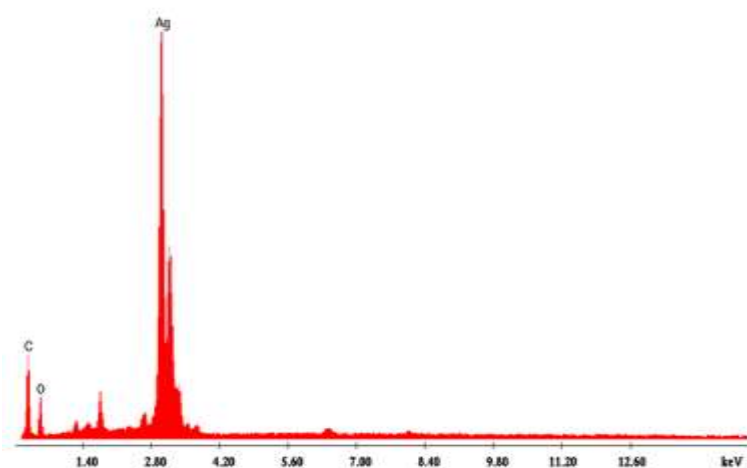


Fig. 6 EDS spectra of silver nanoparticles synthesized by fruit extract of *I. frutescens*

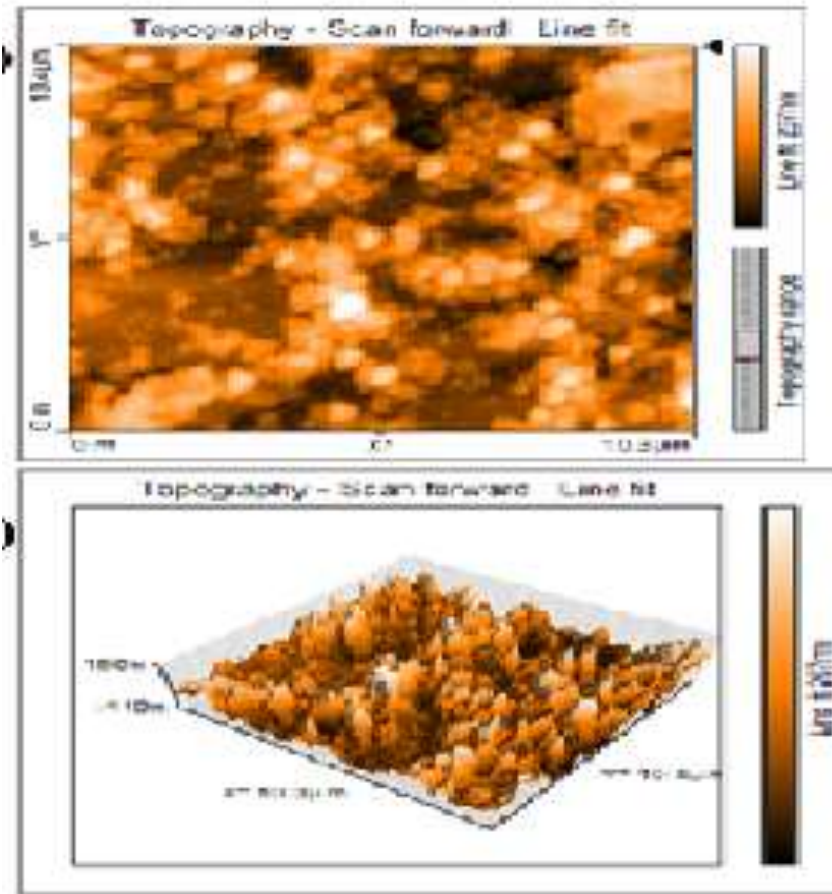


Fig.7 AFM images of Silver nanoparticles synthesized from fruit extract of *Ichnocarpus frutescens* (L.) W.T.Aiton

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 fruit extract bound the nanoparticles as capping agents to hinder further oxidation of nanoparticles.

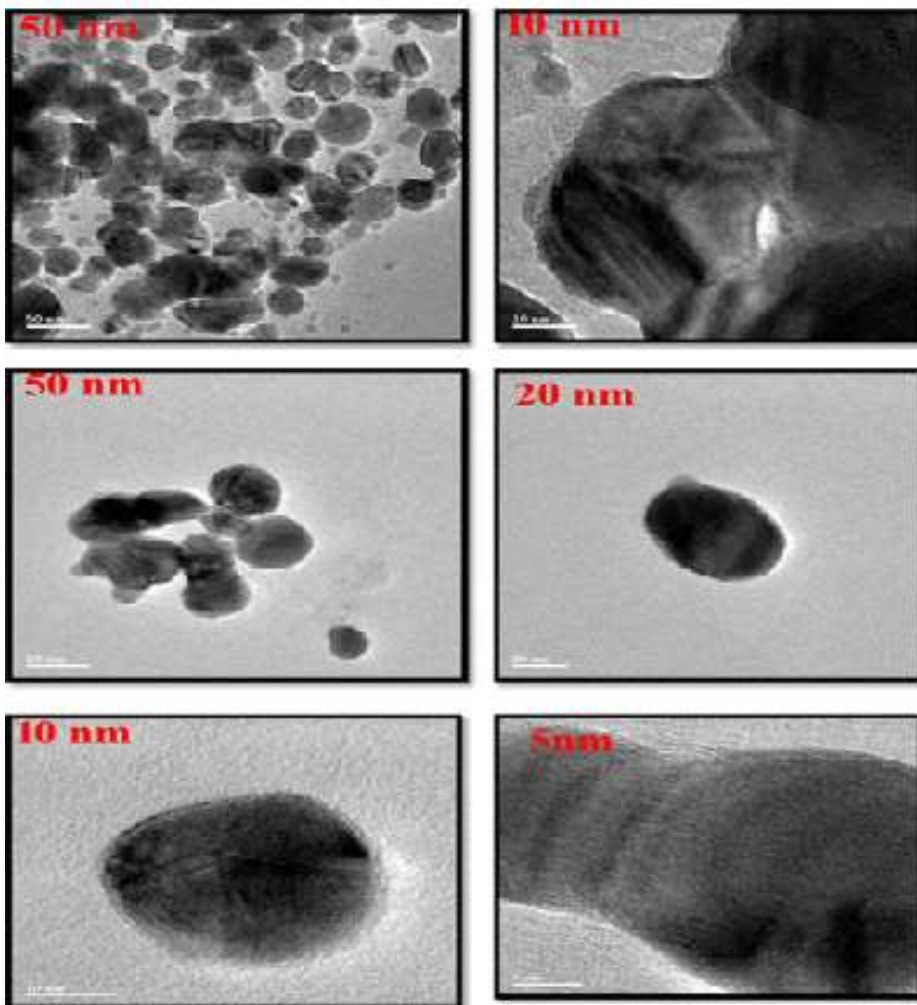


Fig.8 HR-TEM images of silver nanoparticles synthesized from fruit extract of *Ichnocarpus frutescens* (L.) W.T.Aiton

3.3 The Antimicrobial Activity

The antimicrobial activity of *I. frutescens* fruit extract mediated biogenic silver nanoparticles exhibited antibacterial activity against gram positive *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus polymyxa* and gram negative *Escherichia coli*, *Salmonella typhi*, *Vibrio cholera* and antifungal activity against *Aspergillus niger* and *Candida albicans* (fig. 9), while control (fruit extract) didn't show any antimicrobial activity. The antibacterial and antifungal effect of silver nanoparticles at different concentrations (25, 50, 100, 200 & 400 μ l) was quantitatively assessed on the basis of the zone of inhibition (Table 2). It was noticed that the zone of inhibition increases with increased concentration of Ag NPs. The minimum inhibitory concentration (MIC) studies showed varied concentrations of AgNPs against selected microbes. In summary, the present data revealed that the MIC was lowest in *B. subtilis* (25 μ l). In case of fungi *C. albicans* only showed MIC of 200 μ l. Therefore *I. frutescens* fruit extract mediated AgNPs showed good antibacterial activity and are more potential against all selected microbes except *V. cholera* and *A. niger*.

The antimicrobial activity may depend 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 fruit 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 preferably attack the respiratory chain and cell division process and finally leading to cell death.

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

Bacterial species	Zone of inhibition (mm)						
	Control (plant extract)	Silver nanoparticle solution					
	400 μ l	25 μ l	50 μ l	100 μ l	200 μ l	400 μ l	MIC μ l
<i>S. typhi</i>	0	0	5	9	12	14	50
<i>B. polymyxa</i>	0	0	4	6	11	13	50
<i>E. coli</i>	0	0	0	0	0	2	400
<i>V. cholera</i>	0	0	0	0	0	0	0
<i>B. subtilis</i>	0	3	6	9	11	13	25
<i>S. aureus</i>	0	0	9	11	13	17	50

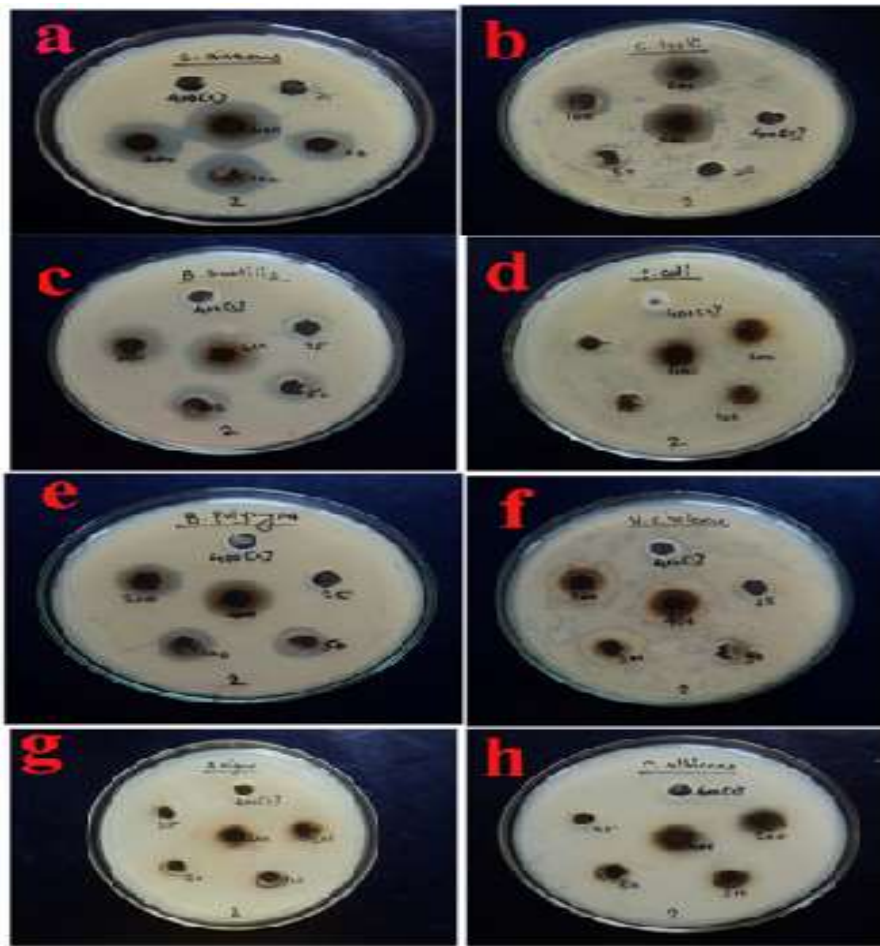


Fig. 9 The antimicrobial activity of silver nanoparticles synthesized from Fruits of *Ichnocarpus frutescens* (L.) W.T.Aiton against – a) *Staphylococcus aureus* b) *Salmonella typhi* c) *Bacillus subtilis* d) *Escherichia coli* e) *Bacillus polymyxa* f) *Vibrio cholera* g) *Aspergillus niger* h) *Candida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

IV. 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 domain of medical, leather and food technologies. 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 exploit the antimicrobial properties of silver nanoparticles.

V. ACKNOWLEDGMENT

We would like to acknowledge SAIF IIT Madras for characterization of nanoparticles, SRM University Madras and Manipal institute of technology for XRD and EDS analysis. I extend my thanks to Biogenics Hubli for their help to conduct antimicrobial experiments. The financial assistance from UGC, New Delhi in the form of UPE fellowship and DSA-I phase program of Department is greatly acknowledge.

VI. REFERENCES

1. Du L, Jiang H, Liu X, Wang E. Biosynthesis of gold nanoparticles assisted by *Escherichia coli* DH5a and its application on direct electrochemistry of haemoglobin. *Electrochemistry Communications* 2007; 9:1165-1170.
2. Sinha S, Pan I, Chanda P, Sen SK. Nanoparticles fabrication using ambient biological resources, *Journal of Applied Biosciences* 2009; 19: 1113 – 1130
3. Tachikawa S, Noguchi A, Tsuge T, Hara M, Odawara O, Wada H. Optical properties of ZnO nanoparticles capped with polymers, *Materials* 2011; 4:1132–1143.
4. Prabhu S, Poulse EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *Inter. Nano letters.* 2012; 2:32.
5. Ayman A. Abdel H., Medhat A., Ghobasy Al., Manal F., Mona B, et al. Phytosynthesis of Au, Ag, and Au-Ag Bimetallic Nanoparticles Using Aqueous Extract of Sago Pondweed (*Potamogeton pectinatus* L.) *ACS Sustainable chem. Eng.* 2013; 1:1520-1529. DOI: 10.1021/sc4000972.
6. Hedaginal B.R., and Taranath T. C. Fruit Mediated Synthesis of Silver Nanoparticles, Characterization and their Antimicrobial Activity using *Thunbergia alata* Bojer ex sims. *Ijppr.Human*, 2016; 7 (3), 52-69.
7. Kiruba S.G. Daniel, G. Vinothini, N. Subramanian, K. Nehru and Sivakumar M. “Biosynthesis of Cu, ZVI, and Ag nanoparticles using *Dodonaea viscosa* extract for antibacterial activity against human pathogens”. *Journal of Nanoparticle Research.* 15 (2013) 1-10.
8. Hedaginal B.R, and Taranath T. C., Phytosynthesis, characterization and antimicrobial activity of silver nanoparticles using *Solanum seaforthianum* Andr. *Int J Pharm Bio Sci.* 2016;7(3),185 – 194
9. Hedaginal B. R, Taranath T. C.. Phytosynthesis of Silver Nanoparticles by *Thunbergia fragrans* Roxb. And Their Characterization. *Int. J. Pharm. Sci. Rev. Res.*, 2016; 39(1), 54-58.
10. Hedaginal B. R., and Taranath T. C. Characterization and antimicrobial activity of biogenic silver nano-particles using leaf extract of *Thunbergia alata* bojer ex sims *Int. J. Pharm. Pharmace. Sci.* 2017; 8(5): 1000-12
11. Ahmad N, Sharma S, Alam MK, Singh VN, Shamsi SF, Mehta BR, Fatma A.,. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. *Colloids Surfaces B: biointerfaces*, 81(1), (2010) 81–86
12. Morones, J., Elechiguerra, JL, Camacho, A, Holt, K, Kouri, JB, Ramirez, JT, Yacaman, MJ. "The bactericidal effect of silver nanoparticles". *Nanotechnology*, 2005; **16**: p. 2346-2353.
13. Raimondi, F., Scherer, G.G., Kotz, R. and Wokaun, A. Nanoparticles in energy technology: examples from electrochemistry and catalysis. *Angew Chem Int Ed Engl.* 2005; 44, 2190–2209.
14. Taranath T. C., Hedaginal B. R., Rajani P Sindhu M. Phytosynthesis of Silver nanoparticles using the leaf extract of *Diospyros malabarica* (desr.) Kostel and its antibacterial activity against human pathogenic gram negative *Escherichia coli* and *Pseudomonas aeruginosa*. *Int. J. Pharm. Sci. Rev. Res.* 2015, 30(2), 109-114.