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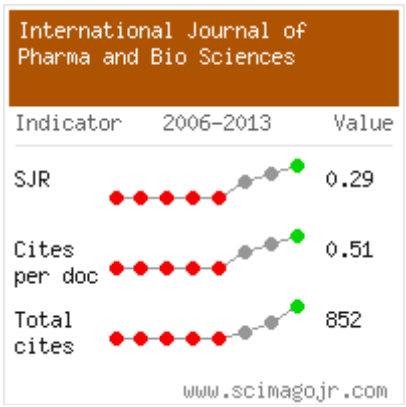

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COMPARATIVE STUDIES ON GREEN SYNTHESIS AND THERAPEUTIC APPLICATIONS OF SILVER NANO PARTICLES USING *FLACOURTIA SEPIARIA* AND *RHINACANTHUS NASUTUS*

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ABSTRACT

The aim of the study is to evaluate the therapeutic applications of silver nanoparticles synthesized using 2 medicinal plants collected from yelagiri hills. The leaves of the plants were used for optimization of silver nanoparticles by varying the time exposure of the reaction mixture to sunlight (5, 10, 15 minutes). The anti-oxidant, anti inflammatory and antimicrobial potentials of samples was studied by different assays. Also, the synthesized nanoparticles were characterized by UV, SEM, XRD and FTIR techniques. The results suggest that nanoparticles synthesis was significant at exposure time of 5 and 10minutes. The synthesized particles were confirmed by UV spectroscopy, which showed a characteristic peak at 427 and 418nm for the 2 samples respectively. The synthesized nanoparticles were found to be in the size range of 60-80nm and possessed characteristic XRD peaks. The results of the study revealed that the synthesized silver nanoparticles possessed significant antioxidant, anti inflammatory, anti-proliferative and antimicrobial properties.

KEYWORDS: **Flacourtia *sepiaria*, *Rhinacanthus nasutus*, SEM, XRD, FTIR, MCF7 cells.**

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INTRODUCTION

In recent years, the research towards nanotechnology has contributed many significant applications towards nanomedicine. Nanopartilces play vital role in the field of nanomedicine due their unique properties which are significantly different from those of different materials. These unique properties attributes to their small sizes and larger surface areas. Therefore, these nanoparticles have found a wide range of applications in the different fields, such as drug delivery, gene manipulations and tissue engineering. [1] Nanoparticles are mostly prepared from the noble metals such as Gold, Silver, Platinum and Lead using chemical methods. Among these metals, silver (Ag) is most evidently used in the field of biological systems, living organisms and medicine. Since silver nanoparticles are widely used in areas of human contact, there is a growing ability to develop environmentally safe processes for nanoparticles synthesis. Biological methods of synthesis silver nanoparticles have proven that it is significant methods due to slower kinetics, better manipulation and control over crystal formation and their stabilization. This upgraded the research on synthesis of silver nanoparticles that allow better control of shape and size for various nanotechnological applications.[2] Silver nanoparticles have been reported on various applications like detection and diagnosis, antimicrobial, anti inflammatory and many other therapeutic uses. These silver nanoparticles are also inducing cancer cells and suggest that nanoparticles synthesized from plants have been reported positively. Therefore, silver nanoparticles are much evident in the treatment of cancer.[3] The research deals with the green synthesis of silver nanoparticles of medicinal plants *Flacourtia sepiaria* and *Rhinacanthus nasutus* using sunlight method. The synthesized nanoparticles were characterized using UV, SEM, XRD, and FTIR techniques. In vitro screening of the anti oxidant activities are evaluated using different assays. Other applications like anti microbial, anti inflammatory and anti proliferative potential of the silver nanoparticles are evaluated.

MATERIALS AND METHODS

PLANT SAMPLE COLLECTION

Flacourtia sepiaria Roxb and *Rhinacanthus nasutus* (L.) Kurz were collected from the Yelagiri hills with knowledge of tribal people living in that region. The plants were collected based on their medicinal properties that were used by the tribal people in Yelagiri hills. The plants were taxonomically identified by Mr. S. Aroumougame, CAS in Botany, University of Madras.

SYNTHESIS OF SILVER NANO PARTICLES

Preparation of leaf extract

In the extraction process direct boiling method was used. Leaves were washed several times with de-ionized water. The extract used for the synthesis of silver nanoparticles was prepared by taking 10g of thoroughly washed finely cut leaves. Then it is boiled in 50ml of distilled water of each sample respectively. It is then filtered through Whatman No 1 filter paper. The filtrate was collected and stored at 4°C for further experiments. [4]

Optimization and production of silver nanoparticles by Sunlight Irradiation method

Silver nanoparticles were synthesized by exposing the reaction mixture containing plant extract and silver nitrate (1mM) in the ratio 1:9(w/v) to sunlight for different time intervals (5, 10, 15 minutes). The reduction of pure silver ions was monitored by UV - Vis spectrum of the reduction media. The reaction mixture was kept for overnight incubation and then centrifuged at 8000rpm for 20minutes to recover the silver nanoparticles. Bulk production of the silver nanoparticles was carried out from the optimized time.[4]

CHARACTERIZATION OF SILVER NANOPARTICLES

The synthesized silver nanoparticles were subjected to various characterization techniques such as UV-Vis spectroscopy, SEM, XRD and FTIR following standard methods.

SCREENING OF ANTIOXIDANT ACTIVITY OF AgNPs

In-vitroDPPH Free Radical Scavenging Assay

To the various concentrations of sample (50-250µg/ml) 1ml of DPPH (0.1mM in ethanol) was added and the reaction mixture was incubated in dark at room temperature for 15 minutes. The absorbance of the resulting solution was measured at 517nm. The reference standard used was tocopherol.

$$\% \text{ RSA} = \frac{\text{Absorbance (Cont.,)} - \text{Absorbance (sample)}}{\text{Absorbance (cont.,)}} \times 100$$

Hydroxyl Radical Scavenging Activity Assay

To various concentrations of sample (50-250µg/ml) 1ml of Fe-EDTA(0.13% ferrous ammonium sulphate + 0.26 % EDTA), 0.5ml of 0.018% EDTA and 1ml of 0.22% Ascorbic acid were added. The sample mixture was incubated at 90^0C for 15 minutes followed by the addition of 1ml of 17.5%ice cold TCA solution and 3ml of NASH reagent (7.5 g ammonium acetate +0.5 ml glacial acetic acid +0.2 ml acetone). The sample mixture was kept at room temperature for 15 minutes and the OD was measured at 412nm. Ascorbic acid was used as standard .[8]

$$\%\text{HRSA} = \frac{\text{Absorbance (Cont)} - \text{Absorbance (sample)}}{\text{Absorbance (cont)}} \times 100$$

SCREENING OF ANTIMICROBIAL ACTIVITY OF AgNPs

Agar well diffusion method

The antimicrobial activity of the synthesized AgNPs was studied using well diffusion assay. The antimicrobial efficacy of the samples was tested in a concentration range of 100-400µg/ml against *Bacillus subtilis* (MTCC 441), *Klebsiella pneumoniae* (MTCC 109), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Candia albicans* (MTCC 183) and *Candida tropicalis* (MTCC 184).

INVITRO SCREENING OF ANTI-INFLAMMATORY ACTIVITY

Inhibition of Albumin Denaturation

To various concentrations of sample GD water was added to make up the volume of the sample to 1ml. 1ml of 1% BSA was added to the mixture. It was incubated at room temperature for 20 minutes at dark condition. Then it was heated at 57°C for 30 minutes. Aspirin was used as the reference standard. The OD was measured at 660nm.[9]

$$\%\text{inhihibiton} = \frac{\text{Absorbance (Cont)} - \text{Absorbance (sample)}}{\text{Absorbance (cont)}} \times 100$$

ANTI PROLIFERATIVE POTENTIAL OF AgNPs

Cytotoxicity Assay on MCF-7 cell lines

Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modification. Briefly, MCF-7cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 hr, in 200µl of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (0.11–100µg/mL) of test compound was added and incubated for 48 hr. After treatment cells were incubated with MTT (10µl, 5mg/mL) at 37 ◦C for 4 hr and then with DMSO at room temperature for 1 hr. The plates were read at 595nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independentexperiments. Doxorubicin was used as reference standard.[10]

$$\text{Cell viability (\%)} = \frac{\text{Mean OD}}{\text{Control OD}} \times 100$$

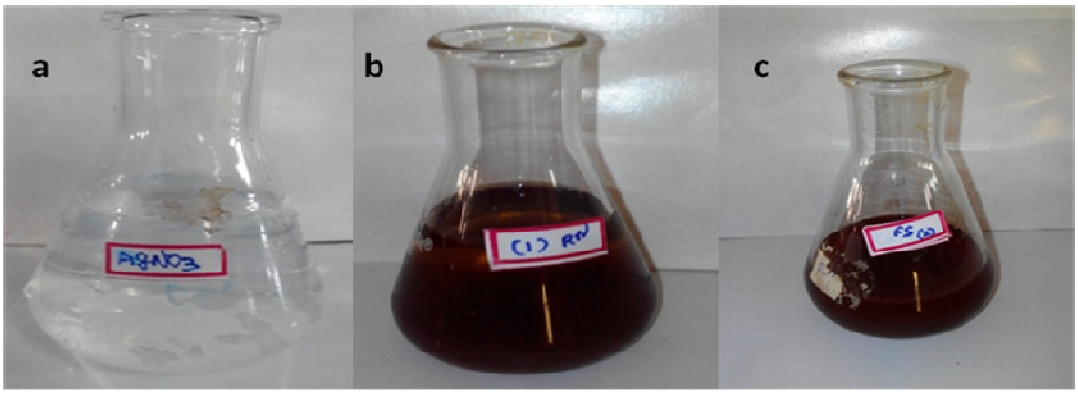
RESULTS

SYNTHESIS OF SILVER NANOPARTICLES

The aqueous extract and silver nitrate were mixed in the ratio of 1:9(v/v).After exposing to sunlight the bioreduction of silver nitrate was noted by the colour change from pale yellow to pale brown. This signifies that silver nanoparticles (AgNPs) were synthesized using different plant extract. The optimal time was recorded as 5 minutes for both plants *F. sepiaria* and *R. nasutus.* The AgNPs thus recovered from *F. sepiaria* and *R. nasutus* were denoted as samples A and B, respectively .

Figure 1

1mM Silver Nitrate Solution and its Bioreduction by F. sepiaria and R. Nasutus



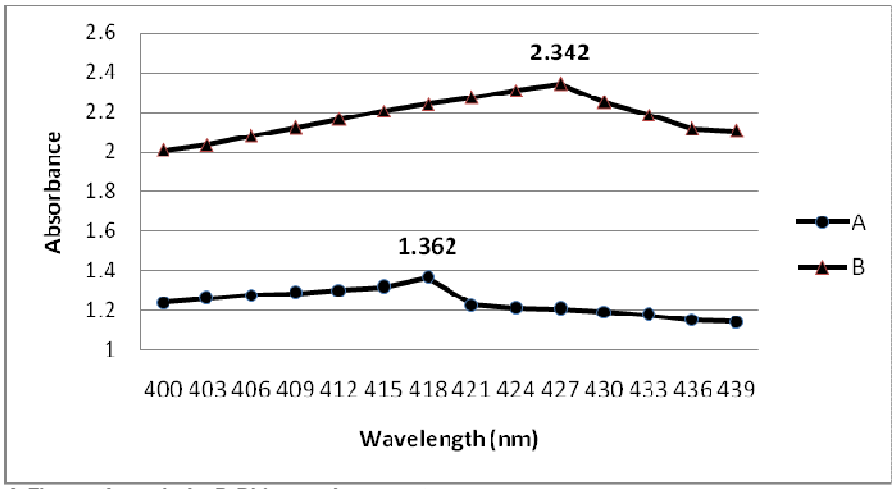
a – Silver nitrate; b, c – Colour change in AgNO3 by addition of F. sepiaria (b) and R. nasutus (c) extract after exposure to sunlight

UV-Vis spectral analysis

Sunlight irradiated reaction mixture showed a strong characteristic absorbance peak at around 427, 418 for the samples A and B respectively.

Figure 2

UV- Vis spectra of Synthesized AgNPs



A-Flacourtia sepiaria, B-Rhinacanthus nasutus

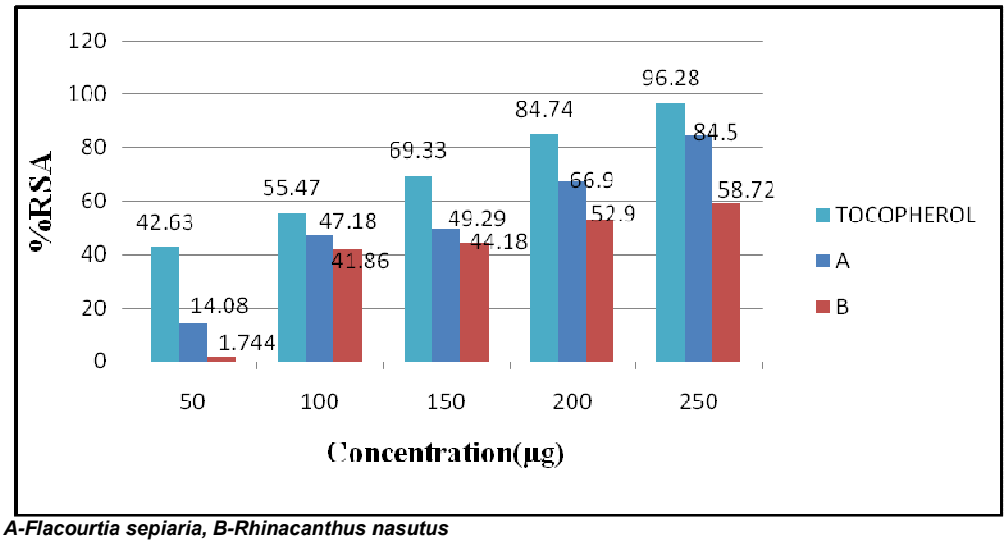
IN-VITRO ANTI OXIDANT ACTIVITY

DPPH free Radical scavenging Activity

The results of DPPH assay revealed that the synthesized AgNPs possessed significant antioxidant potential. The RSA was studied to be in the range of 14-84% and 1-58% for samples A and B respectively. Also, the IC_{50}values for the samples were recorded to be 150, 200µg. The data also

suggests that among the 2 samples, the AgNPs synthesized using sample A was more potent in scavenging free radicals and was much comparable with the standard used.

Figure 3
DPPH Radical Scavenging Activity ofAgNPs

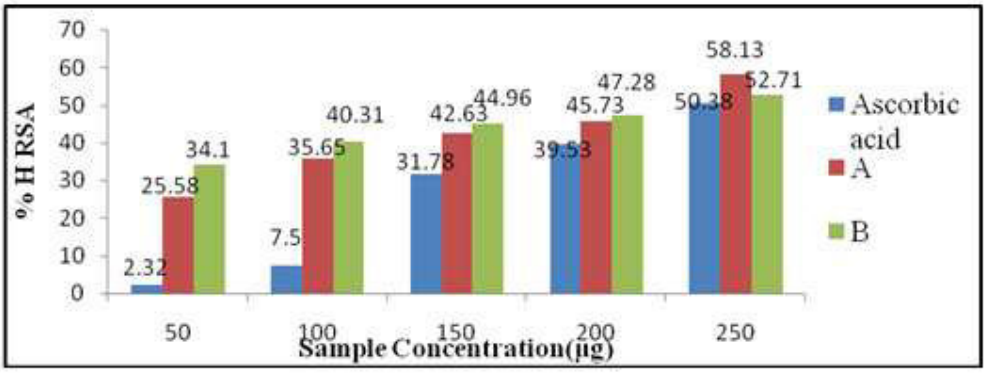


A-Flacourtia sepiaria, B-Rhinacanthus nasutus

Hydroxyl radical scavenging activity

The results of HRSA revealed that the synthesized AgNPs possessed significant hydroxyl radical scavenging potential. The HRSA was studied to be in the range of 25-58% and 34-52%, for samples A and B, respectively. Also, the IC_{50}values for the samples were recorded to be 215, 237µg. The data also suggests that among the 2 samples, the AgNPs synthesized using sample A was more potent in scavenging hydroxyl free radicals and was much comparable with the standard used.

Figure 4
Hydroxyl Radical Scavenging activity of AgNPs



A-Flacourtia sepiaria, B-Rhinacanthus nasutus

ANTI MICROBIAL ACTIVITY OF AgNPs

Anti-bacterial activity

The inhibitory effect of the synthesized nanoparticles on bacterial pathogens was studied. The results indicated that the particles possessed maximum inhibitory activity on all the tested pathogens (Table 1). It was also noted that the antibacterial action of the AgNPs was much greater than that of the standard antibiotic used (Cefotaxime). Among the 2 samples tested, sample B exhibited maximum inhibitory action on the bacterial pathogens with maximum ZOI of 18, 12, 13, and 23.5mm against *B. subtilis*, *E. coli*, *K. pneumonia* and *P. aeruginosa*, respectively. The ZOI of standard antibiotic was recorded as 10, 10.5, 13.5, and 14mm against *B. subtilis*, *E. coli*, *K. Pneumonia* and *P.*

aeruginosa, respectively at a concentration of 250µg.

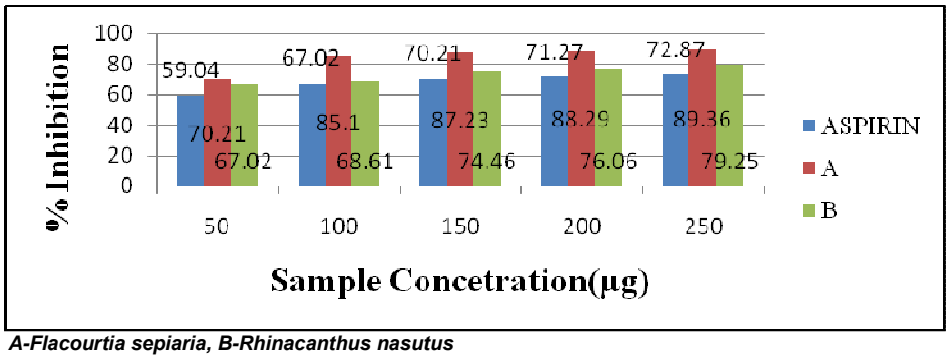
Anti fungal activity

The inhibitory effect of the synthesized nanoparticles on fungal pathogens was studied. The results indicated that the particles synthesized from plants A and B possessed moderate inhibitory action on the fungal pathogens. Among the fungal pathogen *C. albicans* was inhibited by samples A and B. *C. tropicalis* was only inhibited by sample A but it was not inhibited by sample B (Table 2).

INVITRO ANTI INFLAMMATORY ACTIVITY

The results of inhibition of albumin denaturation revealed that the synthesized AgNPs possessed significant anti inflammatory potential. The maximum inhibition was studied to be in the range of 90.42 and 79.25 for samples A and B, respectively. Also, the IC_{50}values for the samples were recorded to be 138, 157µg. The data also suggests that among the 2 samples, the AgNPs synthesized using sample A was more potent in inhibition of albumin denaturation and was much comparable with the standard used.

Figure 5
Anti inflammatory effect of AgNPs



A-Flacourtia sepiaria, B-Rhinacanthus nasutus

CYTOTOXICITY OF THE AgNPs ON MCF7 CELLS

The cytotoxic effect of the silver nanoparticles synthesized from *F. sepiaria* was studied by MTT assay. The results indicate that the sample had significant toxicity on liver cancer cells. The silver nanoparticles from *F. sepiaria* reduced the viability of MCF 7 cells from 82 to 40% in the concentration range of 1ng to 100µg. The IC_{50}value was studied to be 83.3µg/ml and 108.69µg/ml for *F. sepiaria* and standard respectively.

Figure 6
Cytotoxic Effect of AgNPs from F. sepiaria on MCF7 Cells using MTT assay

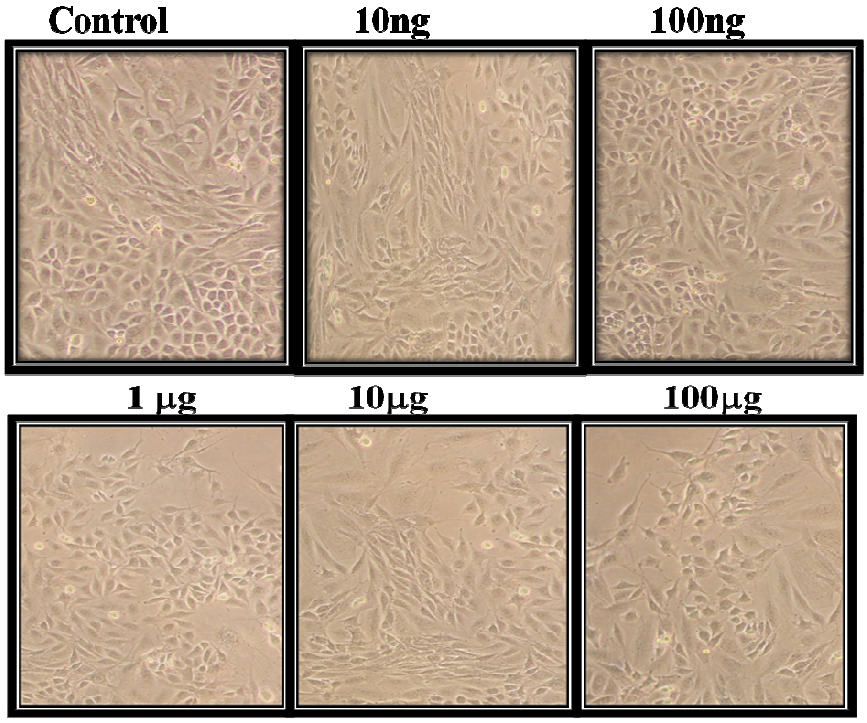
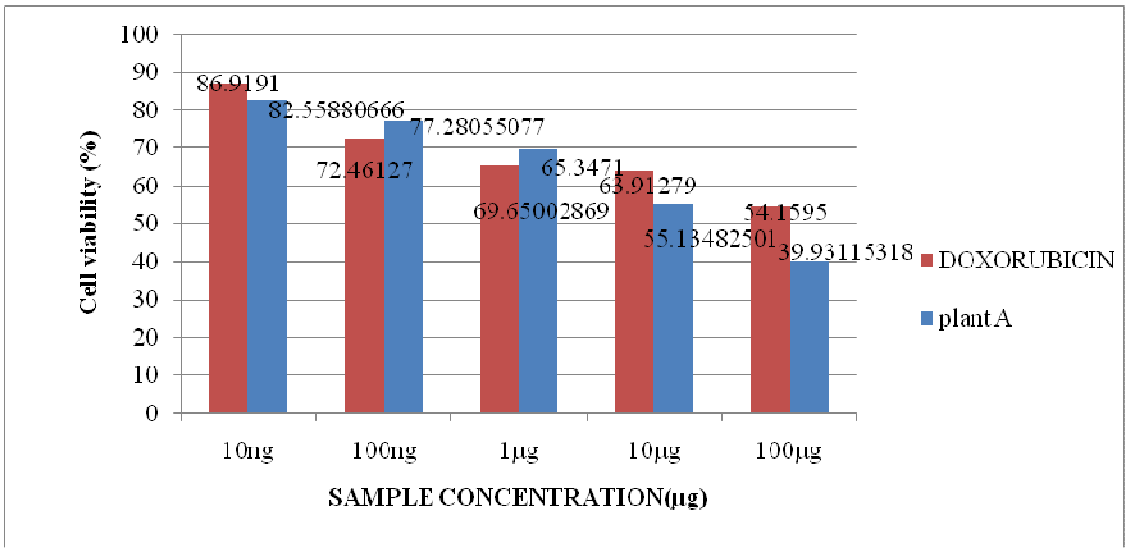
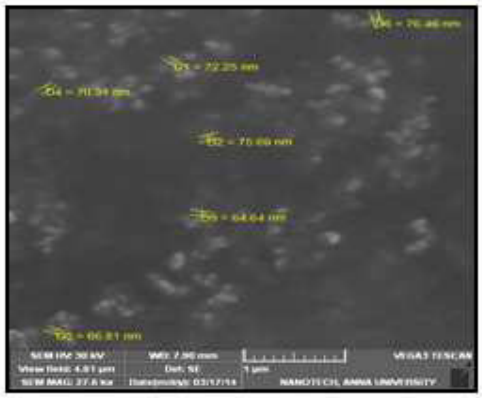


Figure 7
Cytotoxicity caused by AgNPs on MCF7 Cells



CHARACTERIZATION OF SILVER NANOPARTICLES
Scanning Electron Microscopy
SEM image showed relatively spherical shaped particles for the plants *F.sepiaria*in the range of 60-80 nm.

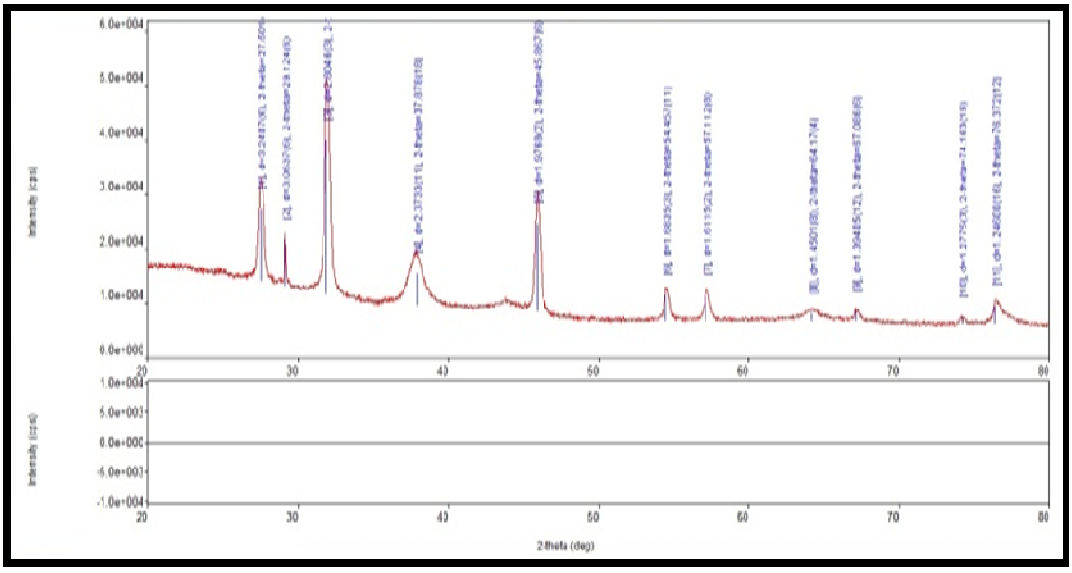
Figure 8
SEM Image of AgNPs from F.sepiaria



X-Ray Diffraction

From the XRD curve it is significant that the synthesized particles are silver nanoparticles which is evident from the characteristic peaks at 37.87 and 45.86 for *F.sepiaria.*

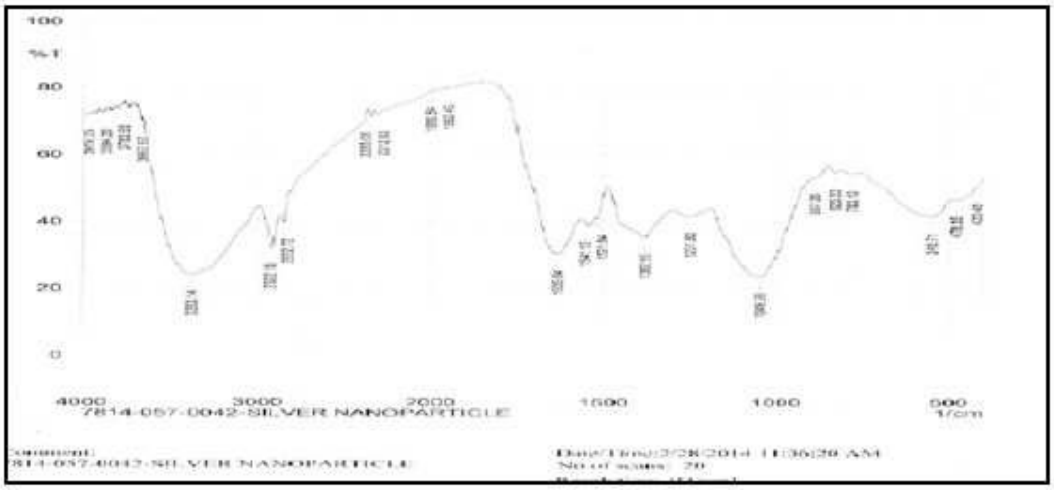
Figure 9
Characterstic Peaks of XRD from AgNPs of F.sepiaria



Fourier Transform Infra Red Spectroscopy

FTIR measurement was carried out to identify possible biomolecules of *F. sapiaria* leaf extact responsible for the formation and stabilization of nanoparticles.

Figure 10
FTIR analysis of AgNPs from F.sepiaria



DISCUSSION

The morphology of the silver nanoparticles was obtained through characterization using SEM. SEM image showed relatively spherical shaped particles in the range 60-80 nm which is comparatively higher than size of AgNPs synthesized using *Cynodon dactylon* which is 30-60nm.[4]The XRD pattern showed two intense peaks in the whole spectrum 2θ values ranging from 20-90 whereas in *Cynodon dactylon* showed three intense diffraction peaks from 10-70. In the FT-IR analysis bands were indicates the presence of alkanes, alkynes, amines, aliphatic amines, alkyl halides whereas in *Cynodon dactylon* showed functional groups such as alkanes, phenols, carboxlic acid groups, nitro compounds, alcohol, esters and ethers.[4]DPPH assay has been widely used to determine the free radical scavenging activity of various plants. The polar fraction of *D. viscosa* has shown potent antioxidant activity with IC_{50} value of 50µg/ml.[11] Similarly methanol extract of *R. nasutus* has also showed significant DPPH radical scavenging activity.[12] It is evident from the results that the antioxidant potential of the silver nanoparticles might be acquired from the plant extract which was used for reducing silver nitrate to elemental silver. Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects. The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria and fungi of selected species.[13] The silver NPs of the selected 2 medicinal plants exhibited maximum antibacterial activity and moderate antifungal activity. The ionic silver strongly interacts with thiol group of vital enzymes and inactivates the enzyme activity. Experimental evidence indicates that DNA loses its replication ability once the bacteria have been treated with silver ions. It is mentioned that the pathogenic effect of nanoparticles can be attributed to their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the pathogen proteins and arrests its growth.It has been reported that Nano-Ag breaks down the membrane permeability barrier of *C.albicans*, it is possible that nano-Ag perturbs the membrane lipid bilayers, causing the leakage of ions and other materials as well as forming pores and dissipating the electrical potential of the membrane.[14] This explains the mechanism behind the antifungal potential of the selected NPs.

CONCLUSION

Thus the plants collected showed significant activities in medicinal aspects yet further mechanistic studies are necessary to prove the results *invivo.* Therefore the synthesized nanoparticles from the plants are environmentally safe which can be considered for use in medicinal applications.

ACKNOWLEDGEMENT

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