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Studies on Green Synthesis, Characterization and Anti-proliferative Potential of Silver Nano Particle using *Dodonaea viscosa* and *Capparis decidua*

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The aim of this study is to evaluate the anti-proliferative activity of silver nanoparticles synthesized using *D.viscosa* and *C.decidua*. 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 potential 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 10 minutes. The synthesized particles were confirmed by UV spectroscopy which showed characteristic peak at 421nm for both the samples. The synthesized nanoparticles were found to be in the size range of 60-90nm 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.

Key words: Silver nanoparticles, Anti inflammatory activity,

Nanotechnology plays a vital role in technologies of new millennium. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine and water treatment[1]. Nanosilver can be used as a colloid and also it is used in the textile industry by incorporating it into the fiber. There are many consumer products and applications utilizing nanosilver in consumer products; nanosilver plays major role in bringing out commercial products[2]. Chemical and physical methods may successfully produce pure, well-defined nanoparticles, but these techniques are more expensive, energy consuming and potentially toxic to the environment. Biosynthetic methods can employ either microorganism cells or plant extract for nanoparticles production. An exciting branch of biosynthesis of nanoparticles is the application of plant extracts to the biosynthesis reaction. Recently, the green processes for the synthesis of nanoparticles are evolving into an important branch of nanotechnology[3].

The study of this paper deals with synthesis of silver nanoparticles from the medicinal plants. The medicinal plants were collected from yelagiri hills with the knowledge of tribal people. The medicinal values of the selected plants were evaluated by biological synthesis of the silver nanoparticles. Silver is used as the reducing factor to synthesis silver nanoparticles. The various

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applications of the selected plants were evaluated using invitro techniques. The plants selected showed much anti proliferative activity. The study also reveals that it has wide range of application against microbial strains, inflammation, etc.

MATERIALS AND METHODS

Plant sample collection

Dodonaea viscosa and *Capparis decidua* these two plants were collected from the yelagiri hills with knowledge of tribal people living in that region. Based on their suggestion of plants materials were collected which were free from pests and disease caused plants. The plants were collected based on their medicinal properties that were used by the tribal people in yelagiri hills

Preparation of leaf extract

For 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 20g of thoroughly washed finely cut of plant leaves. Then it is boiled in 100ml 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].

Synthesis of silver nano particles

Optimization and production of silver nanoparticles by Sunlight Irradiation method

To the 1ml of leaf extract, 9ml of the 1mM silver nitrate solution was added with constant stirring. The conical flask was exposed to the sunlight at different time intervals (5, 10, 15 minutes). The reduction of pure silver ions were monitored by UV - Vis spectrum of the reduction media. The solution mixture was kept for overnight incubation. The overnight samples were centrifuged at 8000rpm for 20 minutes to recover the silver nanoparticles from leaf extracts. 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-vitro DPPH 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.[5]

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

Phosphomolybdenum Assay

To the various concentrations of sample (50-250¼g/ml), 3ml of reagent solution containing 0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate was added. It was incubated at 95^{0}C for 90 minutes and the absorbance of the mixture was then measured at 695nm. Ascorbic acid was used as the standard.[6]

Metal Ion Chelating Assay

To various concentrations of sample (50-250¼g/ml), 100µl of 2mM ferrous chloride and 200µlof 5mM ferrozine were added. The mixture was incubated at room temperature for 10minutes at dark condition. EDTA was used as the reference standard. The OD was measured at 562nm.[7]

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

Hydroxyl Radical Scavenging Activity Assay

To various concentrations of sample (50-250¼g/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^{0}C 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]

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

Screening of antimicrobial activity of AgNPs

Screening of Antibacterial and Anti fungal Activity - Agar well diffusion method

The antimicrobial activity of the synthesized AgNPs was studied using well

diffusion assay as demonstrated by[9]. The antimicrobial efficacy of the samples was tested in a concentration range of 100-400µg/ml.

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 57^0 C for 30 minutes. Aspirin was used as the reference standard. The OD was measured at 660nm.[10]

%inhihibiton= Absorbance (Cont) - Absorbance (sample)×100

Absorbance (cont)

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 æ%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 independent experiments. Doxorubicin was used as reference standard.[11]

Cell viability (%) = Mean OD x 100
Control OD

RESULTS

Preparation of aqueous extract

By Direct Boiling Method the above mentioned medicinal plants extract was prepared and stored in 4^0C for future experiment.

Synthesis of silver nanoparticles

Optimization and Bulk Production 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 were synthesized using different plant extract. The optimized time was recorded as 5 minutes for both plants *D.viscosa* and *C.decidua.*

UV-Vis spectral analysis

Sunlight irradiated reaction mixture showed a strong characteristic absorbance peak at around 421 for both the samples A and B respectively.

A-*Dodonaea viscosa,* B-*Capparis decidua*

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 7-78% and 1-51% for samples A and B respectively. Also, the IC_{50}values for the samples were recorded to be 200, 250µg. 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.

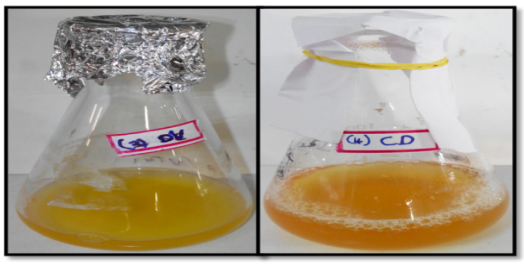

Fig. 1. Aqueous extracts of *D.viscosa* and *C.decidua*

Fig. 2. 1mM Silver Nitrate Solution

Phosphomolybdenum reducing activity

The phosphomolybdenum reducing potential of the tested samples was found to be significant which is evident from the increase in the absorbance values of the test samples.

A-*Dodonaea viscosa,* B-*Capparis decidua*

Metal ion chelating activity

The results of metal chelating assay revealed that the synthesized AgNPs possessed significant metal chelating potential. The metal chelating ability was studied to be in the range of 5-63% and 38-70% for samples A and B, respectively. Also, the IC_{50} values for the samples were recorded to be 200, 100µg. The data also suggests that among the 2 samples, the AgNPs synthesized using sample B was more potent in chelating ferrous metal ions and was much comparable with the standard used.

Fig. 3. Bioreduction of Silver Nitrate By *D.viscosa* and *C.decidua*

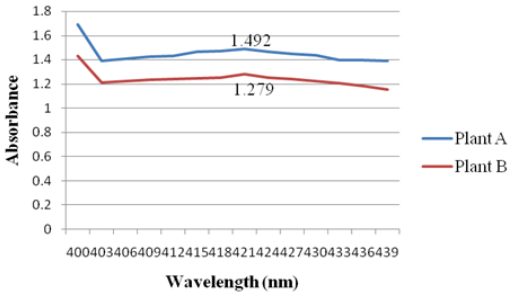

Fig. 4. UV- Vis spectral of Synthesized AgNPs

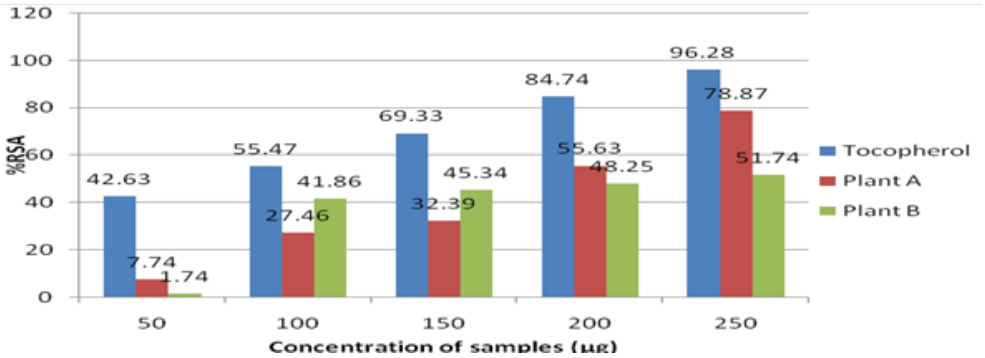

Fig. 5. DPPH Radical Scavenging Activity ofAgNPs

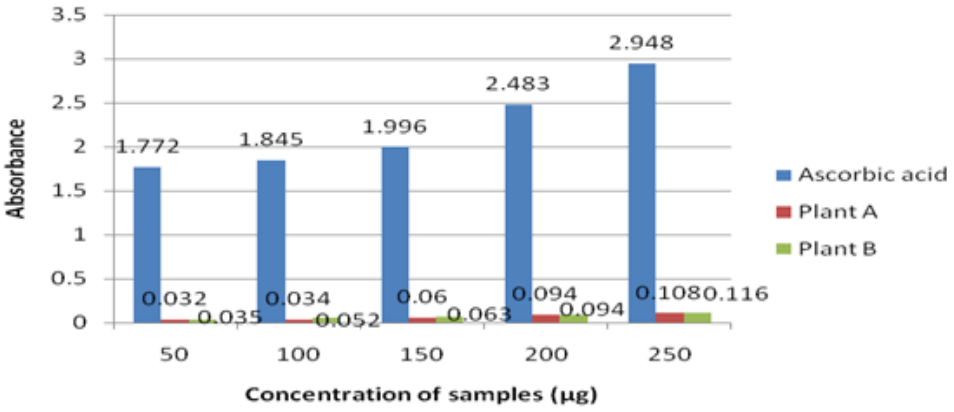

Fig. 6. Phosphomolybendum reducing activity ofAgNP

A-*Dodonaea viscosa,* B-*Capparis decidua*

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 42-70% and 47-72% for samples A and B, respectively. Also, the IC_{50}values for the samples were recorded to be 177, 173µg. The data also suggests that among the 2 samples, the AgNPs synthesized using sample B was more potent in scavenging hydroxyl free radicals and was much comparable with the standard used.

A-*Dodonaea viscosa,* B-*Capparis deciduas*

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. 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 12.5, 13, 10.5, and 13.5mm against *B. subtilis*, *E. coli*, *K. pneumonia* and *P. aeruginosa*, respectively. The ZOI of standard

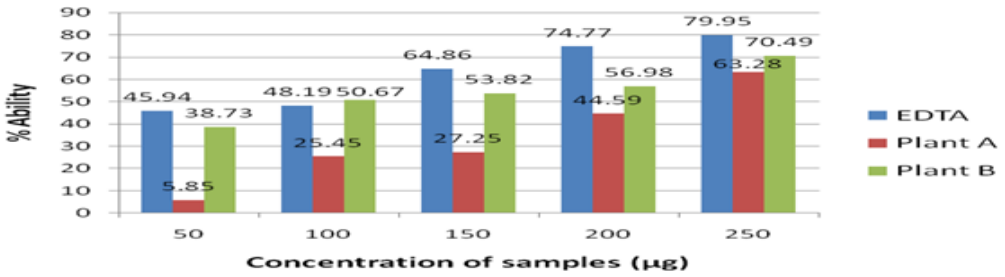

Fig. 7. Ferrous Chelating ability of AgNPs

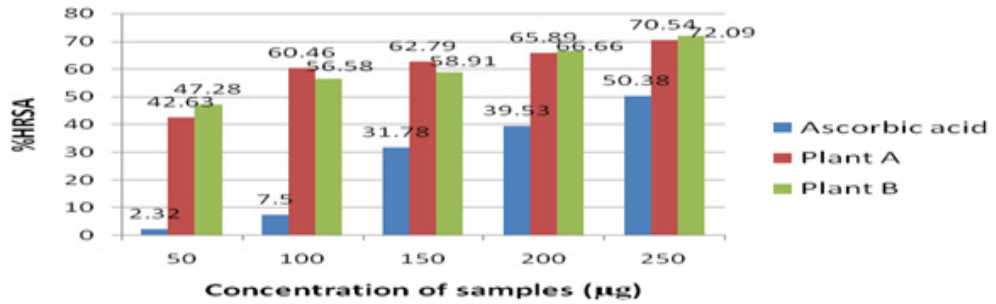

Fig. 8. Hydroxyl Radical Scavenging activity of AgNPs

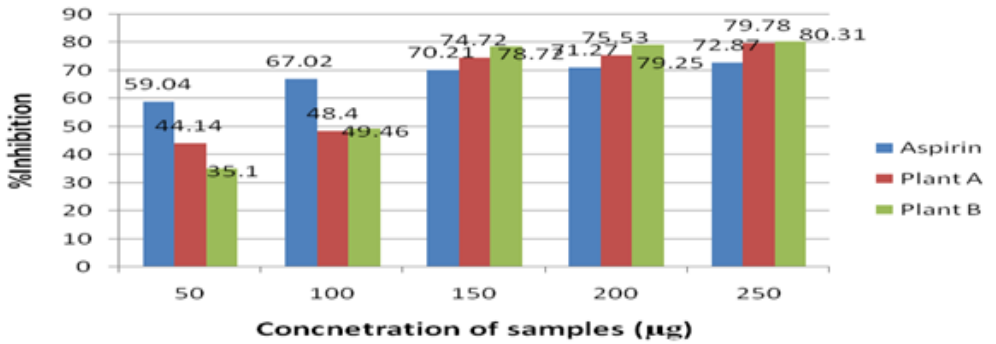

Fig. 9. Anti inflammatory effect of AgNPs

Fig. 10. Cytotoxic Effect of AgNPs from *D.viscosa* on MCF7 Cells using MTT assay

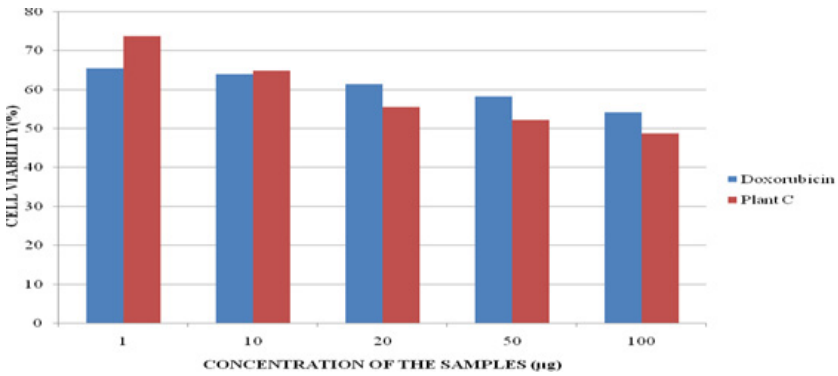

Fig. 11. Cytotoxicity developed by AgNPs on MCF7 Cells

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 plant A possessed moderate inhibitory action whereas plant B didn't show any activity on the fungal pathogens.

A-*Dodonaea viscosa,* B-*Capparis decidua*

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 79.78 and 80.31for samples A and B, respectively. Also, the IC_{50}values for the samples were recorded to be100µg for both the samples. The data also suggests that among the 2 samples, the AgNPs synthesized using both samples was equivalent potent in inhibition of albumin denaturation and was much comparable with the

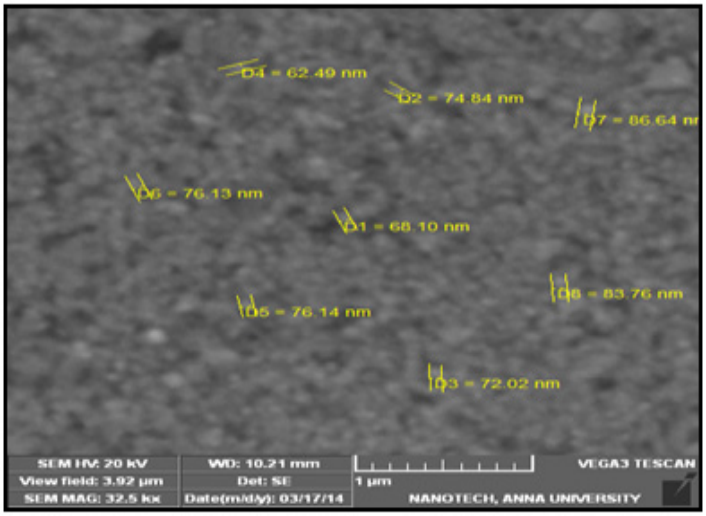

Fig. 12. SEM Image of AgNPs from *D.viscosa*

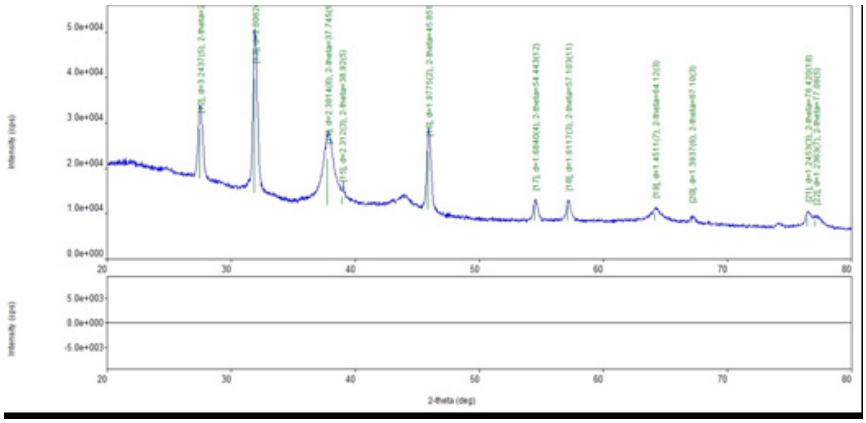

Fig. 13. Characterstic Peaks of XRD from AgNPs of *D.viscosa*

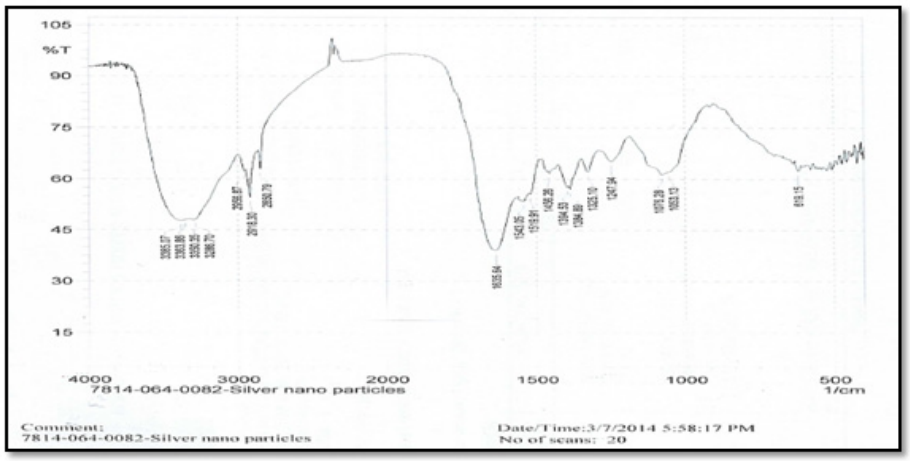

Fig. 14. FTIR analysis of AgNPs from *D.viscosa*

standard used.

A-*Dodonaea viscosa,* B-*Capparis decidua*

Cytotoxicity of the on MCF7 cells

The cytotoxic effect of the synthesized silver nanoparticles was studied by MTT assay. The results indicate that the samples had significant toxicity on liver cancer cells. The silver nanoparticles from *D.viscosa* reduced the viability of MCF 7 cells from 74 to 49% in the concentration range of 1ng to 100µg. The IC_{50}value was studied to be 98.03µg/ml and 108.69µg/ml for *D.viscosa* and standard respectively.

Scanning Electron Microscopy

SEM image showed relatively spherical shaped particles for the plants *D.viscosa* in the range of 60-90 nm.

X-Ray Diffraction

From the XRD curve it is significant that the synthesized particles are silver nanoparticles which was evident from the characteristic peaks at 37.74 and 45.85 for *D.viscosa.*

Fourier Transform Infra Red Spectroscopy

FTIR measurement was carried out to identify possible biomolecules of *D.viscosa* leaf extract responsible for the formation and stabilization of nanoparticles.

DISCUSSION

The morphology of the silver nanoparticles was obtained through characterization using SEM. SEM image showed relatively spherical shaped particles in the range 60-90 nm which is comparatively higher than size of AgNPs synthesized using *Parthenium hysterophorus*[12].

The XRD pattern showed two intense peaks in the whole spectrum 2¸ 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[14].

DPPH assay has been widely used to determine the free radical scavenging activity of various plants. The polar fraction of *M.arundinacea* has shown potent antioxidant activity[13]. Similarly methanol extract of *R. nasutus* has also showed significant DPPH radical scavenging activity[14]. 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[15]. 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[16]. 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 use in the medicinal field.

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