

Biosynthetic Greener Move towards Nanosized Silver by Diospyros Montana Leaf Extract and Their Antimicrobial and Antioxidant Activities



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Abstract: Synthesis of nanomaterials by eco-friendly method is being exploited by chemists because of several advantages over other conventional techniques. The current investigation reports the green approach for Diospyros Montana (DM) silver nanosized particles which has high antimicrobial and antioxidant activities. The synthesized nanosized particles were investigated by several techniques such as IR, Ultra violet, X-Ray Diffraction, Transmission Electron Microscope and AFM studies. Crystalline size of the silver nanoparticle is in the range of 47.05 nm which was clear from the powder X-rd studies. The silver nanoparticles prepared were tested for their antimicrobial potential against *E.coli*, *B.subtilis*, *C.albicans* and *A.flavus* species and was found to reveal excellent activity because of its volume ratio and high surface. The DPPH assay was used to find scavenging activity of free radicals. In this work we report the silver nanoparticles by aqueous Diospyros Montana leaf extract and its potential for free radical scavenging.

Keywords : AFM, antimicrobial studies, leaf extract, silver Nanoparticles, TEM , XRD.

I. INTRODUCTION

Nanotechnology is the most promising technology over the past few decades and it is applied in many fields ranging from cosmetics, electronics, food packaging, chemical and mechanical industries. It is also used in NLO devices¹⁻⁶. Different kind of nanoparticles like metallic nanoparticles, metal oxide nanoparticles and non-metallic nanoparticles are synthesized by different methods like chemical, physical and bioorganic methods. In the biosynthesis metal nanosized particles are either oxidised or reduced⁷. In recent years green synthesis of nanoparticles is attracted by chemists than routine traditional conventional synthesis since this type of synthesis is ecofriendly.

Biosynthesis of nonsized material's by unicellular and multicellular organisms like bacteria, fungi and viruses are also known⁸⁻¹¹. The nanoparticles synthesized by green approach are non-toxic and has extensive uses. The presence of photochemicals in leaf extracts perform the function as reducing agent, oxidizing agent and stabilizing agent for the preparation of nanomaterials.

Metallic nanoparticles find wide applications than non-metallic nanoparticles owing to their unique excellent properties which make them appropriate for vast applications. Depending on their size and distribution both silver and gold nanoparticles possess magnetic, optical, electronic and antibacterial activity¹²⁻¹⁴ which makes them a potential medicinal tool for pharmaceutical researchers. An easier and routine chemical technique is used for synthesis of silver nanosized particles but due to the contamination of the nanoparticles it limits the usage in biomedical applications¹⁵. Hence the non-toxic green synthesis is needed to utilize the antimicrobial properties of silver nanoparticles. Hence green chemical method seems to be advantages for the synthesis on silver nanoparticles.

After synthesis of metal nano particles, characterization of NP's is very important because the physicochemical properties plays a key role in determining biological properties¹⁶⁻¹⁷. A particle size, shape, method of preparation, functional groups are the various parameters which should be determined before considering the toxicity of the synthesized nanoparticles. The presence of redox property present in the leaf extract determines the surface morphology of metal nano particles.

Surgical site infections are due several bacteria and viruses. These infections are mainly associated with joint implants and orthopedic implants. The detection of such kind of infections are very difficult to identify in the early stage because we cannot able recognize the growth of bacteria and viruses. Such microorganisms can infect the surgical devices by three different ways: 1. At the time of implantation through direct inoculation 2. Through hematogenous seeding 3. From adjacent infectious focus¹⁸⁻¹⁹. Silver nano particles are usually having significant antibacterial activities and it prevents any kind of infections & controls spoilage. Silver shows a wide spectrum of activity and it is active for more than 600 pathogens including bacteria's such as Gram positive and negative^{20,21} in the era of multi drug resistance. The increase in oxidative pressure tends to the differences in the level of antioxidant via the oxidation of Deoxyribo nucleic acid and Ribonucleic acid molecule upon exposing the bacteria's to silver nano particles²².

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Sangiliyandi et al showed that bimolecular assisted nanosized particles are proficient against pathogenic bacteria with minimum side effects. Treatment of postsurgical infection is highly impossible without using antibiotics²³⁻²⁴. The antimicrobial activity of electrochemically synthesized silver doped nano composites which shows non-cytotoxicity towards peripheral blood mononuclear cells which shows antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*²⁵.

Non-conventional treatments for antimicrobial infections consist of phyto, naturopathic therapy, antimicrobial peptides, using minute molecular inhibitors and usage of metal nanoparticles. Earlier, the use of AgNPs was recommended against microbial infections. Their antimicrobial effect blocks the enzyme respiratory pathways by interacting with DNA of sulphur-containing proteins. The silver and silver ion-containing materials are consequently, used in number of biomedical applications.

II. MATERIALS AND METHODS

2.1. Chemicals

The tree leaf sample of *Diospyros Montana* were used, for the preparation of the silver nanosized materials. The collected leaves belongs to the region of Kinathukadavu area, Coimbatore, Tamil Nadu, India. Silver nitrate used in this present work were purchased from Aldrich. Double distilled water was used for the whole experiment.. The leaves of the selected species are shown in the Fig-1.



Fig-1(Image of Diospyros Montana leaf)

2.2.Preparation of the sample extract:

Diospyros Montana leaves were given double distilled water wash to avoid the foreign impurities and then cut into fine pieces. About 20 gms of these leaves were weighed and then dried under sunshade and crushed using mechanical grinder and stored in air tight container. About 15grams of the grinded leaf along with 150mL of distilled water was heated to boiling at 80°C for 5hours and cooled slowly to the room temperature. The Whatmann No.1 filter paper were used to filter the extract in order to get pure solution. The sample thus obtained was kept in cooler at 4°C for future work.

2.3.Synthesis of Ag Nano Particles

Silver nanosized particles from Silver nitrate were obtained by adopting the procedure of Shakeel Ahmel et.al.,. The synthesis process involves 2 stages. In the I stage 1mM concentration of AgNO₃ was prepared using deionised water. In the next stage, to the AgNO₃ solution, the plant extract was added. To a series of 10ml of AgNO₃ solution, a volume of 1,2,3,4 & 5 ml of the plant extract was added. All these

reactions were carried out at room temperature at constant pressure in a dark compartment. Upon addition of the extract a yellow color developed immediately showing the reaction progress. The color extends from top to bottom showing the reduction of silver nitrate to silver .

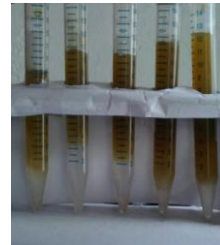


Fig 2. Silver nitrate solution

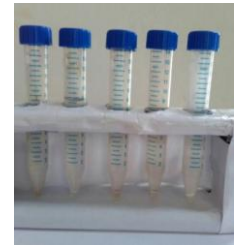


Fig 3. Plant leaf extract + Silver nitrate

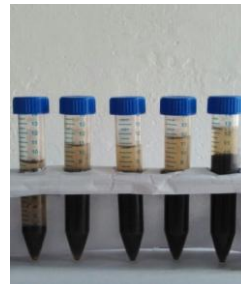


Fig 4. Leaf extract + Silver nitrate



Fig 5. Leaf extract + Silver nitrate (5 min)

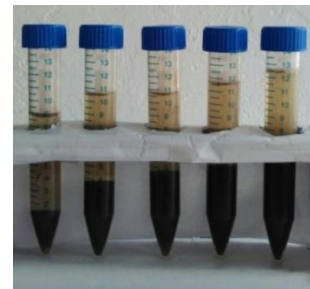


Fig 6. Leaf extract + Silver nitrate after 15 minutes

2.4. Physico Chemical Experimental techniques of Silver Nanoparticles:

The development of the proposed silver nanosized particles were inferred through observations like Optical spectroscopy, FTIR, XRD, EDAX, TEM and AFM studies. Optical spectral study were done by UV-3000⁺, between 190nm and 900 nm with a resolution of 2 nm. The IR spectra of the pure extract, extract with silver nanoparticles and silver nanosized particles were recorded on ATR-FTIR-IR affinity 1, spectrometer, between the wavelength interval of 4000 to 400cm⁻¹.The crystalline nature of prepared Ag nanosized particles were detected using X-Ray Diffractometer which is performed in the 2θ range of 0-100 at 45kV and 30mA with a divergence slit of 0.2177. The powder X – ray diffraction was done by the Analytical analyzer . The crystalline size can be measured using Scherer formula. TEM analysis was taken for the morphological studies using JEOL JEM2100 electron microscope. The elemental analysis done by an analytical technique such as Energy dispersive x-ray(EDX).

Then the antimicrobial studies were performed by the disk diffusion method that was cultured on agar plates with selected samples of *E.coli* and *Basillus subtilis* bacterias and fungal strains as *Canadiaalbicans* and *Aspergillusflavus*. Antioxidant activity was carried for the prepared nano particles.

III. RESULT AND DISCUSSION

3.1. UV-Visible Spectroscopy

The stability and presence of Ag nanosized particles was studied through UV spectrum which is shown in the fig-7. The adding up of *Diospyros montana* sample into aqueous solution of $AgNO_3$ in small quantities led to change the colour of the solution starting from yellow to reddish brown. The optical spectra was recorded, once the silver nanosized particle preparation was over. It is due to the surface Plasmon vibration property of silver nanoparticles. The SPR peak of silver nano particle was appeared at 207nm, which was similar to the results obtained by Nath et al²⁶. By means of raise in particle size, the peak value were found to be the same gradually, due to the oxidation of silver nano particles the surface plasma resonance effect decreases with time²⁷. The absorbtion peak of UV- Vis at 207 nm confirms the presence of silver nanoparticles.

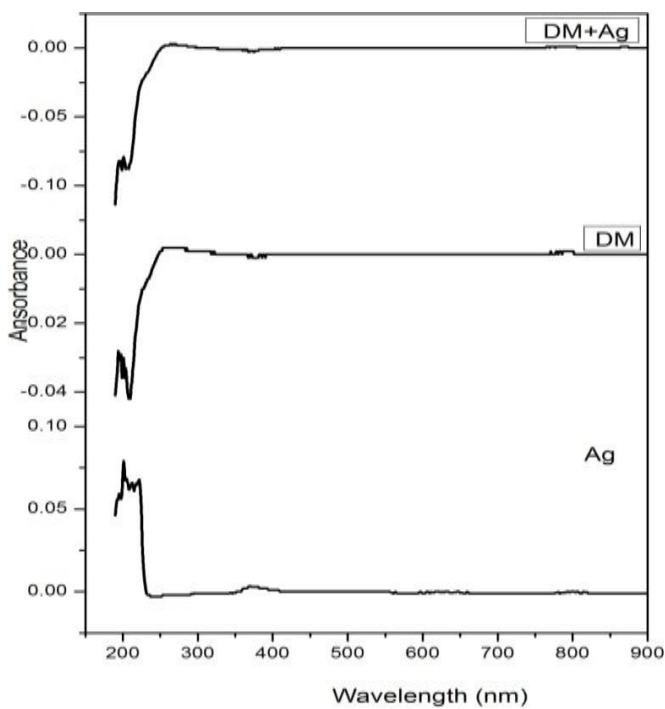


Fig-7 UV- Spectra of the samples

3.2. Fourier-transform infrared spectroscopy

IR measurements of the aqueous *Diospyros Montana*, silver nanoparticles and pure silver were conceded to recognize the metabolites which is accountable conversion of Ag^{2+} to Ag^+ ions and formation of the biosynthesized silver nanoparticles from the *Diospyros Montana* leaf sample. The band at 3202 cm^{-1} and at 1540 cm^{-1} due to the N-H and C=O stretching frequency respectively in the plant extract. It is shifted to 3344 and 1638 cm^{-1} in the spectra of Silver nanoparticles which may be suitable to the existence of high activity multi phenolic groups in the leaf sample. Apart from this occurrence of extra bands at 1372 cm^{-1} and 1227 cm^{-1} shows the configuration of silver nanosized particles. The IR

spectra of Ag nanoparticles, leaf extract, silver are shown in the fig-8.

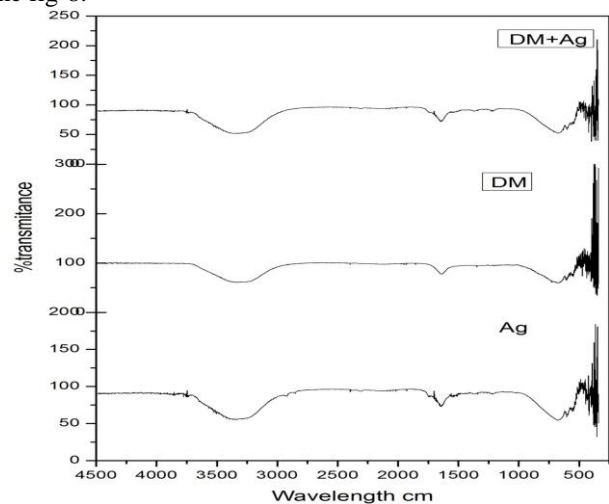


Fig-8 FTIR- spectrum of *Diospyros montana*, *Diospyros montana*+ silver nitrate, Solid silver nanoparticle

3.3. Structural Characterization and morphology

The structural analysis was carried out using powder XRD analysis. The Diffraction pattern with d spacing of the prepared Ag nanoparticles obtained using *Diospyros montana* leaf extract was shown in the fig 9. The diffracted angles at 2θ level from 0° to 100° are shown in (Table-1). The silver nanoparticles corresponds to fcc crystal lattice which was conferred from diffraction peaks of xrd analysis with intense peak at $32.41, 45.37, 64.47, 77.42$ correspond to lattice plane 111, 200, 220, 300 respectively (JCPDS card file no.03-0921). By substituting Full width half maximum values in Scherer's formula the standard particle size of the silver nanoparticles was calculated. The particle size thus calculated was in the range 10 to 35nm which was less than silver nanoparticles prepared from *Ocimum sanctum* (Tulsi) leaf extract²⁸.

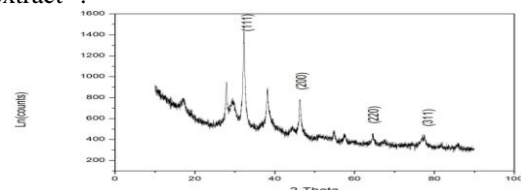


Fig-9 XRD diffraction pattern of Ag nanoparticle

Peak List Pos. [2θ]	Height [cts]	FWHM Left [2θ]	d-spacing [\AA]	Rel. Int. [%]
17.1195	121.27	0.8187	5.1796	13.2
27.8655	361.28	0.2047	3.2018	39.31
29.5269	178.92	0.8187	3.02531	19.47
32.2839	919.08	0.1535	2.77297	100
38.1928	379.89	0.2558	2.35646	41.33
44.3475	60.6	0.8187	2.04267	6.59
46.3016	347.09	0.2558	1.96091	37.77
54.8496	90.1	0.5117	1.67382	9.8
57.5399	80.46	0.614	1.6018	8.75
64.5424	104.42	0.307	1.4439	11.36
85.7445	39.67	0.614	1.13312	4.32

Table-1 X-RD of silver nanoparticles

3.4. Transmission electron microscopy & Energy Dispersive X-Ray Spectroscopy

The TEM images of the synthesized DM silver nanoparticles at 20 nm and 100 nm are exhibited in the Figs. 10 and 11. The biosynthesized silver nanoparticles were in uniformly circular form with particle size in the array of 10 to 30 nm. It was also noticed that uniform distribution of Ag nanoparticles in the whole sample. Energy Dispersive X-Ray Spectroscopy pattern obviously shows that the silver nanoparticles are produced by the conversion of Ag^{2+} ions in $AgNO_3$ to Ag^+ ions by DM extract. The EDAX graph results were shown in fig – 12. Energy Dispersive X-Ray spectrum showed the existence of elemental silver along with carbon and copper impurities

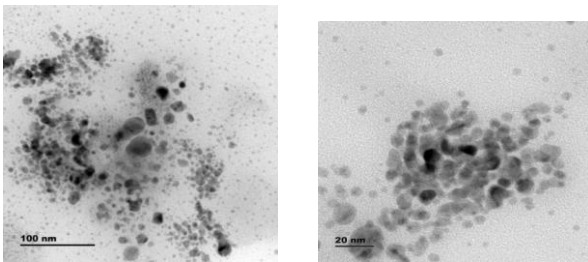


Fig-10 TEM image at 20nm Fig-11 TEM image at 100nm

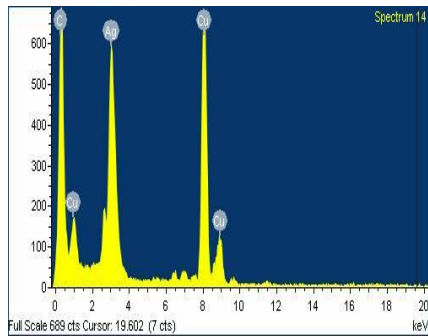


Fig-12 EDAX of silver nanoparticles

3.5 AFM

The surface morphology and dimensions of the silver nanoparticles were measured using AFM. The two and three-dimensional surface morphology and particle size were determined from AFM studies, the structure distribution of the nanoparticles were done with MNOVA software using the line analysis measurements.[Figure 13,14]. The average Ag nano particle size were found to be 40 nanometer.

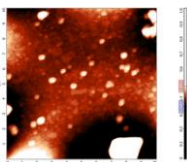


Fig-13 2D image of nanoparticle

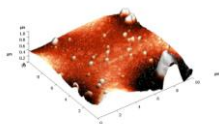


fig-14 3D image of nanoparticles

3.6. Antimicrobial Properties

3.6.1. Antibacterial activity

The silver nanoparticles are exhibited for antimicrobial activity. The bacterial pathogens were resistant to all 4 antibiotics as per Clinical and Laboratory standards Institute.

Nanoparticles is tested against both types of bacteria cells such as *E.coli* and *Bacillus subtilis* as it shows a clear inhibition zone. The growth inhibition zone was measured after the incubation period. Silver nanoparticles show inhibition zone of 5.5mm to 5 mm against *E.coli* and 7.8mm to 7mm against *Bacillus subtilis*. The images are shown in the fig-15 and 16. The inhibition values are shown in the table 2.

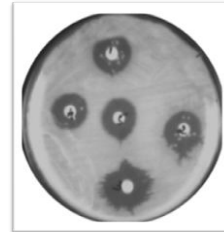


Fig-15 E- coli

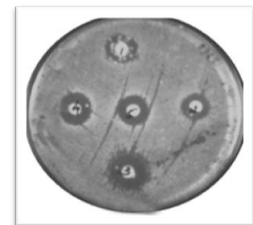


Fig-16 Candida albicans

CONCENTRATION (μG/ML)	12
BACTERIA	1
ESCHERICHIA COLI	5.5
BACILLUS SUBTILIS	7.8
FUNGI	
CANDIDA ALBICANS	7.9
ASPERGILLUS FLAVUS	5.6

Table-2 Zone inhibition of silver nanoparticles

3.6.2. Antifungal activity

The experimental antifungal activity was indicated in the Table. 1 and it clearly shows that silver nano particles has appreciable activity against *Canadiaalbicans*

Antifungal activities of synthesized DM Ag nanoparticles were performed on two fungi, *Canadia albicans* and *Aspergillus flavus*. The zone of inhibition exhibits due to nanosized silver particle is shown in table-2. The nanoparticle shows 8 mm to 7.2 mm diameter of inhibition zone against *Canadiaalbicans* and 5.6 mm to 5 mm against *Aspergillusflavus*. The images are shown in the fig-17 and 18. The inhibition values are shown in the table-2.

In our work, compared to anti bacterial activity, the silver nano particles shows better activity towards antifungal activity. But Yousefzadi et al pointed that for green alga *Enteromorpha flexuosa* silver nanoparticles shows enhanced antimicrobial activity²⁹. This may due to linkage of AgNPs on the plane of cell wall membrane and alters its functions³⁰. The silver nanoparticles might be used as an optional orthodox antibiotics for the treatment of infectious diseases³¹.

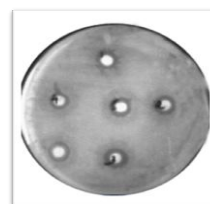


Fig-17 Bacillus subtilis

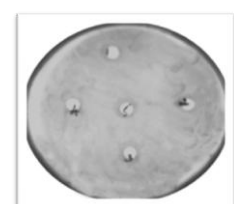


fig- 18 Aspergillusflavus

3.7. Antioxidant activity

Scavenging Activity of DPPH radical

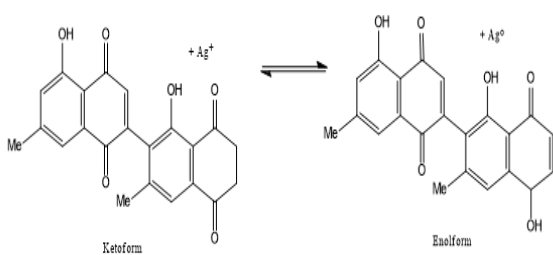
The positive DPPH tests results shows that silver nanoparticles act as free radical scavengers. It shows successful inhibition activities on silver nanoparticles. The DPPH activity of silver nanoparticles were improved. The IC 50 value for DPPH activities of silver nanoparticles were found to be 0.44 which shows that the prepared sample has excellent radical scavenging activity. The values are shown in the Table-3. The silver nanoparticles preformed as hydrogen atoms donors in exchange reactions of DPPH into its another concentrated form of DPPH. Silver nanoparticles showed more scavenging activity against DPPH due to its electrons reduction ability. The results of radical scavenging activity shows that nanoparticles donates electrons or hydrogen atoms in the conversion of DPPH radical to DPPH. The significant antioxidant activity for the plant extract compared to synthesized chemical compounds may due to the occurrence of phenols, flavonoids and polyphenols in DM extract sample.

Conc (mg/ml)	0.2	0.4	0.6	0.8	1	IC50
DMR	11.5	45.66	65.37	90.35	93.44	0.44

Table-3 DPPH radical scavenging activity of silver nanoparticles

IV. CONCLUSION

In this present study we report a biogenic greener synthesis of silver nanosized particles from *Diospyros montana* leaf extract at normal room temperature. It shows to be an ecofriendly and cost effective way for the green synthesis of Ag nanoparticles. This experimental reaction pathway itself proves that, it is an 100% green synthesis process. It is also characterized using a UV, IR, XRD, TEM, AFM and EDAX studies. The synthesized nanoparticles exhibits an considerable antimicrobial activities against phytopathogens like *Escherichia coli* and *Bacillus subtilis*. It can be used for developing good fungicidal formulation for commercial use. Advantages of using DM leaf extract for synthesis of nanoparticles is that it is protecting human health and environment which leads to lesser waste and safer products. The DPPH radical scavenging activity has been carried out and the nano silver particle has good scavenging activity.



Mechanism of silver nanoparticles

Author Contribution Statements

A conceived the original idea, b.c. wrote the manuscript, d performed the analytic calculations and performed the numerical simulations,e carried out the experiment. All authors discussed the results and contributed to the final manuscript.

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