GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *DENDROPHTHOE FALCATA* (L.F) ETTINGSH STEM EXTRACT- AN EPIPHYTIC PLANT: CHARACTERIZATION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY

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Abstract

A phytoextract mediated blend of silver nanoparticles using *Dendrophthoe falcata*stem extract as capping and stabilizing agent without using hazardous toxic chemicals via green route has been calculated. The green synthesized nanoparticles were characterized by Scanning electron microscopy (SEM), UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Atomicforce microscopy (AFM) analysis. By UV-Vis spectra at 484nm, AgNPs formation was checked. FTIR had shown that the phytochemicals were responsible for the reduction and capping material of silver nanoparticles. The size and shape of the AgNPs were regulated by making use of SEM. The XRD results divulged a crystalline nature of AgNPs with ausual size of 33.56nm. The surface topography was supported by AFM techniques. The antioxidant activity of the plant stem extract and formulated AgNPs have been performed by means of 2-2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay employing ascorbic acid as a standard. The antibacterial activity was regulated by disc diffusion method. This method is used to measure the efficiency of both plant stem extract and its mediated silver nanoparticles against six gram positive bacterial strains like *Bacillus thuringiensis, B. subtilis, Streptococcus faecalis, S. pyrogens, Staphylococcus aureus, Enterococcus faecalis* and six gram negative bacterial strains such as *Salmonella paratyphi, S.paratyphi-A, S.paratyphi-B, Proteus vulgaris, P. mirabilis* and *Escherichia coli.* The antibiotic tetracyclineis used as reference standardfor both bacterial strains. The results illustrate that the *Dendrophthoe falcata* stem mediated AgNPs may be employed in many biomedical applications and the production of many antibacterial products in the future.

Keywords: *Dendrophthoe falcata*, Silver nanoparticles, Antibacterial, *Salmonella paratyphi*, Antioxidant activity.

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Introduction

Nanotechnology is seen as a fast growing field of science mainly due to the increasing interest in nanostructures [1]. Nanoparticles (NPs) are structures with atleast one dimension under 100nm and also have high surface to mass ratio. This gives them exclusive properties that their macroscopic counterparts do not hold and allows their use for instance in electronics [2]; bioremediation [3]; biological markers [4] catalysis [5], antimicrobials [6,7]. The current literature review discloses that the NP synthesis by marine plants, microorganisms and algae as source has been not explored and under adventure. The growth of green processes for the synthesis of NPs is developing into an important branch of nanotechnology. It has many benefits like ease with which the process can be scaled up, economic viability, etc. Currently, the researchers are looking into development of cost- effective procedures for producing reproducible, stable and biocompatible AgNPs. [8]. AgNPs play a vigorous role in nanobiotechnology as biomedicine against drug resistant bacteria.

Recent study has reported the plant extracts such as *Melissa officinalis* [9], *Vernonia cinerea* [10];*V.amygdalina* [11]*Satureja hortensis*[12]; *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini,Centella asiatica,Citrus sinenais* [13] *Alocasia odora* [14] *Saraca asoca* [15] *Cinnamomum tamela* [16] and others are environment friendly materials. They also offer anexcellent alternative to obtain AgNPs. On the other hand, many reports designates that the AgNPs exhibits antibacterial activity and compromise an attractive alternative to the develop of antibiotics [17,18].

Dendrophthoe falcata is seen as Vanda in the Indian Ayurvedic System of Medicine. It has been used in traditional old medicine and found to possess antimicrobial, antidiabetic, antioxidant, anticancer, antilithiatic, hypertensive and antiviral properties. *D. falcate* is an epiphytic plant (stem hemiparasite) mainly studied and is employed to control a wide variety of diseases like skin disorder, asthma, paralysis, ulcers, pulmonary tuberculosis, psychic disorders, menstrual disorders and wounds. They are employed as health food for attractive immunity and employed as a pain reliever, aphrodisiac, narcotic and diuretic.Based on the above medicinal uses, this research has achief objective, to suggest a simplified and efficient green synthesis of AgNPs with proven antioxidant and antibacterial properties. In this work, the green synthesis of AgNPs by*Dendrophthoe falcata* stem aqueous extract and the bactericidal effect against six gram positive and six negative bacteria wasauthorized.

MATERIALS AND METHODS

Collection of Plant Material

Healthy fresh epiphytic plant of *Dendrophthoe falcata* (L.F) Ettingsh were gathered from Puttalam, Kanyakumari District, Tamil Nadu India.The collected plant was identified with the aid of local flora. The voucher specimen (EPH762) was deposited in the Ethnopharmacology units ResearchDepartment of Botany, V.O.Chidambaram College, Thoothukudi.

Preparation of Extract of Phytochemical Screening *(Cold Maceration Method)*.

The collected stem samples were cut into small fragments. This was dried under shade. The dried material was granulatedor powdered by making use of a blender. Required quantity of stem powder weighed and transferred to stoppered flask. This was treated with double distilled water (aqueous) until the powder is fully immersed. The flask was shaken infrequently for three days and then the extractsfilter through Whatman No.1filter paper. The aqueous extract subjected to qualitative tests for the identification of numerous phytoconstituents as per standard procedure [19].

Preparation of *D. falcata* Stem Extract

Twenty gram of healthy and freshly collected stem of *D.falcata* were methodically washed with double distilled water and cut into fine pieces. The fine pieces of stem were taken in a 250ml Erlenmeyer flask ; 100ml of double distilled water was added. Further the same was boiled at 80^{o} C for 20min. The stem extract was filteredusing Whatman's No.1 filter paper. The obtained filtrate was stored at 4^{o}C for further use for the green synthesis of AgNPs. In this research, stem extract act as a reducing and protecting agent for the green synthesis of AgNPs.

Synthesis of Silver Nanoparticles

In a representative synthesis of AgNPs, 10ml of *D. falcata* stem aqueous extract was added to 20ml of 1mM $AgNO_3$ aqueous solution and allowed to stir to react. After stirred, the reaction mixture was preserved in a dark place at room temperature. A colour change is observed from transparent yellow to reddish brown after 24hrs. This indicates the formation of AgNPs.

Characterization of Ag Nanoparticles

The synthesized AgNPswere employed for physical characterization alike UV- Visible, XRD, FTIR, SEM and AFM. Characteristic optic properties of the AgNPs were recorded by making use of UV-Vis spectrophotometer (Shimadzu V650 spectral range from 200 nm to 900 nm). FTIR spectra of stem powder and synthesized AgNPs werere corded as KBr pellet on Thermoscientific iS5 model. This is done in the range of 4000 – 400 cm^{-1}. The morphological study of the AgNPs was carried out by making use of SEM images analysis on a Carl Zeiss EVO18). XRD characterization was done by employing Shimadzu XRD 6000/6100 model consists of 30Kv, 30mA with CuKα radiation. Surface topology of the synthesized AgNPs were deliberated by 1µm×1µm Atomic force microscopy (AFM Nanosurf 2).

Antibacterial Activity

Antibacterial activity of synthesized AgNPs was carried out. This is done by disc diffusion method [1]. The test bacteria gram positive, *Bacillus thuringiensis, B. subtilis, Streptococcus faecalis, S. pyrogens, Staphylococcus aureus, Enterococcus faecalis,* gram negative*Salmonella paratyphi, S.paratyphi -A, S. paratyphi-B, Proteus vulgaris, P. mirabilis and Escherichia coli* was acquired from the Research Laboratory, Department of Microbiology, Bharathidasan University, Tiruchirapalli, TamilNadu. The overnight incubated bacterial culture was spread over the recently prepared nutrient agar plates. The 6mm sterile disc (Hi media) was kept at the centre. In addition to that different concentrations of synthesized silver nanoparticles (40 µg/mL., 80 µg/mL and 100µg/mL were poured on disc. Further it was placed on the plate. The tetracycline disc (reference or positive control), silver nitrate solution without extracts and plant aqueous extract were also kept and then incubated at 37°C for 24h. After incubation the zone of inhibition was measured.

***In vitro* Antioxidant Activity**

To assess the scavenging activity on DPPH, dissimilar concentration of synthesized AgNPs /aqueous stem extract /ascorbic acid (12.5, 25, 50, 100 and 200 µg/ml) in water was mixed with 1 ml of methanol solution incorporating DPPH radicals (0.2 mM). The mixture was shaken energetically and left to stand for 30 min in the dark formerly, measuring the absorbance at 517nm against a blank [20]. Then the scavenging capacity was computed by means of the following equation as described below.

Percent inhibition = A blank – A sample/ A blank) × 100here, 'A blank' is the absorbance of the control reaction (containing all reagents except the test compound) and "A sample" is the absorbance of test compound.

Result and Discussion

Phytochemical Screening

Screening of aqueous extract of *D.falcata* stem displays the presence of alkaloid, flavonoid, terpenoid, glycoside, phenol, steroid, saponin, tannin, sugar and xanthoprotein.(Table 1). Phytochemicals act as reducing agent as well as capping agent and helps to minimize the agglomeration of $AgNP_S$ there by controlling the morphology and also protecting and stabilizing the formed NPs. [22,23].

Table 1: Preliminary phytochemical screening of *D. falcata* stem

Phytochemicals	Aqueous
Alkaloid	+
Anthraquinone	-
Catechin	-
Coumarin	-
Flavonoid	+
Phenol	+
Quinone	-
Saponin	+
Steroids	+
Tannin	+
Terpenoid	+
Sugar	+
Glycoside	-
Xanthoprotein	+
Fixed oil	-

+ Present - Absent

Synthesis of Silver Nanoparticles

Synthesized silver nanoparticles were authorized due to the colour change of the reaction mixture (silver nitrate and aqueous extract of *D.falcata* stem) from colourless to reddish brown (Fig 1 a,b,c). The characteristic changes of colour is because of the stem extract acting as a reductant ascribable to the phytochemicals present in the extract [24].

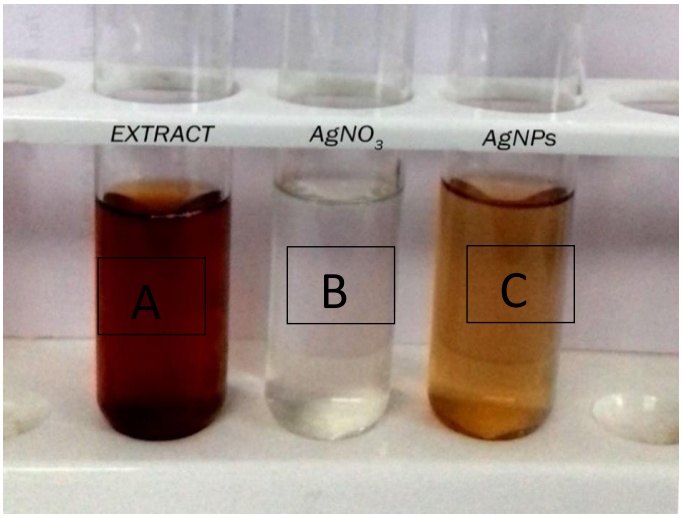


Fig. 1: Synthesis of AgNPs of *D. falcata* stem

Characterization of Synthesized Silver Nanoparticles

UV-Visible Spectroscopy Analysis

UV- Visible absorption study is a simple and most prominent method to evaluate the optical properties of nanoparticles. Fig2 shows the UV-Vis absorption studies of silver nanoparticles seen at the wavelength of 400 – 900 nm. The synthesized silver nanoparticles with *D. falcata* stem extract displayed a strong absorbance at the wavelength of 484nm, checking the formation of silver nanoparticles. It is confirming, with Rajaram et al., [25] who boomed the silver nanoparticles absorption peak at 480nm.

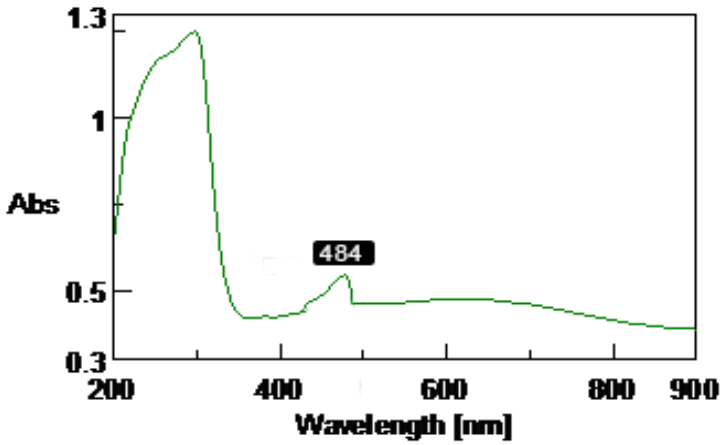


Fig. 2: UV-Visible spectrum analysis of synthesized AgNPs of *D. falcata* stem.

Fourier Transform Infrared Spectroscopy Analysis (FTIR)

FTIR was carried out to classify the possible potential functional groups for biosynthesis and stabilizing of AgNPs using *D.falcata* stem extract. Fig 3a shows the FTIR spectrum of the stem powder of *D.falcata.* The strong peaks at 3823, 3805 and 3752 cm^{-1}areallocated to the O-H group from hydroxyl, peak at 3411cm^{-1} represent O-H stretch of alcohols / phenols, peaks at 2923and 2853cm^{-1} indicate O-H stretch of carboxylic acid, peaks at 2353 and2342 cm^{-1}represent NH^{+} stretch of tertiary amine, peak at 1735 cm^{-1} indicate C=O stretch of ester, andpeak at 1620cm^{-1} represent > N-H group from secondary amine, peaks at 1542and 1511 cm^{-1}indicate NO_2stretch of aromatic nitrocompounds. The peak at 1457 cm^{-1} represent C-C stretch of aromatics,peak at 1380 cm^{-1}

represent C-H rock of alkanes, peak at 1246, 1156 and1107 cm^{-1}designate the presence of C-O stretch of esters/ethers, peaks at 1035cm^{-1} indicate C-N stretch of aliphatic amines, peaks at 822cm^{-1} to 765cm^{-1}represent C-H "oop" of aromatics, peaks at 669and 619cm^{-1} indicate C-Br stretch of alkyl halides. The slight shift in the peaks position from 3412 cm^{-1}, 2923cm^{-1}, 2853cm^{-1},2361cm^{-1}, 2341cm^{-1}, 1616 cm^{-1},1383 cm^{-1}, 1104 cm^{-1},826 cm^{-1}, 879 cm^{-1},765 cm^{-1}, 669 cm^{-1} corresponds to phytochemicals responsible for the synthesis of AgNPs. In AgNPs, nearly eleven peaks disappeared at 3823 cm^{-1}, 3805 cm^{-1}, 3752 cm^{-1}, 1735 cm^{-1}, 1719 cm^{-1},1542 cm^{-1}, 1511 cm^{-1},1457 cm^{-1}, 1246 cm^{-1},1156 cm^{-1} and 1035 cm^{-1}simultaneously only one new peak get appeared at 879 cm^{-1} (Fig 3b and Table 2). Shiftingof these peaks shows the possible involvement of the assessed functional groups of *D.falcata* stem extract in AgNPs biosynthesis. It is probable to carry out reduction of Ag ions by *D.falcata* stem extract and this result confirms the presence of organic compounds associated to the synthesis of AgNPs [26-28].

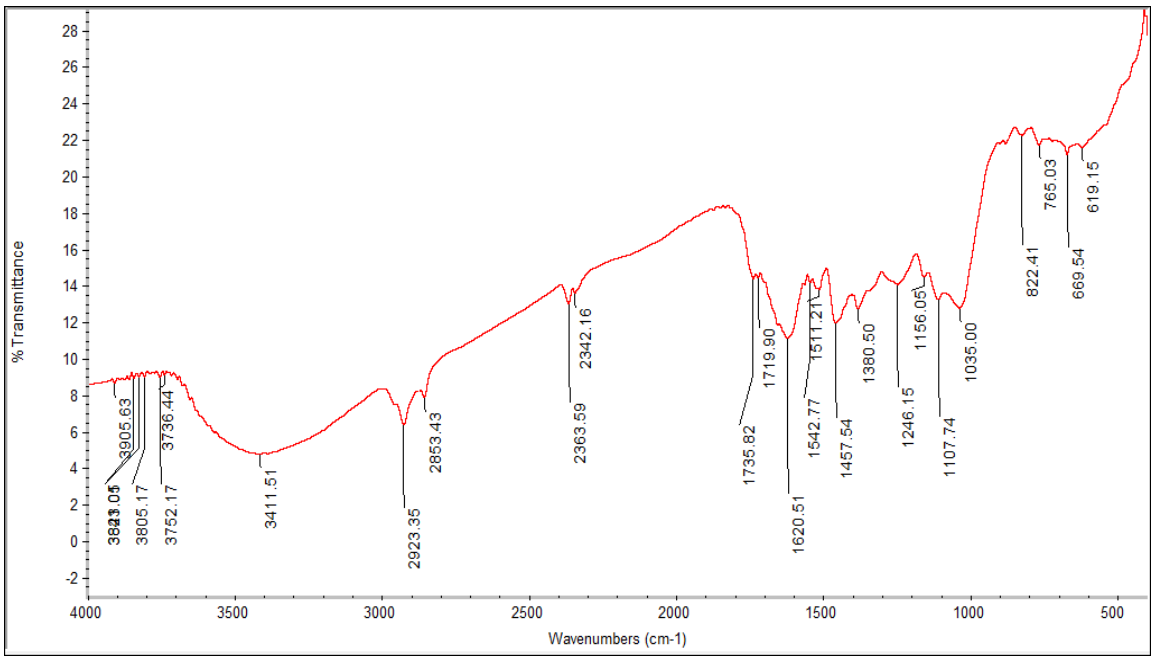


Fig 3a: FTIR spectra of stem powder of *D. falcata.*

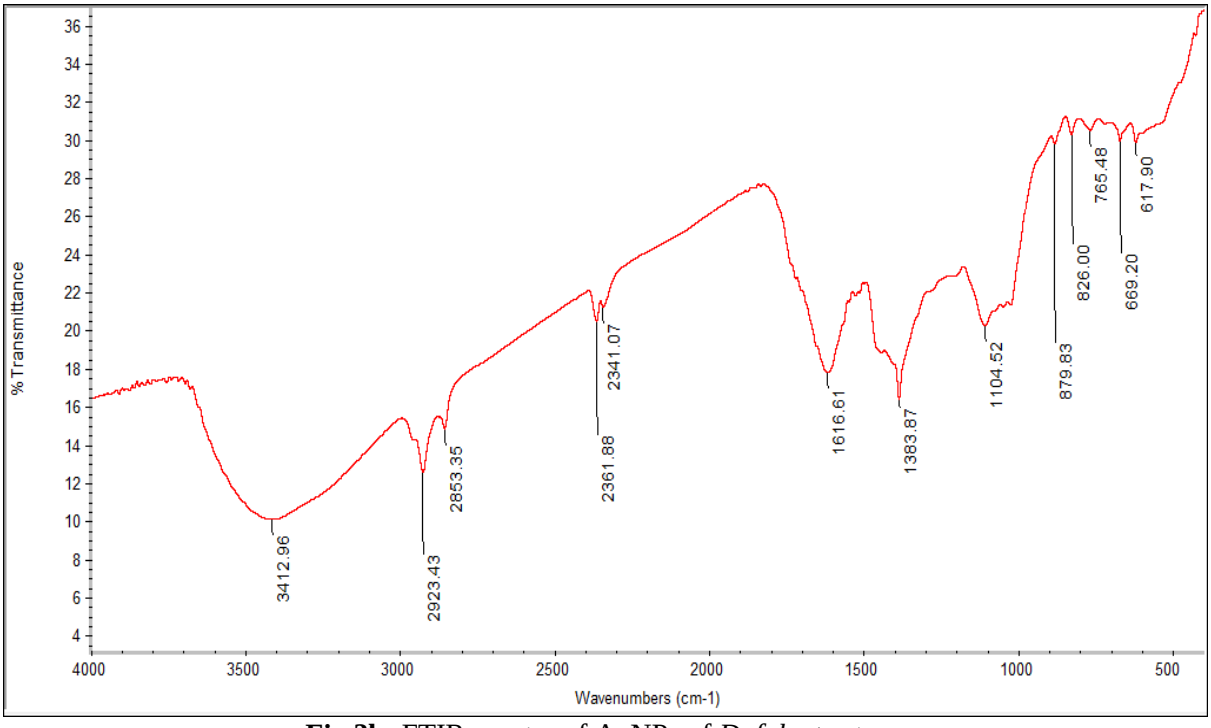


Fig 3b: FTIR spectra of AgNPs of *D. falcata* stem.

Table 2: FTIR analysis of stem powder and synthesized AgNPs of *D. falcata*stem.

S. No.	Frequency(cm^{-1})	Chemical Bond	Phytoconstituents Present	Peak Observed (Stem powder)	Peak Observed (AgNPs)
1.	3850 -3500	O-H Stretch	Hydroxyl group	3823, 3805,3752	-
2.	3500 -3200	O-H Stretch	Alcoholsor Phenols	3411	3412
3.	3300 - 2500	O-H Stretch	Carboxylic acid	2923,2853	2923,2853
4.	3000 -2850	C-H Stretch	Alkanes	-	-
5.	2700 -2250	NH^{+}Stretch	Tertiary amine salt	2353,2342	2361, 2341
6.	1760 -1740	C=O Stretch	Alkyl carbonate	-	-
7.	1750 -1725	C=O Stretch	Ester	1735	-
8.	1650- 1550	>N-H bend	Secondary amine	1620	1616
9.	1555-1485	NO_2 stretch	Aromaticnitro compound	1542,1511	-
10.	1500- 1400	C-C Stretch	Aromatics	1457	-
11.	1390- 1350	C-H rock	Alkanes	1380	1383
12.	1360- 1290	N-O Symmetric Stretch	Nitro compound	-	-
13.	1320- 1000	C-O stretch	Esters, Ethers	1246, 1156,1107	1104
14.	1250- 1020	C-N Stretch	Aliphatic amines	1035	-
15.	995-850	P-O-C stretch	Aromatic phosphate	-	879
16.	900- 675	C-H “oop”	Aromatics	822, 765	826, 765.
17.	690- 400	C-Br Stretch	Alkyl halides	669, 619	669, 617.

Scanning Electron Microscopy (SEM) Analysis

The SEM image displays the morphological character of AgNPs synthesized by making use of stem extract of *D.falcata* (Fig 4).Most of the biosynthesized AgNPs were closely cubic crystal like structure.

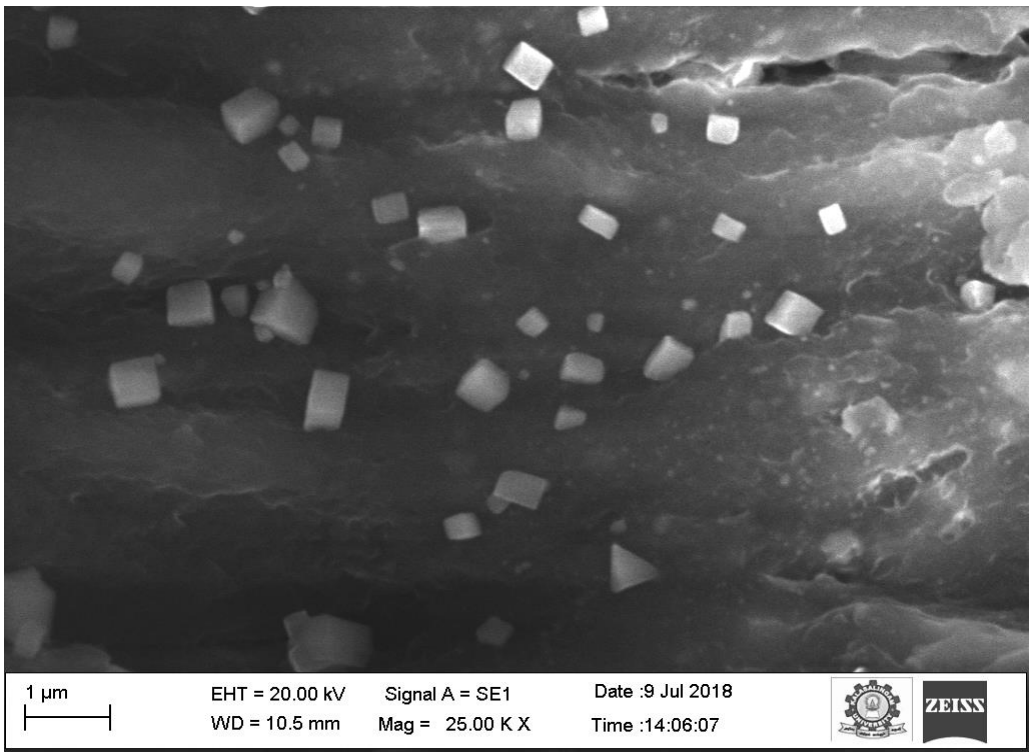


Fig 4: SEM image of AgNPs of *D. falcata* stem

X-Ray Diffraction (XRD) Analysis

XRD spectra provides an insight around the crystallinity of nanoparticle. Fig 5 characterises XRD spectra of AgNPs synthesized using *D.falcata* stem aqueous extract. Size of the nanoparticle was computed using Debye- Scherrer equation. X-ray diffraction peaks attained at 27.56^{o}, 32.18^{o}, 46.22^{o}, 54.82^{o}, 57.31^{o} and 77.20^{o} corresponded to the lattice plane of (111), (200),(211),(220), (311) and (222) suggesting the face- centered cubic (FCC) crystal structure of the nanoparticle. Average size of the synthesized nanoparticle was seen to be 33.56nm.

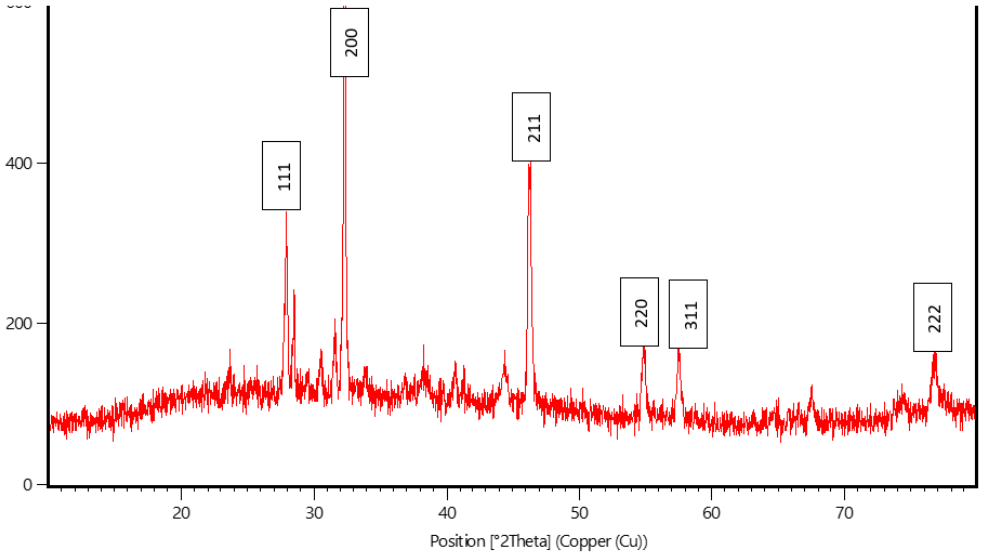
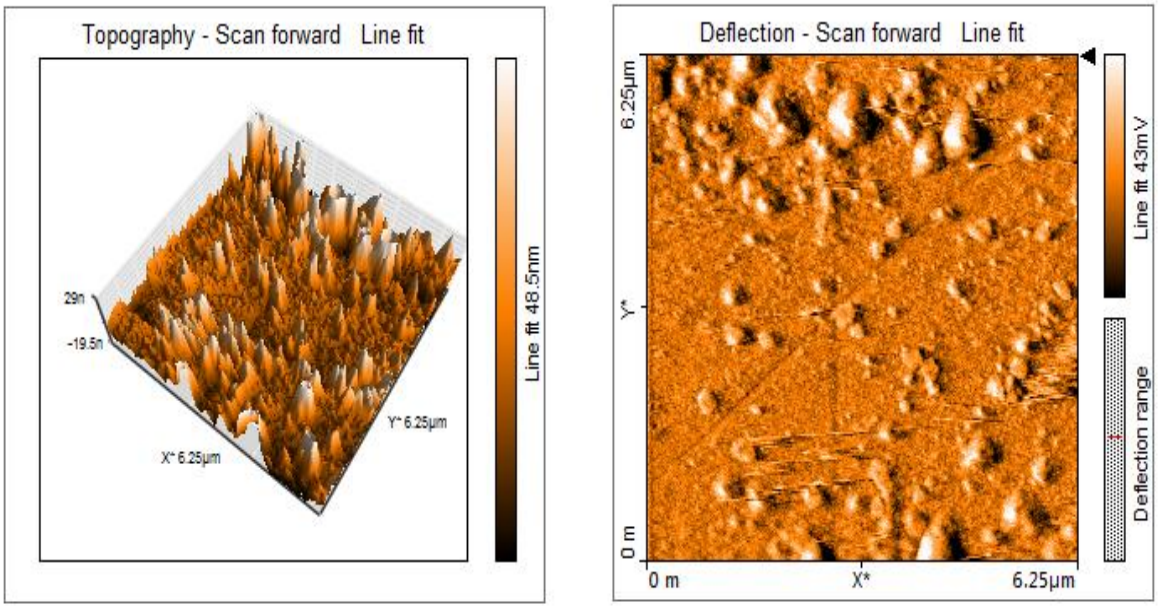


Fig 5: XRD image of synthesized AgNPs of *D. falcata* stem.

Atomic Force Microscopy (AFM) Analysis

The synthesized silver nanoparticles were described by Atomic Force Microscopy (AFM) for its detail morphology of silver. AFM image clearly exemplify that surface topograpy was rough hilly mountains like structure (Fig 6).



a)2D- Topography **b)** 3D- Topography

Figure 6: AFM structure of AgNPs of *D. falcata* stem.

Antioxidant Activity

The results for the effect of synthesized AgNPs / aqueous stem extract on DPPH radical scavenging activity as seen in Fig 7. The DPPH radical scavenging activity tends to increase the concentration of AgNP increases (12.5- 200 µg/ml). At 200 µg/ml concentration of AgNPs, of*D.falcata* stem, the DPPH radical scavenging activity was seen to be 67.56%. The DPPH radical scavenging activity of aqueous stem extract of *D.falcata* was seen to be 51.76% which was lower than that of the ascorbic acid standard (79.31%). The free radical scavenging activity of many synthesized nanoparticles is also reported lately because of the high surface area to volume ratio. [29-31].

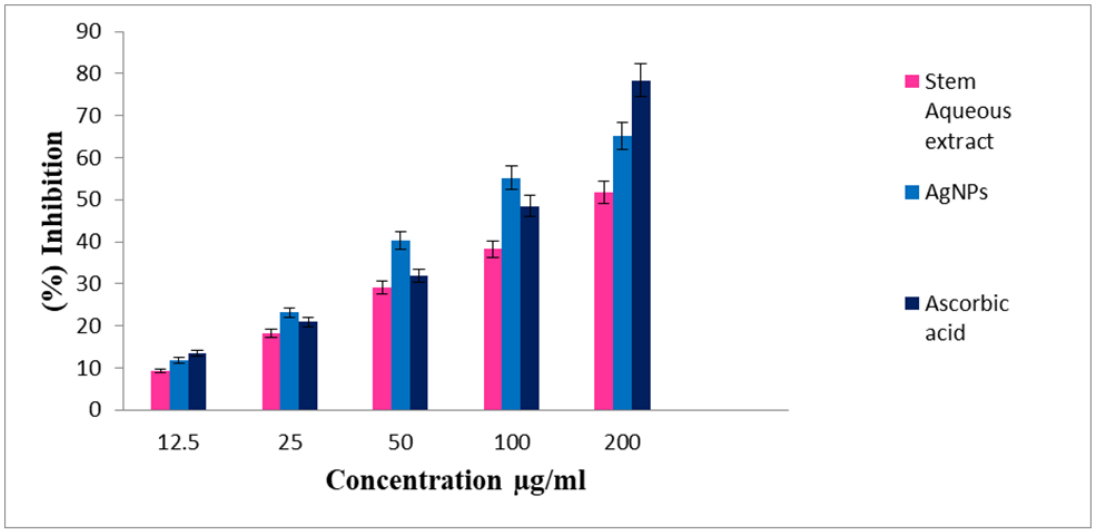


Figure 7: DPPH radical scavenging activity of AgNPs.

Antibacterial Activity

The antibacterial activity of silver nanoparticles synthesized by *D.falcata* stem extract was examined against various pathogenic organisms such as gram positive, *Bacillus thuringiensis, B. subtilis, Streptococcus faecalis, S. pyogens, Staphylococcus aureus, Enterococcus faecalis* gram negative, *Salmonella paratyphi, S.paratyphi-A, S.paratyphi-B, Proteus vulgaris, P.mirabilis and Escherichia coli.* The diameter of inhibition zone (mm) around each disc with silver nanoparticles is shown in Table 3.The silver nanoparticles synthesized by *D.falcata* stem extracts were found to have maximum antibacterial activity against *Salmonella paratyphi* (18mm) and the lesser antibacterial activity of silver nanoparticles synthesized by *D.falcata* stem extract was found against *Proteus mirabilis* (11mm). The silver nanoparticles displayed efficient antibacterial property compared to other nanoparticles due to their tremendously large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrated inside the bacteria. The bacterial membrane contains sulfurcovering proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell itcustoms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates therefore, protecting the DNA from the silver ions. The nanoparticles first attack the respiratory chain, cell division finally leading to cell death [13]. The nanoparticles release silver ions in the bacterial cells, which improve their bactericidal activity [32].

Table 3: Antibacterial activity of synthesized AgNPs of *D. falcata* stem

Name of Bacteria	Zone of Inhibition (mm)						
	Control	Tetracycline 30mcg/disc	Aqueous stem extract 100 µg	Silver nitrate 100µg	Different Concentration of AgNPs		
					40 µg	80 µg	100µg
Bacillus thuringiensis	-	20	8	10	9	13	16
Bacillus subtilis	-	18	7	9	8	12	15
Streptococcus pyogenes	-	22	9	10	9	12	17
Streptococcus faecalis	-	21	8	12	11	13	16
Staphylococcus aureus	-	22	8	11	9	13	15
Enterococcus faecalis	-	19	9	13	10	14	17
Salmonella paratyphi	-	23	8	12	11	15	18
Salmonella paratyphi- A	-	22	7	10	9	13	16
Salmonella paratyphi- B	-	23	6	11	10	12	14
Escherichia coli	-	24	7	10	11	14	16
Proteus mirabilis	-	23	6	11	9	13	15
Proteus vulgaris	-	18	5	7	6	8	11

Conclusion

The silver nanoparticles have been yielded by *D. falcata* stem extract. This is an economical, efficient and eco-friendly process. UV- visible spectrophotometer, XRD, FTIR, SEM and AFM technique have approved the reduction of silver nanoparticles. The zones of inhibition were formulated in the antibacterial screening test indicated, that the AgNPs synthesized in this process has the efficient antibacterial activity against pathogenic bacteria. *In vitro* antioxidant activity carried out by DPPH method showed higher activity of synthesized AgNPs. The biologically synthesized silver nanoparticles could be of huge use in medical field for their efficient biological activities.

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Conflicts of Interest

The authors declare no conflict of interest.

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