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Facile Green Synthesis of Zinc Oxide Nanoparticles Using *Dendrophthoefalcata* (L.F) EttingshStem Extract and Evaluation of their Antioxidant and Antibacterial Activities

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Abstract

The present study was aimed at the biosynthesis of zinc oxide nanoparticles (ZnONPs) using aqueous stem extract of *Dendrophthoefalcata* and testing their antioxidant and antibacterial activity. The green synthesized ZnONPs were characterized using UV-Visible spectrophotometer, Fourier transform infrared (FTIR) spectrophotometer, Scanning electron microscope (SEM), X-ray diffraction (XRD) and Atomic force microscopy (AFM). The ZnONPs showed better antioxidant and antibacterial activity against DPPH radical scavenging and gram positive bacteria respectively.

Key words: Epiphytic plant, Zinc oxide nanoparticles, AFM, DPPH, antibacterial.

INTRODUCTION

The production of nanoparticles can be done through physical, chemical and biological approaches, so that, physical and chemical methods are more expensive and often produce toxic materials especially chemical method that is a common method for producing nanoparticles and its potentially harmful to the environment. Therefore, development of clean, biocompatible, nontoxic and eco-friendly methods and gaining importance in nanotechnology using microorganism, enzymes and plant extracts (Mishra and Sardar, 2012 Cihokriwal*et al*., 2017; Dhandapani*et al*., 2020; Awwad*et al*., 2020). Among the diverse biosynthetic approaches, the use of plant extracts has received much attention as they are safe to handle, easily available and have broad variability of metabolites. The phytochemicals such as carbohydrates, flavonoids, saponins, proteins and amino acids and terpenoids present in the plant extracts play a key role in the synthesis of nanoparticles (Rehana*et al*., 2017).

Biosynthesis of ZnO nanoparticles (ZnONPs) using plant extracts of *Tecomacastaneifolia* (Sharmila*et al*., 2019) *Veronica multifida*(SahinDogan and Kocabas,2019); *Silybummarianum* (Hameed *et al*., 2019); *Phoenix dactylifera* (Barani*et al*., 2019); *Costuswoodsonii* (Khan *et al*., 2019); *Vitexnegundo* (Anbuvarnan*et al*., 2018); *Urticadioica* (Bayrami*et al*., 2020) and *Ocimumtenuiflorum*(Sharma *et al*., 2020) have been reported. Zinc oxide nanoparticles have been described as functionally promising and versatile inorganic materials with a broad range of

applications (Johar*et al*., 2015). Bhuyan*et al*. (2015) reported on the synthesis of ZnONPs where they showed that their material had antimicrobial, anticancer and antioxidant properties. It has also been reported that during the synthesis of ZnONPs, the zinc salt is normally used as an oxidant and the phytochemicals in the plant extract serve as reducing agents or stabilizers (Prabha and Raj, 2016; Agarwal *et al*., 2017).

Dendrophthoefalcata is known as Vanda in the Indian Ayurvedic System of Medicine. It has been used in traditional medicine and found to have antimicrobial, antidiabetic, antioxidant, anticancer, antilithiatic, hypertensive and antiviral properties. *D. falcata*is an epiphytic plant largely studied and is used to control a wide variety of diseases such as skin disorder, pulmonary tuberculosis, psycho disorders, asthma, paralysis, ulcers, menstrual disorders and wounds. They are used as health food for enhancing immunity and used as a pain reliever, aphrodisiac, narcotic and diuretic. Based on the above medicinal uses, this research has a chief objective to suggest a simplified and efficient green synthesis of ZnONPs with proven antioxidant and antibacterial properties. In this work, the green synthesis of ZnONPs by *Dendrophthoefalcata* stem aqueous extract and the bactericidal effect against six gram positive and six negative bacteria was authorized.

MATERIALS AND METHODS

Collection of Plant Material

Healthy fresh epiphytic plant of *Dendrophthoefalcata* (L. F) Ettingsh were collected from Puttalam, Kanyakumari District, Tamil Nadu India.The collected stem samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Preparation of Extracts for Phytochemical Screening (Cold Maceration Method)

The required quantity of stem powder was weighted. This is transferred to stoppard flask. Further it is treated with aqueous. This is carried out until the powder is fully immersed. For the first six hours, the flask is shaken every hour. The extract was filtered by making use of whatman No. 1 filter paper. The extract was allowed to undergo qualitative tests as per standard procedures for the identification of many phytochemical constituents.

Green Synthesis of Nanoparticles

Preparation of Stem Extract (Reducing Agent)

Stem extract was prepared by taking 20 gm of *D.falcata*stem. The stem were washed thoroughly with double distilled water and cut into fine pieces. Fine pieces are boiled in100 ml double distilled water for 20 minutes at 60^{o}C in a glass beaker. After boiling the extract was filtered using Whatman No. 1.

Preparation of Precursors

Precursors for zinc oxide nanoparticles ($ZnCl_2$) were purchased from Hi-media chemicals, India and prepared freshly. Precursor for preparing zinc oxide nanoparticle was 0.02 M of zinc chloride using double distilled water.

Synthesis of Zinc oxideNanoparticles

Ten ml of the extracts of the aqueous stem was slowly added separately into 10 mL of 0.02 mM zinc chloride solution under constant stirring. Afterward, the mixture was treated with 1 M sodium hydroxide drop by drop. As soon as the mixture was changed into pale white color. This was then placed in a magnetic stirrer for 2 hrs. A white precipitate was obtained and dried in a hot air oven overnight at 60°C. Complete conversion of $Zn(OH)_2$ into ZnO nanoparticles took place during drying.

Characterization of the synthesized zinc nanoparticle

UV – Vis Spectroscopy

Ultraviolet-visible spectroscopy (UV-Vis) submits to absorption spectroscopy in the UV-visible spectral area. The zinc oxide nanoparticles were characterized in a Shimadzu V 650 UV- Vis spectrophotometer. The scanning range for the samples was 200-700 nm. The double distilled water is made use as a blank reference.

Fourier Transform Infra-Red Spectroscopy(FTIR)

The nanoparticles were illustrated by means of a Fourier Transform Infra-red Spectrophotometer (FTIR Thermoscientific iS5). Two milligrams of the sample was mixed with 100 mg Potassium bromide (KBr). Compressed to prepare a salt disc roughly 3 mm in diameter and the disc were at once kept in the sample holder. FTIR spectra were recorded in the absorption range between 400 and 4000 cm^{-1}.

Scanning Electron Microscope (SEM) Analysis

Morphological analysis of the synthesized ZnONPs was carried out by making use of scanning electron microscope (SEM) (Carl Zeiss Microscopy GmbH, German Model EV018). Further it is equipped with 15kv acceleration voltage.

X-Ray Diffraction (XRD) Analysis

The particle size and nature of the zinc oxide nanoparticles were determined using XRD. This was done using Shimadzu XRD – 6000/6100 model with 30 kv, 30 mA with Cukα radians at 2θ angle. X-ray powder diffraction is a fast analytical technique. This is chiefly used for phase identification of a crystalline material and can supply information on unit cell dimensions. The analyzed material is thinly ground, and the normal bulk composition is established. The particle or grain size of the zinc oxide nanoparticles was determined using Debye Sherrer's equation.

$$D = 0.94\,\lambda / B\cos\theta$$

AFM Analysis

Surface topology of the synthesized zinc nanoparticles were studied by 1µm x 1 µm Atomic Force Microscopy (AFM Nanosurf 2) analysis, 0.01 g synthesized nanoparticles were mixed with 20 ml of acetone and sonicated for 5-10 minutes using ultrasonicator. The solution was poured on a clean glass slide and was allowed to dry until all the acetone gets evaporated. Now this glass slide is studied using the Atomic Force Microscopy in a non contact mode and the captured image was processed using XEI software.

Antioxidant Activity

2,2,Diphenyl-1-Picrylhydrazyl Assay (DPPH)

DPPH study was performed by ZnONPs was described by earlier, shimada*et al*. (1992). In briefly, 1.0 mL of DPPH solution (0.2 mm) was taken havingZnONPs various dose (12.5, 25, 50, 100 and 200 µg/mL) are used and stand for 30 min under dark condition. After 30 min the absorbance was read at 517 nm.

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

A_0 – control, A_1 - ZnONPs

Antibacterial Activity

Antibacterial activity of synthesized nanoparticles was determined using disc diffusion method. The test bacteria *Bacillus thuringiensis, B. subtilis, Streptococcus faecalis, S. pyogenes, Staphylococcus aureus, Enterococcus faecalis, Salmonella paratyphi, Salmonella paratyphiA, S.paratyphi B, Proteus vulgaris, P.mirabilis* and *Escherichia coli*was obtained from the Research Laboratory,

Department of Microbiology, Bharathidasan University, Tiruchirapalli, Tamil Nadu. The overnight incubated bacterial culture was spread over the freshly prepared nutrient agar plates. The 6 mm sterile disc (Hi media) was kept at the centre and different concentrations of synthesized nanoparticles (40 µg/mL, 80µg/mL and 100 µg/mL) was poured on disc and placed on the plate. The tetracycline disc (reference or positive control), $ZnCl_2$ solution without extracts and plant aqueous extract were also kept and then incubated at 37° C for 24h and after incubation the zone of inhibition was measured.

RESULTS AND DISCUSSION

Phytochemical Screening

Screening of stem aqueous extract of *D.falcata* shows the presence of alkaloid, flavonoid, phenol, steroid, saponin, tannin, terpenoid, glycoside, sugar and xanthoprotein (Table 1). The stem extract contain phenol and flavonoid acts a reducing and capping agent for the synthesis of zinc oxide nanoparticles.

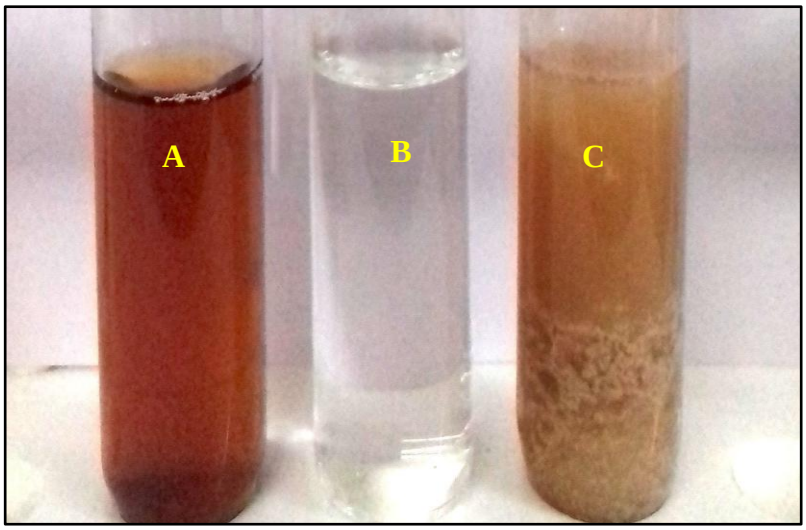
Table 1: Preliminary Phytochemical Screening of *Dendrophthoefalcata*stem

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

+ Present - Absent

Synthesis of Zinc OxideNanoparticles

The *D.falcata* aqueous stem extract was used in the reduction of zinc chloride into ZnONPs and the bioreduction was visually confirmed by the colour in the reaction mixture. The colour change of zinc chloride solution from yellowish to white precipitate indicated the formation of ZnONPs (Fig. 1a, b, c). The colour change is due to the excitation of surface plasmon vibration in the NPs (Sastry*et al*., 1997).



A - Plant extract B - $ZnCl_2$C - ZnONPs

Fig. 1 Synthesis of zinc oxide nanoparticles from *D. falcata* stem

Characterization of Synthesized ZnONPs

UV-Vis Spectroscopy Analysis

UV-Vis spectra of the synthesized ZnONPs using *D.falcata* aqueous stem extract are shown in Fig. 2. The synthesized ZnONPs with *D.falcata* stem extract showed a strong absorbance at the wavelength of 298nm conforming the formation of ZnONPs. It is confirmed with previously reported plant extracts (Abel *et al*., 2021; Loganathan*et al*., 2021).

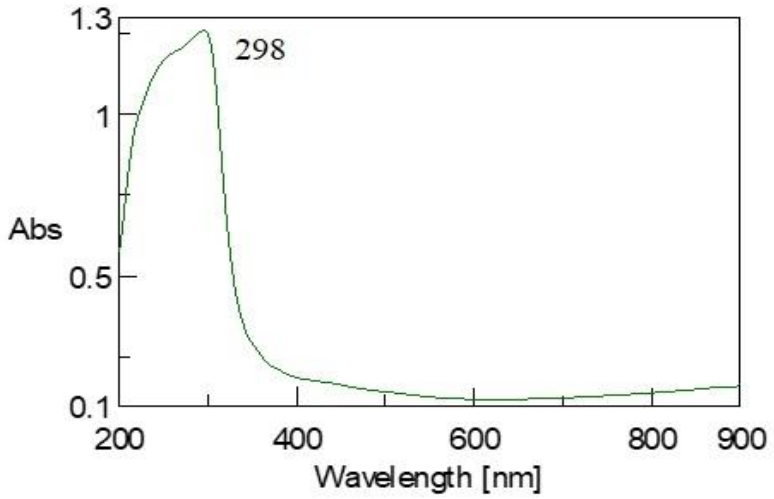


Fig. 2 UV-Vis spectrum of synthesized zinc oxide nanoparticles of *D. falcata* stem

Fourier Transform Infrared Spectroscopy (FTIR)Analysis

FTIR analysis is very cardinal in ascertaining the functional group of the biomolecules responsible for the capping and stabilizing during the synthesis of nanoparticles. Figure 3a shows the FTIR spectrum of the stem powder of *D.falcata*. The peak appearing at 3823, 3805 and 3752 cm^{-1} is due to the O-H stretching of hydroxyl group while the band at 3411cm^{-1} is due to O-H stretching of phenol/alcohol. The peaks appearing at 2923 $cm^{-1}$and 2853cm^{-1} is due to O-H stretching of carboxylic acid while the band at 2363 cm^{-1} and 2342 $cm^{-1}$is due to NH^{+} stretching of tertiary amine salt, whereas the band at 1735 cm^{-1} is due to C=O stretching of alkyl carbonate. The peak appearing at 1719 cm^{-1} is due to C=O stretching of ester while the band at1620cm^{-1} is due to > NH band of secondary amine. The multiple peaks appearing at 1542 and 1511cm^{-1} is due to C-C stretching (in ring) of aromatics, whereas the band at 1457cm^{-1} is due to C-C stretching vibrations of aromatics. The peak appearing at 1380$cm^{-1}$is due to C-H rock of alkanes while the band at 1246$cm^{-1}$is due to N-O symmetric stretching of nitro compound. The multiple peaks appearing at 1156 and 1107 cm^{-1} is due to C-O stretching vibrations of esters and ethers while the band at 1035 is due to C-N stretching of aliphatic amines. The peaks appearing at 822 and 765cm^{-1} is due to C-H "oop" of aromatics whereas the peak appearing at 669 and 619 cm^{-1} is due to C-Br stretching of alkyl halides. The slight shift in the peak position from 3848cm^{-1},3832cm^{-1},3795cm^{-1},3655 cm^{-1}, 3666 cm^{-1}, 3241 cm^{-1}, 2356cm^{-1}, 1553 cm^{-1}, 1435 cm^{-1},1088cm^{-1},1035 cm^{-1}, 842 cm^{-1}, 774 cm^{-1}, 714 cm^{-1}, 510 cm^{-1} and 482 cm^{-1} corresponds to phytochemicals responsible for the synthesis of ZnONPs. In ZnONPs nearly twelve peaks disappeared at 2923cm^{1}, 2853cm^{-1}, 2342cm^{-1}, 17.35 cm^{-1},17.19 cm^{-1},1620 cm^{-1}, 1511 cm^{-1},1380 cm^{-1}, 1246 cm^{-1}, 1156 cm^{-1}, 1107 cm^{-1} and 1088 cm^{-1}. Simultaneously four new peaks get appeared at 3655 cm^{-1}, 3666 cm^{-1}, 1088 cm^{-1} and 714 cm^{-1} (Fig 3b). Shifting of their peaks indicates the possible involvement of the assured functional groups of stem extract in ZnONPs biosynthesis. The phytochemical screening of *D.falcata* stem extract reflected the presence of phenols, flavonoids, alkaloids and proteins. In fact, the plant extracts may be play dual role as reducing and stabilizing agents of biosynthesized ZnONPs.

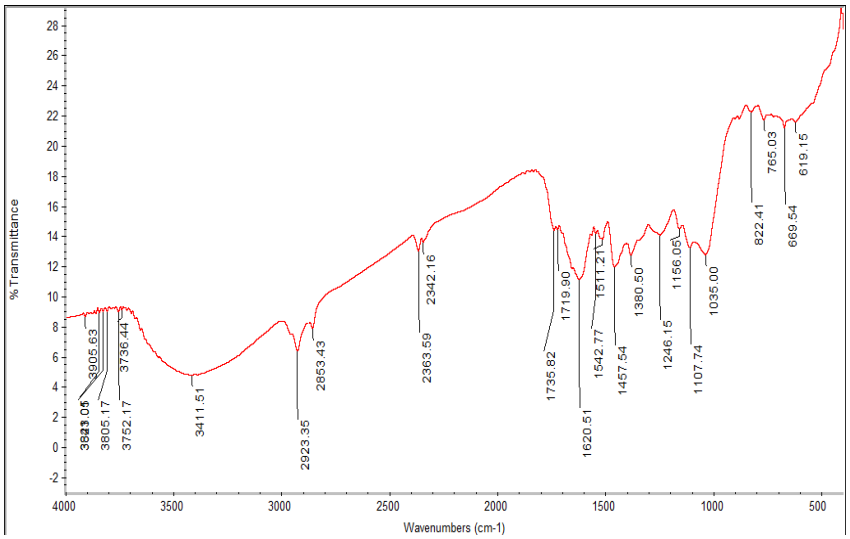


Fig. 3aFTIR Spectrum of *D. falcata* stem powder

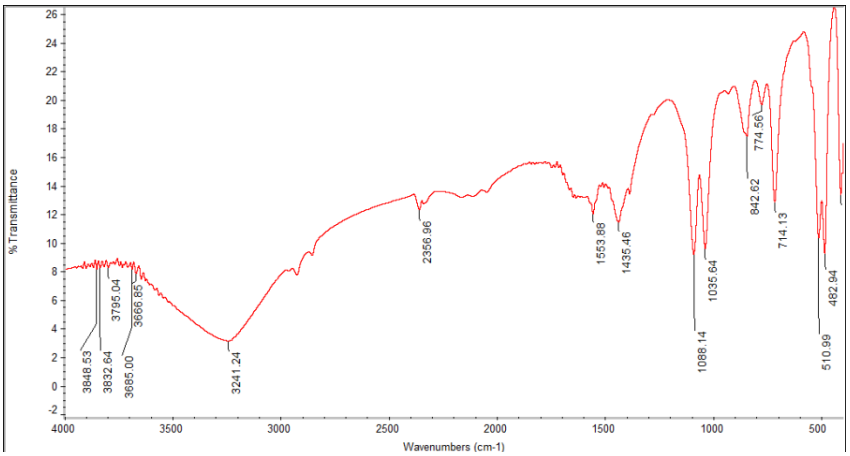


Fig. 3b FTIR spectrum synthesized zinc oxide nanoparticles of *D. falcata* stem

Scanning Electron Microscope (SEM) Analysis

The SEM images show the morphological character of ZnONPs synthesized by using stem extract of *D.falcata* (Fig 4). Most of the biosynthesized ZnONPs were nearly feathery scales like structure. The average particle size of ZnONPs was seen to be 52 nm.

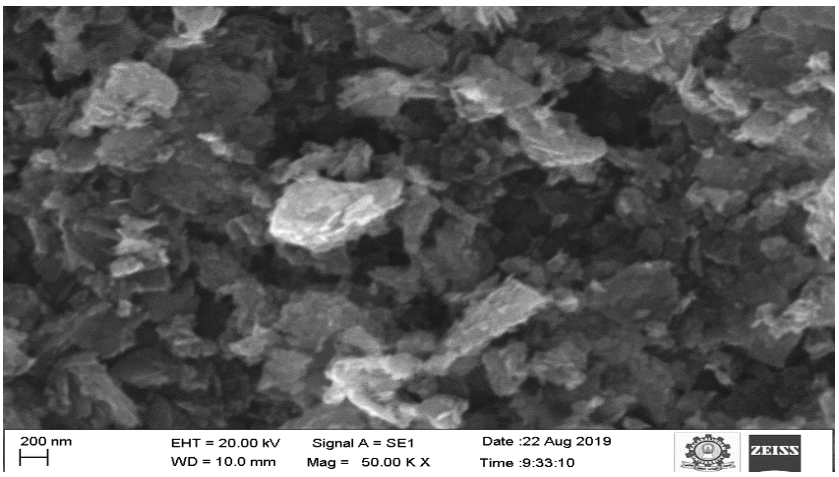


Fig. 4SEM image of synthesized zinc oxide nanoparticles of *D. falcata* stem

X-Ray Diffraction (XRD)Analysis

The XRD patterns of ZnONPs are shown in Fig 5. The XRD pattern shows peak in the whole spectrum of 2θ values ranging from 20° to 70°. The XRD pattern of *D. falcata* stem extract synthesized ZnONPs showed eleven distinct peaks of 32.94, 35.18, 36.24, 47.39, 56.51, 62.73, 66.14, 67.74, 69.08, 72.41 and 76.72 which can be indexed as (100), (002), (101), (110), (103), (200), (112), (201), (004) and (202) reflection plants respectively. All the peaks due to ZnONPs are well matched with the standard diffraction pattern of hexagonal crystal structure. The sharp and well designed peaks designate the crystalline nature of the synthesized ZnONPs.

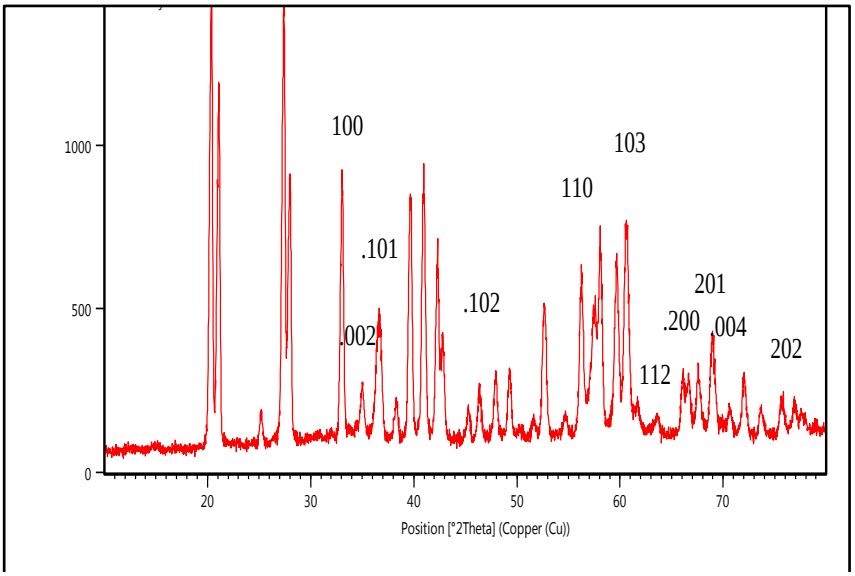


Fig. 5XRD image of synthesized zinc oxide nanoparticles of *D. falcata* stem

Atomic Force Microscopy (AFM) Analysis

AFM analysis is gives us insight about the topography, roughness of nanoparticles. AFM image (Fig 6) clearly demonstrate that streak like structure.

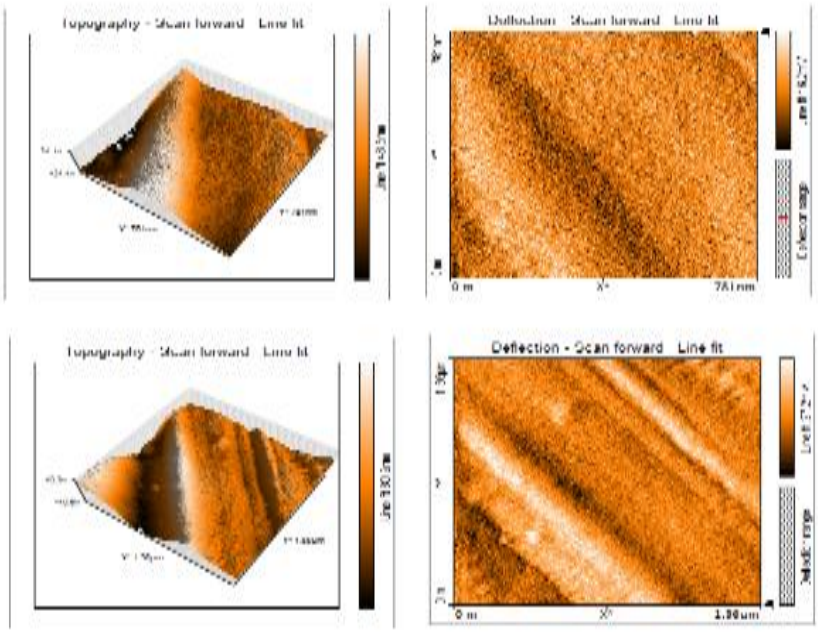


Fig. 6AFM of synthesized zinc oxide nanoparticles of *D. falcata* stem

Antioxidant Activity

The antioxidant activity of synthesized ZnONPs and aqueous extract of *D.falcata* stem was evaluated by DPPH radical scavenging assay. The results indicate that the scavenging property increases with increase in concentration by using ascorbic acid as a standard. The recorded

scavenging ability for the lowest concentration of the synthesized ZnONPs aqueous extract was 11.84% and this scavenging ability was increased to 65.16%, when concentration was increased to 200µg/mL.However, the scavenging ability was recorded for aqueous stem extract at lowest concentration 9.32% and standard ascorbic acid was 79.31% (Fig 7). The antioxidant activity is generally due to the phenolic and flavonoid compounds (Kharatand Mendhulkar 2016).

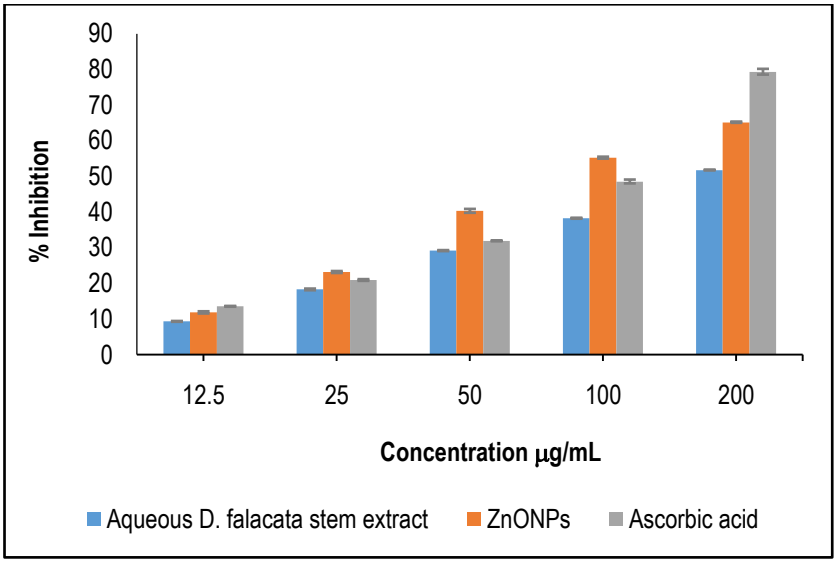


Fig. 7DPPH Radical Scavenging Activity of ZnONPs*D. falcata* Stem

Antibacterial Activity

In the present study, biosynthesized zinc oxide nanoparticles were analysed by six grampositive and six gram negative bacterial strains in disc diffusion method. The acquired zone of inhibition of various extracts were compared with standard drug tetracycline (30mcg/disc). The highest inhibition zone of synthesized zinc oxide nanoparticles of *D.falcata* stem were observed in *Enterococcus faecalis* (18mm), followed by *Bacillus subtilis* (17mm), *Streptococcus faecalis* (17mm) and *Salmonella paratyphi* (17mm) (Table 2). Generally, the gram positive bacteria are more susceptible than gram negative bacteria due to having only an outer peptidoglycan layer which is not an effective permeability barrier while the gram negative bacteria possesses an outer phospholipidic membrane carrying the structural lipopolysaccharide compound. Thus, the cell wall is impermeable to drug constituent in gram negative bacteria because of the presence of multi-layered of peptidoglycan and phospholipidic bilayer. (Ravikumar*et al*., 2010). Besides,factors such as particles size and morphology also play a role in determining the antibacterial potential of a material.

Table 2: Antibacterial Activity of Synthesized Zinc Oxide Nanoparticles of *D.falcata*Stem

Name of Bacteria	Zone of Inhibition (mm)						
	Control	Antibiotic (Tetracycline) 30mcg/disc	stem extract µg	Zinc chloride µg	Different Concentration of ZnONPs		
					60 µg	80 µg	100µg
Bacillus thuringiensis	-	20	6	8	9	12	14
Bacillus subtilis	-	21	5	7	10	13	17
Streptococcus faecalis	-	19	4	7	11	15	17
Staphylococcus aureus	-	18	5	8	9	13	15
Streptococcus pyogenes	-	18	4	9	12	14	15
Enterococcus faecalis	-	20	6	8	10	14	18
Salmonella paratyphi	-	21	6	9	10	15	17
Salmonella paratyphi-A	-	19	7	7	9	12	16
Salmonella paratyphi -B	-	20	6	8	9	11	16

Proteus vulgaris	-	18	5	9	8	11	14
Escherichia coli	-	19	4	8	9	12	16
Proteus mirabilis	-	18	4	7	9	11	14

In this study, the dominance of the smaller particle sizes as compared to other ZnO particles that have been derived from plants, played a role. It has been shown that when the nanoparticle size decrease, the microbial interaction between the cell membrane and Zn^{+} released from the ZnO nanoparticles become strong, thus increasing the antibacterial activity (Ngoepe*et al*., 2018).

Over all this study, the green synthesized ZnONPs using *D. falcata* stem aqueous extract is eco-friendly approached and to effectively control of tested gram positive bacteria. Green synthesis of ZnONPs was primarily confirmed by the position of SPR band at 298 nm in UV-Vis spectrum. The particle size was 92 nm with Leathery scales like structure. XRD spectrum displays crystalline nanostructured ZnONPs and AFM techniques show the surface roughness. The ZnONPs exhibited strong antioxidant and antibacterial activity.

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CONFLICT OF INTERNET
Nil.

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