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PLANT MEDIATED SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING LEAF AND TWIG EXTRACT OF *PREMNA BARBATA*: A COMPARATIVE APPROACH ON ITS PHYSICAL CHARACTERISTIC AND ANTIBACTERIAL PROPERTY

Prachi, Aayasha Negi and Devendra Singh Negi*

Department of Chemistry, H.N.B. Garhwal (A Central) University, Srinagar, Uttarakhand, India, 246174.
Department of Chemistry, Birla Campus, Hemvati Nandan Bahuguna Garhwal University, (A Central University), Srinagar Garhwal-246174.

Abstract

The comparative antibacterial activity of green synthesized ZnO-NPs of the leaf and twig extract of *Premna barbata* investigated in this study. X-ray Powder Diffractometer, Scanning Electron Microscopy and Energy Dispersive analysis of X-rays used to characterize the size and morphology of ZnO-NPs. ZnO-NPs of the leaf and twig extract with 22.1 and 81.5 nm respectively and SEM image revealed that both the nanoparticles are spherical in shape. Antibacterial activity tested against gram positive (*Micrococcus luteus, Staphylococcus aureus, Streptococcus pneumonia*) and gram negative (*Escheria coli and Pseudomonas aeruginosa*) bacteria. These ZnO-NPs showed antibacterial activity against both the gram positive and gram negative bacteria. Broad spectrum of these NPs could be made them very effective bactericide.

Key words : Green synthesis of Zinc Oxide Nanoparticles (ZnO-NPs), XRD, SEM, EDAX, Antibacterial activity, *Premna barbata.*

Introduction

The term "Nanotechnology" has been coined by "Norio Taniguchi", a researcher at the University of Tokyo, Japan in 1974. Nanotechnology may be defined as the manipulation of the particle with the size dimension smaller than 100nm and having specific properties, which can be used in particular applications (Kurkure *et al.,* 2016). Nanotechnology is the technology, which deals with nanometer-sized objects. It has been expected that at materials, devices and systems are the levels at which nanotechnology will be developed. The levels of nanomaterials are the most advanced levels scientific knowledge and in commercial applications (Salata, 2003).

In the past decade, it has been perceived that the field of the fabrication of nanoparticles with controlled morphologies and noteworthy features increased and making it an extensive area of research. In chemistry, the synthesis of nanoparticles with crystalline nature, control shape and particle size has been one of the main objectives that could be used for potential applications i.e. catalyst for bacterial biotoxin elimination, bio-medical, a lower cost electrode, biosensor, separation science, cancer treatment, biosensors, targeted drug delivery, magnetic resonance imaging [MRI], gene therapy, antibacterial agents, enhancing reaction rates and DNA analysis (Sharma *et al.,* 2015; Li *et al.,* 2011). In modern medical science, it has been seen that nanotechnology became the newest and one of the most promising areas of research. Nanoparticles exhibited pioneering and enhanced properties as compared to larger particles of their bulk materials from which they are made. These properties are based on dimension, morphology and distribution. The surface area to volume ratio of NPs is inversely proportional to the size of NPs. The biological efficacy of nanoparticles is directly proportional to their specific surface area (Dipankar *et al.,* 2012).

Zinc oxide nanoparticles has become a great interest of the researches and scientific or industrial society because of miscellaneous applications in sensors,

***Author for correspondence :** E-mail : devendra_negi@yahoo.com

catalysis, solar energy conversion, paints, fibers, cosmetics, and drug delivery efficient antibacterial (Kumar, *et al.* 2017; Narasimha *et al.,* 2014) and antifungal properties. Recently, it has been reported that zinc oxide nanoparticles have been used to make sunscreens, antibacterial creams, self-cleaning glass, deodorants, ointments and lotions, food packaging and ceramics. Zinc oxide became interesting among all the metals due to its unique properties: stability, high catalytic activity, luminescence properties, large surface area, and intensive IR and UV-Vis absorption (Varghese and George, 2015; Geetha *et al.,* 2016; Jamdagni *et al.* 2016; Vaishnav *et al.,* 2017; Espitia *et al.,* 2012; Li *et al.,* 2008; Marcous *et al.,* 2017; Marcous *et al.* 2017).

Premna barbata belongs to genus *Premna* and *Verbenaceae* family. Generally, genus *Premna* is trees and shrubs. A rich amount of iridoid, flavonoids, glycosides, and diterpenoids are found in the *Premna* genus, which indicated by phytochemical literature review. Furthermore, sesquiterpenoids, isoflavones, xanthones, triterpenoides, and ligans are also known to be isolated secondary metabolites from many species of genus *Premna*. These isolated classes of secondary metabolites have been found to show biological application such as antioxidant, anti-inflammatory, hepatoprotective, antibacterial and cytotoxic (Rekha *et al.,* 2015). In this study, we have been firstly reported the eco-friendly, non-toxic and cost-effective green synthesis of the leaf and twig extract of *Premna barbata*. These synthesized nanoparticles further carried out for the comparative antibacterial activity against *Micrococcus luteus, Staphylococcus aureus, Streptococcus pneumoniae, Escheria coli* and *Pseudomonas aeruginosa*.

Material and Method

Twig and leaves of *Premna barbata* Wall. were collected from the Depatment of Botany and Microbiology, H.N.B. Garhwal university, Srinagar, Uttarakhand, India (Fig. 1). Plant has been identified by Garhwal University Herbarium and given accession number is 20763. Zinc nitrate and silver nitrate were purchased from Sigma Aldrich and NaOH and methanol were purchased from Fischer Scientific. Double distilled water was used for the experiment.

Preparation of plant extract

Twig and leaves of *Premna barbata* were cut into small pieces and dried at room temperature. 5 gm of each part of the plant washed with distilled water and boiled with 50 ml of the double distilled water at 50-60^{0}C for 20 min. The extract filtered through Whattman filter No. 1 and filtrate stored in refrigerator.

Green synthesis of zinc oxide nanoparticles

20 ml of the plant extract is heated at 60^{0}C for 10 min under magnetic stirring and then 50 ml solution of the $Zn(NO_3)_2.6H_2O$ in double distilled water added drop wise to the plant extract. The mixture was heated under continuous stirring for 15 min and few drops of NaOH added for maintaining the pH of solution. The reaction solution centrifuged at 3000 rpm and pale white precipitate kept at room temperature for drying. After drying the precipitate, pale white NPs are washed with methanol to remove the unreacted material and used for the further characterization (Fig. 2) (Bala *et al.,* 2015; Ghorbani, 2015).

XRD (X-Ray Powder diffraction spectroscopy)

This analysis was carried out using X-ray diffractometer (PANalytical, X*PERT PRO), using a radiation of CuKα with 1.54 Å wavelength (Azizietal, 2014). XRD was used to analyze the crystalline nature of nanoparticles and for providing information us on unit cell dimensions. XRD also determined the crystalline size of nanoparticles using Debye Scherrer's equation:

$$D = K\lambda / \beta\cos\theta$$

Where D= Crystallite size, K= shape factor, λ = wavelength, β = full width at half maxima and θ = angle.

SEM (Scanning Electron Microscopy) analysis

Scanning Electron Microscope (CARI ZEISS, MA15/EVO18) was used to determine the morphology of the green synthesized zinc oxide nanoparticles (Jagtap and Bapat, 2013).

EDAX (Energy Dispersive analysis of X-rays) Pattern

Chemical composition of the nanoparticles was determined by EDAX pattern followed by SEM analysis (Jagtap and Bapat, 2013).

Antimicrobial assay

Zinc oxide nanoparticles of the leaf and twig extract were tested for their antimicrobial activity against three gram positive and two gram negative pathogens *viz*, *Micrococcus luteus*, *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* (clinical isolates and got from the Department of Botany and Microbiology, H.N.B. Garhwal university, Srinagar (Uttarakhand) India) respectively by using the agar well diffusion method (Andrews, 2001). A suspension of zinc nanoparticles of varying concentrations of 70ug/ml, 100ug/ml and 150ug/ml in 0.5% DMSO was used for screening the antimicrobial activity of nanoparticles against the test microorganisms. DMSO was used as a control and

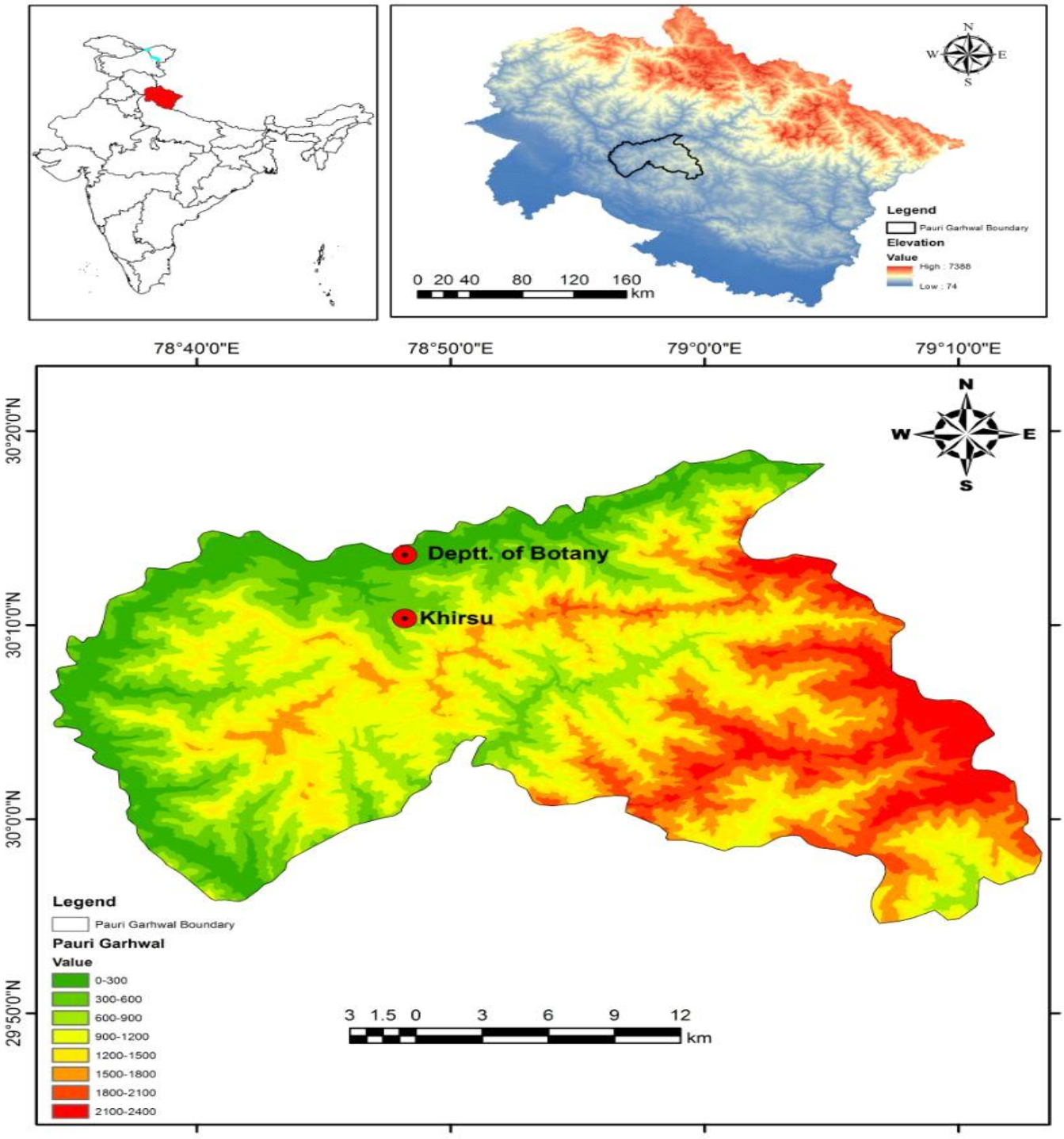


Fig. 1: Field location map.

erythromycin has been used as a standard antibiotics. A 100ul of overnight culture of all the test microorganisms was spread on Muller Hinton agar plates aseptically and allowed to dry for 15minutes. Subsequently four adequately spaced wells of 8mm in diameter were punched into the plates using a sterile cork-borer. A 70ul zinc oxide nanoparticles solution of each concentration was poured into each well under aseptic conditions. The plates were kept at room temperature to allow the extract to diffuse into agar medium. The plates were then incubated at 37°C for a period of 24h and observed for the zone of inhibition. The antimicrobial activity of the nanoparticles was determined by measuring the zone of inhibition surrounding the wells. The assay was performed in triplicates for the accuracy of the results.

Result and Discussion

XRD analysis determined the crystallite nature of

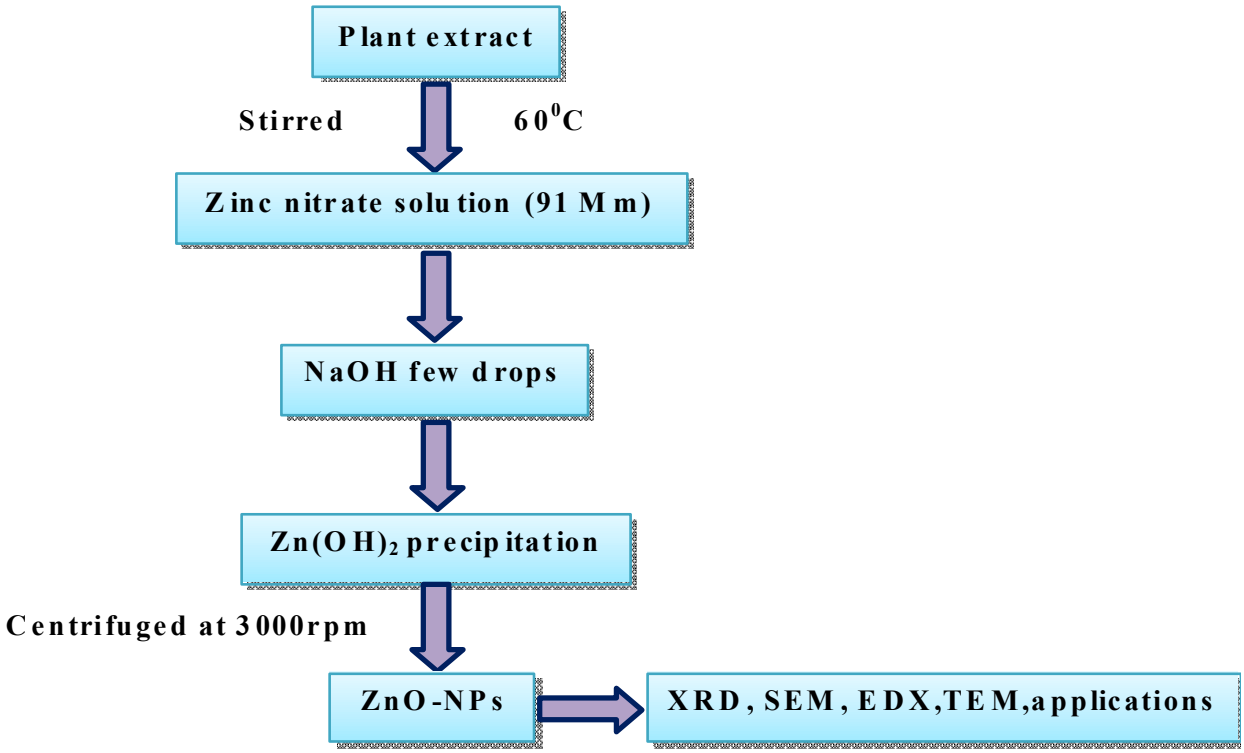


Fig. 2: Flow chart of the ZnO-NPs synthesis.

the synthesized zinc oxide nanoparticles. Sharp peaks showed the highly crystalline nature. High intense peak at 2θ of 36.33 for the ZnO-NPs of leaf extract (Fig. 3a) and 36.40 for the ZnO-NPs of twig extract (Fig. 3b), correspond to (101) lattice parameter showed the hexagonal crystal nature of the synthesized zinc oxide nanoparticles. K. Elumalai and S. Velmurugan, (2015) reported the hexagonal wurtzite structure of the zinc oxide nanoparticles (Elumalai and Velmurugan, 2015). The crystalline size of ZnO-NPs of the leaf and twig extract of *Premna barbata* are 22.1 and 81.5 nm respectively.

SEM images revealed the spherical shape of both the zinc oxide nanoparticles (Fig. 4a and 4b). Haritha Meruvu *et al.,* (2011) demonstrated the spherical shape of zinc oxide nanoparticles with the size 30-63 nm (Meruvu *et al.,* 2011).

EDAX pattern showed that the synthesized nanoparticles contain Zn and O in good quantity (Fig. 5a and 5b). Other peaks may be due to the biological compounds present in the plant material. In figure 5a signal of the Zn found at 0.9 and 1 keV and the signal of O found at 0.5 keV and in figure 5b signal of the Zn found at 0.9 and 1 keV and the signal of oxygen found at 0.5 keV. These signals confirmed the presence of ZnO.

Antibacterial activity of the synthesized zinc oxide nanoparticles has been tested on both the gram positive and gram negative bacteria. The zone of inhibition of

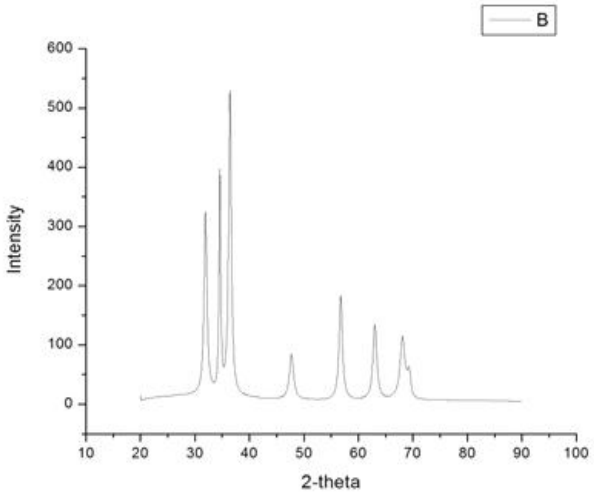


Fig. 3b: XRD pattern of ZnO-NPs of the twig extract of *P. barbata.*

ZnO-NPs of the leaf extract against these bacterial pathogen shown in the table1 and (Fig. 6a). The calculated zone of inhibition of ZnO-NPs of the twig extract has shown in the table 2 and in the figure 6b. ZnO-NPs of the leaf extract showed more antibacterial activity as compare to ZnO-NPs of twig extract. ZnO-NPs of leaves extract shown antibacterial activity against *M. luteus, S. aureus, E. coli* and *S. pneumonia* while ZnO-NPs of the twig extract shown antibacterial activity against *M. luteus* and *E. coli* only. Both the ZnO-NPs showed a broad spectrum, which enhance their applications in the

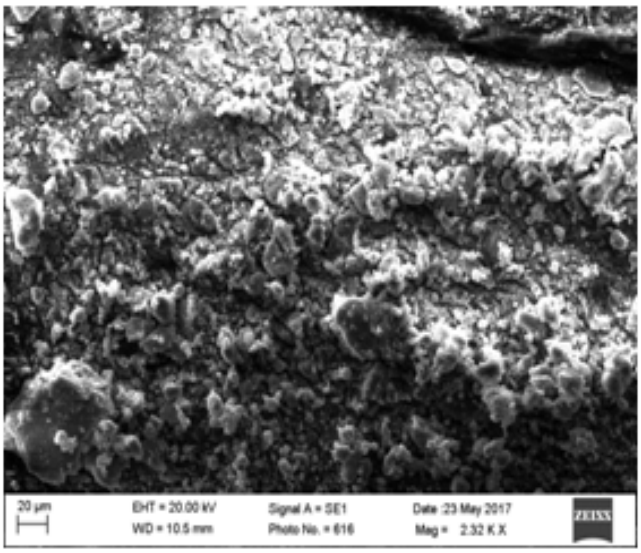


Fig. 4a: SEM image of ZnO-NPs of the leaf extract of *Premna barbata.*

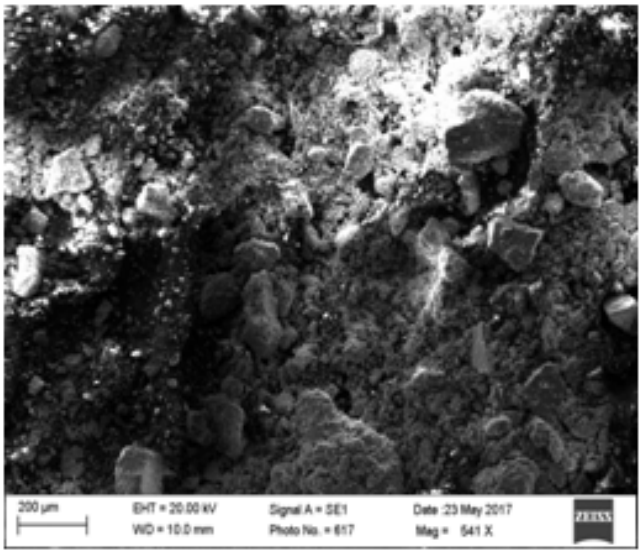


Fig. 4b: SEM image of ZnO-NPs of the twig extract of *P. barbata.*

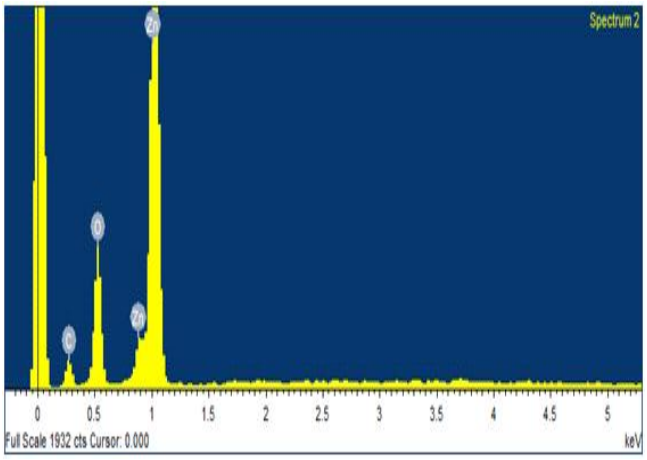


Fig. 5a: EDAX pattern of ZnO-NPs of the leaf extract of *P. barbata.*

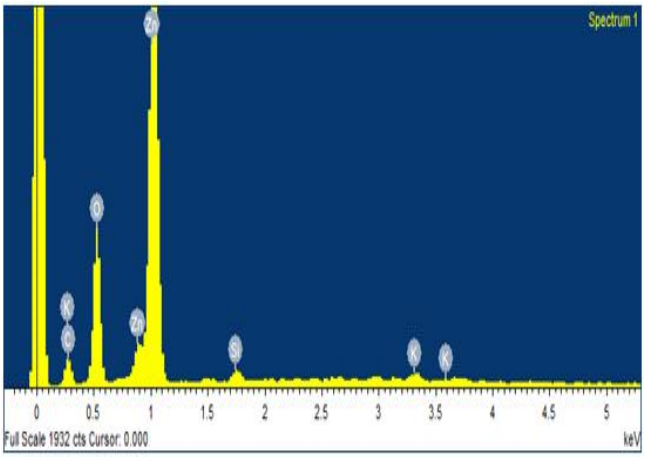
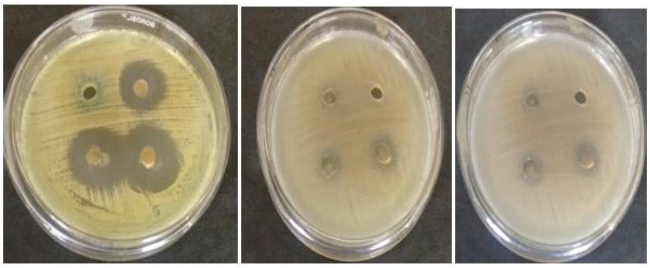
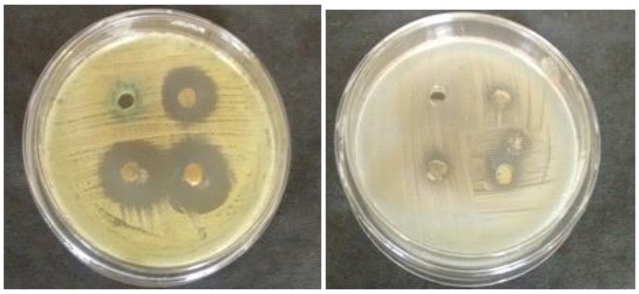


Fig. 5b: EDAX pattern of ZnO-NPs of the twig extract of *P. barbata.*



M. luteus *E. coli* *S. pneumoniae*

Fig. 6a: Antimicrobial assay of the leaf extract of *P. barbata.*



M. luteus *E. coli*

Fig. 6b: Antimicrobial assay of ZnO-NPs of the twig extract of *P. barbata.*

biomedical field ZnO-NPs of the leaf extract showed more activity against *M. luteus, S. aureus* and *E. coli* as compare to the standard antibiotics and ZnO-NPs of the twig extract have good antibacterial activity against *E. coli*, while standard antibiotics having no zone of inhibition against *E. coli*.

There are many ZnO-NPs with their antibacterial property in the literature. Some are given in the table: 3.

Conclusion

Zinc oxide nanoparticles have been synthesized via green method using leaf and twig extract of *Premna barbata*. The characteristic techniques (XRD, SEM and

EDAX) confirmed the formation of Zinc oxide nanoparticles. These nanoparticles showed antibacterial activity against both the gram positive and gram negative bacteria; due to the broad spectrum these nanoparticles have good antibacterial activity. These ZnO-NPs could be used as effectual bactericide in the field of biomedical and study may be useful for the further studies in the field of nanotechnology.

Table 1: Antimicrobial assay of ZnO-NPs of the leaf extract of *P. barbata*.

Cork diameter- 8mm

S.N.	Organism	Control	Erythromycin (15 µg)	70 µL	100µL	150µL
1.	*M. luteus*	-	23 mm	21 mm	24 mm	28 mm
2.	*S. aureus*	-	14.5 mm	12 mm	15 mm	17 mm
3.	*E. coli*	-	-	-	13 mm	15 mm
4.	*S. pneumoniae*	-	20 mm	11 mm	15 mm	17 mm
5.	*P. aeruginosa*	-	10 mm	-	-	-

Table 2: Antimicrobial assay of ZnO-NPs of the twig extract of *P. barbata*.

Cork diameter- 8mm

S.N.	Organism	Control	Erythromycin (15 µg)	70 µL	100µL	150µL
1.	*M. luteus*	-	23 mm	-	13 mm	19 mm
2.	*S. aureus*	-	14.5 mm	-	-	-
3.	*E. coli*	-	-	11 mm	15 mm	18 mm
4.	*S. pneumoniae*	-	20 mm	-	-	-
5.	*P. aeruginosa*	-	10 mm	-	-	-

Table 3: Antibacterial assay of some zinc oxide nanoparticles.

Plant	Part	Particle size	Antibacterial Activity	Reference
Trifolium pratense	Flower	60-70 nm	*S. aureus* - 22 mm *P. aeruginosa*- 21mm *E. coli* – 22 mm	[24]
P. caerulea L.	Leaf	37.67 nm	*E. coli* – 13 mm *Enterococcus sp.*- 9.33mm *Klebsiella sp.*- 11 mm *Streptococcus sp.*- 11.67mm	[25]
Costus pictus D. *Don*	Leaf	29.1 nm	*S. aureus* – 10 mm *B. subtilis*- 17 mm *E. coli* – 10 mm *S. paratyphi* – 12 mm	[26]
Camellia sinensis	leaf	16 nm	*K. pneumonia*- 10.3 mm *P. aeruginosa*- 3.3 mm *E. coli*- No inhibition *S. aureus*- 5.3 mm	[27]
Premna barbata	Leaf	22.1 nm	*M. luteus*- 24 mm *S. aureus*- 15 mm *E. coli*- 13 mm *S. pneumoniae*- 15 mm *P. aeruginosa*- no inhibition	Present study
Premna barbata	Twig	81.5 nm	*M. luteus*- 19 mm *S. aureus*- no inhibition *E. coli*- 18 mm *S. pneumoniae*- no inhibition *P. aeruginosa*- no inhibition	Present study

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