

Biosynthesis, Characterization And Antimicrobial Activities Of Zinc Oxide Nanoparticles From Leaf Extract Of *Acmella Oleracea*

K. Sowmiya, J. Thomas Joseph Prakash*

Abstract: The potential for the synthesis of metallic and semiconductor nanoparticles has expanded research applications in the development of novel technologies. In the present study, a simple and eco-friendly package. Zinc oxide nanoparticles (ZnO NPs) use *Acmella oleracea* leaf extract. ZnO NPs are characterized by various techniques such as UV-Vis, XRD, DLS, FE-SEM, EDX and FT-IR. XRD data showed the crystallinity of nanoparticles and EDX measurements indicates higher zinc content 67.63% and 32.37% of oxygen. FT-IR confirmed the presence of functional groups of leaf extract and ZnO NPs. DLS results confirmed successful synthesis of ZnO nanoparticles. Size and morphology of particles determined from FE-SEM and UV visible absorption spectra ZnO NPs exhibited absorption band at 314 nm. Combined ZnO nanoparticles are potentially antibacterial agents have been studied in *Enterococcus aerogenes*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. Antifungal agent have been studied in *Candida albicans* and *Candida vulgaris*. These results indicate aqueous extract *Acmella oleracea* Effective reducing agents for green synthesis of ZnO NPs with significant antimicrobial potential.

Key word: *Acmella oleracea*, Zinc Oxide nanoparticles, FTIR, XRD, FE-SEM, Antibacterial Activity, Antifungal Activity.

1. INTRODUCTION

Nowadays, nanotechnology is one of the most active fields of research, it is related to physical, chemical, biological and engineering Nanotechnology [1]. Nanoparticles (NPs) obtained Significant interest due to their very low size (1–100 nm) The largest surface to volume ratio that causes both physical and chemical Variations in their properties such as catalytic activity, heat and the like Electrical conductivity, biological and steric properties, melting point, Mechanical properties and optical absorption compared to most The same chemical compound [2]. Preparation of semiconductor nanoparticles (NPs) is one of the most emotionally studied subjects. Their novel optical, chemical, and optical sciences over the last two decades. Photo electrochemical and electronic properties are different in total [3]. Zinc oxide (ZnO) is one of the most exploited n-type semi-behaviors Due to its uniformity and multi-functional of metal oxide materials Morphological, photonic and spintronics properties [4,5]. It is classified With a wide live band gap and high excitation of 3.37 eV 60 MeV energy [6]. It has many positive properties such as high electron mobility, high room temperature luminosity, good transparency etc. These properties are used in applications such as solar cells [7,8], Photovoltaics [9], photosynthesis [10], anti-bacterial material [11,12], Catalyst [13], drug delivery and nanoparticle [14,15], anti-biofilm [16,17] and anti-diabetic activities [18]. Zinc oxide nanoparticles can Sol-gel [19] can be prepared by several techniques such as hydrothermal [20], solvothermal [21] and pulsed laser deposition [22]. In addition, Such methods are generally expensive, labor-intensive and feasible harmful to the environment and organisms [23]. In recent years, research has focused on increasing methods aimed at producing environmentally friendly nanoparticles, they are not use toxic substances [24]. Green sources can stabilize and function reduce the agent for the synthesis of form and size controlled metal and metal oxide nanoparticles [25,26]. This biological approach appears be a cost effective alternative to regular body and chemistry package methods. Enzymes [27], leaf extract

[28,29] and bacteria [30] play an important role in the green synthesis of nanoparticles. The genus *Acmella*, class pinopsida, order Asterales, family Asteraceae and species *Acmella oleracea* [31]. The leaves of *Acmella oleracea* (Tooth ache plant) have therapeutic significance. These are effective in all respiratory diseases. [32]. Anti-inflammatory and anti-oxidant [33], antigenotoxic [34]. There are also antibacterial [35,36] effects of pulegium Demonstrated. Key components Pulcon (40.5%), Mentone (35.4%) and Piperidone (5.2%) and Phytochemical Study Showed that flavonoid, terpenoid and phenolic compounds were important Elements of methanol and water extracts [37]. In this regard, antibacterial activity of ZnO was tested and the effect was greater than that of Gram-positive and Gram-negative bacteria [38]. Usually, smaller amounts of components reveal more surface area. The biodegradable ZnO nanoparticles per volume ratio were investigated for antimicrobial activities. Other proposed anti-bacterial resistance mechanisms of ZnO NPs include the direct interaction between ZnO NPs and bacterial cells that affect cell membrane permeability altering membrane irregularity and protein structure.. The biodegradable ZnO nanoparticles showed enhanced antimicrobial activity than the chemically synthesized one [39,40]. In the present study we aimed to investigate the antibacterial properties of the synthesized ZnO NPs It is conceived by *Acmella oleracea* leaf via green system.

2. Material and Method

2.1. Synthesis of zinc oxide nanoparticles

10g of *Acmella oleracea* leaf extract (fig.1) mixed with 100 ml of double distilled water. The mixture was boiled for 30min to 60 ° C. The mixture was cooled to room temperature and filtered whatman No 1 paper. The filtered compound was used for the extraction and synthesis of ZnO NP nanoparticles. 0.01 M zinc oxide precursor and 5 mL leaf extract was used as a reducing agent. Within a certain period, the color of the solution was chaged (fig 2). The mixture was stirred in a magnet plate at 80 ° C for 20

min. The mixture is 2 hours and ultraviolet-visible The measurements were recorded in which a strong peak was observed. The mixture was centrifuged at 5000 rpm for 10 min . The particle was collected and dried overnight in a hot-air oven running at 80 ° C. White powder Obtained and used for characterization.

2.2 Characterization of ZnO NP synthesis

The synthesis of nanoparticles was confirmed by ultraviolet-visible measurements after visual color change. Infrared spectroscopy (FTIR) analysis was used to identify the activity Groups of plant extracts that cause zinc ion to be reduced Nanoparticles are formed. X-ray diffraction analysis (XRD) was used to identify Crystal structure of lattice plane, ZnO NP. DLS of this suspension was then performed using Nano Plus to analysis particle size. Shape and size were determined using field emission scanning electron microscopy (FESEM). The basic composition energy distribution of the combined nanoparticles was calculated using X-ray Analysis (EDAX).

2.3 Anti bacterial Activity

To investigate the antibacterial activity of a sample of zinc nanoparticles, three bacterial strains were *Enterococcus aerogenes* (MTCC 29212), *Pseudomonas aeruginosa* (MDCC 27853), and *Proteus vulgaris* (MDCC 7299) were prepared as test organisms. All strains were purchased from Microbial Culture and Collection (MDCC), Chandigarh, India. Bacterial strains were cultured at 37°C and maintained on a gradient in nutrient agar (Difco, USA) for 4°C.

2.4 Antifungal Activities:

Culture media The medium used for the mildew test is Sabou Rot's Dextrose Agar / Broth Hi Media Pvt. Bombay, india . Inoculum

Fungal strains were individually vaccinated for 6 h in Sabou Rat's dextrose broth, and the suspensions were tested to provide approximately 10⁵ CFU / ml. Fungal strains are used The clinical fungal test organisms used for study are *Candida albicans* (MTCC-3498) and *Candida vulgaris* (MTCC 227), were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

3 RESULT AND DISCUSSION

3.1. UV-vis spectrum

The optical properties of biodegradable ZnO NPs were investigated using ultraviolet-vis absorption methods. As can be seen from Fig 3, a sharp absorption band around the wavelength of 314 nm is evident in the ultraviolet-vis absorption spectrum.

3.2. FTIR Analysis.

The FTIR spectrum shows a peak at 3843 cm⁻¹ which is associated to stretching vibration of OH (hydroxyl group) while the broad peak at 3451 cm⁻¹ belongs to -OH unit of -COOH group of galacturonic acid . A small peak at 2896 cm⁻¹ belongs to the methylene group (-CH₂ asymmetric stretching) in gum peak at 2831 cm⁻¹ ia associated with the carbonyl stretching vibrations. The vibrational extension of the di-galacturonic acid unit of cumine carboxylate anion appears at 2075 cm⁻¹. The peaks appeared at 1817 and

1642 cm⁻¹ are related to C-C bending vibrations and C-O-C stretching vibration respectively . The absorption peak at 1399 - 1267 cm⁻¹ corresponds to the saturated primary alcohol C-O stretching. The prominent stages of double absorption observed at 1180 - 712 cm⁻¹ reveal the presence of C-H stretching vibrations of an aromatic aldehyde. The peak of stretching vibration of C-O of polyol and other group is appeared at 601 cm⁻¹. A small peak at 561 cm⁻¹ may be attributed to the pyranose ring of polysaccharide (fig.4). There are soluble elements from the FTIR end *Acemella oleracea* leaf extract as a capping agent Aggregate and related to nanoparticles in solution Their additional cellular synthesis and role in shaping [48,49].

3.3 XRD Analysis

XRD method of bio-synthesized ZnO nanoparticles from leaves the extract of *acemella oleracea* is shown in Fig 5. Distinctive distinction $2\theta = 31.74^\circ, 34.41^\circ, 36.23^\circ, 47.53^\circ, 56.60^\circ, 62.86^\circ, 66.43^\circ$ and 67.96° were assigned to (100), (002), (101), (102), (110), (103), (200), (112) planes, respectively. Sharp and narrow peaks indicate the presence of nanoparticles Crystallized. The crystal size of the nanoparticles was calculated by the Debye-Scherrer formula.

3.4 DLS Analysis

DLS is a growing and widely used technique for calculating the hydrodynamic diameter of nanoparticle suspensions based on the Brownian motions exhibited by the particles. The average hydrodynamic diameter calculated by DLS is 74.36 nm (Fig. 6).

3.5 FE-SEM and EDX analysis

The FE-SEM image of the prepared ZnO NPs is presented in Fig.7. The nanoparticles are semi-spherical and diameter The particles are 60-80 nm. Further insights into the features of ZnO NPs Quantitative analysis was performed using EDS, which revealed the presence Significant quality ZnONPs (Fig.8). The results showed high values Zinc (66.17%) and oxygen (42.12%)(Table 1), respectively [50,51].

3.6 Antibacterial activity of zinc nanoparticle sample (disc diffusion method)

The antibacterial activity of the zinc nanoparticle sample was determined using the disk diffusion method. Petridishes (60 mm in diameter) were prepared with Muller Hinton agar and vaccinated with test organisms. A sterile disk of six millimeters wide was inserted into 10 µl of different samples, respectively. The prepared discs were placed on the top layer of agar plates and left to mix for 30 min at room temperature. Positive control was prepared using 10 µl of amoxicillin fixed antibiotic disc. The dishes were incubated at 37°C for 24 h, and the inhibition zone was recorded in millimeters and the test was repeated twice. The results of the antibacterial activity of various samples were tested against pathogens by disk diffusion method are shown in (Table 2). The Sample D showed growth inhibitory activity against *Enterococcus aerogenes* (6 mm) and *Proteus vulgaris* (7 mm). At sample C exhibited the antibacterial activity all the four bacteria, but was more susceptible against *Enterococcus aerogenes* (4 mm) and *Proteus vulgaris* (5 mm).. However, the crude extract and

synthesized nanoparticles showed better inhibitory actions against pathogens (fig.9).

3.7 Antifungal activity

Antifungal activity of sample was determined using the disc diffusion method. The petridishes (diameter 60 mm) was prepared with Sabouraud's dextrose agar (SDA) and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of various samples. The prepared discs were placed on the top layer of agar plates and left to mix for 30 min at room temperature for compound diffusion. Positive control was prepared using the 10 µl of Fluconazole as standard antibiotic disc. The dishes were incubated for 24 h at 37°C and the zone of inhibition was recorded in millimeters (fig.10). Table 3 shows results of the antifungal susceptibility test of the different samples and against the test organisms. From the result, the sample D was the most effective and the highest activity was demonstrated against *Candida albicans* (2 mm zone of inhibition).

4. CONCLUSION

Green synthesis of zinc oxide nanoparticles using fresh leaf extract of *Acmella oleracea* provides an eco-friendly, rapid, simple, non-toxic and efficient means for the synthesis of nanoparticles. Synthesized ZnO NPs were further characterized using UV-Vis absorption spectroscopy, XRD, DLS, FE-SEM and EDAX, FTIR spectroscopy. The UV-Visible spectra suggested the presence of a strong peak at 314 nm that would stabilize the nanoparticles synthesis. The FE-SEM micrograph demonstrated the presence of spherical nanoparticles with a size range of 60–80 nm. There was crystal size was further confirmed using XRD method analysis. EDAX and XRD demonstrate the purity of the formed nanocrystals. DLS studies show the size distribution of nanoparticles. FTIR spectra depicted at 3843 cm⁻¹ correspond to the vibration of the hydroxyl group, the characteristic peak for the synthesis of zinc oxide nanoparticles. Biodegradable ZnO nanoparticles exhibited strong antibacterial activity against *Enterococcus aerogenes*, *Pseudomonas aeruginosa* (Gram-positive bacteria) and *Proteus vulgaris* (Gram-negative bacteria). *Candida vulgaris* exhibited stronger mildew behavior than the *Candida albicans* strain. Biodegradable ZnO nanoparticles have been detected to protect against bacterial and fungal phyto pathogens, suggesting that they may be used as commercial antimicrobial agents for commercial biomedical applications.

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FIGURE CAPTIONS

Fig.1 *Acmella oleracea* Leaf

Fig.2 The colour change of *Acmella Oleracea* plant extract after the addition of ZnO

Fig.3 UV-Vis spectra of zno-Nanoparticles

Fig.4 FTIR spectra of zno-Nanoparticles

Fig.5 XRD spectra of zno-Nanoparticles

Fig.6 DLS spectra of zno-Nanoparticles

Fig.7 FE-SEM spectra of zno-Nanoparticles

Fig.8 EDX spectra of zno-Nanoparticles

Fig.9 Antibacterial image of ZnO nanoparticles

Fig.10 Antifungal image of ZnO nanoparticles

TABLE CAPTION

Table 1. EDAX results for ZnO nanoparticles.

Table 2. Antibacterial results for ZnO nanoparticles.

Table 3. Antifungal results for ZnO nanoparticles.



Fig.1 Acemella oleracea Leaf

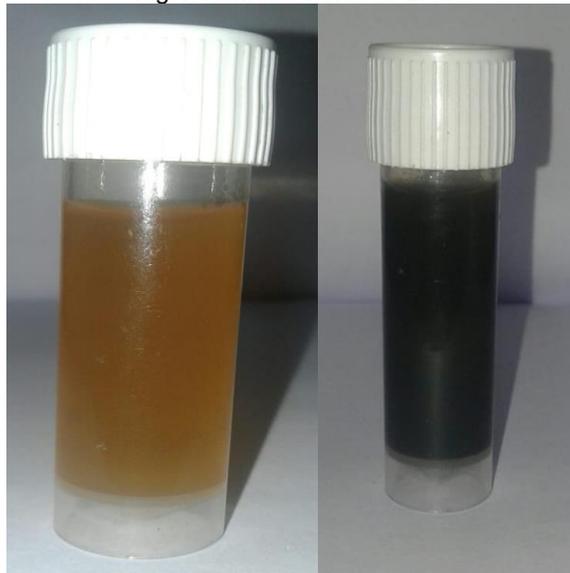


Fig.2 The colour change of Acemella Oleracea plant extract after the addition of ZnO

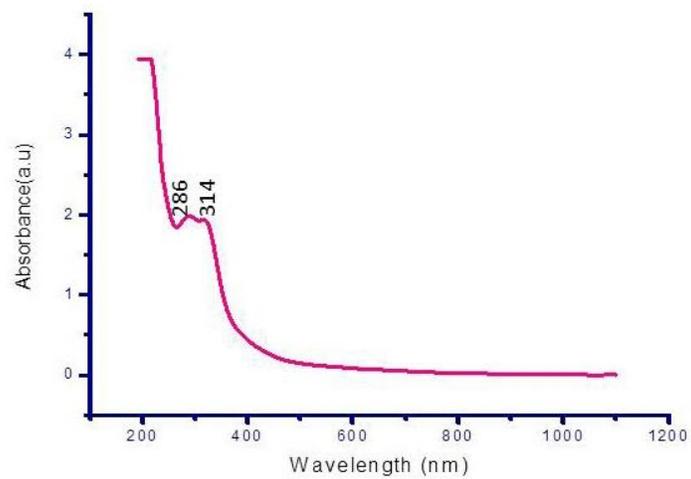


Fig.3 UV-Vis spectra of ZnO-Nanoparticles

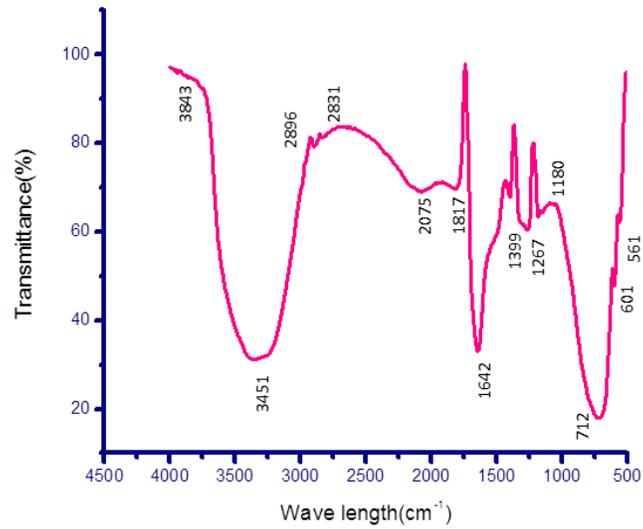


Fig.4 FTIR spectra of znO-Nanoparticles

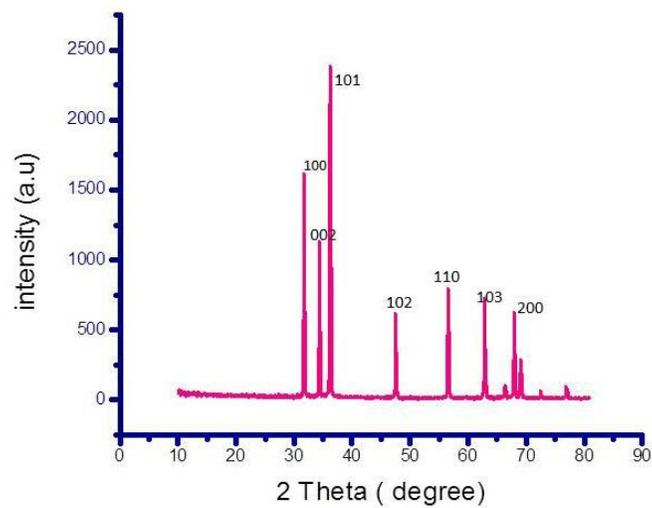


Fig.5 XRD spectra of znO-Nanoparticles

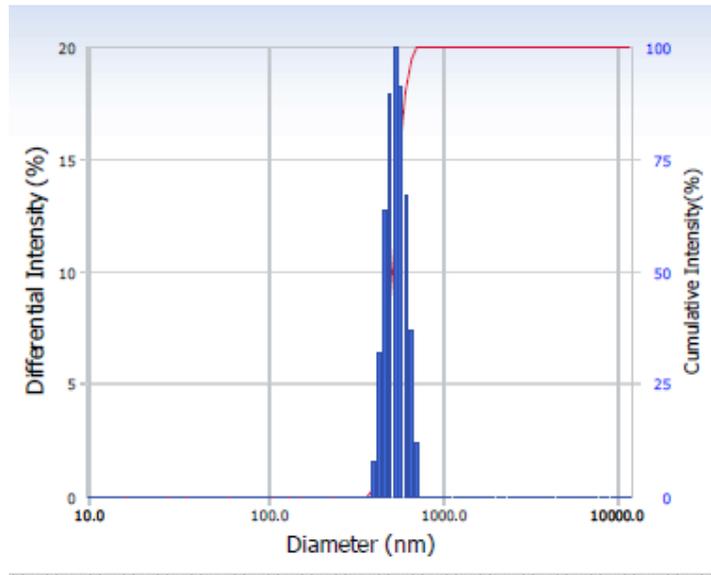


Fig.6 DLS spectra of znO-Nanoparticles

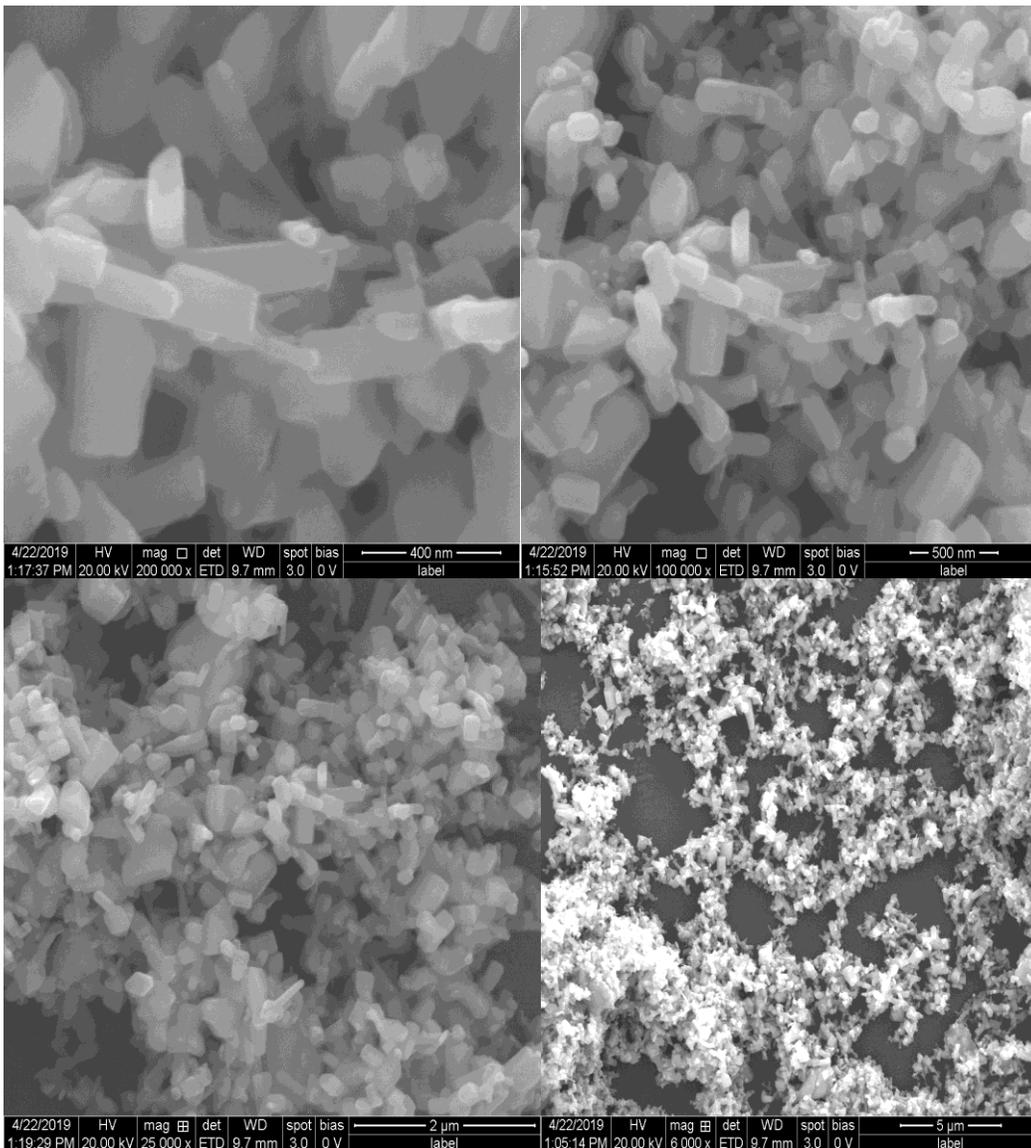


Fig.7 FE-SEM spectra of znO-Nanoparticles

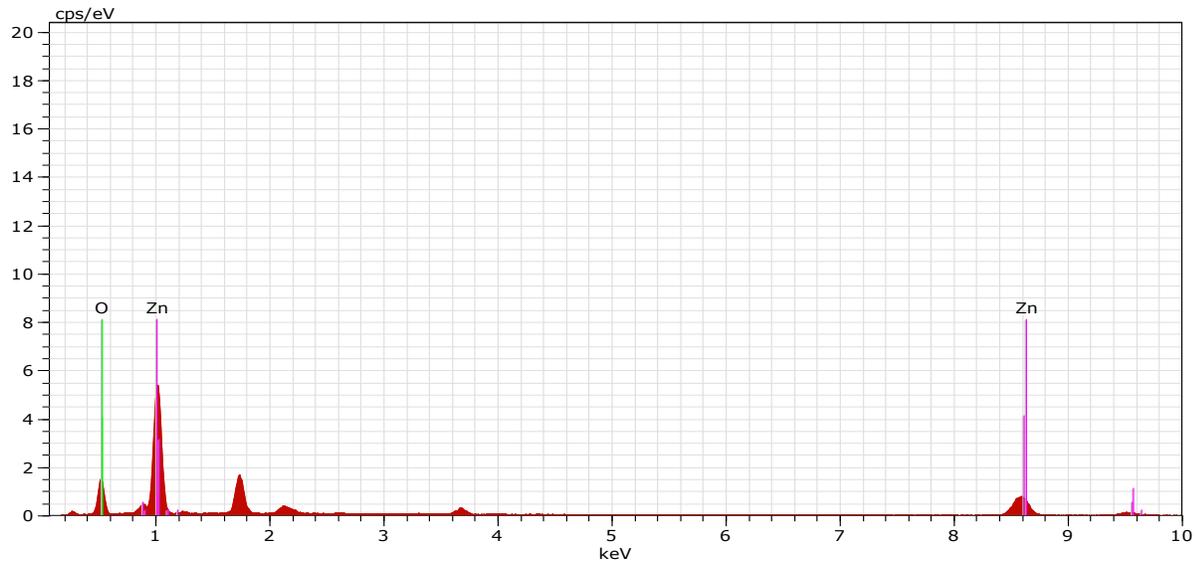
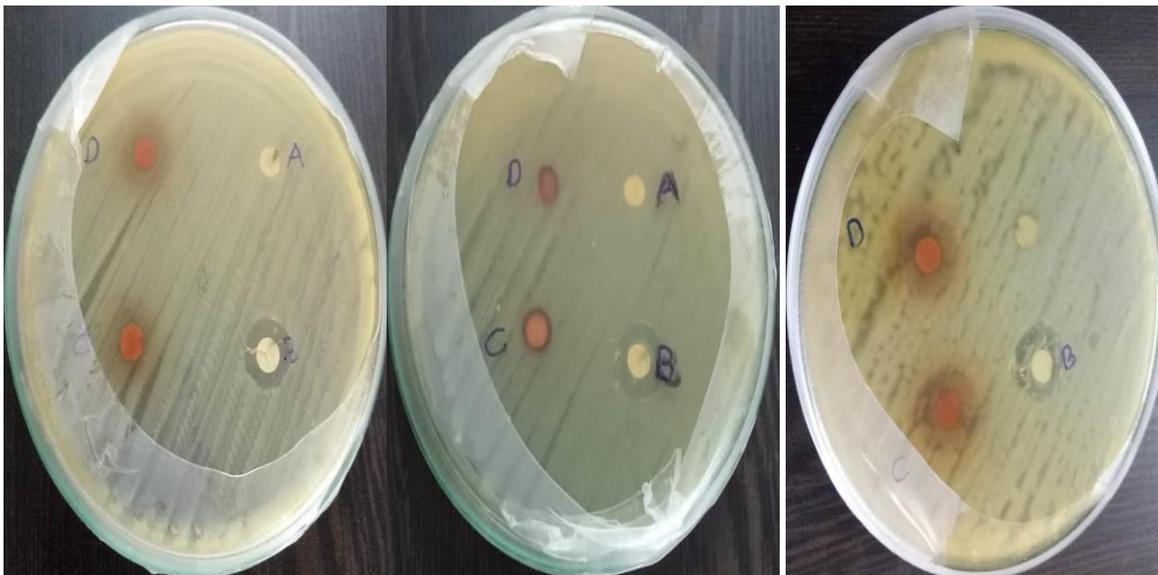


Fig.8 EDX

spectra of zno-Nanoparticles



Enterococcus aerogenes

Pseudomonas aeruginosa

Proteus vulgaris

Fig.9 Antibacterial image of ZnO nanoparticles

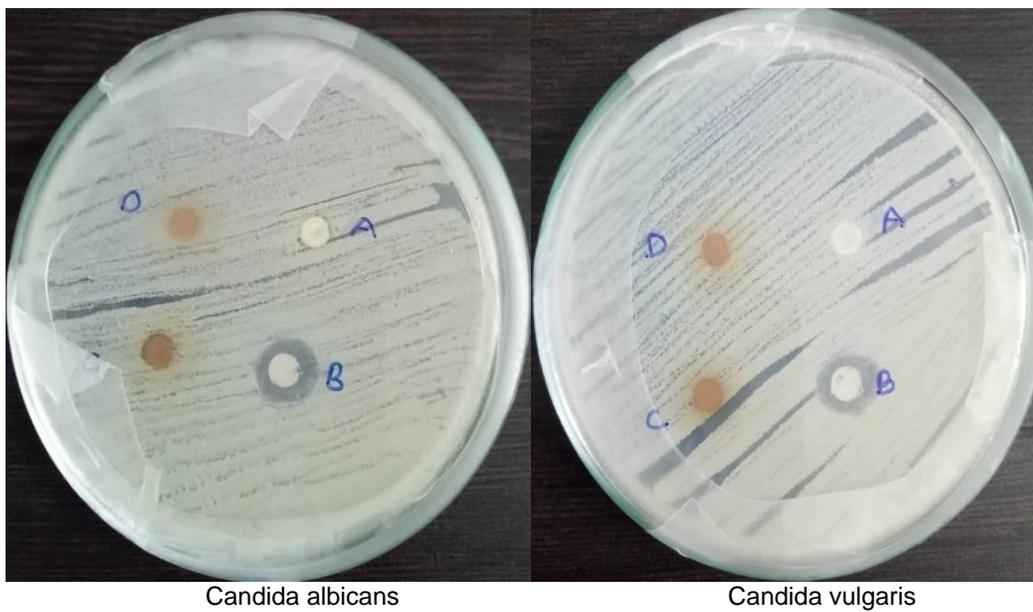


Fig.10 Antifungal image of ZnO nanoparticles

Element	Weight%	Atomic%
Zn	67.63	33.83
O	32.37	66.17
TOTAL	100.00	100.00

Table 1: EDAX results for ZnO nanoparticles

Samples	Concentrations ($\mu\text{l/ml}$)	Organisms/Zone of inhibition (mm)		
		Enterococcus aerogenes	Pseudomonas aeruginosa	Proteus vulgaris
A (Amoxicillin)	10	8	8	8
B (Zinc Oxide)	10	0	0	0
C (Plant extract)	10	4	2	5
D (Nanoparticles)	10	6	0	7

Table 2: Antibacterial results for ZnO nanoparticles

Samples	Concentrations ($\mu\text{l/ml}$)	Organisms/Zone of inhibition (mm)	
		Candida albicans	Candida vulgaris

A (Fluconazole)	B	10 µl	8	8
(Zinc Oxide)		10 µl	0	0
C(Plant extract)		10 µl	3	2
D(Nanoparticals)		10 µl	2	5

Table 3 : Antifungal results for ZnO nanoparticles