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ORIGINAL ARTICLE

Characterization and Antibacterial activity of Green Synthesized ZnO Nanoparticles from *Ocimum basilicum* Leaf Extract

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ABSTRACT
The Zinc Oxide nanoparticles are mostly used in the field of pharmaceutical sciences and it has more number of applications which plays an important role on antibacterial activity. In modern era, the nanoparticle synthesized by biological methods using micro organisms, enzymes, and plant extracts has been suggested as ecofriendly to environment. The nanostructured and highly stable zinc oxide nanoparticles are produced by Zinc nitrate and Ocimum Basilicum leaf Extract. Morphological, Structural and antimicrobial properties of synthesized nanoparticles are characterized by using XRD analysis, SEM, UV-Vis Spectrophotometer, FTIR and Disc diffusion Method (Antimicrobial activity). SEM and XRD analysis shows that synthesized nanoparticles are found to be predominantly hexagonal in shape and the average particle size range from 9 to 18nm. While increasing the concentration of the leaf extract of Ocimum Basilicum, the ZnO nanoparticles size can be increased.
Keywords: *Zinc Oxide, Ocimum basilicum, Antimicrobial activity, SEM, XRD, UV, FTIR*

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INTRODUCTION

Nanoparticles attracted tremendous interest that have been extensively used in biological applications. Nanomaterials are classified into three different groups as '0', '1' and '2' dimensional nanostructures [3]. These Nanoparticles are having noticeable performance in bioelectronics and biomedical applications. The Engineering technology and scientific field of nanosystems are one of the most quickly and exigent developing discipline of nanotechnology [3].

Zinc Oxide is an inorganic metal oxide and has a characteristic of exhibiting a wide range of nanostructures. The photo oxidizing and photocatalytic properties are used against biological and chemical species, which are used to characterize bio-synthesized metal oxides.

In this work we used environmentally benign leaf extract of *Ocimum Basilicum* which has more medicinal properties [9]. While it is a surface stabilizing agent it acts as a biotemplate for the synthesis of Zinc Oxide nanoparticles. The Morphological, structural and antibacterial properties of zinc oxide nanoparticles are also evaluated.

MATERIALS AND METHODS

Synthesis of Zinc Oxide Nanoparticles using *Ocimum Basilicum* leaf Extract

The *Ocimum Basilicum* leaves are collected from the foot hills of Yercaud, Salem, TamilNadu, India. The fresh plant leaves were washed with normal tap water and should be shadow dried. The leaves are crushed and coarsely powdered by mortar and pestle. The Coarsed powders of 25g were subjected to successive extraction in 250 ml of methanol (solvent) with the help of Soxhlet apparatus. Finally, the obtained leaf extract solution was stored and used for the further research. Further 50 ml of *Ocimum Basilicum* methanol extract has taken from prepared stock solution (stored already) and boiled at 60^0 to 80^0C by using stirrer-heater. When temperature reached 60^0C, 5g of Zinc nitrate hexahydrate was added. The mixture was boiled until the formation of deep yellow coloured suspension. The obtained paste was

transferred to ceramic crucible and annealed at 400^{0}C for 2 hours. Finally, the obtained light white coloured powder was used for antibacterial activity, Structural and for other characterization.

Antimicrobial assay

Inoculums are prepared by, that the stock cultures are stored at 4^{0}C on slope of nutrient agar. Active cultures of experiment was prepared by shifting a loopful of cells from the stock cultures to the test tube of Muller-Hinton broth(MHB), that are incubated without agitation for 24 hours at 37^{0}C and 25^{0}C. Then the cultures are diluted with the fresh Muller-Hinton broth to gain optical densities approximately to 2.0 x 10^6 CFU/ ml for bacteria.

Here the disc diffusion method (Bauer *et al.,* 1966) was used to screen the antibacterial activity. In *vitro*, the microbial activity has been screened by using Muller-Hinton Agar (MHA) from Hi-media, Mumbai. The MHA plates are prepared using 15 ml of molten media into sterile petri plates. The plates are dried for 5 minutes and 0.1 %, inoculums suspension was swabbed throughout the plate and dried for 5 minutes. The concentration of extracts is 4 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of the medium and extract was allowed to diffuse for 5 minutes and the plates are kept in incubator for 24 hours at 37^{0}C. As a result, the incubation zones on the disc were measured with the help of transparent ruler. The inhibition zones are obtained in the range of millimeter.

RESULTS AND DISCUSSION

Macroscopic characteristics

The *Ocimum Basilicum* leaf is a compound and opposite bipinnateparipinnate with oblong shape mucoranate apex and has an average length of 15–20 cm. Single leaflet is 2–3 cm long and 10–15 mm breadth, oblong, linear, mucronate, setaceous deciduous. On average, in a mature compound leaf**,** there are 8– 10 paired leaflets.

Physicochemical analysis

The Physicochemical analysis of *Ocimum Basilicum* is shown in the **Table 1**. It shows the soluble and insoluble ash in percentage (%).

Table 1: Physicochemical Properties

S.No	Parameter	Values obtained (%)
1	Total Ash (%)	11.31
2	Water Soluble ash (%)	14.6
3	Water Insoluble ash (%)	10.3
4	Acid Soluble ash (%)	7.6
5	Acid Insoluble ash (%)	0.47
6	Sulphated ash (%)	17.3

Phytochemical analysis

The qualitative phytochemical analysis of the *Ocimum Basilicum* leaf is shown in the **Table 2**. It shows the presence of alkaloids, flavonoids, Terpenoids, phenols and carbohydrates and hence steroids, anthroquinone, saponins, tannin, oils and resins were found to be absent in the extract [9].

Table 2: The qualitative phytochemical analysis

Phytochemicals	Observations	Sample B
Alkaloids Mayer's test Wagner's test	Cream color Reddish brown solution/ precipitate	+ +
Flavonoids Lead acetate test H_2SO_4 test	Yellow orange Reddish brown / Orange colour precipitate	+ +
Steroids Liebermann-Burchard test	Violet to blue or Green color formation	-
Terpenoids Salkowski test	Reddish brown precipitate	+
Anthroquinone Borntrager's test	Pink color	-
Phenols Ferric chloride test Lead acetate test	Deep blue to Black colour formation White precipitate	+ +
Saponin	Stable persistent	-
Tannin	Brownish green / Blue black	-
Carbohydrates	Yellow / brownish / blue / green color	+
Oil and Resin	Filter paper test	-

(+) - Presence, (-) - Absence

X-Ray Diffraction

By scattering the X-ray beam on the sample, we get the information about the crystallographic structure, chemical and physical properties of the ZnO nanoparticles. The 2θ values 32.75, 34.66, 37.20, 57.41, 62.85, 66.14, and 68.03 is corresponding to the plane of (100), (002), (101), (110), (103), (112), and (201) respectively according to JCPDS No. 036 1451 shown in **Figure 1**. The particle sizes are obtained in the range of 13.86, 9.25 and 18.38 nm. The nanoparticle sizes are calculated by using Debye Scherrer's formula.

$$D = \frac{K\lambda}{\beta \operatorname{Cos} \theta} Å$$

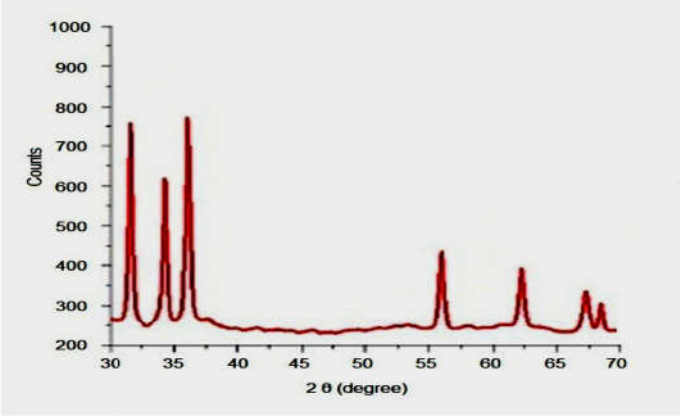

Figure 1: XRD Pattern

UV-Vis Spectrum

The obtained UV-Vis Spectrum result was shown in **Figure 2**. The result shows that the maximum absorption has takes place at 305.01nm.

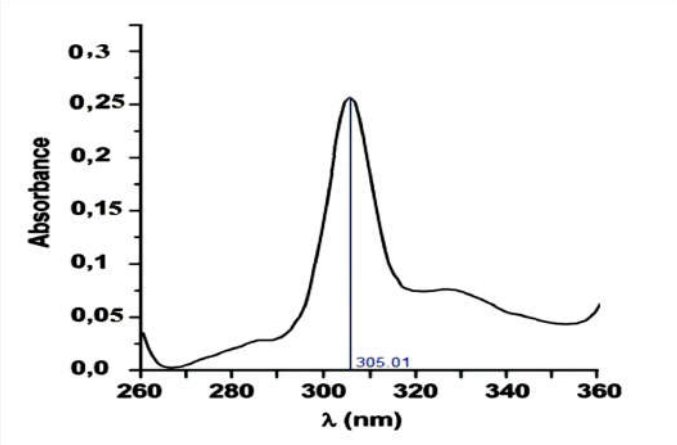

Figure 2: UV-Vis Spectrum

SEM imaging

This analysis was performed by using Hitachi S-4500 Scanning Electron Microscope. SEM image **Figure 3** shows that the ZnO nanoparticles are hexagonal in shape and the particle sizes are < 50nm in range.

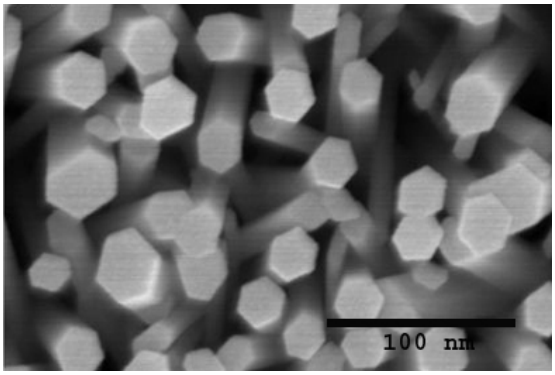

Figure 3: SEM Image

FTIR Spectrum

The FTIR Spectrum of synthesized ZnO nanoparticles has shown in **Figure 4**. Form this result we analyzed that C-O stretching (R-O-R, H_3C-OH, etc.) at 1065.42 cm^{-1}, Amine group (NH_2) at 3400 cm^{-1}, Scissoring, N-H bending at 1615 cm^{-1}, Keto group (C=O) at 1742 cm^{-1}, Presence of carbonyl group stretching (C=O) at 1427.94 cm^{-1} and Carbon-Carbon stretching in ring (medium) in Aromatic ring occurs at 1586.63 cm^{-1}. C-H stretching in aromatic ring (Ar-H) occurs at 3043.17 cm^{-1}. 721.58 cm^{-1} peak showed that it is finger print region. Hence these variations are occurred due to the presence of metal oxides.

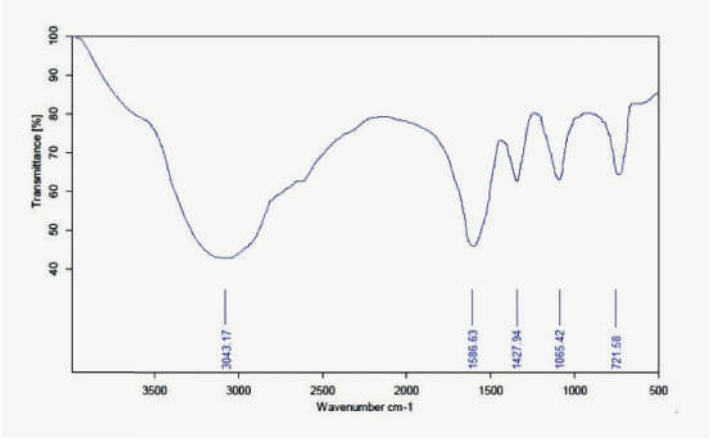

Figure 4: FTIR Spectrum

Antibacterial Activity

The antibacterial activity of synthesized ZnO nanoparticles shows better effect against *S.typhi, S.aureus, B.subtilis, E.coli* and *P.aeruginosa* shown in **Figure 5**. The maximum zone of inhibition obtained for 50µl concentration was observed that *E. coli* (16 mm), *S.aureus* (14 mm), *S.typhi*and *B.subtilis* (13 mm) shown in **Table 3**. The methanol extracts has reported that it is more effective than aqueous and ethanol extracts against for all the organisms [17].

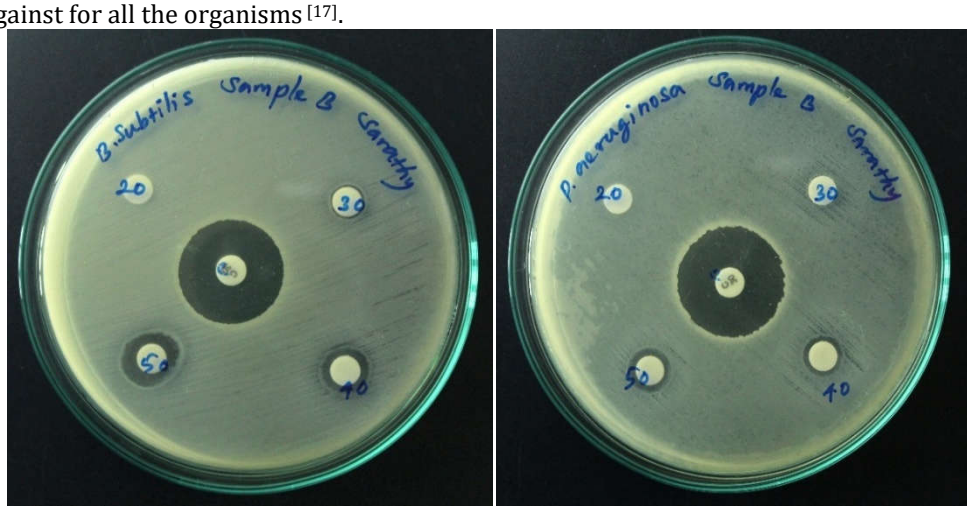

Fig.5 (a) **Fig.5 (b)**

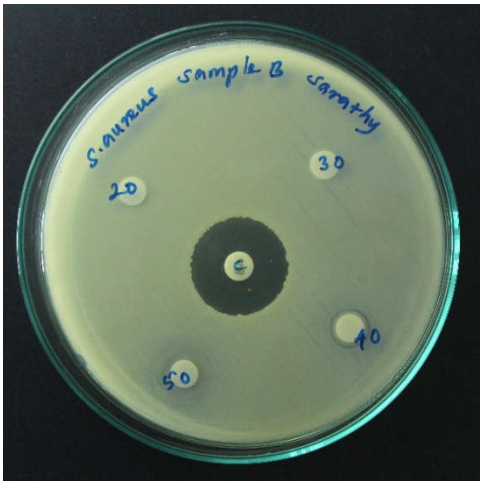

Fig.5 (c)

Fig.5 (d)

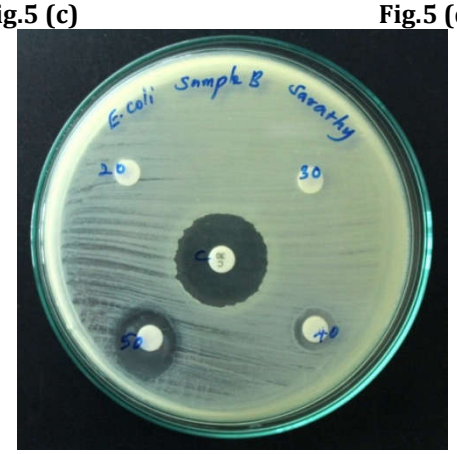

Fig.5 (e)

Figure 5: Antibacterial activity of ZnO Nanoparticles

Table 3: Antibacterial activity

S.NO.	*Organisms*	*Zone Of Inhibition (mm)*				
		Control	Concentration of Sample *20µl*	Concentration of Sample *30µl*	Concentration of Sample *40µl*	Concentration of Sample *50µl*
1	***S.typhi***	23mm	00mm	07mm	11mm	13mm
2	***S.aureus***	22mm	00mm	00mm	09mm	14mm
3	***B.subtilis***	22mm	00mm	07mm	10mm	13mm
4	***E.coli***	23mm	00mm	00mm	11mm	16mm
5	***P.aeruginosa***	24mm	00mm	00mm	09mm	10mm

CONCLUSION

Green Synthesis of ZnO nanoparticles are safe and eco-friendly to environment while comparing to chemical synthesis. The Morphological studies and Characterizations are confirmed that the presence of ZnO nanoparticles and also the particle size are obtained in the range of 13.86nm, 9.25nm and 18.38nm. The antibacterial activity against *S.typhi, S.aureus, B.subtilis, E.coli* and *P.aeruginosa* has exhibited good results. Hence the ZnO nanoparticles are synthesized using the plant extract will overcome the chemical methods [23].

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Conflict of Interest

There are no conflicts of interest.

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