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Green Synthesis of Zinc Oxide Nanoparticles from Ocimum Tenuiflorum L. Leaves and Its Antimicrobial Activity

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Abstract:
Green synthesis of Nanoparticles is an important area in the field of Nanotechnology, which has cost effective and environment friendly benefit over physical and chemical methods. Nanoparticles exhibit distinct features compared to traditional physico-chemical synthesis and they have many applications in a wide range of fields of life sciences such as surface coating agents, catalysts, food packaging, corrosion protection, environmental remediation, electronics, biomedical and antimicrobial. Green-synthesized metal Nanoparticles, mainly from plant sources, have gained a lot of attention due to their intrinsic characteristics like eco-friendliness, rapidity and cost-effectiveness. In this study, green synthesis of Zinc Oxide Nanoparticles from leaves extract of *Ocimum tenuiflorum* L. The prepared Zinc Oxide Nanoparticles were characterized by Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) method. The crystalline structure of the Zinc Oxide Nanoparticles was determined by X-ray diffractometer and to identify the functional groups and chemical bond present in the green synthesized Zinc Oxide Nanoparticles were studied using FT-IR. The average particle crystalline size, is calculated as 18 nm by using Scherrer's formula. Then, Zinc Oxide Nanoparticles were tested for antimicrobial activity against *E. coli*, *Streptococcus sp.* and *Kappa casein* pathogenic microorganism's strains at concentrations of 100µl, 250µl, 500µl, and 1000µl. The E. coli shows highest inhibition zone of 3.4mm in 500µl concentration, while, the zone of inhibition was observed less for *Kappa casein* is 1 mm in 100µl concentration. The antimicrobial study revealed that Zinc Oxide Nanoparticles confirmed an excellent zone of inhibition against pathogenic microorganisms. Through the findings of this study, it has been shown that *Ocimum tenuiflorum* L. leaves extract can be used in a green synthesis approach to prepare Zinc Oxide Nanoparticles, which can be employed as alternatives to antibiotics and a tool to eliminate drug-resistant microbes in the future.

Keywords: *Ocimum tenuiflorum* L., Zinc Oxide Nanoparticles, XRD, FT-IR, Antimicrobial

I. INTRODUCTION

Nanotechnology is a research hot spot in modern materials science. This technology is capable of provi-

ding miscellaneous novel applications that range from innovative fabric compounds, food processing, and agricultural production to sophisticated medicinal techniques [1]. It is considered as the synthesis, characterization, and exploration of materials in the nanometre region (1–100 nm). At this level, the properties and functions of living and anthropogenic systems are defined [2]. In this technology, the pertinent materials are those whose structures exhibit new and considerably enhanced physicochemical and biological properties as well as distinct phenomena and functionalities as a result of the nanoscale size [3]. This nanoscale size generally confers larger surface areas to nanoparticles compared with macro-sized particles [4]. Nano Molecules are able to show some unique properties in their nano size range which can be used in different field of sciences including agriculture. Nanoparticles have already revolutionized the different field of science such as textiles, industry, information and communication technology, energy and electronic sector.

Now focuses is on the application of nanotechnology in agriculture sector. The need of the day is to increase crop productivity from the limited natural resources to assure the food security. Among the key nanoparticle materials with successful practices is the metal oxide nanosized such as Zinc Oxide Nanoparticles. Zinc is the most widespread deficient micronutrient in the soil world over. In India, 40-42 percent cultivated lands show Zinc deficiency which is causing considerable reduction in yield. So, there is need to supplement crop plants with zinc nutrient. Zinc Oxide is an inorganic compound which occurs rarely in nature. It is generally found in crystalline form. Naturally occurring Zinc Oxide has manganese impurities that give it a typical red or orange color appearance [5]. When purified, Zinc Oxide appears as white crystalline powder which is nearly insoluble in water. Due to their low toxicity and size dependent properties, Zinc Oxide Nanoparticles have been widely used for various applications in textiles, cosmetics, diagnostics and even in micro-electronics. Because Zinc Oxide is generally recognized as safe (GRAS) and exhibits antimicrobial properties, Zinc Oxide Nanoparticles hold greater potential to treat infectious diseases in humans and animals [6]. Zinc Oxide has been found to be potentially useful and efficient than other metals for Biosynthesis of Nanoparticles for clinical purposes. Several studies have demonstrated the synthesis of Zinc Oxide Nanoparticles using different plant extracts. For example, flower extract of the medicinal plant *Cassia auriculata* [7] and leaf extract of *Hibiscus rosasinensi* [8] were used as reducing agents for Zinc Nitrate to synthesize Zinc Oxide Nanoparticles. Zinc Oxide have many and very impressive properties like large binding energy, wide band gap, high piezoelectric property etc. It is used in large number of applications like laser devices, optoelectronic devices, electromagnetic coupled sensor, surface acoustic wave devices [9], [10], [11], [12], [13], [14]. Zinc oxide nanoparticles have been used to eliminate sulphur, arsenic from water because bulk Zinc Oxide cannot remove arsenic because Nanoparticle have great surface area than bulk material [15]. Zinc oxide have amazing application in diagnostics, biomolecular detection, microelectronics [16]. Zinc Oxide Nanoparticles are effective adsorbates [17], coating elements for cellulose fibres [18], and magnetic materials used in information storage devices [19]. Besides, the small size and hence great surface area and surface energy of Zinc Oxide Nanoparticles allow different pharmacological and biomedical activities as antibacterial, fungicidal, and anticancer agents [20], [21], [22], [23]. The synthesis of Zinc Oxide Nanoparticles was reported by several research workers including in the applications of solar cell and biological sciences. This aspect necessitated their usage as antibacterial agents, noxious to microorganisms and hold good biocompatibility to human cells. Synthesis of nanoparticles can be performed using a number of routinely used chemical methods such as chemical precipitation, sonochemical, solvothermal, sol-gel process, hydrothermal

decomposition and so on. The biological method of the synthesis of Zinc Oxide Nanoparticles is gaining importance due to its simplicity, eco-friendliness and extensive antimicrobial activity [24], [25].

Ocimum tenuiflorum L. also known as *Ocimum sanctum*, Tulsi or Holi basil, it is belonged to Lamiaceae family. It is mostly present in tropical region. It has been used as medicine for more than thousands of years in Indian traditional medicine, Ayurveda and its allied verbalism disciplines for its diverse healing properties. The plant is considered sacred and is worshipped in a sanctorum of its own in traditional Hindu temples, sacred groves, and households throughout the subcontinent and therefore its taxonomical synonym *O. sanctum* L. is more popular in Indian scientific literature [26]. It relieves

people of stress, restore and improve body immunity and digestion. The chemical constituents of *Ocimum tenuiflorum* L. are linalool, alkaloids, ursolic acid, glycosides, carvacrol, tannins, rosmarinic acid, aromatic compound. Recently Leaves extract of *Ocimum tenuiflorum* L. plant have been utilized in the synthesis of copper nanoparticles, gold nanoparticles, and silver nanoparticles [27], [28]. To the best of our knowledge, the use of leaf extract *Ocimum tenuiflorum* L. plant for green synthesis of Zinc Oxide Nanoparticles has not been revealed. In the present study, Zinc oxide Nanoparticles are synthesized by green synthesis method using *Ocimum tenuiflorum* L. leaves extract and its antimicrobial activity.

II. MATERIALS AND METHODS

A. Area of study

This study was conducted at the Department of Biochemistry, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar. All experiments were accomplished aseptically in the Biochemistry Microbial Research laboratory.

B. Collection of Plant Material (Fig.1)

The *Ocimum tenuiflorum* L. plant leaves sample was collected from Tahshil Partur, District Jalna, Maharashtra. The plant leaves sample are collected in early morning at 7.30 am in a sterilized paper bag with the help of sterilized Hand gloves and clean cutter. The plant sample locality takes the GPS camera photograph and paper bag was labelled properly by indicating the site of collection, Date, time and then sample were taken to the laboratory for further analysis.

C. Chemicals

The precursor chemicals used for the synthesis were included: Zinc acetate dihydrate (99% purity), sodium hydroxide (pellet 99%) and Distilled Water.

D. Preparation of *Ocimum tenuiflorum* L. Leaves extract (Fig. 2)

For the preparation of *Ocimum tenuiflorum* L. plant leaves extract, fresh leaves were obtained in a beaker and washed several times with running tap water to eliminate dust and other impurities. and finally wash with double distilled water. The plant leaves were properly dehydrated in the shade at room temperature in blotter paper for removing excess amount of water. After dehydrated, weigh 10 g leaves and cut into fine pieces and crushed with the help of mortar and pestle by adding 50ml distilled water. Then leaves are squeeing with help of four folded muslin cloth in beaker. The extract was boiled 15 minutes [29], then the extract colour is changed to reddish colour. The extract was filter using Whatman filter paper No. 1 after warming to room temperature. The filtered extract was refrigerated at 4 ºC.

E. Green synthesis of Zinc Oxide Nanoparticles (Fig.3)

For the Zinc Oxide nanoparticles synthesis, 50 ml extract of *Ocimum tenuiflorum* L. leaves was taken boiled to 60-80 ºC. The Zinc acetate dihydrate (99% purity) and Sodium hydroxide (pellet 99%) are utilized as the precursor material. Zinc acetate dihydrate (Zn (CH3COO)2·2H2O) is added to distilled

water under vigorous stirring and after 10 minutes stirring,the aqueous leaf extract of *Ocimum tenuiflorum* L. is introduced into the above solution followed by the addition of aqueous 2.0 M Sodium hydroxide (NaOH); it results in a white aqueous solution at 12 pH. The pH of medium greatly influences the size of Zinc Oxide Nanoparticles. Then the above solution will be positioned in a magnetic stirrer for 2 hours. Finally, the precipitates are taken out and washed repetitively with distilled water followed by ethanol to remove the impurities of the obtained product. A white powder of Zinc Oxide nanoparticles will be obtained after drying at 60 ºC in vacuum oven overnight [30].


Fig. 3: Green synthesis of Zinc Oxide Nanoparticles

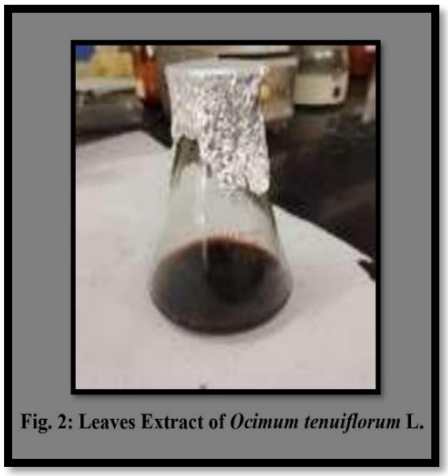
Fig. 2: Leaves Extract of *Ocimum tenuiflorum* L.

Fig. 1: Collection of *Ocimum tenuiflorum* L. plant leaves

F. Collection of Tested Microorganisms

The tested microorganisms *E. coli*, *Streptococcus sp.* and *Kappa casein* bacteria were isolated from the garden soil of Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar by using Nutrient agar media. After the growth of Bacteria on Petri Plates, on the basis of Morphological and Microscopic character of Bacteria were to identify. The bacterial strains were belonging to the gram negative (*E. coli*) and gram-positive (*Streptococcus sp.* and *Kappa casein*) were taken for tests. All the above bacterial strains were prepared in Nutrient Broth were maintained 40ºC. Active culture inoculums for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes containing nutrient broth and incubated for 24 h at 37 ºC.

a) Composition of Nutrient Agar Media

Sodium chloride (NaCl) 10.0g, Peptone 10.0g, Yeast Extract 1.0g, Agar-Agar 15.0g, p^H was maintained 7.5, Distilled water 1000ml.

b) Preparation of Microbial media

Nutrient agar was used for antimicrobial Activity. This was prepared according to the manufacturer's specification. The nutrient agar was prepared by dissolving 7 g of the agar in 250 ml of distilled water contained in a 500 ml sterile conical flask. The media was then autoclaved at 121°C for 15 min. The sterilized media were allowed to cool to a temperature of 45°C and then approximately 20 ml was poured into sterile Petri-dish and allowed to solidify.

F. Antimicrobial activity of Zinc Oxide Nanoparticles

The antibacterial activity of the Zinc Oxide Nanoparticles solution was determined by agar-well diffusion method [31]. The Microbial isolates were first grown in a nutrient broth for 24 h before used. Freshly prepared inoculum was inoculated all over the surface of the agar plate using sterile Nichrome Wire loop. Well of 5 mm diameter were bored in the medium with the help of sterile cork-borer having 5 mm diameter

and were labeled properly and different concentrations of Zinc Oxide Nanoparticle solution (100 µl, 250 µl, 500 µl, 1000 µl) were filled in the wells with the help of micropipette as compare to control, then incubated for 24 h at 37°C. The plates were observed for zones of inhibition after 24 h incubation period at 37°C. The zone of inhibition was quantified in millimetres (mm).

G. Characterization Methods

a. XRD analysis of Zinc Oxide Nanoparticles

The crystalline structure of the Zinc Oxide Nanoparticles was determined by X-ray diffractometer (Bruker D8 DISCOVER, Bruker, Germany). The relative intensity data were collected over a 2θ range of 20º - 80º, 2θ values and relative intensities (I/Io) were determined from the chart. The average crystalline size of Zinc Oxide Nanoparticles was calculated by Debye-Scherrer's formula.

$$Dp = k\lambda/\beta\cos\theta$$

Where, Dp is diffraction peaks, k is the shape of constant (0.9), β is the full width at Half Maximum, λ is the wavelength of X-Ray (1.5406 A), θ is the angle of diffraction.

b. FT-IR Spectroscopy analysis of Zinc Oxide Nanoparticles

To identify the functional groups and chemical bond present in the green synthesized Zinc Oxide Nanoparticles were studied using Fourier transform spectroscopy analysis. The IR spectrum was recorded in the range of 1000- 3500cm^{-1} wavelength. FT-IR spectroscopy is widely used in industrial research for quality control and dynamic measurement.

III. RESULT AND DISCUSSION:

a. Antimicrobial activity of Zinc Oxide Nanoparticles

The antimicrobial potential of the green synthesized Zinc Oxide Nanoparticles using *Ocimum tenuiflorum* L. leaves extract is tested against *E. coli*, *Streptococcus sp.* and *Kappa casein* bacteria shown in Table 1 (Fig. 4). The green synthesized Zinc Oxide Nanoparticles showed antimicrobial activity on all the tested microorganism's strains. The microbial effect of Zinc Oxide Nanoparticles was found higher for Gram-negative bacteria than Gram-positive bacteria, was based on the difference in the structural composition of Gram-positive and Gram-negative bacteria [32], *Ocimum tenuiflorum* L. leaves extract-derived Zinc Oxide Nanoparticles showed a highest zone of inhibition in all concentration 500µl for *E. coli* is 3.4mm and *Streptococcus sp.* show 3mm inhibition zone in 1000µl concentration, whereas the zone of inhibition was observed less for *Kappa casein* is 1 mm in 100µl concentration. Similar results on antimicrobial effect for *P. aeruginosa* and *E. coli* by Zinc Oxide Nanoparticles were reported previously in the literature [33]. The mechanism of antimicrobial activity of Zinc Oxide Nanoparticles may be attributed to the penetration and disintegration of the membrane by smaller sized Nanoparticles which lead to cell lysis [34], [35]. The release of H_2O_2 from the surface of Zinc Oxide also reported as the possible mechanism for microbial activity. The generation of H_2O_2 is highly depended on the surface area of Zinc Oxide and the generated H_2O_2 penetrates the cell membrane and cause damage to kill the microorganism [36], [29]. Due to the presence of alkaloids, terpenoids, flavonoids, tannins, carbohydrates, sterols, saponins, proteins, and amino acids in using *Ocimum tenuiflorum* L. leaves extract showed potential bioreducing activity and also antimicrobial activity against the tested microorganisms which could be useful for biomedical applications.

Table 1: Antimicrobial Activity of Zinc Oxide Nanoparticles

Name of Microorganisms	Different Concentration Zinc Oxide Nanoparticles			
	100 µl	250 µl	500 µl	1000 µl
E. coli	3mm	3.1mm	3.4mm	3.2mm
Streptococcus sp.	2mm	2.2mm	2.5mm	3mm
Kappa casein	1mm	1.3mm	1.5mm	2mm

Fig. 4: Antimicrobial Activity of Zinc Oxide Nanoparticles

b. X-ray Diffraction (XRD) Analysis of Zinc Oxide Nanoparticles

The XRD pattern of green synthesized Zinc Oxide Nanoparticle using leaves extract of *Ocimum tenuiflorum* L. is illustrated in Fig. 5. The XRD diffraction peaks existed at 2θ angles of 31.77º, 34.41º, 36.25º, 47.51º, 56.52º, 62.84º, 66.84º, 66.49º, 68º and 69.06º corresponding to lattice planes (100), (002), (101), (102), (110), (103), (200), (112), and (201), respectively (Table 2) [37]. These peaks are indicating the confirmation of the hexagonal wurtzite structure of Zinc Oxide Nanoparticles formation [38]. The average crystalline size (ACS) of green synthesized Zinc Oxide Nanoparticles was calculated using Deby-Scherrer's formula [39] and the Average crystalline size (ACS) of the Zinc Oxide Nanoparticles was estimated to be 18 nm, which is derived from the full width at half maximum (FWHM) of the most intense peak corresponding to (101) plane located at 36.25º. Furthermore, the XRD pattern revealed no additional peaks other than the characteristic Zinc Oxide peaks, confirming the purity of the produced Zinc Oxide Nanoparticles. Additionally, the narrow and strong diffraction peak clearly indicates that the Zinc Oxide Nanoparticles have an optimal crystalline structure [40], [41].

Table 2: d-spacing calculation for Zinc Oxide Nanoparticles

Sr. No.	Peak 2 hr.	θ	cosθ	d (nm)	Hkl
1	31.77	15.885	0.273638	0.2811	100
2	34.41	17.07	0.295725	0.2600	002
3	36.25	18.125	0.310971	0.2480	101
4	47.51	23.755	0.409692	0.191	102
5	56.52	28.26	0.474031	0.162	110
6	62.84	31.42	0.521132	0.148	103

7	66.49	33.245	0.548031	0.141	200
8	68.0	34	0.559065	0.138	110
9	69.06	34.53	0.566635	0.136	201

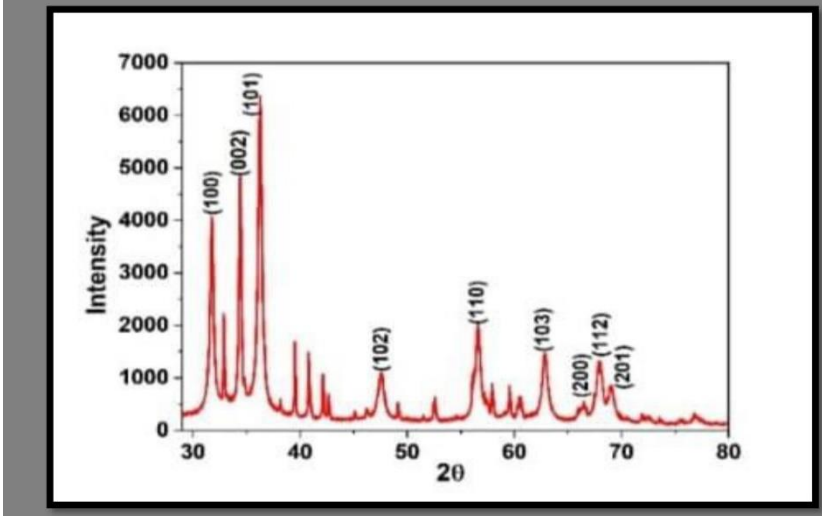

Fig. 5: X-ray Diffraction (XRD) Analysis of Zinc Oxide Nanoparticle

c. FT-IR Spectroscopy analysis of Zinc Oxide Nanoparticles

The FTIR technique was used in order to detect possible Function group and chemical bond present in the leaves extract of *Ocimum tenuiflorum* L. that contribute to the reduction in and stabilization of Zinc Oxide Nanoparticles. Fig. 6 represents the FTIR spectra of green synthesized Zinc Oxide nanoparticles of *Ocimum tenuiflorum* L leaves extract. The peaks of *Ocimum tenuiflorum* L leaves extract green synthesized Zinc Oxide nanoparticles are displayed in Table 3. The FTIR spectra resulted in various peaks at 3783.18, 3690.96, 3402.44, 2790.68, 2346.03, 1577.59, 1414.37,1234.01, 1011.97 ,857.41, 643.51 cm^{-1}. The broad stretch peak at 3690.96 and 3402 correspond to H bonded OH stretch and proteins and carbohydrate stress [42], [43], for the extract of Zinc Oxide Nanoparticles. The low intensity peaks that arise at 2790.68 cm^{-1} were assigned to CH and CH_2 stretching vibration of the aliphatic group [44], [45]. The peaks observed at 2345.03 cm^{-1} indicate results from the stretching bands of C=C variation of the functional groups system [46], [47]. The absorption peak at 1577.59 cm^{-1} correspond to the C-N stretching vibration stretch in protein amide linkages [44]. The peak results from C-N amide II band and the two peaks at, 1011.97 C-O-C Polysaccharides. The 857.41 peaks correspond to bending C = O inorganic carbonate and CH out of plane aromatic band [45], respectively. The absorption band observed at 643.51 cm^{-1} confirmed the successful formation of Metal- Oxygen (Zinc Oxide). The Zinc Oxide absorption peak obtained by FTIR analysis of green synthesized Zinc Oxide Nanoparticles has been detected at wavelengths 643.51 cm^{-1} [48], 652 cm^{-1} [49], 660 cm^{-1} [50] and 685 cm^{-1} [51] in the range 600 to 700 cm^{-1} [52], which are consistent with our findings.

Table 3: FT-IR Spectra of green synthesized Zinc Oxide Nanoparticles from *Ocimum tenuiflorum* L. leaves extract

Sr. No.	Functional groups	Absorption Bands in Zinc Oxide Nanoparticles (Cm^{-1})
1	-OH Stretch	3690.96
2	-CH Stretch	2790.68
3	-C=C Stretch	2345.03
4	-C-N Stretch	1577.59
5	-C-O-C Stretch	1011.97
6	-C-H Stretch (Aromatic)	857.41
7	ZnO	643.51

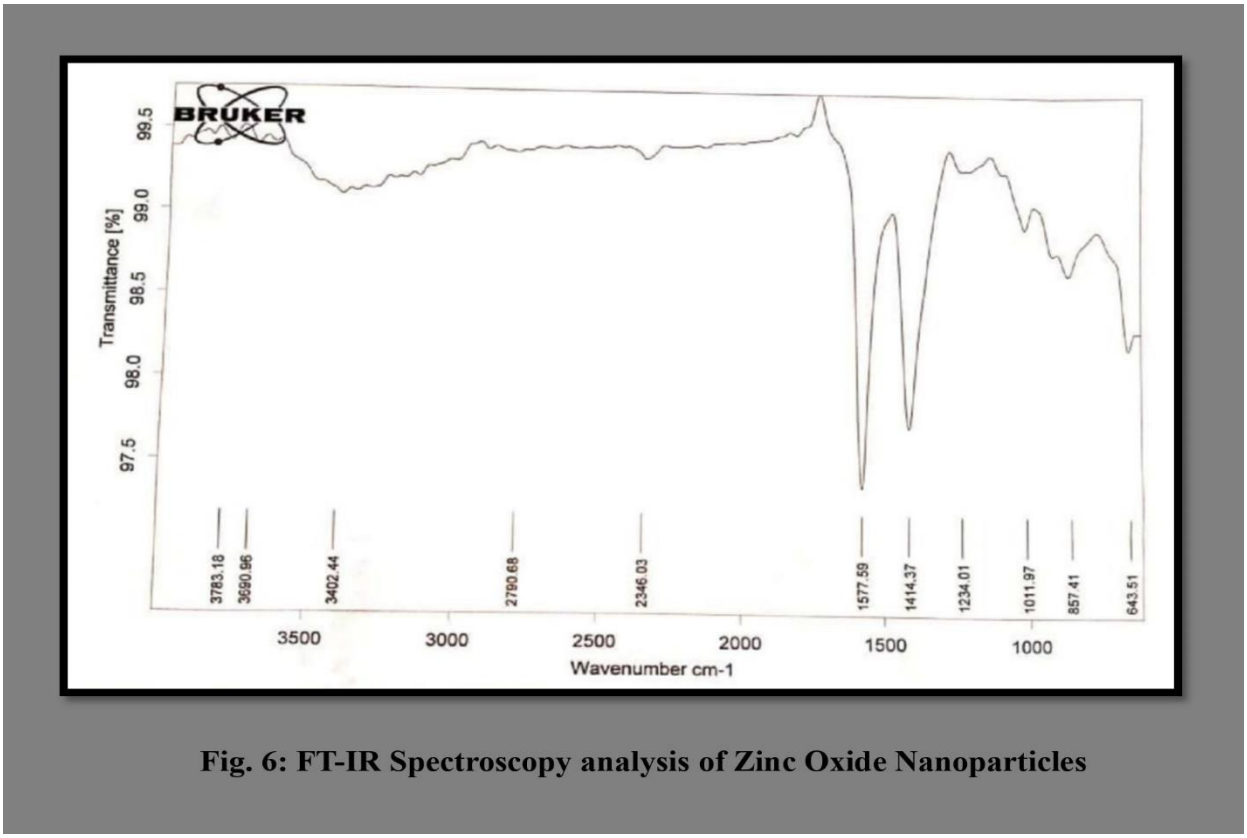

Fig. 6: FT-IR Spectroscopy analysis of Zinc Oxide Nanoparticles

IV. CONCLUSIONS

This study presents the green synthesis Zinc Oxide Nanoparticles for the using leaves extract of *Ocimum tenuiflorum* L. Green synthesis method is simple, nontoxic, rapid method. The size and structure of nanoparticles is confirmed with the XRD technique. The synthesized Zinc Oxide Nanoparticles average particle size is calculated as 18 nm by using Scherrer's Formula. The characteristic peak of Zinc Oxide at 643.51in FT-IR absorption spectra is also noticed and carboxylic acid group, alkynes group, amino group are present in leaves extract of *Ocimum tenuiflorum* L. Furthermore, Zinc Oxide Nanoparticle showed excellent antimicrobial activity against *E. coli*, *Streptococcus sp.* and *Kappa casein* bacteria. These Zinc Oxide nanoparticles can be used in various industrial applications like active medium for lasers, luminescent material for fluorescent tubes, paints, and so forth. Our findings suggest the possibility of

using the leaves extract of *Ocimum tenuiflorum* L. for green synthesizing stable Zinc Oxide Nanoparticles. The green synthesized Zinc Oxide nanoparticles possess a significant antimicrobial activity against pathogenic bacteria that can be used as a safe and stable alternative to synthetic substances in the fields of pharmaceutical and biomedical research.

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Disclosure of conflict of interest:

The authors declare no conflict of interest, financial or otherwise

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