Telosma cordata and *Zephyranthes candida* mediated Green Synthesis of Zinc Oxide Nanoparticles using Plant Extract

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Abstract

The current research is focused on the production of zinc oxide nanoparticles utilizing eco-friendly bio-reductants derived from the leaves of Telosma cordata and Zephyranthes candida. Ultraviolet-visible (UV-VIS) spectroscopy is employed for quantitative analysis based on the absorption of light by the analyte. FT-IR is utilized to characterize the zinc oxide nanoparticles, identifying bio-functional groups present on their surface. X-ray diffraction (XRD) patterns confirm the hexagonal phase and crystalline size of the nanoparticles. Thermal gravimetric analysis was conducted to assess the thermal stability of the nanoparticles.

Studies used optical profilometer for the topography and roughness of nanoparticles. The-synthesized nanoparticles also demonstrate notable antibacterial activity. The results of the study show that the plant extracts have several naturally occurring bioactive compounds that could be effectively utilized in the production of nanoparticles.

Keywords: Zinc oxide nanoparticles, Green synthesis, Characterisation, Antibacterial activity.

Introduction

Nanotechnology is the technological innovation in the 21st century. Research and developments in this field are growing rapidly throughout the world. A major contribution of this field is the development of new materials in the nanometer scale[3,11]. Nanoparticles exhibit completely new or improved properties with larger particles of the bulk materials and these novel properties are derived due to the variation in specific characteristics such as size, distribution and morphology of the particles[30,35]. Compared to other 1-D semiconductors, ZnO has received its popularity due to the wide band gap and high excitation binding energy (3.37 eV and 60 meV). The synthesis methods of diverged zinc oxide nanostructures can broadly be classified as two main categories namely gas phase synthesis and solution phase synthesis[31].

Vapour solid, vapour liquid solid, chemical or physical vapour deposition are commonly used gas phase methods while solution phase techniques are the sol-gel, templates assisted and spray pyrolysis[13,15]. It is usually insoluble in water and appears as a white powder. The powder is widely used as an additive into numerous materials and products including plastics, ceramics, glass, cement, rubber (e.g. car tyres), lubricants, paints, ointments, adhesives, sealants, pigments, foods (source of Zn nutrient), batteries, ferrites, fire retardants etc[33]. Zinc oxide (ZnO) nanoparticles have received considerable attention due to their antimicrobial, UV blocking, high catalytic and photochemical activities[8].

Material and Methods

Materials: *Zephyranthes candida* and *Telosma cordata* leaves were gathered in the Dindigul District (Fig. 1). Solvent and chemicals were purchased from Merk (India) Ltd. Fresh double-distilled water was used to prepare all needed samples.

Figure 1: *Zephyranthes candida* and *Telosma cordata*

Extract Preparation: Fresh leaves of *Zephyranthes candida* (ZCL) and *Telosma cordata* (TCL) underwent a thorough cleaning process with distilled water. To prepare the aqueous extract, 10g of freshly cut leaves were steamed with 100 ml of distilled water at 60°C for approximately half an hour. The obtained extract was cooled and filtered through Whatmann No.1 filter paper. The filtered extract was stored in a refrigerator for subsequent use[37].

Synthesis of Zno nanoparticles: To synthesize ZnO nanoparticles, freshly prepared 0.5M zinc acetate dihydrate solution (50 mL) was combined with 1 mL of the leaf extract in a beaker. The mixture was stirred for ten minutes and to maintain a pH of 12, NaOH solution was added. Following the color change to a pale white aqueous solution, the solution was subsequently subjected to stirring on a magnetic stirrer for duration of 2 hours. Centrifuged the solution and washed to obtain zinc oxide nanoparticles. Subsequently, the obtained pale white powder of ZnO nanoparticles was dried in an oven[38].

Characterisation: The UV-Visible spectra of the zinc oxide nanoparticles were recorded in the range of 200-800 nm using ethanol as the solvent, employing a double-beam spectrophotometer. Infrared spectra of the zinc oxide nanoparticles were recorded within the range of 4000-400 cm^{-1} using a Shimadzu model FTIR (IR Tracer-100) spectrometer. Powder X-ray diffraction (XRD) analysis of the zinc oxide nanoparticles was conducted using a Bruker D8 Advance Instrument with CuKα radiation. Thermal gravimetric analysis (TGA) of ZnO NPs was performed on an EXSTAR/6300 thermogravimetric analyzer at a heating rate of 10 °C/min.

Phytochemical screening test: Standard methods were employed to conduct phytochemical examinations on the extract[27].

Antibacterial study: The Kirby-Bauer technique was employed to assess the antibacterial activity of the ZnO particles that were produced[16]. From an agar plate culture, three to five well-isolated colonies of the same morphological type were chosen. Following an incubation period of 35°C, the broth culture produced a suspension of 1 to 2 x 10^8 CFU/ml for *Staphylococcus aureus,Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. By swabbing the entire sterile agar surface, a nutrient agar plate's dry surface was infected. 50 µl of the material was added to a 6 mm diameter well that had been punched in the media. The Petri plates were inverted to allow for complete diffusion, after 24 hours of incubation at 37°C and the diameter (mm) of the inhibition zones that developed around the well was measured with a typical Hi-Media scale. Teicoplanin served as the industry standard.

Results and Discussion

Qualitative Phytochemical Analysis: Table 1 presents the phytochemical evaluation of the plant extracts from TCL and ZCL, indicating the existence of several phytochemicals such as carbohydrates, alkaloids, glycosides, phenolic compounds, tannins, saponins, flavonoids, phytosterols, proteins and amino acids. The presence of hydroxyl (OH) groups in flavonoids is capable of reducing zinc compounds into ZnO nanoparticles and serves as a capping or stabilizing agent for the nanoparticles.

Consequently, separate capping or stabilizing agents are deemed unnecessary in this nanoparticle synthesis approach[29]. Based on the outcomes of the phytochemical screening, it is inferred that the formation of ZnO nanoparticles utilizing the plant extracts is primarily attributed to the presence of flavonoids, glycosides and saponins.

UV-Visible Spectroscopy: The green-synthesised zinc oxide nanoparticles' UV-visible spectra are illustrated in fig. 2. An absorption peak, associated with the photoexcitation of electrons from the valence band to the conduction band, was observed at 355 nm (for TCL-ZnO NP) and 251 nm (for ZCL-ZnO NP), indicating a significant blue shift from the absorption value of bulk ZnO, where the band gap is nearly at 370 nm and 3.3 eV.

Table 1
Phytochemical constituents of TC and ZC aqueous leaf extract

S.N.	Chemical constituent	Phytochemicals Test	TCL	ZCL
1.	Carbohydrates	Fehling's test	-	-
2.	Alkaloids	Drangendroff's test	+	+
3.	Glycoside	Legal's test	-	+
4.	Phenolic test	Ferric chloride test	+	-
5.	Tannins	Gelatin test	-	+
6.	Saponins	Foam test	+	+
7.	Flavonoids	Alkaline reagent test	+	+
8.	Proteins	Xanthoproteic test	-	-
9.	Amino acid	Ninhydrin test	-	-

'+' represents the presence of compounds;
'-' represents the absence of compounds

The reduction in absorption value may be attributed to the potential agglomeration of particles[18,32]. For TCL and ZCL-ZnO nanoparticles, the optical energy band gap (Eg) was calculated to be 3.53 eV and 4.6 eV respectively (Fig. 3)[7,19,34].

FT-IR Spectroscopy: FT-IR spectroscopy was utilized to identify the plant extract present in the synthesized nanoparticles, with analysis conducted within a frequency range of 4000-400 cm^{-1} at room temperature. A wide peak noticed at 3454 cm^{-1} and 3353 cm^{-1} is associated with the O-H stretching vibration of ZCL-ZnO NP and TCL-ZnO NP. In TCL-ZnO NP and ZCL-ZnO NP, strong and intense absorption peaks at 1550 cm^{-1} and 1543 cm^{-1} indicate the aromatic stretching vibrations of C-C. Peaks at 1408 cm^{-1} (TCL) and 1401 cm^{-1} (ZCL) indicate that C-H alkene may be present. C-H bending is responsible for the peaks in TCL-ZnO NP and ZCL-ZnO NP which are positioned at 835 cm^{-1} and 711 cm^{-1} respectively. The possible source of absorption peaks between 400 and 700 cm^{-1} is stretching vibrations of Zn-O (Fig. 4 and table 2)[1,4,12,21,26].

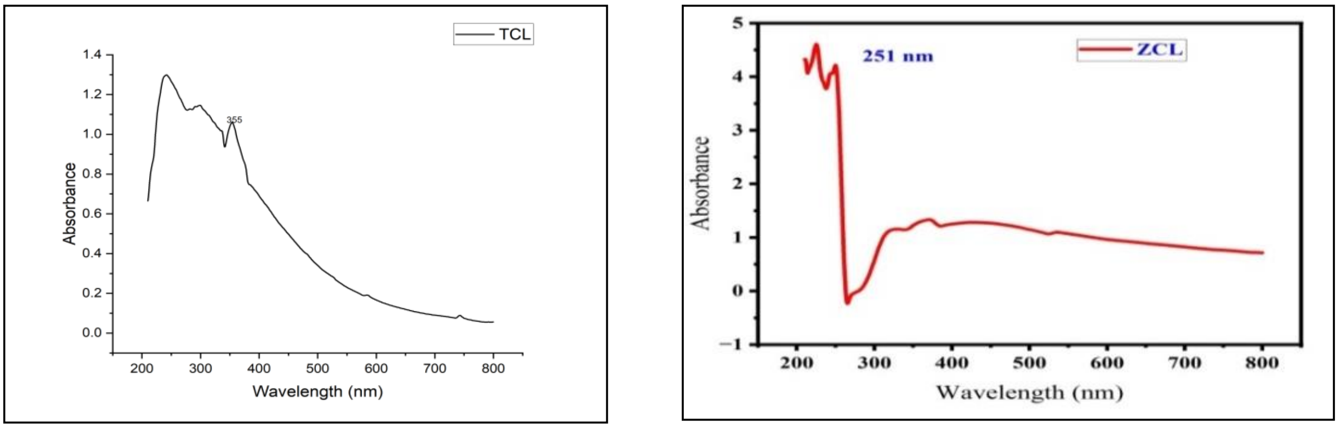


Figure 2: UV-Visible spectrum of ZnO nanoparticles

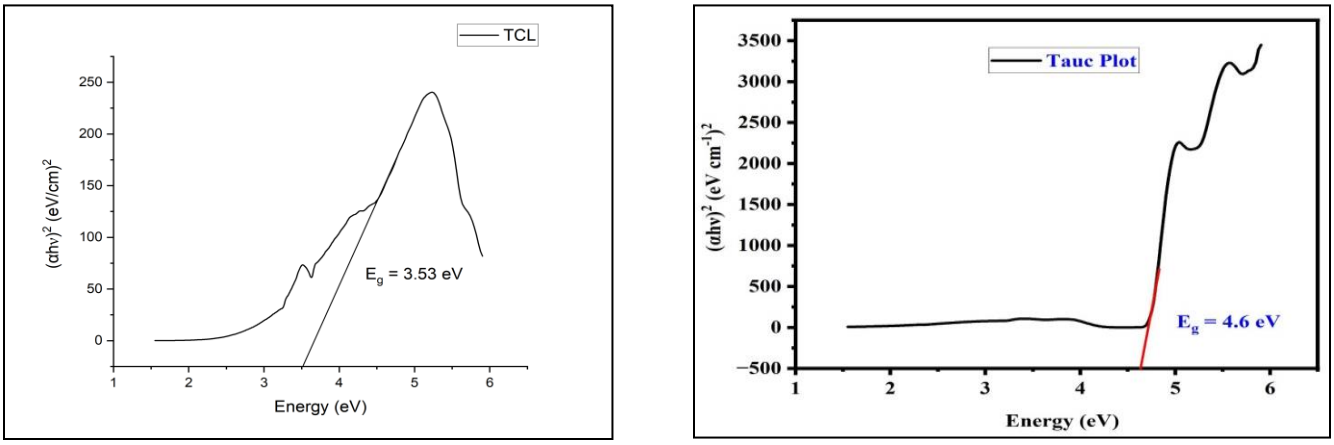


Figure 3: Band gap energy of TCL-ZnO NP and ZCL-ZnO NP

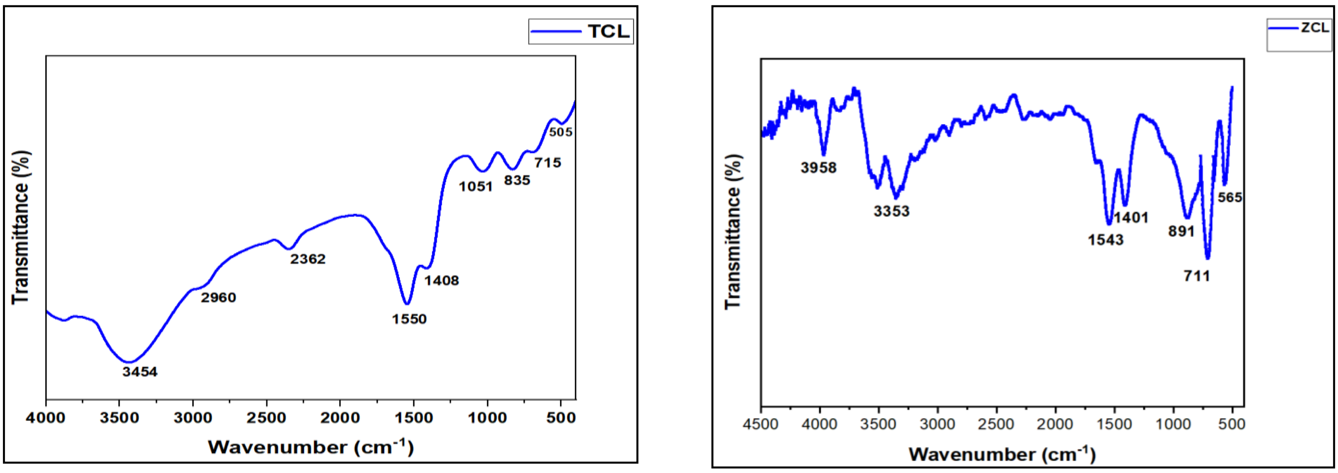


Figure 4: FT-IR spectrum of ZnO NPs

X-Ray Diffraction: The XRD pattern of TCL-ZnO NP exhibited 2 theta values at 31.5°, 34.1°, 36.1°, 47.9°, 57.8°, 59.2°, 66.1°, 68.25° and 69.1° which correspond to the planes (100), (002), (104), (102), (110), (020), (200), (112) and (201) respectively. Similarly, ZCL-ZnO NP demonstrated good crystallinity with peak positions at 31.6°, 34.3°, 36.1°, 47.4°, 56.5°, 62.7° and 68° corresponding to the planes (100), (002), (101), (102), (110), (103) and (112) respectively[17,23]. These results indicate sharp and narrow diffraction peaks in the XRD spectrum, suggesting that the synthesized nanoparticles are pure and crystalline. With the help of Scherrer equation, the average crystallite size of TCL-ZnO NP was found to be 13.3 nm while for ZCL-ZnO NP, it was 10.4 nm illustrated in fig. 5[2,24,25].

Thermogravimetric Analysis: The thermal gravimetric analysis of ZnO NPs performed using an EXSTAR/6300 Thermo Gravimetric Analyzer, reflected a weight decrease up to 600°C. The total weight loss observed was 35.8% for TCL-ZnO NP and 8% for ZCL-ZnO NP was shown in fig. 6. The thermal desorption of carbon dioxide and water from the surface of ZnO particles may be the cause of the weight loss that was seen between 100°C and 200°C. Furthermore, the TG curve of ZnO formed between 200°C and 600°C suggests the formation of nanocrystalline ZnO[14,22]. Weight loss between 500°C to 600°C could be due to the thermal decomposition of plant bioorganic molecules present on the surface of ZnO NPs. No additional weight loss was observed in the TGA curve, signifying that ZnO NPs were found to be stable in the temperature range of 600°C and above[20].

3-D Optical Profilometer: Optical profilometer studies provide valuable insights into the topography and roughness of nanoparticles. The images obtained clearly illustrate the smoothness of the nanoparticles, attributed to the capping of phytochemicals over the nanoparticle (Figs. 7 and 8)[28].

Table 2
Spectral range and functional group of ZnO NPs.

Vibrational frequency (cm^{-1})		Functional group	Reference
ZCL	**TCL**		
3353	3454	O-H stretching	1
1543	1550	Aromatic C-C stretching	26
1401	1408	C-H alkene	26
711	835	C-H bending	21
400 to 700	400 to 700	Zn-O stretching vibrations	12,4

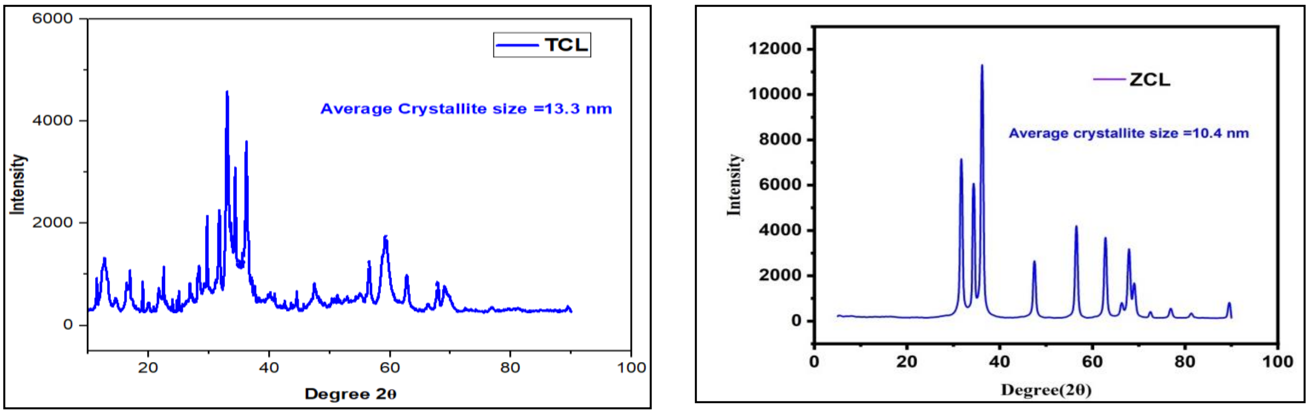


Figure 5: XRD image of green synthesized ZnO NPs

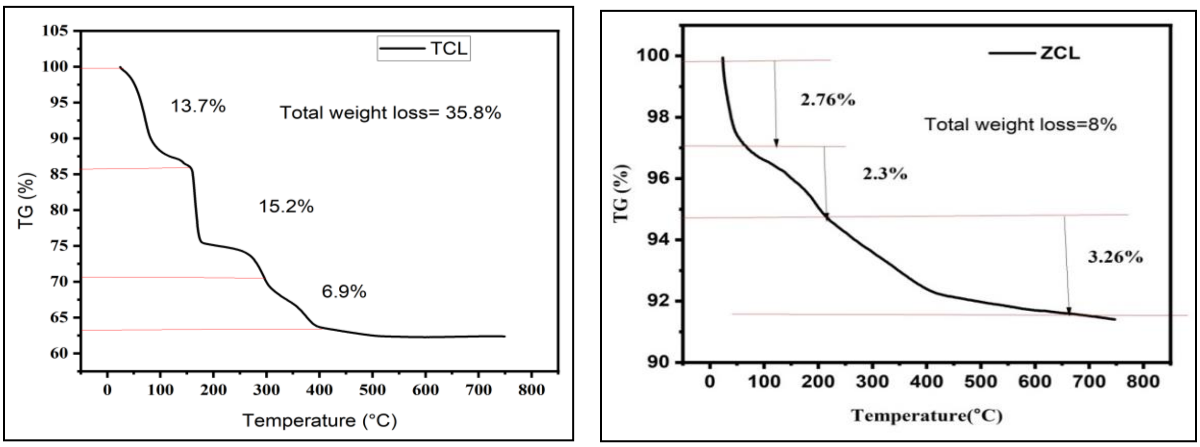
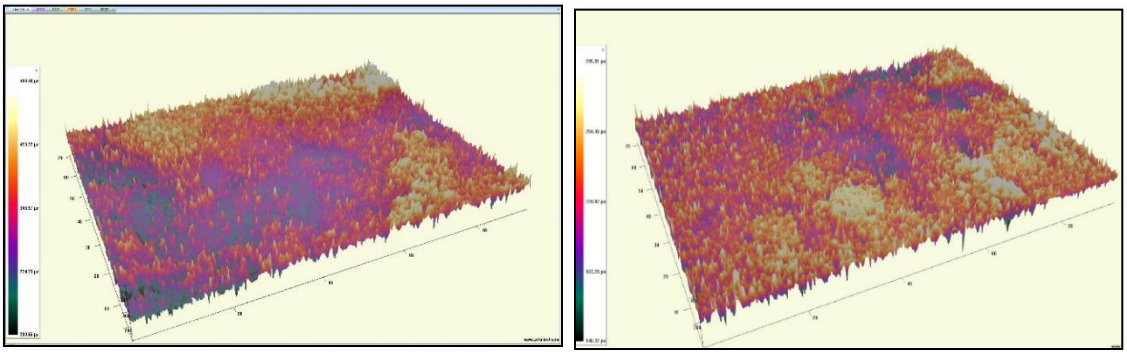


Figure 6: TG image of green synthesized ZnO NPs



TCL -ZnO Np: Ra(nm) :19.14, Rq(nm):23.03   ZCL -ZnO Np: Ra(nm) :34.77, Rq(nm):44.24

Figure 7: 3D Optical Profilometer image of green synthesized ZnO NPs

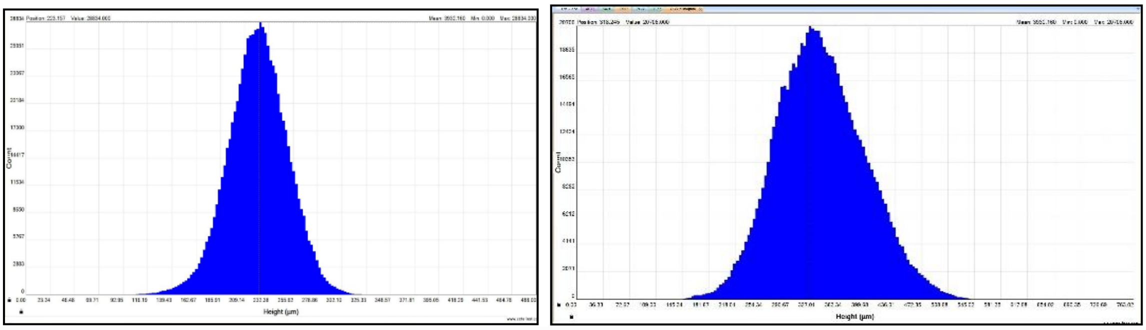


Figure 8: Histogram image of TCL-ZnO NP and ZCL-ZnO NP

Table 3
Comparison of Zone of inhibition (mm) of the as-synthesised ZnO NP

Sample	**Zone of Inhibition (mm)**			
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***	***Bacillus subtilis***
Teicoplanin(Standard)	15	17	16	14
ZCL ZnO NPs	11	16	15	12
TCL ZnO NPs	14	15	16	11

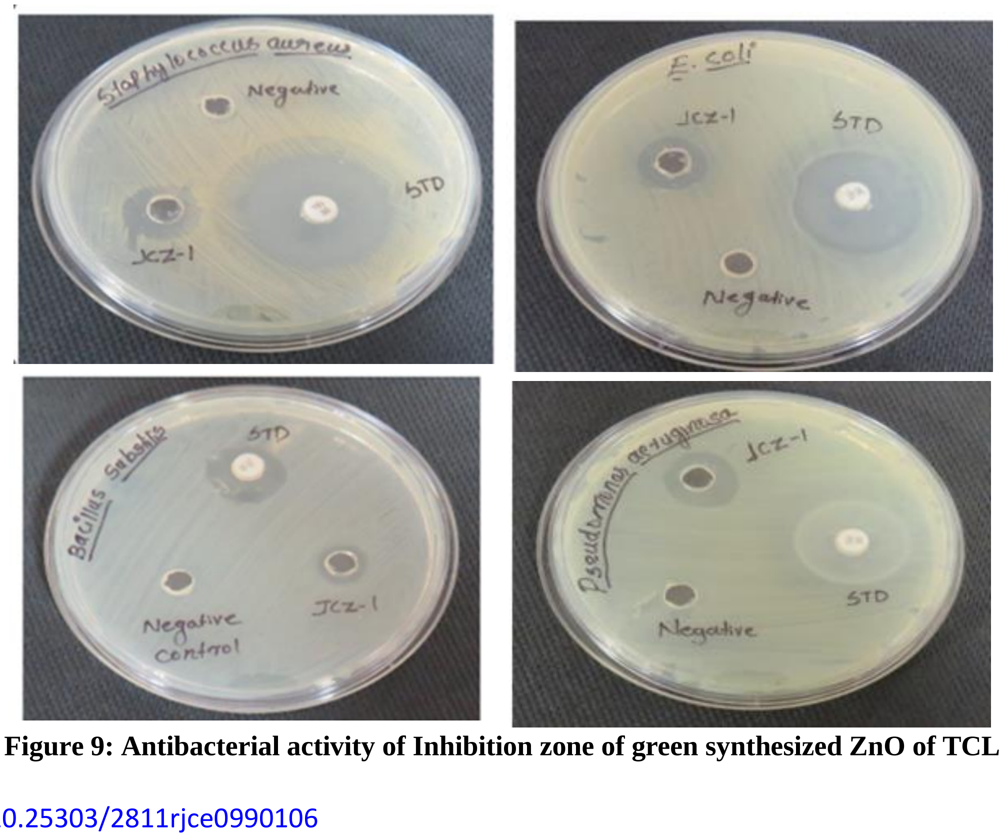


Figure 9: Antibacterial activity of Inhibition zone of green synthesized ZnO of TCL

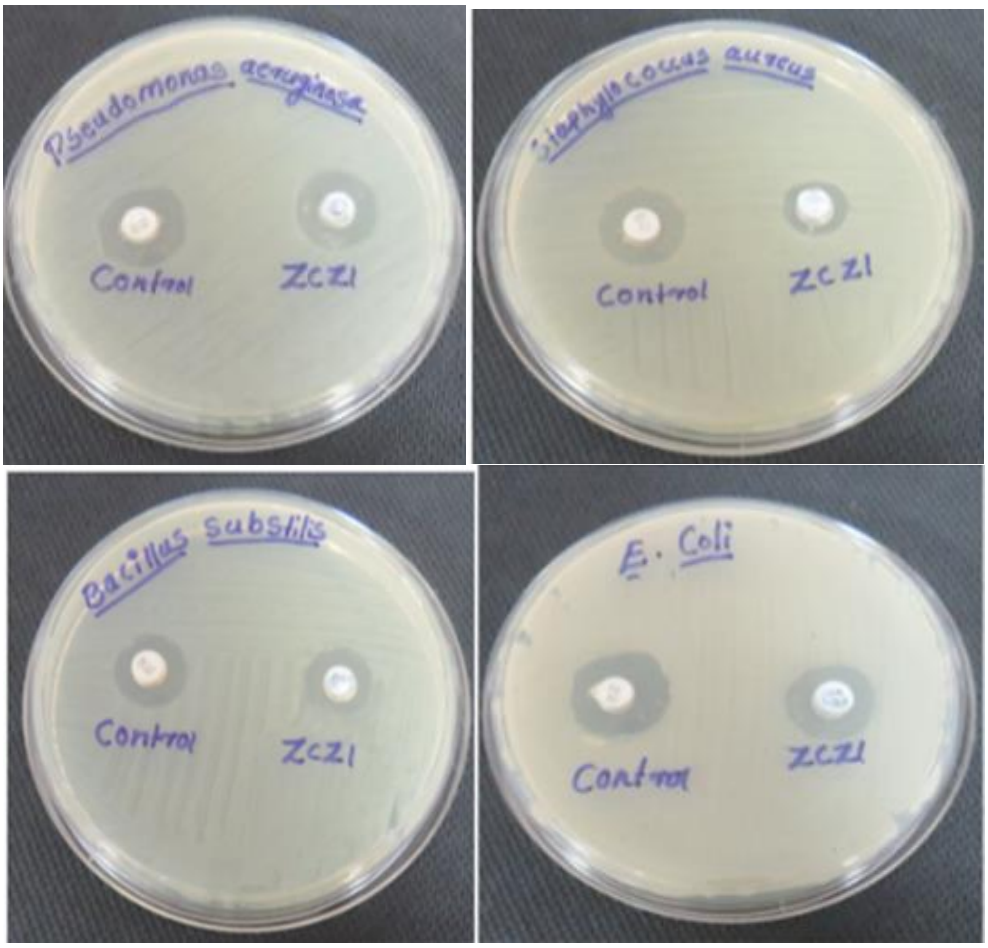


Figure 10: Zone of inhibition against various bacterial strains- ZCL ZnO NP

Antibacterial Activity: The green-synthesized ZnO NPs were evaluated for their antibacterial efficacy against human pathogenic bacterial strains (Table 3, figs. 9 and 10). The results revealed significant antibacterial activity with minimum inhibition zones of 11 mm, 16 mm, 15 mm and 12 mm observed for *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*, respectively for ZCL ZnO NPs.

In the case of TCL ZnO NPs, the minimum inhibition zones for *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were 14 mm, 15 mm, 16 mm and 11mm. It was observed that the inhibition zones were only slightly smaller than those of the standard disc Teicoplanin[5,6,10,24,36]. Hence, the findings substantiate the efficacy of green-synthesized ZnO NPs against the studied bacterial strains, suggesting their potential as effective antibacterial agents in various industrial and pharmaceutical applications including food packaging for preservation purposes.

Conclusion

The objective of this work was to use leaf extracts from *Telosma cordata* and *Zephyranthes candida* to synthesize zinc oxide nanoparticles. These extracts served as reducing agents which helped to lower metal ions and produce nanoparticles. Alkaloids, glycosides and flavonoids were among the water-soluble natural compounds that were engaged in the reduction process.

The produced nanoparticles were characterised by means of UV-Visible, FTIR, XRD, TG and Optical Profilometer for structural, morphological and antibacterial activity evaluations. The study's findings demonstrate that the plant extract contains a number of naturally occurring bioactive chemicals that could be successfully used in the manufacturing of nanoparticles.

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