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Green Synthesis of Zinc Oxide Nanoparticles using Seed Extract of *Murraya koenigii* and Their Antimicrobial Activity against Some Human Pathogens

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ABSTRACT

In the present report, simple and green protocols for the synthesis of ZnO nanoparticles under ambient conditions from the aqueous extracts of *Murraya koenigii* seeds were reported. The synthesized nanoparticles are characterized by UV spectrometer, FT-IR spectroscopy, powder XRD pattern, scanning electron microscope (SEM) and transmission electron microscopy (TEM) analysis. The synthesized ZnO nanoparticles tested for antimicrobial activities by Bauer-Kirby method using human pathogenic bacterial and fungal species. The synthesized nanoparticles exhibited good antimicrobial agent for all the four bacterial and three fungal species.

1. Introduction

The research of nano-biotechnology deals with the development of different eco-friendly and bio-reduction process for the synthesis of stable nanoparticles possessing well-defined shapes and controlled narrow sizes [1]. The metal nanoparticles have played vital role in medicine and pharmacy. For instant, zinc oxide nanoparticles are used in medicine for antibacterial, antifungal agents, drug delivery and bio imaging probes [2]. Kavithaa *et al.* had synthesized zinc oxide nano rods using *Santalum album* leaf extract and studied their activity on human breast cancer (MCF-7) cells [3]. Recently Patil and Taranath had synthesized zinc oxide nanoparticle using *Limonia acidissima* leaf extract and studied for mycobacterium tuberculosis activity [4].

The physicochemical methods for synthesis of metal nanomaterials are costly; require grand labour and large time. In addition, large quantities of secondary waste are generated resulting from the addition of chemical agents for precipitation and reduction in the processes [5]. Biosynthetic methods are simple, eco-friendly reaction ethics for the synthesis of nanoparticles by using plant extracts that give a biological synthesis route of many metallic nanoparticles which are more solvent-friendly and allows reduction, capping and control with well-shapes and defined size of nanoparticles. To avoid the use of toxic organic chemicals, solvents and severe reaction conditions (pressure, temperature and long refluxing time) for the preparation of nanomaterials, several researchers have been analysis the possibilities of synthesizing metallic nanomaterials in aqueous medium with the help of capping or stabilizing agents [6]. Singh *et al.* had synthesized ZnO (zinc oxide) nanoparticles using calotropis provera seaweed by green synthetic method [7]. This bio-synthetic method found to be simple and viable alternative to chemical and physical methods, environmental friendly, scaled up, economically feasible for mass-scale production without any complexity [8].

In this present work, the biosynthesis of ZnO nanoparticles from *Murraya koenigii* seed extract. The synthesized nanoparticles have been extensively characterized by UV, FT-IR, powder XRD pattern, scanning electron microscope (SEM), transmission electron microscopy (TEM) and their antimicrobial activity was evaluated against various bacterial species namely *Bacillus subtilis*, *Staphylococcus aureus* (Gram-positive) and fungal species namely *A. flavones*, *A.niger* and *T. veride*.

2. Experimental Methods

2.1 Materials and Methods

From Sigma Aldrich Chemical Company, Bengaluru-100, Himedia, Mumbai, the chemicals viz., zinc nitrate ($ZnNO_2 \cdot 6H_2O$), potato dextrose agar, Mueller Hinton agar, nutrient broth, Tween-80 solution and other relevant materials required for in the analysis. All the glass wares are used in the present investigation (conical flask, beaker, measuring cylinder, test tubes, petri dishes, glass column) were purchased from Borosil (India).

2.2 Preparation of Seed Extract

Murraya koenigii seed was collected from the area of Chidambaram surroundings. The *Murraya koenigii* seeds have been washed several times with deionised water to remove the unwanted dust particles and then dried light. The dried seeds have been grinded to form powder. The powder was dissolved in deionised distilled water. The seed extract used for the reduction of Zn^{2+} (zinc ions) to ZnO NPs (zinc oxide nanoparticles) was synthesized by placing 6 g of washed dried fine powdered *Murraya koenigii* seeds with 100 mL of deionised water in 250 mL glass beaker. The extract was boiled for 40 minutes until the colour of the aqueous solution changes from watery to light green by using magnetic stirrer. The extracts were then filtered thrice through Whatman No. 1 filter paper. The prepared seed extract was stored in the Erlenmeyer flasks for further experiments.

2.3 Preparation of Zinc Oxide Nanoparticle

In a 250 mL conical flask, 20 mL of *Murraya koenigii* seed extract mixed with 80 mL of zinc nitrate ($ZnNO_2$) and few drops of 2.0 M NaOH solution was added with continuous vigorous stirring for 3–5 hrs, finally black coloured solution is formed and then incubated overnight at room temperature. The white coloured precipitate was formed at the bottom of conical flask. This synthesized zinc oxide nanoparticles (white precipitate) was washed with distilled water (3–4 times), ethanol (4–5 times) and dried at hot air oven at room temperature. The synthesized zinc oxide (ZnO) nanoparticles have been scrapped out for further analysis. The synthesized zinc oxide nanoparticle shown in Fig. 1.

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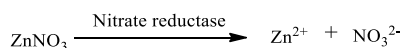


Fig. 1 (a) zinc nitrate solution and (b) color change during the reduction of zinc nitrate into zinc oxide nanoparticle by the extract of *Murraya koenigii* seeds after 120 hrs of incubation

3. Results and Discussion

3.1 UV-Visible Absorption Spectra

The UV spectroscopy is a useful study to confirm the bio-reduction of zinc oxide nanoparticles. Bio-reduction of zinc nitrate into zinc oxide nanoparticles in the presence of *Murraya koenigii* seed extract was confirmed from UV-Vis spectral measurements. Zinc oxide nanoparticles contain free electrons, these free electrons have the probability to give rise to a SPR (surface Plasmon resonance) absorption band [9], and this is due to the combined vibration of electrons of metal nanoparticles in resonance with the light wave. The biosynthetic reduction process is extracellular and fast leading to the development of easy bio-synthesis of ZnO NPs (zinc nanoparticles). At the time zinc nitrate is breakdown into zinc (Zn^{2+}) and nitrate ions (NO_3^{2-}), a nitrate ions (NO_3^{2-}) reductase enzyme to take place the reaction.



The UV-visible spectral results of zinc oxide with different time of incubation are given in Fig. 2. As shown in Fig. 2, the broad spectrum of zinc oxide with absorption maximum at 330 nm was seen due to the SPR (surface plasmon resonance) absorption bond. The incubation time of zinc nitrate increases from 12 hrs to 120 hrs and also increases the reduction process of ZnO nanoparticles. The presence of amides and acid groups in the extract of *Murraya koenigii* seeds may play an important role in reduction and stability of zinc oxide Nanoparticle.

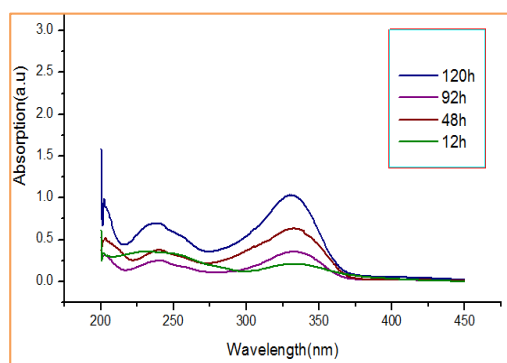


Fig. 2 UV-visible absorption spectra of zinc oxide nanoparticles with different incubation time

3.2 FT-IR Spectral Analysis

The FT-IR spectrum of *Murraya koenigii* seed extract and zinc oxide nanoparticles stabilized by the extract is shown in Fig. 3(a). For the seed extract, the FT-IR spectra shows different major peaks positioned at 3420.01, 2926.42, 2849.30, 1652.22, 1506.13, 1416.98 and 1078.71 cm^{-1} . The presence of peak at 3421.01 cm^{-1} could be due to O-H group in polyphenols or proteins/enzymes or polysaccharide [5, 10, 11]. A small peak observed at 2925.42 cm^{-1} is due to CH-stretching of alkanes. The bands positioned at 2849.30 cm^{-1} characteristic of C-CH₂ groups in chitosan or oleic acid. The (NH)C=O group appeared sharp and intense peak at 1653.22 cm^{-1} . The peaks at 1416.99 cm^{-1} and 1079.83 cm^{-1} may be ascribed to -C-C- and -C-O-C stretching modes. On the other hand, FT-IR spectrum of synthesized zinc oxide nanoparticles in Fig. 3(b) shows the presence of major peaks at 3421.21, 2925.24, 2852.32, 1622.61, 1430.22, 1010.64 and 526.34 cm^{-1} , which are associated with -OH stretching

vibrations, CH-stretching of alkanes, C-CH₂ groups in chitosan or oleic acid, (NH) C=O group, -C-C- and -C-O-C stretching modes, respectively. The IR spectra peak shows the bulk zinc oxide showing a high intensity broad band at 525.35 cm^{-1} is due to the stretching mode of the zinc and oxygen bond. After bio-reduction, there is a shift in the absorption band of 3420.01-3421.21, 2926.42-2925.24, 2849.30-2852.32, 1652.22-1622.61, 1416.98-1430.22 and 1078.71-1010.64 cm^{-1} . The shifting of the band increases and decrease is due to the various functional groups such as alginic acid, ascorbic acid, protein, carbohydrates, flavanoids, tannins, lipids and mannitol acted as both stabilizing and reducing agents for the zinc oxide nanoparticle formation reaction.

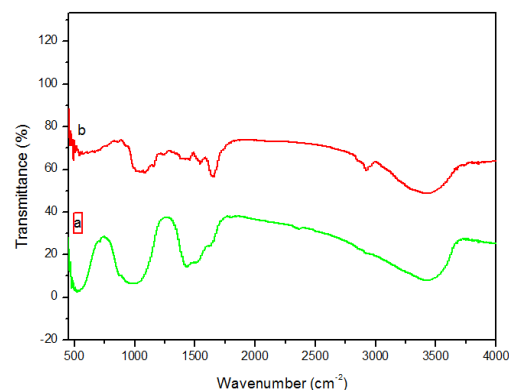


Fig. 3 FT-IR spectra of (b) *Murraya koenigii* seed extract and (a) zinc oxide nanoparticles (ZnNPs)

3.3 X-Ray Diffraction Pattern

The XRD diffraction patterns of synthesized ZnO nanoparticles are shown in Fig. 4. From the XRD diffraction pattern whole spectrum 2 hrs ranging from 10 to 90, the XRD diffraction pattern obtained at 31.80°, 34.4°, 36.2°, 47.5°, 56.6°, 67.9° and 69.07° are observed corresponding to (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 1 2) and (2 0 1) planes. This planes suggesting fcc (face-centered cubic) crystalline structure of the ZnO nanoparticle. The XRD diffraction pattern shows strong and narrow diffraction peaks indicate the well crystalline nature of ZnO nanoparticles.

$$d_{hkl} = K\lambda/\beta\cos 2\theta$$

where, D – the crystal size; λ – the wavelength of the X-ray radiation, for CuK α ; K – is the Scherrer constant; θ – is the Bragg diffraction angle; β – the line width at half-maximum height [12].

The characteristic peaks are higher in intensity which indicates that the products are of good crystalline nature. Average size of the synthesized nanoparticle was found to be 70–100 nm.

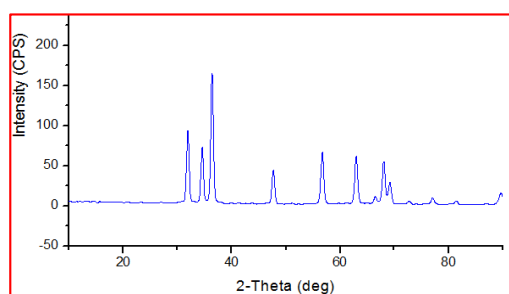


Fig. 4 XRD patterns of zinc oxide nanoparticles synthesized after 120 hrs of incubation

3.4 Scanning Electron Microscopy (SEM) and EDS Analysis

SEM and EDX analysis is done to visualize shape, size and purity of the bio-synthesized zinc oxide (ZnO) nanoparticles was studied. Fig. 5(a) shows the scanning electron microscope of synthesized zinc nanoparticles. SEM images were measured and topographical analysis was performed based upon the surface study. The spherical, triangle, radial, hexagonal, rod and rectangle shaped. The typical scanning electron microscope (SEM) image of the zinc oxide size about 100 nm obtained by the bio-synthesis process. The resulting zinc nano particles shape was quite similar to transmission electron microscopy (TEM) analysis. In general, small morphology is preferred to biomedical field [13]. The EDX (energy-dispersive X-ray) analysis shows the chemical composition for the synthesized ZnONPs. Fig. 5(b) shows the EDX spectrum ZnO nanoparticles after 120 h incubation. The Fig. 5(b) shows strong signal for zinc and oxygen.

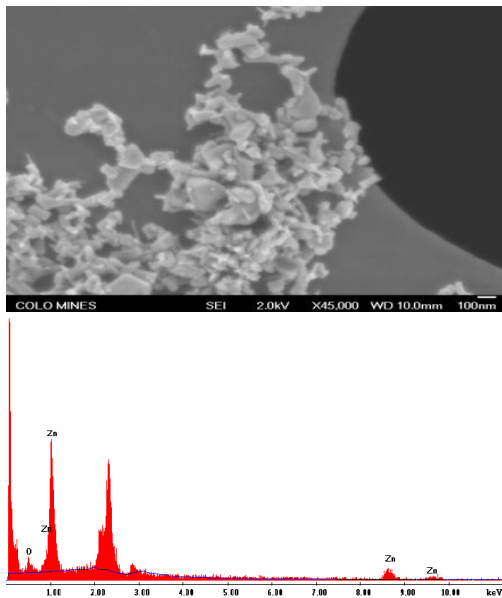


Fig. 5 (a) FESEM micrographic image of synthesized ZnO NPs (b) EDX of the zinc oxide nanoparticles showing chemical composition

3.5 Transmission Electron Microscope Analysis

The UV spectral studies provided strong evidence for the formation of ZnONPs and their growth kinetics, the shape and size of the synthesized ZnONPs (zinc oxide nanoparticles) were analysed by transmission electron microscopy (TEM). The TEM image of synthesized ZnONPs after 120 hrs of incubation is given in Fig. 6. The figure exhibits the TEM image of ZnONPs being different sizes are radial, triangle, rod, hexagonal, and rectangle with size of 100 nm.

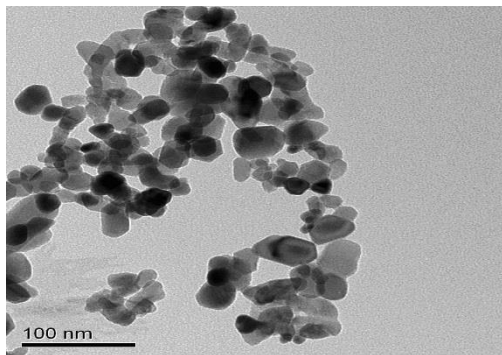


Fig. 6 TEM images of synthesized zinc oxide nanoparticles using extract of *Murraya koenigii* seed extract

3.6 Antimicrobial Activity

3.6.1 Antibacterial Activity

The antibacterial activity of the synthesized zinc oxide nanoparticles was investigated against the Gram-negative bacterial species (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram-positive bacterial species (*Bacillus subtilis*, *Staphylococcus aureus*) through disc diffusion method. The positive control (ciprofloxacin) against all the pathogens and the zone of inhibition values was measured as shown in Fig. 7 and the corresponding cluster column charts are shown in Fig. 8. The results are given in Table 1 from the table, indicate that the synthesized zinc oxide nanoparticles (ZnO NPs) have shown a considerable antimicrobial activity for all the four pathogens studied; however, the highest anti-bacterial activity of zone of inhibition values recorded for *Bacillus subtilis* (18 mm). The difference in antibacterial activity is due to their difference in membrane structure (Gram-positive and Gram-negative). The *Bacillus subtilis*, *Staphylococcus aureus* (Gram-positive) bacteria have a thick peptidoglycan layer, whereas, peptidoglycan layer in the *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative) bacteria is thinner but surrounded by a lipid layer outside [14]. In the antimicrobial activity, initially zinc oxide nanoparticles attach to the surface of the bacterial cell membrane and then penetrate into the bacteria. After penetration, they inactivate the enzymes of the microbes, generating hydrogen peroxide. Hence bacterial cells were dead.

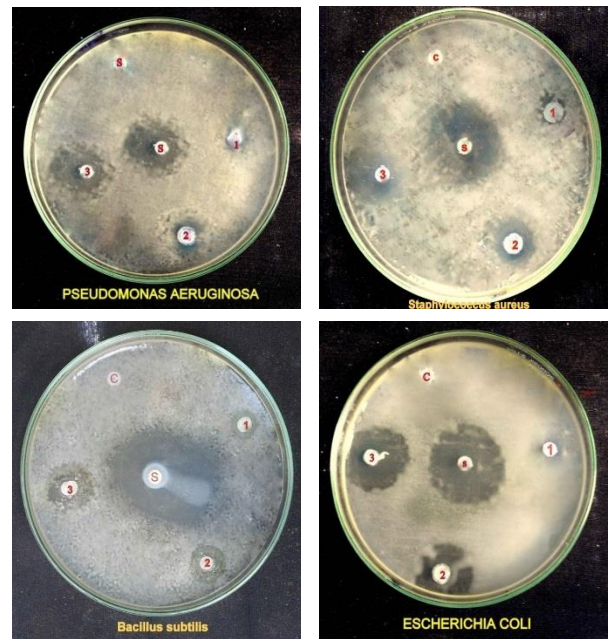


Fig. 7 Antibacterial activity of ZnO Nps

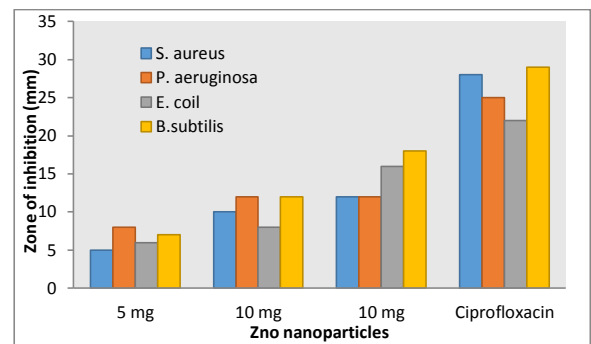


Fig. 8 Antibacterial activity of ZnO Nps (Cluster column-chart)

Table 1 Antibacterial activity of synthesized ZnO Nps [Disc diffusion method]

S. No	Bacteria	Standard drug	Zone of inhibition (mm)			Control
			5 mg	10 mg	15 mg	
1	<i>Staphylococcus aureus</i>	28	5	10	12	0
2	<i>Pseudomonas aeruginosa</i>	25	8	12	12	0
3	<i>Escherichia coli</i>	22	6	8	16	0
4	<i>Bacillus subtilis</i>	29	7	12	18	0

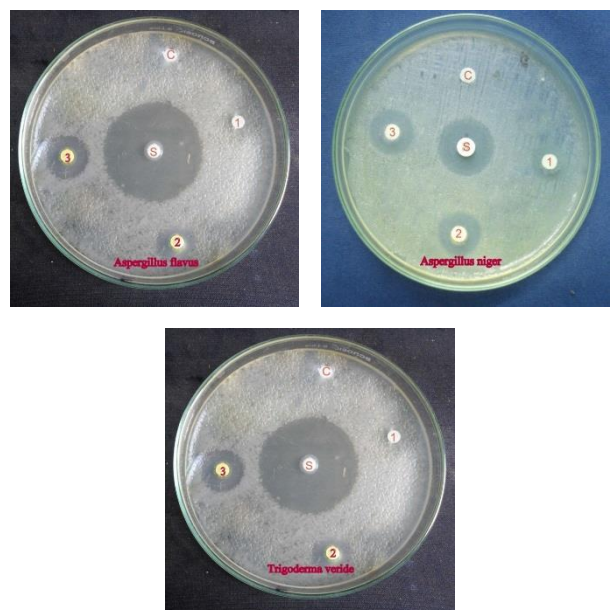


Fig. 9 Antifungal activity of ZnO Nps

3.6.2 Antifungal Activity

The antifungal activity of the synthesized zinc oxide nanoparticles was investigated against three fungal species namely *A. flavones*, *A. niger* and *T. veride* through disc diffusion method. The antifungal activity of the zinc oxide nanoparticle and the positive control (Miconazole) against the pathogens subjected for analysis for Kirby-Bauer method. The zone of inhibition values was measured as shown in Fig. 9 and the corresponding cluster column charts are shown in Fig. 10. The results are given in Table 2. From the results of antifungal activity indicate that the synthesized zinc oxide nanoparticles showed a considerable antifungal activity for all the pathogens. From the results of antifungal activity of synthesized zinc oxide nanoparticles suggest their usage in medical devices.

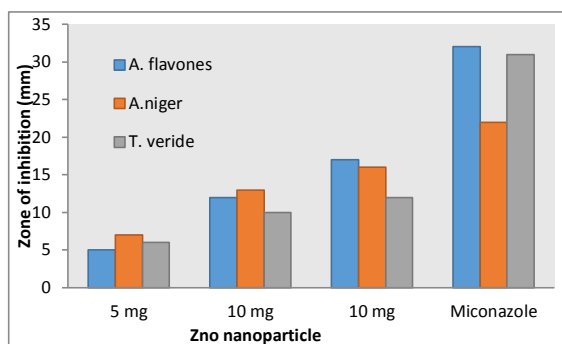


Fig. 10 Antifungal activity of ZnO Nps (Cluster column-chart)

Table 2 Antifungal activity of synthesized ZnO Nps [Disc diffusion method]

S. No	Fungal	Standard drug	Zone of inhibition (mm)			Control
			5 mg	10 mg	15 mg	
1	<i>A. flavones</i>	32	5	12	17	0
2	<i>A. niger</i>	22	7	13	16	0
3	<i>T. veride</i>	31	6	10	16	0

4. Conclusion

In conclusion, a "green" approach to synthesis of ZnONPs through bio-reduction of zinc nitrate using aqueous seed extract of *Murraya koenigii* was demonstrated. The synthesized products were characterized by FT-IR, UV-Vis Spectrophotometer, SEM, EDS, and TEM. The Bio-synthesized ZnONPs was confirmed by the presence of surface plasma resonance peak at 330 nm. The crystalline nature of the ZnO NPs was confirmed by XRD analysis. The EDX analysis also confirmed the amount of zinc and oxide ions present in the product. FT-IR results suggest that proteins present in the extract were largely responsible for the bio-synthesis of the zinc nitrate into zinc oxide nanoparticles. The SEM analysis of ZnO NPs

demonstrated the size of ZnO NPs 100 nm. The TEM image of synthesized zinc oxide nanoparticles (ZnO NPs) shows rod, triangle, radial, hexagonal, and rectangle shape with the size of nanoparticle 100 nm. Also the antimicrobial activity of synthesized ZnO NPs has confirmed that these can be used as potent antimicrobial agent against urinary tract infection.

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