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Green synthesis of zinc oxide nanoparticles using *Moringa oleifera* leaf extract and evaluation of its antimicrobial activity

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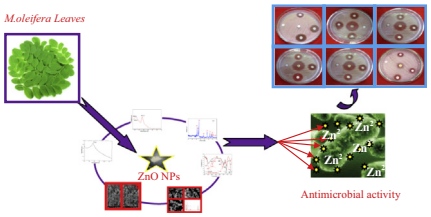
HIGHLIGHTS

- ZnO nanoparticles were prepared from *M. oleifera* leaf extract.
- Particle sizes and structure were determined by XRD analysis.
- FT-IR study determined functional groups of nanoparticles.
- FE-SEM and EDX revealed that the topographical and chemical compositions.
- Microbial activity of ZnO NPs has more susceptible against *S. aureus* than the other micro organisms.

GRAPHICAL ABSTRACT



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ABSTRACT

The development of semiconductor materials made a considerable progress of catalytic technologies. In the present study, a simple and eco-friendly chemical direction for the synthesis of zinc oxide nanoparticles (ZnO NPs) using leaf extract of *Moringa oleifera* has been used. The prepared ZnO NPs were characterized various techniques such as UV–Vis absorption spectroscopy, X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), energy dispersive X-ray analysis (EDX), Fourier transform infrared spectroscopy (FT-IR) and photoluminescence spectroscopy (PL). XRD analysis revealed the wurtzite hexagonal structure of ZnO NPs. FT-IR confirmed the presence of functional groups of both leaf extract and ZnO NPs. The particles size, morphology and topography determined from FE-SEM. The intense and narrow width of zinc and oxygen have high purity and crystalline were identified using EDX. UV–Vis absorption showed the characteristic absorption peak of ZnO NPs. The results of antimicrobial activities revealed that maximum zones of inhibition was observed Gram (+ve) positive bacteria and followed by the Gram (−ve) negative bacteria and fungal at concentration of 200 μg/mL of ZnO NPs.

Introduction

The area of Nanotechnology is one of the most dynamic fields in advanced material science [1]. Nanoparticles exhibit completely improved properties based on specific characteristics such as size, distribution and morphology [2]. Nanomaterials significantly affect the areas of physics, chemical science, electronics, optics, materials science and biomedical science [3]. There are several methods reported for the synthesis of ZnO nanoparticles, which include chemical vapor deposition, gas-phase method, spray pyrolysis, hydrothermal synthesis, micro emulsion, electrochemical method, pulsed laser deposition, microwave synthesis and the sol gel method [4]. The green synthesis are more advantageous over chemical and physical method as it is cost efficient and eco-friendly [5]. The synthesis of nanoparticles using plant

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extracts are often termed as green synthesis method that reduces or eliminates the generation of hazardous substances [6]. The Mechanism of biosynthesis of nanoparticles in plants may be associated with the phytoremediation concept [7]. Metal oxide nanoparticles (NPs) are viewed as a potential next generation or disinfecting agents, which are finding applicable in the field of clinical concern, consumer products and in other industrial applications [8]. Zinc oxide nanoparticles (ZnO NPs) also have considerable attention to their unique antibacterial, antifungal, UV filtering properties, high catalytic and photochemical activity [9]. However, most ZnO NPs are produced synthetically and has the advantage of low cost and white appearance over the silver nanoparticles [10].

Moringa oleifera (L.) belongs to the single genus of family Moringaceae. It is a small fast-growing ornamental tree widespread over the tropical regions of Africa and Asia [11]. The young leaf, flowers, and green pods are commonly used as vegetables in the Filipino diet. The medicinal value of the seeds and the different parts of the plant has long been recognized in folklore medicine [12,13]. Moringa leaf have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids. [14].

In recent decades *M. oleifera* leaf, flowers, gums, roots and seeds were extensively used for treatment of many diseases including inflammation, cardiovascular and liver diseases and for immune boosting agent and regulator for blood sugar and cholesterol [15,16]. In various parts of the world *M. oleifera* termed as miracle tree that used in medicine because it is rich in amino acids, K, Ca, Fe, ascorbate, and growth regulating hormones like zeatin which will promotes cell division, cell elongation and also copious antioxidant properties [17]. The bark extract has been shown to possess antifungal, antitubercular activity and the ethanolic extract (50%) of *M. oleifera* (whole plant excluding roots) were also showed anticancer activity in mice [18]. In recent decades, the extracts of leaf, seeds and roots of *M. oleifera* have been broadly studied for many potential uses including wound healing antihepatotoxic, antifertility, hypotensive and analgesic activity [19]. In this regard, ZnO NPs are effective as they exhibit antibacterial activity under visible light illumination [20]. The antibacterial properties of ZnO NPs were investigated and developed as antibacterial agents against a wide range of microorganisms to control and prevent the spreading and persistence of bacterial infections [21].

Antibacterial activity of ZnO was tested and the effect was more pronounced with the Gram-positive than the Gram-negative bacteria and also ZnO NPs exhibited a preferential ability to kill cancerous HL60 cells as compared with normal peripheral blood mononuclear cells [22]. The most likely mechanism of antibacterial activity of ZnO NPs is attributable to photochemical property of ZnO NPs. The photoinduced charge carriers (electrons and holes) interact with oxygen and H_2O molecules that are adsorbed on the surface of ZnO NPs to produce reactive oxygen species (ROS) like, singlet oxygen, hydroxyl radical, etc, [23,20,24]. These reactive oxygen species might trigger membrane lipid peroxidation and cause antibacterial effect [25]. In addition, dissolution of zinc ions from ZnO NPs as Zn^{2+} or as complex hydroxide anions of Zn in the culture medium has been attributed to enhanced antibacterial activity of ZnO NPs [26,27]. In the present study we aimed to investigate the antibacterial and antifungal properties of ZnO NPs synthesized from *M. oleifera* leaf via green method, which consider being the low cost, simple procedure and eco–friendly to the environment.

Materials and methods

Materials

M. oleifera leaves were collected during the month of January 2014 from the Annamalai university campus, Annamalai Nagar, India. The leaf were identified and authenticated by an herbalist, Department of Botany, Annamalai University. Chemicals and glassware were procured from Sigma Aldrich, Pondicherry, India. The Clinical isolates of bacterial strains *viz., Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli* and fungi strains such as *Candida albicans* and *Candida tropicalis* were obtained from the Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamil Nadu, India. These strains were maintained on nutrient agar slant at 4 °C. Twenty-four hours old culture of selected bacteria was mixed with physiological saline and turbidity, and was adjusted by adding sterile physiological saline until a 0.5 McFarland turbidity standard 10^8 colony forming units (CFU) per mL was obtained.

Synthesis of zinc oxide nanoparticles using M. oleifera leaf extract

The collected *Moringa oleifera* were washed with tap water and after that followed with distilled water for removing the unwanted impurities such as scum, dust and other materials. The leaf sample was allowed to dry in room temperature (32 °C) and 20 g was taken for synthesis purpose. The weighed of 20 g leaf were boiled with 100 mL of double distilled water for 20 min at 60 °C. During the procedure of boiling, a light yellow colored solution was formed and which was cool at room temperature. After that, the yellow colored extract was filtered with filter paper (Whatman No. 1) and stored at refrigerator.

Further, 20 mL of *M. oleifera* leaf aqueous extract was taken from the stock solution (stored at refrigerator) and boiled at 60–80 °C by using magnetic stirrer. When the temperature of the solution was reached at 60 °C, 2 g of zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$) was added. Then the mixture was boiled until it becomes deep yellow colored paste. Then, it transferred to a ceramic crucible cup and heated in furnace at 400 °C for 2 h. Finally, obtained light yellow colored powder. This powdered product (ZnO NPs) was used for the further studies. The flowchart used for the preparation of ZnO NPs is shown in Fig. 1.

Antimicrobial assay

In the present study *in vitro* antimicrobial activity were carried out by the using of disc-diffusion method (Bauer, 1966) [28]. This method followed the following procedure: First of all, Petri plates were prepared with 20 mL of sterile Muller Hinton Agar for bacteria and Sabourdad Dextrose Agar for fungi. The standard inoculums using bacterial suspension containing 10^8 (CFU) per mL, yeast suspension containing 10^4 (CFU) per mL were swabbed on the top of the solidified media and allowed to dry for 10 min. Previously, prepared ZnO NPs impregnated discs at the concentrations of 200 μg/mL for bacteria and fungi were placed aseptically on sensitivity plates with appropriate controls. The tests were conducted with 20 per disc with three replicates. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using 10% DMSO. Methicillin (5 μg/disc) for *S. aureus,* Ciprofloxacin (10 μg/disc) for bacteria was used as positive control and Amphotercin-B (100 units/discs) was used as positive control for *Candida.* All the plates were then incubated for 24 h at 37 °C for bacteria and 28–35 °C for *Candida* respectively. The sensitivity