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Bio-fabrication of zinc oxide nanoparticles using leaf extract of curry leaf (*Murraya koenigii*) and its antimicrobial activities

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ABSTRACT

The present study focused on the development of zinc oxide nanoparticles (ZnO NPs) from the leaf extract of *Murraya koenigii* where zinc nitrate acts as the precursor. The X-ray diffraction (XRD) analysis showed the crystalline structure, and atomic force microscopy (AFM) showed the morphology of the ZnO NPs to be spherical with an average size of 12 nm. Functional groups of the sample were identified by using Fourier transmission infrared (FT-IR) spectroscopy. Their shape, structure and composition were assessed by Field emission scanning electron microscopy (FE-SEM) and energy dispersive spectroscopy (EDS). The results depicted that synthesized ZnO NPs were moderately stable and hexagonal shape, spherical shape with maximum particle size less than 100 nm. The green-synthesized ZnO NPs had prominent activities against *Staphylococcus aureus* (14.0 ± 0.50 mm) and followed by *Bacillus subtilis* (13.8 ± 0.76 mm) at the concentration of 200 μg/mL.

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1. Introduction

Nanotechnology has huge potentiality virtually in every area of science and engineering, principally due to its size and shape dependent intrinsic, optoelectronic, catalytic, biological properties and greater surface area [1]. Generally, metal and metal oxide nanoparticles are synthesized and stabilized through chemical and mechanical methods [2,3], electrochemical technique [4], photochemical reactions in reverse micelle [5] and now-a-days via a Green chemistry method [6]. The biological synthesis process elucidates the importance of metal microbe interaction in several biotechnological applications including the field of bioremediation, biomineralization, bleaching and microbial corrosion [7].

Nanobiotechnology is an important and emerging technical tool for development of eco friendly and reliable methodology for the synthesis of nanoscale materials by using biological sources [8]. The plants phytochemicals like terpenoids, flavonoids and alkaloids present in the aqueous leaf extract with antioxidant properties are accountable for the preparation of metal oxide nanoparticle [9]. Recently nanoparticles synthesis is achieved with bacteria, fungi and actinomycetes [10–12] and also from plants leaf extract such as *Neem*, *Camellia sinensis*, *Corriandrum*, *Nelumbo licifera*, *Ocimum sanctum* and several others which are compatible with the green chemistry principles [13,14].

The considerable antimicrobial activities of inorganic metal oxide NPs, such as ZnO, MgO, TiO_2 and SiO_2 and their selective toxicity to biological systems suggest a potential application as therapeutics, diagnostics, surgical devices and nanomedicine based antimicrobial agents [15]. In this regard using “green” methods in the synthesis of ZnO NPs has increasingly become a matter of interests since conventional

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chemical methods are expensive and require the use of chemical compounds/organic solvents as reducing agents [16]. Green synthesize is an eco-friendly technology for the synthesis of ZnO NPs. They believe to be nontoxic, bio-safe and biocompatible and are used as drug carriers, cosmetics, and fillings in medical materials [17]. Synthesis of zinc oxide has attracted considerable attention owing to its diverse properties like catalysis, magnetic, optical, electrical conductivity and antimicrobial activity [9].

The plant *Murraya koenigii* (*M. koenigii*) belongs to the family Rutaceae is native to India and now circulated in most of southern and South-East Asia. The different parts of *M. koenigii* plant like the leaves, bark and the base are used intensively in indigenous medicine from ancient time, as a tonic for stomachache, stimulant and carminative [18,19]. Recent studies reveal that carbazole of *M. koenigii* has strong antioxidative activities [20]. The plant also reports to have hypoglycemic [21] and anti-fungal effects [22] as well as tried out its efficacy against colon carcinogenesis [23]. In addition to that curry leaf is fed to the rats equal to normal human. It does not cause any adverse effect on food efficiency ratio (FER), red blood cell count (RBC) and white blood cell count (WBC) [24]. *M. koenigii* leaves have rich source of polyphenols, flavonoids and glycosides [25].

The main active plant has multifunctional agents like polyphenols and flavonoids that have strong roles in the synthesis and stabilization of metal NPs [26,27]. Alam et al. [28] reported that the contents of polyphenol and flavonoids present in the leaf of *M. koenigii* are 81.9 mg Gallic acid equivalent g^{-1} and 39.98 mg of quercetin g^{-1}, respectively. These chemical components are acting as reducing agents and also as the stabilizing agents by adhering on the surface of the NPs formed, thereby preventing their aggregation and controlling the particle size [28]. However the possible mechanism of formation ZnO NPs is still not clear and need further investigation.

Moreover, the metal oxide nanoparticles have good antibacterial activities and antimicrobial formulations comprising nanoparticles which can be used as an effective bactericidal agent [29]. The ZnO NPs are effective as they exhibit antibacterial activities under visible light illumination and mechanism of antibacterial activities attributable to photochemical properties of ZnO NPs [30]. Recent studies suggest that an antibacterial activity of ZnO NPs is enhanced only when sizes of ZnO NPs are reduced [31]. Azam et al. found the antibacterial activity of CuO nanoparticles to be size-dependent [32]. The unique properties of ZnO NPs such as a small size and large surface area render them to exhibit greater antibacterial activity than bulk ZnO [33].

Hence, the present work aims to develop eco-friendly fabrication of ZnO NPs by using *M. koenigii* plant leaf extract and also evaluates antimicrobial activity against five bacterial strains such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *E. coli* and two fungal strains such as *Candida albicans* and *Candida tropicalis*.

2. Materials and methods

2.1. Materials

The fresh leaves of *Murraya koenigii* (*M. koenigii*), free from disease, were collected early in the morning during the month of July 2014 from Kalpattu, Villupuram district, Tamil nadu, India. The leaves were identified and authenticated by the Department of Botany, Annamalai University. The chemicals and glassware were procured from SigmaAldrich, Pondicherry, India. The clinical isolates of bacterial strains *viz*., *S. aureus*, *B. subtilis*, *P. aeruginosa*, *P. mirabilis*, *E. coli* and two fungal strains such as *C. albicans* and *C. 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 slants 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 10^6 colony forming units (CFU) per mL was obtained.

2.2. Synthesis of zinc oxide nanoparticles using M. koenigii leaf extract

The collected leaves were washed thoroughly 2–3 times with running tap water and sterilized with double-distilled water. The leaves sample was allowed to dry in room temperature (32 °C) and 20 g was taken for synthesis purpose. 20 g weighed leaves 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 it was cool at room temperature. After that, the yellow colored extract was filtered with filter paper (Whatman No.1) and stored in refrigerator until further use.

Further, 20 mL of *M. koenigii* aqueous extract was taken from the stock solution (stored at refrigerator) and boiled at 60–80 °C. When the temperature of the solution reached at 60 °C, 2 g of zinc nitrate hexahydrate crystals ($Zn(NO_3)_2 \cdot 6H_2O$) was added. The mixture was boiled until the formation of deep yellow colored paste. The paste was transferred to a ceramic crucible cup and heated in muffle furnace which was maintained at 400 °C for 2 h. The obtained light yellow colored powder was used for structural, antibacterial and antifungal activities. The flow chart used for the preparation of ZnO NPs is shown in Fig. 1.

2.3. Antimicrobial assay

In the present study, in vitro antimicrobial activities were carried out by using a disc-diffusion method [34]. This method followed the following procedure: first of all, Petri plates were prepared with 20 mL of sterile Muller Hinton Agar for bacteria and 20 mL of Sabourdad Dextrose Agar for fungi. The 24 h prepared test cultures of inoculums 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, 100 and 50 μg/mL for bacterial and fungal were placed aseptically on sensitive plates with appropriate controls.

The tests conducted with 20 μg/disc were placed on the surface of the agar medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Ciprofloxacin (5 μg/disc) was used as positive control for bacteria and Amphotercin-B (100 units/discs) was used as positive control for *Candida*. All

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