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Antimicrobial Efficacy of Zinc Nanoparticles Synthesized from Bitter Gourd Extract

Flora-Glad Chizoba Ekezie[1], W. Jessie Suneetha[1*], K. Uma Maheswari[1], T. N. V. K. V. Prasad[2] and T. G. Krishna[2]

[1]Department of Foods and Nutrition, Professor Jayashankar Telangana State Agricultural University, Rajendra Nagar, Hyderabad, 500030, India.
[2]Nanotechnology Laboratory, Institute of Frontier Technology, Regional Agricultural Research Station, Acharya N. G. Ranga Agricultural University, Tirupati, 517502, India.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of the present study was to investigate the *in-vitro* antimicrobial activity of zinc nanoparticles synthesized from ethanol extract of *Momordica charantia* fruit. Antimicrobial activity was evaluated for *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus flavus* by using disc diffusion method. The result obtained showed that the zinc nanoparticles had a potent antimicrobial activity against all the bacterial species. However no growth inhibitory activity was observed against *Aspergillus flavus*.

Keywords: Bitter gourd extracts; antimicrobial activity; zinc nanoparticles; Disc diffusion method; E. coli; S. aureus; A. flavus.

**Corresponding author: E-mail: wjsuneetha@yahoo.com;*

1. INTRODUCTION

Recent research areas have been channeled to the development of antimicrobial agents against diseases caused by microbes. Microorganisms such as *Escherichia coli* can cause food poisoning in several intestinal and extra intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and gram-negative pneumonia. *Staphylococcus aureus* causes furuncles (boils), carbuncles (a collection of furuncles). Now-a-days, Multiple Drug Resistance (MDR) has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of these infectious diseases. Adding to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic response [1].

Owing to the side effects and the resistance that pathogenic microorganisms build against antibiotics as well as diseases arising from oxidative stress, many scientists have recently started paying attention to medicinal plants with biologically active compounds that possess antimicrobial, antioxidant, nutraceutical properties like anti-diabetic/anti-hyperglycemic effects, lowering blood pressure and anti-inflammatory activity due to polyphenolic compounds [2].

Nanoparticles can be synthesized by various conventional methods. Some of these are chemical and physical but biological methods of nanoparticle synthesis using microorganisms, enzymes and plant extracts are possible and eco-friendly alternatives to chemical and physical methods [3]. Living organisms have huge potential for the production of nanoparticles of wide applications. Use of plants for nanoparticles synthesis can be advantageous over other biological processes by eliminating elaborative process of maintaining cell cultures. The use of environmentally benign materials like plant extracts, bacteria and fungi for the synthesis of zinc nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical, biomedical and agricultural applications as they do not use toxic chemicals in the synthesis protocols [4].

One of the established benefits of nanoparticles is their effective antimicrobial potency against a wide strain of microorganisms. They have the remarkable ability to inhibit the propagation of microbes thus impede their various biological activities in numerous systems. NPs have emerged as effective weapons in our antimicrobial arsenal owing to their unique nanoscale physical and chemical properties. NP size is commensurate with biomolecular and bacterial cellular systems, providing a platform where nanomaterial-bacteria interactions can be fine-tuned through appropriate surface functionalization [5]. Moreover, the high surface area to volume ratio of nanomaterials enables high loading of therapeutics, with promising synergy arising from multivalent interactions. NPs thus, provide a way to address the common mechanisms of antibiotic resistance, such as permeability regulation, multi-drug efflux pumps, antibiotic degradation and target site binding affinity mutations [6]. NPs also create alternative pathways to combat biofilm / MDR infections and immensely lower bacteria resistance over time. NPs utilize multiple mechanisms to kill bacteria, making it difficult for them to adapt existing strategies for developing resistance [7].

In this study, the antimicrobial properties of zinc nanoparticles synthesized from ethanol extracts of bitter gourd were investigated and characterization studies reported previously [8].

2. MATERIALS AND METHODS

2.1 Synthesis and Characterization of Nanoparticles

Nanoparticles were prepared according to the method described by [9]; 90 mL aqueous solution of 1.0×10^{-3} M Zinc nitrate was mixed with a 10 mL of 10% aqueous solution of *Bitter gourd* extract. The samples were then centrifuged using REMI K_{70} at 4000 rpm (2146 g) for 15 min to get clear supernatant. They were subsequently characterized using U.V-Visible Spectrophotometer (UV-2450, SHIMADZU Spectrophotometer) to record the localized surface plasmon resonance of zinc nanoparticles at 200 - 800 cm^{-1}. The size and morphology was examined using Scanning Electronic Microscopy (SEM) (Hitachi's SU6600) and Transmission Electron Microscopy (TEM) (JEM-1010). FTIR (Tensor 27, BRUKER) spectrum was recorded in mid IR region in the range of 400 - 4,000 wave number (cm^{-1}). The phyto-reduced zinc nanoparticles were characterized to reveal their crystal structure using X-ray diffraction technique. The XRD pattern was recorded using computer-controlled XRD-system (JEOL, and Model: JPX-8030) with CuK α-radiation (Ni filtered = 13418 Å) in the range of 40 kV, 20 A. The aqueous suspension of the synthesized nanoparticles was filtered through a 0.22 µm syringe driven filter unit, and the size and distribution of the nanoparticles were measured using dynamic light scattering technique (Nanopartica, HORIBA, SZ-100).

2.2 Antimicrobial Assay

In disc diffusion method utilized, a loop of bacteria from the agar slant stock was cultured in nutrient broth overnight and spread with a sterile cotton swap into petri plates containing 10 ml of nutrient agar medium. Sterile filter paper discs (9 mm in diameter) impregnated with the plant extract at different concentrations were placed on the cultured plates and incubated at 37°C for 24 hrs. Standard antibiotic ampicillin was employed as positive control. After 24 hrs of incubation the antibacterial activity was determined by measuring the diameter of inhibition zones. The diameters of the zones of inhibition by the samples were then compared with the diameters of the zones of inhibition produced by the standard antibiotic discs. Each experiment was carried out in triplicate and the mean diameter of the inhibition ones was recorded. The test was carried out at a concentrations of 100 µg/mL prepared from the raw extract and synthesized nanoparticles [10].

3. RESULTS AND DISCUSSION

The result pertaining to the characterization of the synthesized zinc nanoparticles have been previously published by the authors [8]. However, the antimicrobial activity of the crude extracts as well as mediated zinc nanoparticles were investigated using disc diffusion method (measuring the zone of inhibition) at a known concentration of 100 µg/mL against bacterial and fungal species i.e. *Staphylococcus aureus, Pseudomonas aeurogenosa, Escherichia coli* and *Aspergillus flavus* respectively. The result is presented in the Table 1.

Result of anti-microbial activity against *Staphylococcus aureus* showed inhibitory zones of 5.07, 4.97 and 15.03 mm respectively for crude extract, ampicillin and ZnNps. On the other hand, the result for *Pseudomonas aeurogenosa* showed that ethanol extract ZnNps had significantly higher zone of inhibition (15.05 mm) when compared to the other samples (P < 0.05) while the least inhibition of was exhibited by crude extract (2.07 mm). However, the standard drug ampicillin (19.80 mm) had higher antimicrobial activity against the organism than ZnNps ethanol extract. Similarly, the antimicrobial activity against *Escherichia coli* was also screened with the various extracts. The result obtained showed that the standard ampicillin had higher zone of inhibition (14.03 mm) compared to the other samples and differed significantly (P<0.05) from ZnNps ethanol (3.48 mm). The ethanol extracts and its mediated zinc nanoparticles were observed to be weakly active on *E. coli* with only 2.83 and 3.48 mm of zone of inhibition respectively which implies low potency as inhibition zone of 7.0 mm and above is generally acceptable as being active [11]. The result of the assay showed no antimicrobial activity against *Aspergillus flavus*.

Previous studies have demonstrated that *M. charantia* is very rich in triterpenes, proteins and steroids. Those of major interest include momordin, α - and β - momorcharin, cucurbitacin B_1 and oleanolic acid [12]. It is speculated that the antimicrobial activities of triterpenes depend on interactions between their lipid components with the net surface charge of microbial membranes. Furthermore, the bioactives might penetrate the cell membranes and interacting with intracellular sites critical for antibacterial activity [13]. This probably results in dilution effect or chemical antagonism of the various cell constituents on each other [14].

Table 1. Antimicrobial activity of extracts using disc diffusion method

S. no	Sample	*S. aureus* zone of inhibition (mm)	*P. aerogenosa* zone of inhibition (mm)	*E. coli* zone of inhibition (mm)	*A. niger* zone of inhibition (mm)
1	Standard ampicillin	4.97 ± 0.06 ^{e}	19.80 ± 0.2 ^{a}	14.03 ± 0.04 ^{a}	-
2	ZnNps ethanol extract	15.03 ± 0.05 ^{b}	15.05 ± 0.07 ^{b}	3.48 ± 0.04 ^{gh}	-
3	Crude ethanol extract	5.07 ± 0.02 ^{e}	2.07 ± 0.05 ^{l}	2.83 ± 0.03 ^{hi}	-

Key – No Inhibition. Values are expressed as mean ± standard deviation of three determinations. Mean values with similar superscripts within a column row do not differ significantly (P< 0.05).

Several investigations have suggested that the possible mechanisms involved in the interaction of positively charged nano-materials with the negatively charged biological macromolecules. This creates an ‘‘electrostatic’’ attraction between the microbe and treated surface. The enhanced antimicrobial activity of nanoparticles can be attributed to their increased surface area available for interactions, which enhances bactericidal effect than the large sized particles; hence, they impart cytotoxicity to the microorganisms [15]. The mechanism by which the nanoparticles are able to penetrate the bacteria is not understood completely, but studies suggest that when bacteria were treated with zinc nanoparticles, changes took place in its membrane morphology that produced a significant increase in its permeability affecting proper transport through the plasma membrane [16], leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting into cell death. It is observed that zinc nanoparticles have penetrated inside the bacteria and have caused damage by interacting with phosphorus- and sulfur-containing compounds such as DNA [17]. In addition, we speculate that the absence of antimicrobial efficacy against *Aspergillus flavus* might be due to insufficient dosage or concentration (100 µg/mL was utlised). This could also be associated to the resistance of the fungi to the synthesized zinc nanoparticle.

But to understand the mechanisms of action of these agents, more detailed chemical structure and explicate studies of the bioactive components followed by therapeutic investigations and toxicological assessment are required. The findings in this study may lead to the development of nanoparticles based new antimicrobial systems with eco-friendly applications in packaging, preservation and storage of food as well as bactericidal, wound healing, other medical and electronic applications.

4. CONCLUSION

Nanoparticles are versatile and magical tool for various application its conventional preparation methods physical, chemical and biological are somewhat, somewhere not feasible for safety and stability purpose thus green synthesis method for Nps production have developed which overcome the limitations related with traditional methods. Plant synthesized metal Nps are more stable, safe and easily scaled up. These nanoparticles have futuristic applications as antimicrobial agents as established in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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