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Research Article

IN VITRO ANALYSIS: THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF ZINC OXIDE NANOPARTICLES FROM *CURCUMA LONGA*

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ABSTRACT

Objective: *Curcuma longa* is a known natural medicine for inflammation from ancient times. It has a low absorption rate and poor solubility. Hence, it is used for the green synthesis of nanoparticles. Zinc oxide nanoparticle (ZnO NPs) is famous nanoparticles which are economical, less toxic, and brilliantly biocompatible. They have potential biomedical properties, mainly anticancer, antidiabetic, and antimicrobial.

Methods: The present study was designed to investigate *in vitro* analysis of the antimicrobial activity against pathogenic bacteria and fungi and its ability to scavenge reactive oxygen radicals of ZnO NPs.

Results and Conclusion: The results indicated that ZnO NPs produced from *C. longa* had higher antimicrobial activity against *Escherichia coli, Staphylococcus aureus*, *Streptococcus pyogenes,* and *Candida albicans*. Therefore, we suggest that ZnO NPs can be used as the antimicrobial agent. It is a good scavenger of superoxide radical, nitric oxide, and hydrogen peroxide and has reducing power, which is greater than ascorbic acid at a higher concentration.

Key words: Green synthesis, Zinc oxide nanoparticles, Rhizome, *Curcuma longa,* Antibacterial, Antifungal, Antioxidant.

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INTRODUCTION

Nanotechnology represents innovation and facilitates the platform to fabricate novel nanomaterials for a wide range of biological and biomedical applications [1]. Biosynthesis or green synthesis of nanoparticles/nanomaterials is becoming increasingly popular as safer, cost-effective, easy to use, timesaving, free from toxics and pollutants, and simple without many environmental concerns. It is an alternative to the usual physical and chemical process [2,3]. Therapeutic nanomaterials for biomedical and pharmaceutical applications are being carried out by different green synthesis technologies using macro- and micro-scopic organisms (bacteria, fungi, microalgae, seaweeds, and plants) [4].

A zinc oxide nanoparticle (ZnO NP) has created a great interest due to its large bandwidth and high excitation binding energy. It possesses potential biological applications such as antimicrobial (bacterial and fungal), antioxidant, anticancer, wound healing, anti-inflammatory, and antidiabetic [5]. ZnO NP offers simple and easy fabrication and is considered biosafe and biocompatible making them ideal for biomedical applications such as biomolecular detection, nanodiagnostics, nanomedicine, luminescence, and photocatalytic photodiode response [6].

Antibacterial and antifungal activity of ZnO NPs against various pathogenic microbes among humans and plants (bacteria and fungi) such as *Bacillus subtilis*, *Salmonella* sp., *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus flavus*, and *Aspergillus niger* using well diffusion method has been reported by various researchers [7,8]. Some researchers have investigated the antioxidant activity of green synthesized ZnO NP from *Ceropegia candelabrum* L. [9], *Polygala tenuifolia* [10], *Cassia fistula* [11], and *Eucalyptus globules* [12]. *Curcuma longa* is a medicinal plant abundantly available in South India. *C. longa* (turmeric) has curcumin which is a polyphenolic pigment. It is often used as traditional medicine with a wide range of potent medicinal activities for the treatment of inflammation, asthma, wounds, and Alzheimer disease [13]. ZnO NPs were produced through Zn (II) complex formation using *C. longa* extract as reducing agent [14]. Raghad *et al*. [15] demonstrated the green synthesis of titanium dioxide NPs (TiO_2 NPs) from the aqueous extract of *C. longa* and their biological activities. Extracts of *C. longa* have been used for the synthesis of silver nanoparticles by the simple method of green synthesis [16].

The purpose of this investigation was to develop the biogenic zinc oxide nanoparticles from *C. longa* extract as stabilizing and reducing agent and an analysis of their antibacterial, antifungal and antioxidant activity at *in vitro* level.

METHODS

In this investigation, all chemicals and reagents were obtained from Sigma, Aldrich. Zinc oxide nanoparticles were synthesized from *C. longa* rhizome by an eco-friendly method which was characterized using X-ray diffraction and transmission electron microscopy. Healthy and fresh *C. longa* rhizomes were collected from the local market, Coimbatore. *C. longa* rhizomes were verified and authenticated by the Botanical Survey of India, Coimbatore, Tamil Nadu, India, and the voucher specimen was deposited for future reference at the same institute.

Antibacterial activity

An analysis of antibacterial activity was done following agar well diffusion method according to Rajasekar *et al.* [17] incorporating a few modifications. Gram-positive organisms (*Staphylococcus aureus* and *Streptococcus pyogenes)* and Gram-negative organisms (*E. coli, Salmonella typhi,* and *Klebsiella pneumonia)* were used in this investigation. The media were prepared using nutrient agar, and 100µl of overnight pure culture of the pathogenic organism (10^4 cells/ml) was spread on the plates. Five wells (5 mm) were made in each plate after a few minutes. Different concentrations of ZnO NPs (25–100 µg/ml) were poured into each well. Tetracycline having/ml was loaded in one well, and it was used as a control during the experiment. The plates were then kept for incubation at 37°C for a period of 24 h. The zone of inhibition was obtained by measuring the diameter in millimeters.

Antifungal activity

An analysis of antifungal activity was carried out by well diffusion method according to Magaldi *et al.* [18]. The fungal pathogens such as *A. niger, Aspergillus oryzae,* and *Candida albicans* were used in this assay. Potato dextrose agar (PDA) media were prepared and poured in the plates. Fungal pathogens were inoculated carefully after the solidification of PDA. Five wells (5 mm of size) were cut out on the agar plates. Various concentration of ZnO NP (25–100 µg/ml) and antifungal agent (positive control) ketoconazole (20 µg/ml) was introduced in well. The plates were incubated for 2–3 days at room temperature. After 3 days, the zone of inhibition was obtained measured in millimeter.

Analysis of antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging activity was analyzed following the method of Malterud *et al.* [19]. Varied concentrations (50–1000 µg/ml) of ZnO NP and ascorbic were prepared. 2.96 ml of 0.1 mM DPPH solution was added to 3 ml of the prepared solutions. The mixture was stirred thoroughly and was kept in the dark room for 20 min to incubate. Shimadzu UV-2450 spectrophotometer was used to read the absorbance of the reaction mixture at 517 nm. Ascorbic acid was used as a standard solution while 0.1 mM DPPH was used as a control.

Hydrogen peroxide assay

An assay of hydrogen peroxide was performed following the method of Gocer *et al.* [20]. 1 ml of ZnO NP (50–1000 µg/ml) and standard ascorbic acid were mixed with 0.6 ml, 50 mM hydrogen peroxide (phosphate buffer, pH 7.4). The mixture was incubated at room temperature for 10 min. Absorbance was detected by Shimadzu UV-2450 spectrophotometer at 230 nm.

Reducing power assay

An analysis of the reducing power of green synthesized ZnO NP was obtained using the method of Oyaizu's [21]. Varied concentration (50–1000 µg/ml) of 1ml ZnO NP and ascorbic acid was added together with 2.5 ml of 200 mM phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was kept for incubation at 50°C for 20 min and allowed to cool rapidly. 10% of 2.5 ml trichloroacetic acid was added for 10 min. The mixture was centrifuged at 3000 rpm for 10 min. The supernatant was obtained and added to 2.5 ml of de-ionized water and 1 ml of ferric chloride (0.1%). The absorbance of the mixture was detected using Shimadzu UV-2450 spectrophotometer at 700 nm.

Nitric oxide scavenging activity

Sodium nitroprusside was used for the nitric oxide generation of Griess reaction using the spectrophotometric method [22]. 100 µl of sodium nitroprusside (5 mM and pH 7.4) was added to 100 µl varied concentrations of ZnO NP and ascorbic acid (50–1000 µg/ml). The mixture was incubated at 25°C for 30 min. The preparation of control was made without test nanoparticles. 1.5 ml of the incubated solution was diluted with 1.5 ml Griess reagent (2% phosphoric acid, 1% sulfanilamide, and 0.1% N-1-naphthylethlene di-amine di-hydrochloride). The absorbance of the mixture was measured using Shimadzu UV-2450 spectrophotometer.

superoxide radical scavenging assay

The scavenging activity of superoxide radical was analyzed by the nitroblue tetrazolium reduction assay following the method [23]. 1 ml nitroblue tetrazolium solution (1 M NBT in 100 mM phosphate buffer, pH 7.4), NADH solution (1 M NADH in 100 mM phosphate buffer, pH 7.4) 1 ml of the varied concentration of samples (ZnO NP and ascorbic acid), and 0.1 ml of 50 mM phosphate buffer were added. 100 µl phenazine methosulfate solutions were also added. The absorbance of the mixture was measured using Shimadzu UV-2450 spectrophotometer at 530 nm.

Statistical analysis

Three independent tests performed under the same experimental condition were used to obtain the results as a mean±standard deviation.

RESULTS AND DISCUSSION

Characterization of zinc oxide nanoparticles

The synthesized zinc oxide nanoparticles are spherical in shape with an average size of 25 nm (Figs. 1 and 2).

Antibacterial activity

Fig. 3 shows the antibacterial activity of the synthesized ZnO NP against pathogenic bacteria. At a different concentration of samples, the distinct zone of inhibition was formed around the wells. Tetracycline used as a control to compare the zones. Significant results were observed in *E. coli, S. aureus,* and *K. pneumonia*. *S. typhi* and *S. aureus* showed less inhibition. Maximum zone of inhibition was observed in *E. coli* at a higher concentration (100 µg/ml) when compared to others. Minimum zone of inhibition was observed in *S. typhi* at 25 µg/ml concentration. *E. coli* and *S. aureus* showed a higher zone of inhibition than tetracycline (control). This result indicates that ZnO NP can be used as an antibacterial agent. ZnO NP is a less toxic and low-cost nanoparticle that can be used in many fields that include antibacterial, antifungal, antioxidant, and anticancer ones [24,25]. The antibacterial activity of ZnO NP was dependent on their capability to activate excess ROS generation such as superoxide anion, hydrogen peroxide, and hydroxyl radical generation [26]. ZnO NP antibacterial activity may associate with the accretion in bacterial cells cytoplasm and promote the release of Zn^{2+}. It leads to the fragmentation of bacterial cell membrane, damage in membrane protein and genomic weakness resulting in the death of bacteria [27-29].

Antifungal activity

Fig. 4 shows the antifungal activity of ZnO NP against the chosen pathogenic fungus. Agar well diffusion method was used for this study. In different concentration of zinc oxide, nanoparticles showed efficient antifungal activity for *C. albicans* and *A. oryzae* than *A. niger*.

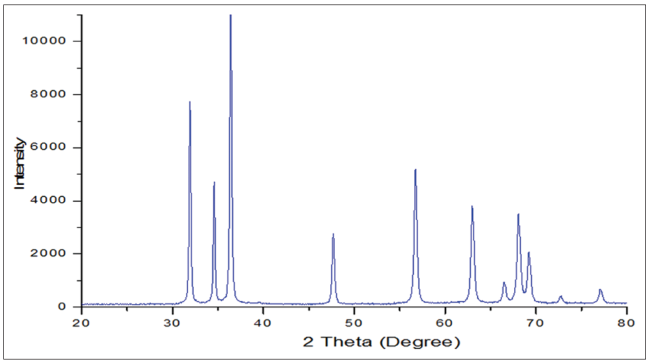

Fig. 1: X-ray powder diffraction spectra of *Curcuma longa* rhizomes mediated zinc oxide nanoparticles

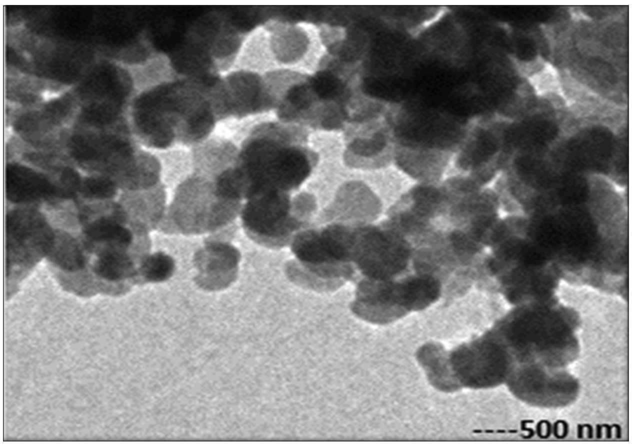

Fig. 2: Transmission electron microscopy images of *Curcuma longa* rhizomes mediated zinc oxide nanoparticles

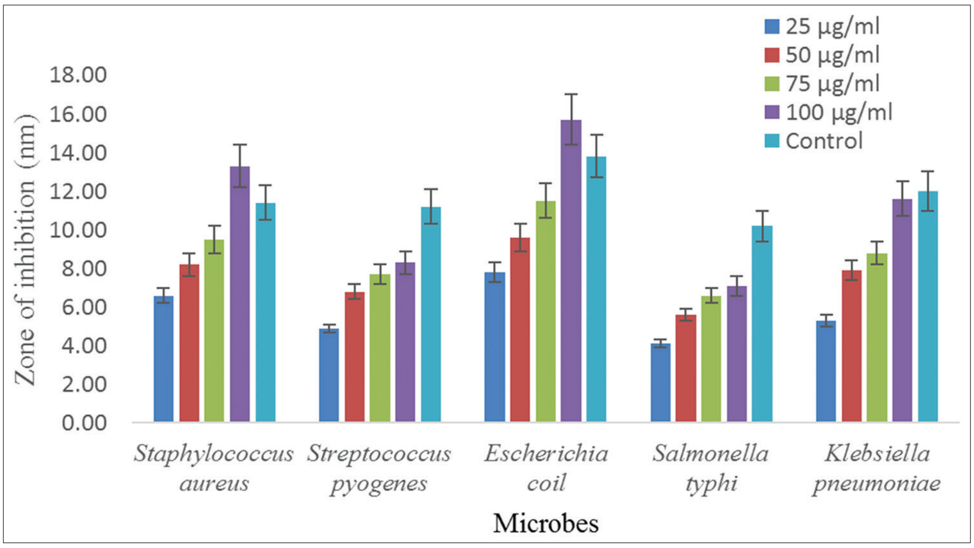

Fig. 3: Antibacterial activity of *Curcuma longa* mediated zinc oxide nanoparticles. Data represent mean±standard error

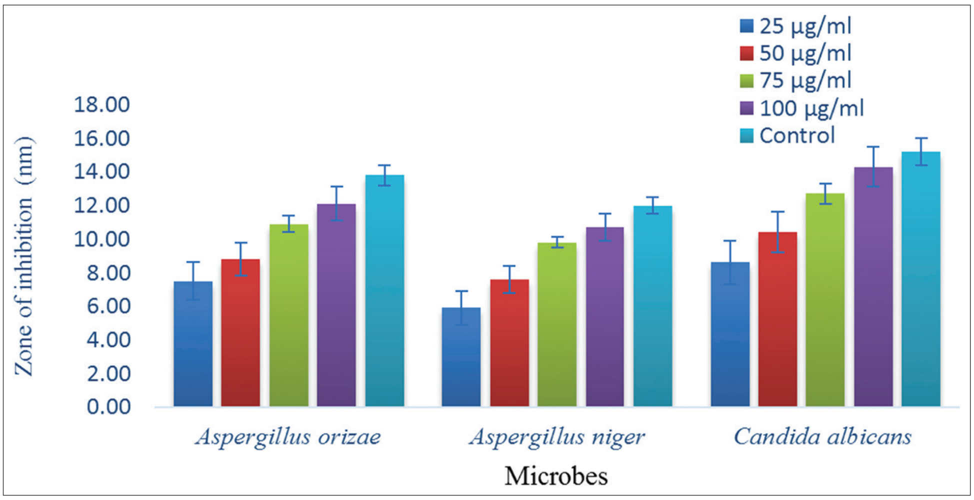

Fig. 4: Antifungal activity of *Curcuma longa* mediated zinc oxide nanoparticles. Data represent mean±standard error

At 100 µg/ml concentration, *C. albicans* showed the highest zone of inhibition, which was higher than control. The lowest inhibition was observed at 25 µg/ml concentration against *A. niger*. Some commercial antifungal agents create side effects [30,31]. This problem leads to the development of a natural antifungal agent [32]. This study suggests that ZnO NP can be the best naturally available antifungal agent.

Antioxidant activity

Fig. 5 shows DPPH radical scavenging activity of ZnO NP. By getting hydrogen or electron from donor atom 1, 1- diphenyl-2- picrylhydrazyl free radical is reduced [33]. The odd electron of DPPH accepts the hydrogen atom from the antioxidants and changes to identical hydrazine [34]. DPPH is an easy and fast way to evaluate antioxidant property using spectrophotometer [35]. According to the dose, the radical scavenging activity of ZnO NP was increased. A significant result was observed at 200 µg/ml, which is a bit less than ascorbic acid. Similar result revealed that nanoparticles could increase the antioxidant property [36]. Lipid peroxidation and cyclooxygenase activity were inhibited by curcumin, which had higher antioxidant activity [37].

ZnO NP hydroxyl radical scavenging activity is shown in Fig. 6. The ZnO NP activity was increased based on the concentration. Higher activity was observed at IC_{200} - 200 µg/ml of ZnO NP than ascorbic acid. The maximum level of hydrogen peroxide caused oxidation in protein, nucleic acid, and lipids of cells which led to mutagenesis and lethal condition to cells. The cells could be protected by removing H_2O_2 using catalase [38]. Naturally synthesized ZnO NP was good hydroxyl radical scavengers. Phenolic was an essential compound for antioxidant property [39] that was present in ZnO NP.

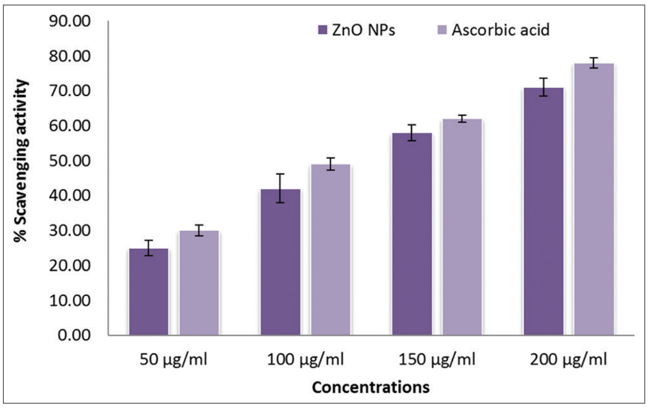

Fig. 5: 1,1-diphenyl-2-picryl hydrazyl scavenging activity of *Curcuma longa* mediated zinc oxide nanoparticles. Data represent mean±standard error

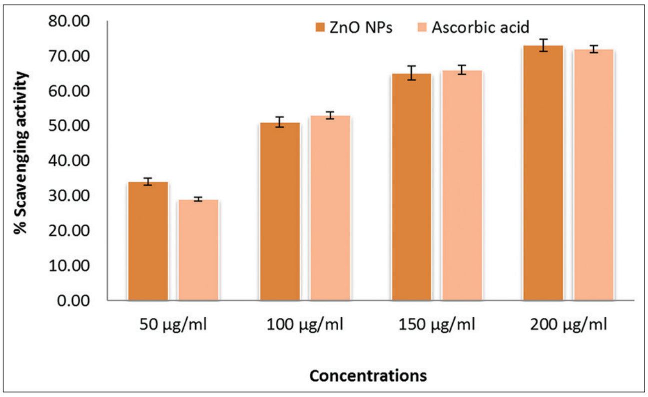

Fig. 6: Hydrogen peroxide scavenging activity of *Curcuma longa* mediated Zinc oxide nanoparticles. Data represent mean±standard error

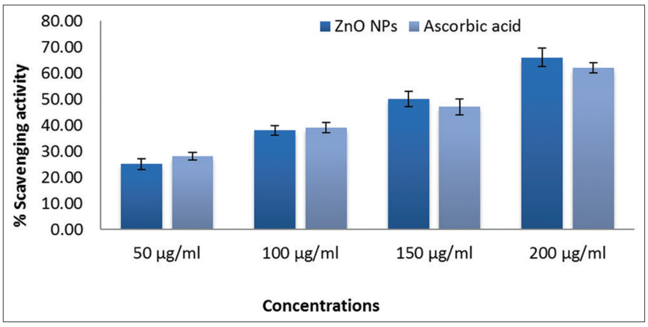

Fig. 7: Reducing power assay of *Curcuma longa* mediated zinc oxide nanoparticles. Data represent mean±standard error

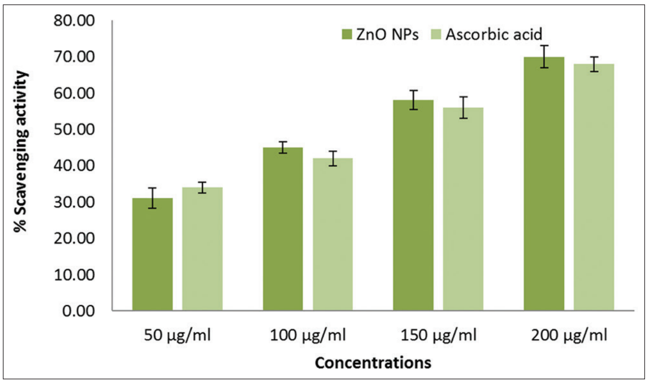

Fig. 8: Nitric oxide scavenging activity of *Curcuma longa* mediated zinc oxide nanoparticles. Data represent mean±standard error

The reducing power of a compound (Fig. 7) is linked to the antioxidant activity [40]. The reducing power of zinc oxide nanoparticles increased in a dose-dependent manner. The value ZnO NP was 200 µg/ml, and it showed nearly the same efficacy as the standard ascorbic acid. This implied that ZnO NP had significant ability to react with free radicals to make them stable. The scavenging activity (Fig. 8) of nitric oxide was determined by the ability to inhibit the formation of nitrite by direct competition with oxygen and oxides [41]. ZnO NP showed more nitric oxide scavenging activity of 200 µg/ml compared to ascorbic acid. Superoxide radical scavenging ability (Fig. 9) was determined by spectrophotometer. ZnO NP showed increased scavenging activity of 200 µg/ml compared to ascorbic acid. Superoxides were dangerous to the body cell as they had the ability to oxidize DNA and protein [41].

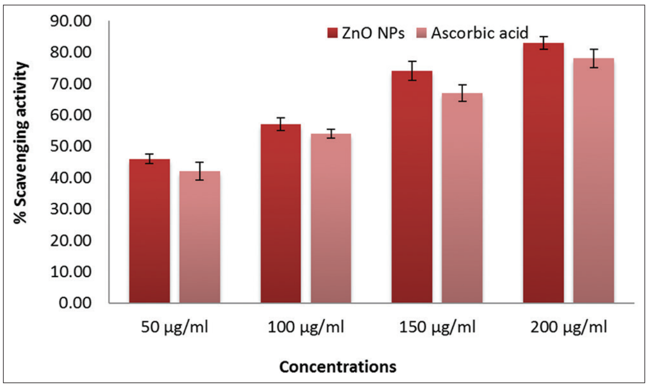

Fig. 9: Superoxide radical scavenging activity of *Curcuma longa* mediated zinc oxide nanoparticles. Data represent mean±standard error

CONCLUSION

It can be concluded that ZnO NP synthesized from *C. longa* possesses strong antibacterial, antifungal, and antioxidant activity based on the above *in vitro* analysis. In higher concentrations, it has significant antimicrobial activity against pathogenic bacteria and fungi. It is also a good source of antioxidant property.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to this manuscript.

CONFLICTS OF INTEREST

No conflicts of interest.

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