Research Article

Antimicrobial Activity of Green Synthesized Zinc Oxide Nanoparticles from *Emblica Officinalis*

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Accepted on: 07-06-2015; **Finalized on:** 31-07-2015.

ABSTRACT

The present investigation was aim to study the antimicrobial potential of methanol extract and zinc oxide nanoparticles synthesized from *Emblica officinalis (E. officinalis).* The antimicrobial activity was performed by agar well diffusion method against six bacterial pathogens namely, *Bacillus subtilis, Streptococcus pneumonia, Staphylococcus epidermidis, Klebsiella pneumonia, Salmonella typhi* and *Escherichia coli,* and two fungal pathogens such as, *Aspergillus niger* and *candida albicans*. The minimal inhibitory concentration (MIC) was evaluated by micro-broth dilution method against selected pathogens and also minimum bactericidal concentration (MBC) reported. The results showed both methanol extract and zinc oxide nanoparticles harbor significant antimicrobial activity on most of the tested organisms. Highest antimicrobial activity was observed in zinc oxide nanoparticles against *S. epidermidis* (35 mm) with minimal MIC and MBC values (7.81 µg/ml) followed by *E. coli*. Both methanol extract and zinc oxide nanoparticles were showed nil activity on fungal pathogens. The present results concluded that green synthesized zinc oxide nanoparticles from *E. officinalis* may be severed as an effective antibacterial agent in traditional medicine.

Keywords: *Emblica officinalis,* zinc oxide nanoparticles, agar well diffusion, MIC, MBC.

INTRODUCTION

Infectious diseases are major causes of morbidity and mortality in worldwide. Nowadays, several pathogenic microorganisms have been developed resistance to currently available commercial antibiotics and also cause adverse impact on health[1].

Hence, there is an urgent need to discover an alternative new, broad spectrum, more active and safer antimicrobial agents. Recent attention has been paid to the use of nanoparticles of metal oxide to stop infectious diseases due to the antimicrobial properties of these nanoparticles including sliver[2,3], copper[4], titanium dioxide[5], zinc oxide[6].

Among these metal oxide nanoparticles, zinc oxide is interesting because it has vast applications in the field of biotechnology, sensors, medical diagnostic, catalysis, high performance engineering material, magnetic recording media, optical devices, diagnostic, catalysis, DNA labeling and drug delivery[7].

The advantage of using zinc oxide as antimicrobial agents is that they contain mineral elements essential to humans and more potential even at low concentrations[8].

Synthesis of zinc oxide nanoparticles can be performed using a number of routinely used chemical and physical methods. However, green synthesis of zinc oxide nanoparticles is an interesting issue of the nanoscience and nanobiotechnology[9].

Nanoparticles produced by plants are more stable, and the rate of synthesis is faster.

Moreover, the nanoparticles are more various in shape and size in comparison with those produced by other methods[10]. The toxicological and environmental hazard of phytochemicals mediated synthesized nano materials are also low[11]. Phytochemical paved nano materials are expected to act as better antimicrobial agents than synthetic and natural compounds due to their structural compatibility and interactions with surrounding environment. Several earlier reports revealed that green synthesized ZnO nanoparticles exhibited strong antimicrobial activities on pathogenic microbes[12-15] of ZnO, MgO and CaO nanoparticles were already reported.

Emblica officinalis used in various traditional medicinal system like, Ayurvedha, Tibetan and Siddha to treat various ailments[16]. *E. officinalis* exhibits several important biological activity such as, antimicrobial,[17-19] anti-inflammatory,[20-22] antidiabetic activity,[23] antiproliferative activity on MCF7 and MDA-MB-231 breast cancer cell lines,[24] antitussive activity,[25] anticancer[26,27] and induces apoptosis in Dalton's Lymphoma Ascites and CeHa cell lines[28]. The present investigation was aimed to evaluate antimicrobial potential of fruit methanol extract and green synthesized zinc oxide nanoparticles from fruit methanol extract of *E. officinalis.*

MATERIALS AND METHODS

Preparation of Extract

Fresh fruit of *Emblica officinalis* were collected from Periyamanali, Namakkal district, Tamil Nadu, India.

The plant material was authenticated by Botanical Survey of India (BSI) (reference number: BSI/SRC/5/23/2013-14/Tech/2085), Coimbatore, Tamil Nadu, India. Fruits were washed with distilled water, sliced, deseeded, shed dried for 15 days and powdered. The powdered plant

material was extracted with methanol (in 1: 5 ratio) for 72 hours in a soxhlet apparatus. The solvent was evaporated under reduced pressure (40°C) in vacuum.

The extract was stored in an air tight container for further study.

Preparation of Nanoparticles

Fruit methanol extract of *E. officinalis* (25%) was treated with 0.1 M of zinc nitrate at 80°C (pH 6.5) for 2 hours resulted the synthesis of zinc nanoparticles (unpublished data).

Antimicrobial Activity

Test Microorganisms

Clinically isolated six bacterial strains such as *B. subtilis, S. pneumonia, S. epidermidis, K. pneumonia, S. typhi* and *E. coli* and two fungal strains namely, *A. niger* and *C. albicans* were collected from clinical laboratories in and around Salem district, Tamil Nadu, India.

Test cultures were prepared by inoculating a loop full of mother culture into the test tubes containing 5ml of broth (Muller Hinton broth for bacteria and Sabourd Dextrose broth for fungal cultures), which were incubated at appropriate time and temperatures (37°C for 24 hours for bacterial cultures and 28°C for 72 hours for fungus.

Agar Well Diffusion Assay

Antimicrobial activity was determined by agar well diffusion method as described by Srinivasan[29] with minor modifications.

Test microbial suspension culture (20 µl) was spread on per-molten media (MHA for bacteria and SDA for fungus) using a sterile cotton swab.

Six wells were made in each seeded plates using sterile cork borer (5mm diameter). Different concentrations (10µg, 20µg, 40µg, 60µg and 80µg) of methanol extract and zinc oxide nanoparticles was separately introduced into wells and allowed to diffuse at room temperature.

50 µl of DMSO was served as negative control and 25 µl of standard antibiotics solution like ciprofloxacin (for bacteria) and fluconazole (for fungus) was used as positive control (µg/µl).

The bacterial and fungal plates were incubated at 37°C for 24 hours and at room temperature for 72 hours, respectively. After the incubation period, the clear zone of growth inhibition diameter was recorded in mm.

Minimum Inhibitory Concentration (MIC)

The minimal inhibitory concentration was determined by micro broth dilution method in 96 well plates[30].

Based on the antimicrobial (agar well diffusion method) results in which organism shows high sensitive to the methanol extract and zinc oxide nanoparticles were chosen for MIC test.

100µl of different concentrations (0.976 to 1000 µg/ml) of extract/nanoparticles and 20 µl of bacterial suspension were added to the wells containing 200 µl of Muller Hinton broth.

After 24 hours of incubation period, 40µl of *p*-iodonitro tetrazolium choloride (0.2 mg/ml) was added to the wells which serve as an indicator for bacterial growth again incubated for 1 hour.

The MIC was recorded as the lowest concentration of the extract/nanoparticles shows nil growth of microorganism.

Minimum Bactericidal Concentration (MBC)

About 10µl of MIC test solution which showed no color change was sub cultured on freshly prepared MHA plates and incubated at 37°C for 24 hours.

The lowest concentration in which has no single colony bacterial growth was taken as MBC[31].

Statistical Analysis

All the experiments were performed in triplicates and results were expressed mean ± standard deviation.

One way ANOVA (in a completely randomized design) and Tukey's multiple tests ($P < 0.05$) were used to compare any significant differences between samples.

RESULTS AND DISCUSSION

The antimicrobial activity results of methanol extract and zinc oxide nanoparticles of *E. officinalis* show broad spectrum antibacterial activity in the range between 7 and 35 mm (Tables 1 and 2).

Both methanol extract and zinc oxide nanoparticles were showed concentration dependent growth of inhibition on all tested microbes.

However, highest zone of growth inhibition was observed in nanoparticles against *S. epidermidis* (32 mm) followed by *E. coli* (31 mm).

Methanol extract expressed significant high antibacterial potential against *S. epidermidis* (29 mm) followed by *B. subtilis* (18 mm).

The MIC and MBC results (Table 3) revealed that the methanol extract and zinc oxide nanoparticles of *E. officinalis* significantly inhibits the growth of selected bacterial pathogens.

The lowest MIC and MBC value was noticed in zinc oxide nanoparticles against *S. epidermidis* (7.81 µg/ml) followed by *E.coli.*

Methanol extract expressed minimal MIC and MBC value against *S. epidermidis* (125µg/ml). Methanol extract and zinc oxide nanoparticles did not inhibit fungal pathogens growth at all tested concentrations.

Table 1: Antimicrobial activity of methanol extracts of *E. officinalis*

Concentration (µg)	Diameter of zone of inhibition (in mm)*							
	B. subtilis	***S. pneumoniae***	***S. epidermidis***	***K. pneumoniae***	***S. typhi***	***E. coli***	***A. niger***	***C. albicans***
10	11.00 ± 2.00^{a}	00.0 ± 0.000^{a}	18.67 ± 1.52^{a}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}
20	$15.00 \pm 1.00^{a,b}$	08.33 ± 1.53^{b}	$22.67 \pm 1.52^{a,b}$	07.66 ± 1.52^{b}	9.66 ± 1.52^{b}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}
40	$15.67 \pm 1.52^{a,b}$	10.33 ± 1.53^{b}	$25.33 \pm 1.52^{b,c}$	11.67 ± 1.52^{c}	$11.67 \pm 1.5^{b,c}$	10.0 ± 2.00^{b}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}
80	18.00 ± 2.00^{b}	16.33 ± 1.53^{c}	29.33 ± 1.52^{c}	15.67 ± 1.52^{d}	$15.67 \pm 1.5^{c,d}$	17.0 ± 2.00^{c}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}
Control[#]	23.00 ± 2.00^{c}	22.00 ± 2.0^{d}	35.33 ± 1.52^{d}	19.67 ± 1.52^{e}	19.67 ± 1.50^{d}	35.66 ± 1.52^{d}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}

#-Ciprofloxacin (25 µg) for bacteria and fluconazole (25 µg) for fungus; *-The values are presented as mean ± standard deviation (n=3). Different superscript letters (a–e) in column indicates significant differences ($P < 0.05$) when subject to Tukey's multiple comparison test.

Table 2: Antimicrobial activity of zinc nanoparticles of *E. officinalis*

Concentration (µg)	Diameter of zone of inhibition (in mm)*							
	B. subtilis	***S. pneumoniae***	***S. epidermidis***	***K. pneumonia***	***S. typhi***	***E. coli***	***A. niger***	***C. albicans***
10	13.00 ± 2.00^{a}	08.33 ± 1.53^{a}	25.33 ± 1.52^{a}	09.60 ± 1.52^{a}	09.33 ± 1.52^{a}	14.66 ± 1.52^{a}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}
20	$16.33 \pm 1.52^{a,b}$	11.00 ± 2.00^{a}	$28.33 \pm 1.52^{a,b}$	11.00 ± 2.00^{a}	11.00 ± 2.00^{a}	20.33 ± 1.52^{b}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}
40	$18.0 \pm 2.0^{a,b,c}$	$13.00 \pm 2.0^{a,b}$	30.33 ± 1.52^{b}	$13.00 \pm 2.0^{a,b}$	$13.33 \pm 1.52^{a,b}$	28.33 ± 1.52^{c}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}
80	$21.0 \pm 2.00^{b,c}$	16.67 ± 1.53^{b}	$32.00 \pm 2.00^{b,c}$	$16.00 \pm 2.0^{b,c}$	$17.67 \pm 1.52^{b,c}$	$31.66 \pm 1.52^{c,d}$	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}
Control[#]	22.67 ± 2.51^{c}	22.33 ± 2.08^{c}	35.00 ± 2.00^{c}	19.00 ± 1.00^{c}	20.33 ± 1.52^{c}	35.00 ± 2.00^{d}	00.0 ± 0.00^{a}	00.0 ± 0.00^{a}

#-Ciprofloxacin (25 µg) for bacteria and fluconazole (25 µg) for fungus; *-The values are presented as mean ± standard deviation (n=3). Different superscript letters (a–e) in column indicates significant differences ($P < 0.05$) when subject to Tukey's multiple comparison test.

Table 3: MIC and MBC (µg/ml) for methanol extract and nanoparticles of *E. officinalis* against selected pathogens

Microorganisms	Methanol		Nanoparticles		Ciprofloxacin	
	MIC	MBC	MIC	MBC	MIC	MBC
B. subtilis	250[a]	500[a]	62.5[b]	250[b]	7.81[c]	15.62[c]
S. epidermidis	125[a]	125[a]	7.81[b]	7.81[b]	1.95[c]	3.9[c]
E. coli	>1000[a]	>1000[a]	31.25[b]	62.5[b]	3.9[c]	3.9[c]

The values are mean of triplicates. Different superscript letters (a–c) in rows indicate significant differences (at $P < 0.05$) when subject to Tukey's multiple comparison test.

Nowadays green medicines are gaining great interest in health compared with synthetic drugs because of high potential and safety. Several plant extracts and phytochemicals have been served as a source of antimicrobial agents[32]. Previous reports on antibacterial activity of various extracts of different parts of *E. officinalis* strengthen the present findings[33-37]. Earlier studies have shown that ethanol extract of *E. officinalis* harbor promising antimicrobial activity which strengthens the present outcome[38]. Several reports revealed that methanol is potent solvent for extracting variety of important phytochemicals, like alkaloids, phenols, tannins, fatty acids, and flavonoids, which harbor antimicrobial potential which support the findings of present study[39]. Both methanol extract and zinc oxide nanoparticles failed to show antifungal activity it's may be due to the resistance of the tested fungal pathogens[29].

Zinc oxide nanoparticles expressed superior antibacterial activity than methanol extract this may be due to the synergistic effect of the Zinc oxide nanoparticles with phytochemicals which supported by several previous studies[40,41]. Green synthesized zinc oxide nanoparticles showed greater antimicrobial potential when compared with chemically synthesized zinc oxide nanoparticles[40].

From the above results green synthesized zinc oxide nanoparticles showed highest activity it was explained that, metal oxides are produce ions it bind to the cell wall of host cells through surface proteins and enter into the cell then changed their metabolism and leads to cell death[42]. Clearly, zinc is an essential process for cellular process and act as nutrient[12]. Zinc oxide produced hydrogen peroxide during generation of oxygen species on its surface and after several process it cause damage to microorganisms[43].

The process of hydrogen peroxide formation is followed as; zinc oxide can be activated by UV and visible light and then electron hole will be formed. In that electron hole water molecules were splited. Dissolved oxygen from the water molecule, transformed to superoxide radical anions (O_2), it cannot enter into the cell membrane because it has been a negatively charged. Then superoxide anions react with H^+ to generate HO_2 radicals which then produce HO_2^- and then react with H+ ions to form hydrogen peroxide it is a strong oxidizing agent and enter into the cell membrane leads to cell death[44,14]. After death of bacteria in the medium, zinc oxide nanoparticles cannot stop the hydrogen peroxide production because bacteria are completely covered by zinc oxide nanoparticles, so that it showed high bactericidal activity. Finally these results suggested that zinc oxide nanoparticles showed best activity compared to the plant fruit extract of *Emblica officinalis.* So it may used in the control of microorganisms and may also used in drug delivery in the form coating nanoparticles against micro organisms.

CONCLUSION

The findings of present study was explored that green synthesized zinc oxide nanoparticles from fruit methanol extract of *E. officinalis* harbor potent antibacterial activity. This is first time report on antimicrobial activity of zinc oxide nanoparticles from *E. officinalis*.

Acknowledgement: The authors are gratefully thankful to the Periyar University, Salem and Dr. T. Selvenkumar, Head, Department of Biotechnology, Mahendra Arts and Sciences College, Kallipatti, Namakkal for providing the necessary facilities.

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Source of Support: Nil, **Conflict of Interest:** None.