EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 10
304-308
Year: 2017

ANTIMICROBIAL POTENTIAL OF ZINC OXIDE NANOPARTICLES SYNTHESIZED FROM BROWN SEAWEED (*TURBINARIA CONOIDES*)

Khoushika Raajshree R. and Brindha Durairaj*

Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India.

***Corresponding Author: Brindha Durairaj**
Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India.

Article Received on 27/07/2017 **Article Revised on 16/08/2017** **Article Accepted on 05/09/2017**

ABSTRACT
Green synthesis of metal nanoparticles is a novel method in the production of eco-friendly nanoparticles. In the present investigation, zinc oxide nanoparticles (ZnO-NP's) are produced by zinc acetate utilizing the biocomponents of crude extract of *Turbinaria conoides*. Formation of ZnO-NP's has been confirmed by UV-Vis absorption spectroscopy and Scanning Electron Microscope with the Energy Dispersive X-ray studies (EDX). The antibacterial activity of Zinc oxide nanoparticles and the crude algal extract against gram positive (*Staphylococcus aureus* and *Bacillus subtilis*), gram negative (*Escherichia coli* and *Pseudomonas aeroginosa*) organisms and the antifungal activity against *Candida albicans* and *Fusarium oxysporum* were evaluated. Minimum inhibitory concentration (MIC) was also measured. The results revealed that the synthesized ZnO-NP possesses antimicrobial activity against the tested strains and MIC values showed maximum inhibition at lowest concentration.

KEYWORDS: *Turbinaria conoides*, MIC values, antifungal, antibacterial.

INTRODUCTION
Nanotechnology is emerging as a rapidly growing discipline with its application in Science and Technology for the purpose of manufacturing new materials at the nanoscale level. It is one of the most promising areas of research in modern medical science. Synthesis of metal nanoparticles attract an increasing interest due to their novel characteristics as compared with those of macroscopic phase and they find attractive applications in different fields such as medicine, biotechnology, optics, cosmetics, health care and food industries.[1] In recent years a number of physical, chemical and biological methods were used to synthesise zinc oxide nanoparticles for effective antimicrobial activity. The biological methods have an advantage over chemical and physical methods, as it is cost effective and eco-friendly.[2] Therefore, there is a need for biological approach to synthesize nanoparticles.[3]

Antimicrobial effects of zinc oxide nanoparticles depend on the size and the shape of the particle.[4] Nanoparticles can act as antibacterial and antifungal agents, due to their ability to interact with microorganisms. It has been established that different sources affect the production of nanoparticles in different ways due to the presence of specific complex phytochemicals.[5]

Algae are the major source of the marine organism with a lot of applications in various fields. Among the marine sources, the seaweeds occupy a major place as a source of biomedical compounds. The compounds derived from brown seaweeds are reported to have a broad range of biological activities such as antibacterial, antifungal, anticoagulant and antifouling effects.[6] In the present study, the marine brown seaweed (*Turbinaria conoides*) were used for the green synthesis of zinc oxide nanoparticles and were evaluated for its antimicrobial effect against various strains of bacteria and fungi.

MATERIALS AND METHODS
Collection and Preparation of Seaweed
The brown seaweed, *Turbinaria conoides*, was collected from Mandapam coastal region, Gulf of Mannar, Southeast coast of India. The algal samples were washed thoroughly with running tap water followed by distilled water to remove adhering salts and associated biota. The washed samples were dried under shade at room temperature for a week. The dried materials were ground to fine powder using mixer grinder and stored in an airtight container for further analysis.

Preparation of algal extract
The crude algal extract (CAE) was prepared by adding 10 g of algal powder into 100 ml of 50% ethanol and kept in the rotatory shaker for 24 hours. Filtered, collected the solvent and was used for further analysis.

Green Synthesis of Zinc Oxide Nanoparticles from Crude Algal Extract

20 ml of the crude algal extract was heated at 50˚C for 10 min and 50 ml of 91 mM of zinc acetate solution (1 g of zinc acetate was dissolved in 50 ml of distilled water) was added drop wise. This was then placed on a magnetic stirrer for 2 hrs. Then the precipitate was collected by centrifugation at 16,000 rpm for 10 min at 4˚C. The pale white precipitate was then taken out and washed with distilled water followed by ethanol to get free of the impurities. The zinc oxide nanoparticles were obtained after drying at 60˚C in an oven overnight and the sample was stored for further studies.

Characterization of Zinc Oxide Nanoparticles

The obtained zinc oxide nanoparticles were measured for its maximum absorbance using UV-Vis spectrophotometry. The optical property of zinc oxide nanoparticles was determined via ultraviolet and visible absorption spectroscopy in the range of 280 – 420 nm. External morphology i.e. the shape of the nanoparticles was characterized by Scanning Electron Microscope (SEM). Elemental analysis was obtained from energy dispersive X-ray diffraction (EDX), which was attached with SEM.

Antimicrobial activity

The antimicrobial activity of the synthesized ZnO-NP and the crude algal extract was evaluated against different strains of bacteria and fungi.

Culture and maintenance of microorganisms

Pure cultures of all experimental bacteria and fungi were obtained from the Department of Microbiology, PSG College of Arts & Science, Coimbatore. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Each bacterial and fungal culture was further maintained by sub culturing regularly on the same medium and stored at 4°C for use in experiments.

Preparation of inoculum

The antibacterial assay was carried out by the microdilution method.[7] The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 × 107 CFU/ml. The inoculum was prepared and stored at 4°C until use. For the fungal inoculum preparation, spore suspension was prepared according to the procedure of Murugan *et al.*,[8] as modified. Briefly, 1 cm^2 of seven-day old spore producing cultures was dropped in sterile distilled water and vortexed for 30 s to release the fungal spores. The spore density of each fungus was adjusted with a spectrophotometer (A_{595}nm) to obtain a final concentration of approximately 10^5 spores/ml. For the *Candida* spp., the inoculum was prepared by adding 1 ml of overnight *Candida* culture to 9 ml of sabouraud dextrose broth to yield 10^4 colony forming units (CFU) per microliter of the inoculum.

Determination of antimicrobial activity using well diffusion method

In vitro antimicrobial activity of the synthesized ZnO-NP and the crude algal extract was studied against pathogenic microbial strains by the agar well diffusion method.[9] The plates were swabbed (sterile cotton swabs) with an inoculum of respective bacteria and fungi. Wells (8mm diameter and about 2 cm apart) were made in each of these plates using sterile cork borer. The wells were filled with 50 µl of samples prepared with 10% DMSO at 200 µg/ml concentration. A well with 10% DMSO served as control and 30 µl of gentamycin and nystatin at 10mg/ml concentration served as positive control for bacteria and fungi respectively. The plates of bacteria and fungi were incubated overnight at 37°C and 28°C for 24 h and 48 h respectively. The development of inhibition zone around the sample loaded well was recorded.

Determination of minimum inhibitory concentration

The micro broth dilution test was performed to determine minimum inhibitory concentration (MIC) using the procedure as described by Buatong *et al.*[10] 100 µl of Mueller-Hinton broth for bacteria and Sabouraud dextrose for fungi were added to each well of a microtiter plate. Then 100 µl aliquots of the stock solution of the samples (200 µg/ml in 10% DMSO) were added and subsequently 2-fold serially diluted with broth. 20 µl of inoculum was added to each well and incubated for 24 h (37°C) for bacteria and 48 h (28°C) for fungi. The final concentrations of the samples ranged from 100 to 0.049 µg/ml. The negative control was also performed using 10% DMSO. The broth with the microbial strain was used as a positive control. Antimicrobial activity was detected by adding 20 µl of 0.5% TTC (triphenyl tetrazolium chloride, Merck) in aqueous solution.

RESULTS AND DISCUSSIONS

Biosynthesis of ZnO-NPs using Brown seaweed

Zinc oxide nanoparticles (ZnO-NP's) were synthesized from the crude algal extract of *Turbinaria conoides* by green synthesis, which is more reliable and less toxic when compared with chemical synthesis. The formation of pale white colour within 3 hours of preparation indicated the synthesis of ZnO nanoparticles.

UV-Visible Spectral Analysis

The optical absorption spectra of ZnO-NPs were recorded using UV/VIS 3000+ Double Beam UV-Visible Ratio-Recording Scanning Spectrophotometer from Lab India (SKU: 174-0020) with dimensions of (W × D × H)/Weight = 540 × 440 × 390 mm/36kg. The spectral bandwidth of Spectrophotometer is 0.5, 1, 2, 5 nm and wavelength is in the range of 190 to 1100 nm. Figure 1 shows the UV-Visible absorption spectrum of ZnO-NPs.

The absorption spectrum was recorded for the sample in the range of 280 - 420 nm. The spectrum showed the absorbance peak at 360 nm corresponding to the

characteristic band of ZnO-NPs. The UV-Visible spectrum showed the absorbance peak at 340 nm corresponding to the characteristic band of zinc oxide nanoparticles.[11]

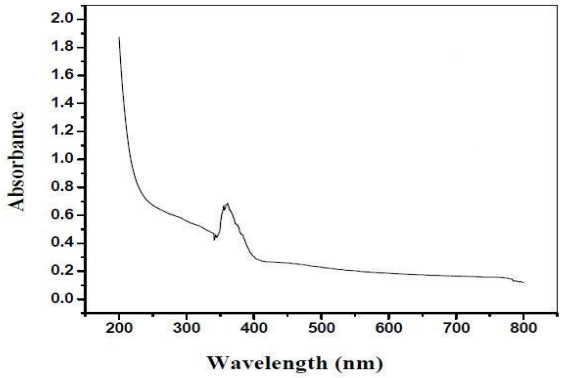

Figure 1: UV-Visible spectrum of synthesized ZnO-NPs.

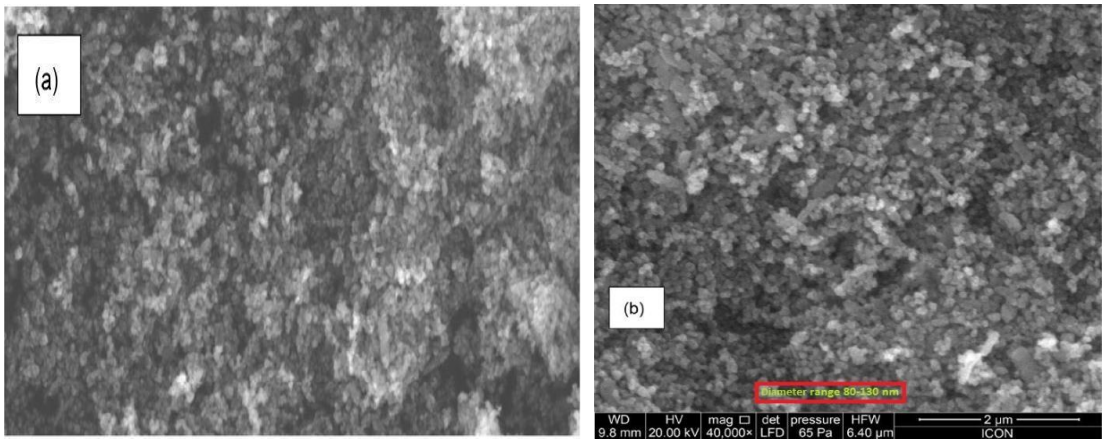

Figure 2[a] & [b]: SEM image of the synthesized zinc oxide nanoparticles.

Energy Dispersive X-Ray Diffractive (EDX) Analysis

The Energy Dispersive X-ray Diffractive (EDX) study was carried out for the synthesized zinc oxide nanoparticles to elucidate the elemental composition. EDX confirms the presence of zinc and oxygen signals of zinc oxide nanoparticle as depicted in Figure 3.

The results revealed that the peaks correspond to the optical absorption of the produced nanoparticle. The elemental analysis of the nanoparticle yielded 77.32% zinc and 22.68% oxygen which proved that the produced nanoparticle is in its highest purified form.

The observed results are in good rapport with the SEM-EDX analysis of ZnO nanoparticles synthesized using *Spathodea campanulata*.[12]

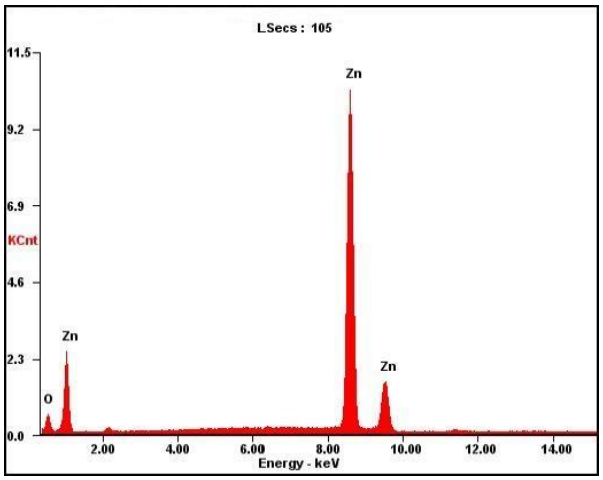

Figure 3: EDX spectrum of synthesized zinc oxide nanoparticles.

Antimicrobial activity

The antibacterial activity of ZnO-NP and the algal extract against gram positive (*S. aureus* and *B. subtilis*) and gram negative (*E.coli* and *P.* aeroginosa) organisms in terms of zone of inhibition (Table 1).

Table 1: Antibacterial activity of ZnO-NP and the algal extract

Strain	Zone of inhibition (diameter in mm)			
	ZnO-NP	Algal extract	Gentamycin (30µg/ml)	10% DMSO
Staphylococcus aureus	19	9	25	-
Bacillus subtilis	17	8	23	-
Escherichia coli	16	8	22	-
Pseudomonas aeroginosa	21	16	26	-

S. aureus was found to be more sensitive to ZnO-NP with the maximum zone of inhibition (19 mm) while it was less sensitive (9 mm) to the algal extract. The zone of inhibition of ZnO-NP and the algal extract against *B. subtilis* was found to be 17 mm and 8 mm respectively. The standard antibiotic gentamycin exerted a zone of inhibition which was found to be 25 mm for *S. aureus* and 23 mm for *B. subtilis*.

Among the gram negative strains tested, *P. aeroginosa* was found to be more sensitive to ZnO-NP at 200 µg/ml concentration with the maximum zone of inhibition (21 mm) while it was less sensitive (16 mm) to the algal extract with the same concentration. The zone of inhibition of ZnO-NP and the algal extract against *E.coli* was found to be 16 mm and 8 mm respectively. Gentamycin, the standard antibiotic exerted a zone of inhibition of 26 mm for *P. aeroginosa* and 22 mm for *E.coli*. From the above results, it was revealed that ZnO-NP exhibited maximum antibacterial effect when compared to that of algal extract. Photosynthesized silver nanoparticles using *Solanum seaforthianum* exhibited antibacterial activity against the bacterial strains.[13]

The antifungal activity of ZnO-NP and the algal extract was tested against *Candida albicans* and *Fusarium oxysporum* organisms in terms of zone of inhibition (Table 2).

Table 2: Antifungal activity of ZnO-NP and the algal extract

Strain	Zone of inhibition (diameter in mm)			
	ZnO-NP	Algal extract	Nystatin (30µg/ml)	10% DMSO
Candida albicans	18	12	21	-
Fusarium oxysporum	12	8	18	-

From the table 2, it was evident that *C. albicans* was found to be more sensitive to ZnO-NP with the maximum zone of inhibition (18 mm) while it was less sensitive (12 mm) to the algal extract. The zone of inhibition of ZnO-NP and the algal extract against *F. oxysporum* was found to be 12 mm and 8 mm respectively. The standard antifungal drug nystatin exerted a zone of inhibition of 21 mm for *C. albicans* and 18 mm for *F. oxysporum*. From the above results, ZnO-NP exerted maximum antifungal activity when compared to the algal extract. Our results are in good accordance with the work done by *Nisha et al.,*[14] *and* Hafez *et al.,*[15] who reported the antifungal activity of silver nanoparticles synthesized from leaf extracts.

The MIC values of ZnO-NP against various microbial strains were presented in Table 3. The MIC values of ZnO-NP against the bacterial and fungal strains ranged between 12.5-25 µg/ml and 25 to 50 µg/ml respectively. Our study is in good agreement with the work of Hedaginal and Taranath, 2016 who reported that the silver nanoparticles synthesized from *Solanum seaforthianum* showed minimum MIC values and exhibited antibacterial activity. The silver nanoparticles synthesized from *Sida acuta* leaf extract[14] and *Morus nigra* leaf extract[15] showed minimal inhibitory concentration value and possessed antifungal activity against *C. albicans and F. oxysporum.*

Table 3: Minimum Inhibitory Concentration of ZnO-NP against microbial strains.

S.No	Strain	MIC (µg/ml)
1.	*Staphylococcus aureus*	12.5
2.	*Bacillus subtilis*	25
3.	*Escherichia coli*	25
4.	*Pseudomonas aeroginosa*	12.5
5.	*Candida albicans*	25
6.	*Fusarium oxysporum*	50

CONCLUSION

The development of reliable and eco-friendly process for the synthesis of metallic nanoparticles is of great importance in the field of nanotechnology. In the present study, we have proposed a green approach to synthesize ZnO-NP using marine brown seaweed *Turbinaria conoides* as a reducing mediator and the synthesized nanoparticles exhibited broad-spectrum biocidal action towards the microbial strains. A minimum MIC value exhibited by ZnO-NP against the strains is of great significance for application in bionanomedicine, biosensors and food industries.

ACKNOWLEDGMENT

Authors wish to acknowledge the management of PSG College of Arts and Science, Tamil Nadu, India, for providing the necessary laboratory facilities.

CONFLICT OF INTERESTS

Declared none.

REFERENCES

1. Priyadarshini S, Gopinath V, Priyadarshini NM, MabarokAli D and Velusamy P. Synthesis of anisotropic Silver nanoparticles using novel strain, *Bacillus flexus* and its biomedical application. Collides Surf B, 2013; 102: 232-7.
2. Nabikhan A, Kandasamy K, Raj A and Alikunhi NM. Synthesis of antimicrobial silver nanoparticles by callus and leaf extract from salt marsh plants, Sesuvium portulacastrum L. Colloid Surf B, 2010; 79(2): 488-93.
3. Kaushik N, Thakkar MS, Snehit S, Mhatre MS, Rasesh Y and Parikh MS. Biological synthesis of metallic nanoparticles. Nanomed Nanotechnol Bio Med, 2010; 6: 257–62.
4. Grace AN and Pandian K. Quinolone antibiotic capped gold nanoparticles and their antibacterial efficacy against gram positive and gram negative organisms. Journal of Bionanoscience, 2007; 1: 96-105.
5. Hernandez JF, Ruiz F, Pena DC, Martinez-Gutierrez F, Martinez AE, Guillen Ade J, Tapia-Perez H and Castanon GM. The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide, and gold. Nanomedicine, 2008; 4(3): 237-40.
6. Rajeshkumar S, Malarkodi C, Vanaja M, Gnanajobitha G, Paulkumar K, Kannan C and Annadurai G. Antibacterial activity of algae mediated synthesis of gold nanoparticles from *Turbinaria conoides.* Der Pharma Chemica, 2013; 5(2): 224-9.
7. Sen A and Batra A. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L. Int J Curr Pharm Res, 2012; 4(2): 67-73.
8. Murugan S, Anand R, Uma Devi P, Vidhya N and Rajesh KA.Efficacy of *Euphobia milli* and *E. pulcherrima* on aflatoxin producing fungi (*Aspergillus flavus* and *Aspergillus parasiticus*). Afr J Biotechnol, 2007; 6: 718–9.
9. Chanda S, Baravalia Y, Kaneria M and Rakholiya K. Fruit and vegetable peels – strong natural source of antimicrobics. Current Research Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. A.Maendez vilas (ed.), 2010; 444-50.
10. Buatong J, Phongpaichit S, Rukachaisirikul V and Sakayaroj J. Antimicrobial of crude extracts from mangrove fungal endophytes. World J Microbiol Biotechnol, 2011; 27(12): 3005–8.
11. Imitan S, Albonetti S, Forni L, Peri F and Lazzari D. Solvothermal Synthesis and Properties Control of Doped ZnO Nanoparticles. Journal of Colloid and Interface Science, 2009; 329: 73-80.
12. Ochieng PE, Iwuoha E, Michira I, Masikini M, Ondiek J, Githira P and Kamau GN. Green route synthesis and characterization of ZnO nanoparticles using *Spathodea campanulata*. International Journal of Bio Chemi Physics, 2015; 23: 53-61.
13. Hedaginal B and Taranath TC. Phytosynthesis, Characterization And Antimicrobial Activity Of Silver Nanoparticles Using *Solanum seaforthianum* Andrews. Int J Pharm Bio Sci, 2016; 7(3): 185–94.
14. Nisha C, Bhawana P and Fulekar MH. Antimicrobial Potential of Green Synthesized Silver nanoparticles using Sida acuta Leaf extract. Nano Sci Nano Technol, 2017; 11(1): 111.
15. Hafez1 RA, Abdel-Wahhab MA, Sehab AF and Karam El-Din AA. Green synthesis of silver nanoparticles using *Morus nigra* leave extract and evaluation their antifungal potency on phytopathogenic fungi. Journal of Applied Pharmaceutical Science, 2017; 7(02): 041-8.