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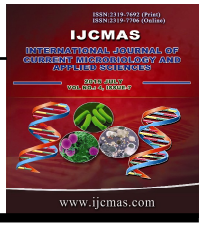

Original Research Article

Synthesis and Characterization of ZnO Nanoparticles using Leaf Extract of *Camellia sinesis* and Evaluation of their Antimicrobial Efficacy

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Keywords

Green-synthesis, Zinc nanoparticles, *Camellia sinensis*, Scanning Electron Microscopy

A B S T R A C T

Nanotechnology is a developing interdisciplinary field of research interspersing material science, bionanoscience, and technology. Nanoparticles are studied extensively for their specific catalytic, magnetic, electronic, optical, antimicrobial, wound healing and anti-inflammatory properties. The main aim of the present study was to synthesize Zn nanoparticles using the aqueous extract of green tea (*Camellia sinensis*) leaves and to evaluate their antimicrobial efficacy against some selected microbes. The synthesis Zn nanoparticles were characterized by UV/VIS spectroscopy, particle size analyzer and Scanning Electron Microscopy. The synthesized Zn nanoparticles showed significant antimicrobial activity against Gram positive and Gram negative bacteria as well as against a fungal strain. The maximum zone of inhibition had been found against *Pseudomonas aeruginosa* (32 ± 0.050) where as the minimum is found against *Staphylococcus aureus* (25 ± 0.100). Thus from this study it can be concluded that green tea leaf extracts can be effectively used for synthesizing Zn nanoparticles. This study also suggests that green synthesized Zn nanoparticles can be used as an alternative to existing antimicrobial agents

Introduction

Nanotechnology is a developing interdisciplinary field of research interspersing material science, bionanoscience, and technology (Gnanajobitha *et al.,* 2013). Metal nanoparticles are known to exhibit various functions which are otherwise not observed in bulk phases (Sosa *et al.,* 2003 and Sun *et al.,* 2003)**.** These nanoparticles are studied extensively for their specific catalytic, magnetic, electronic, optical and antimicrobial (Duran *et al.,* 2005 and Ingle *et at.,* 2008) wound healing and anti-inflammatory properties (Taylor, 2005). Plant mediated synthesis of nanoparticles are preferred over chemical synthesis due to its simplicity, eco-friendliness and extensive antimicrobial activity, non-toxic by-products and large-scale synthesis (Khandelwal, *et al.,* 2010; Vanaja *et al.,* 2013; Iravani, 2011; Satyavani *et al.,* 2011; Rao and Savithramma, 2011; Malabdi *et al.,* 2012; Saxena *et al.,* 2010 and Awwd *et al.,* 2013). Zinc oxide nanoparticles are known to be one of the multifunctional inorganic nanoparticles with effective antibacterial

activity (Gunalana *et al.,* 2012). Antibacterial and antifungal activities of ZnO nanoparticles are observed even at very lower concentrations and also the antifungal activity does not affect soil fertility compared to the conventional antifungal agents (Feris *et al.,* 2010).

The main aim of the present study was to synthesize Zn nanoparticles using the aqueous extract of green tea (*Camellia sinensis*) leaves and to evaluate their antimicrobial efficacy against some selected microbes.

Materials and Methods

Preparation of aqueous leaf extract

Fresh leaves of *Camellia sinensis* were collected and washed in running tap water followed by double distilled water. The aqueous extract of sample was prepared by boiling the freshly collected leaves (10g), with 100ml of distilled water, at 60°C for about 20 minutes, until the colour of the aqueous solution changes from watery to light yellow. Then the extract was cooled to room temperature and filtered using filter paper and used for further experiments.

Preparation of zinc nanoparticles

For the synthesis of nanoparticle, Zinc acetate was dissolved in the extract and the solution was stirred constantly using magnetic stirrer. After complete dissolution of the mixture, the solution was kept under vigorous stirring for 5–6 h at about at 150°C. The solution was then cooled at room temperature and the supernatant was discarded. The pale white solid product obtained was centrifuged twice at 4500 rpm for 15 min after thorough washing and dried at 80°C for 7–8 hours (Gunalana *et al.,* 2012).

Characterization of Zn nanoparticles

The synthesis of Zn nanoparticle was evaluated by taking the absorbance in the range of 300–500 nm using the UV/VIS spectrophotometer. The particle size of synthesized nanoparticles was obtained by particle size analyzer. The dried Zn nanoparticles were also subjected to SEM analysis for characterization.

Test organisms

Microorganisms were procured from Microbial Type Culture Collection and Gene bank (IMTECH, Chandigarh, India). The antimicrobial activity was carried out against both Gram positive and Gram negative bacteria viz. *Staphylococcus aureus* (Gram positive, MTCC 87), *Bacillus cereus* (Gram positive, MTCC 1305), *Escherichia coli* (Gram negative, MTCC 10312), *Pseudomonas aeruginosa* (Gram negative, MTCC-3542), *Proteus mirabilis* (Gram negative, MTCC-3310), as well as against the fungus *Aspergillus niger* (MTCC-9652). The bacterial strains were maintained on Nutrient Agar and fungi on Potato Dextrose Agar. The microbes were sub-cultured at an interval of one month.

Antibacterial activity

Zn nanoparticles synthesized using aqueous leaf extract of *Camellia sinensis* was tested for its potential antimicrobial activity against some selected microbes. To analyze the antimicrobial activity of the sample, the samples were subjected to Agar well Diffusion method (Kelly *et al.,* 2003). 25 ml of media was poured in Petri dishes and allowed to solidify. 0.1 ml of standardized inoculum was introduced and evenly spread onto the surface of sterile agar plates. After drying, well of 5 mm diameter were made on the agar plates with the help of sterilized

cork borer. 100 µl of test sample was poured in the respective well and the plates were incubated for 24 hours at 37°C. Ampicillin and Cefotaxime were used as positive control ns Zinc nitrate solution was taken as negative control. Diameter of the zone of inhibition was measured in mm and expressed as Mean ± Standard Deviation

Result and Discussion

When the leaf extract incubated with Zn nitrate, the colour changed from pale yellow to pale brown after one hour of incubation at room temperature (Fig. 1). The change in colour indicates the formation of nanoparticles. UV spectroscopy analysis showed maximum absorption at about 330 nm. The size of the particles was determined by particles size analyzer (Fig. 2). The sizes of particle in diameter were found to be 853 nm. SEM characterizations of the synthesized Zn nanoparticles are shown in (Fig. 3) which reveals much lesser diameter as compared to particle size analyzer. The nanoparticles were examined under various magnifications. SEM image has showed individual zinc particles as well as a number of aggregates. The synthesized Zn nanoparticles showed significant antimicrobial activity against Gram positive and Gram negative bacteria as well as against a fungal strain. The results are expressed as zone of inhibition (mm) ± SD (Table 1).

Table.1 Antimicrobial activity of the Zn nanoparticle against selected microbes

Sl.No.	Name of Test Organism	Zone of inhibition in mm ± SD			
		ZnO Nanoparticle	Zn Nitrate	Ampicillin 60 µg/ml	Cefotaxime 60 µg/ml
1	*Staphylococcus aureus*	25 ± 0.100	-	25 ± 0.383	24 ± 0.231
2	*Escherichia coli*	29 ± 0.500	-	17 ± 0.058	11 ± 0.058
3	*Pseudomonas aeruginosa*	32 ± 0.050	-	-	21 ± 0.100
4	*Proteus mirabilis*	30 ± 0.116	-	20 ± 0.141	13 ± 0.071
5	*Bacillus cereus*	30 ± 0.188	07±0.090	26 ± 0.435	28 ± 0.289
6	*Aspergillus niger*	30 ± 0.225	-	-	28 ± 0.354

Table.2 Antimicrobial activity of different leaf extracts of *Camellia sinensis* against microbes.

Sl.No.	Name of Test Organism	Zone of inhibition in mm ± SD			
		Methanolic Extract	Ethanolic Extract	Chloroform Extract	Aqueous Extract
1	*Staphylococcus aureus*	25 ± 0.283	16± 0.141	-	14 ± 0.283
2	*Escherichia coli*	27 ± 0.071	16± 0.212	-	14 ± 0.071
3	*Pseudomonas aeruginosa*	24 ± 0.071	15 ± 0.212	-	15 ± 0.071
4	*Proteus mirabilis*	26 ± 0.212	16± 0.141	-	17 ± 0.071
5	*Bacillus cereus*	25 ± 0.071	18± 0.141	-	16 ± 0.283
6	*Aspergillus niger*	26 ± 0.212	17± 0.071	-	13 ± 0.071

Fig.1 Biosynthesis of Zn nanoparticles using plant leaf extract of *Camellia sinensis*

(a) Before synthesis **(b)** After synthesis of Zn nanoparticle

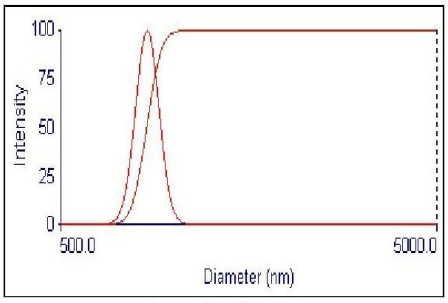
Fig.2 Particles size analysis

Brookhaven Instruments Corp.
90Plus Particle Sizing Software Ver. 5.27

Date: Jan 22, 2015
Time: 14:55:48
Batch: 0

Sample ID **Sample T**
Operator ID **mkd**
Notes

Elapsed Time	00:03:00
Median Diam.	853.4 nm
Mean Diam.	855.6 nm
Polydispersity	0.005
GSD	1.073



Lognormal Size Distribution

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
759.8	26	5	838.3	97	40	895.0	80	75
779.6	44	10	845.9	99	45	905.7	70	80
793.2	58	15	853.4	100	50	918.2	58	85
804.2	70	20	861.1	99	55	934.3	44	90
813.8	80	25	868.8	97	60	958.6	26	95
822.4	87	30	877.0	93	65			
830.5	93	35	885.6	87	70			

Fig.3(a-d) Scanning Electron Microscopic images of ZnO nanoparticles

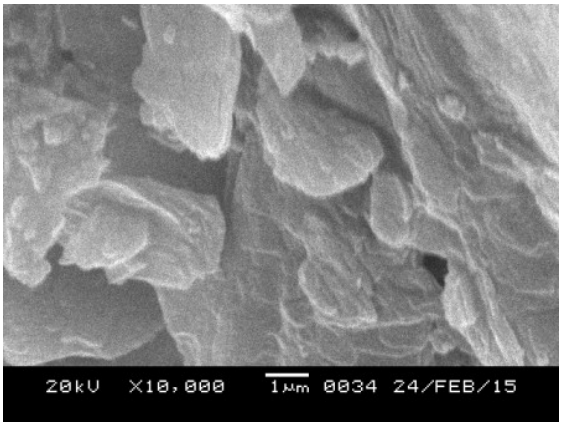


Fig.3(a)

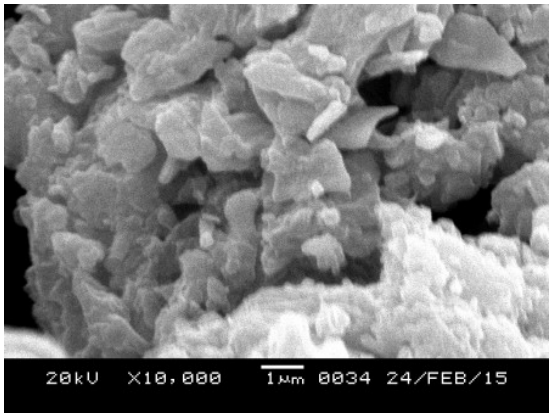


Fig. 3(b)

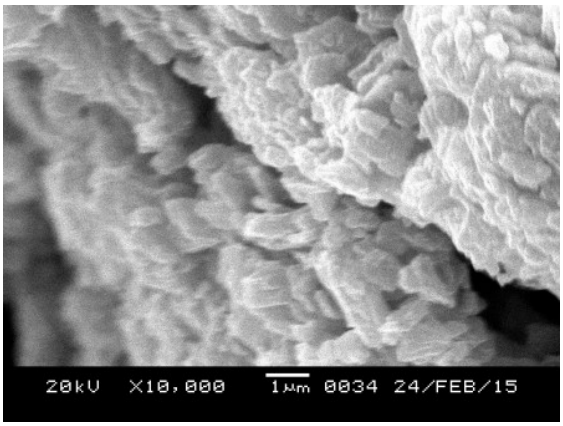


Fig.3(c)

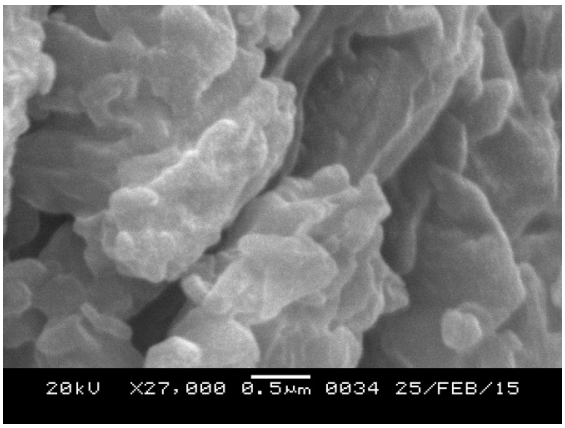


Fig.3(d)

The maximum zone of inhibition had been found against *Pseudomonas aeruginosa* (32 ± 0.050) where as the minimum is found against *Staphylococcus aureus* (25 ± 0.100). The solvent extract of the leaves were also found to be very significant against the microbes. The methanolic extract showed highest activity against the tested microbes. The ethanolic and aqueous extract showed nearly similar activity after methanolic extract however no activity was recorded in the chloroform extract (Table 2). Several research confirming antimicrobial activity of ZnO nanoparticles against the food related bacteria *Bacillus subtilis*, *Escherichia coli Pseudomonas fluorescens Salmonella typhimurium* and *Staphylococcus aureus* had been reported (Russell and Hugo, 1994 and Ip *et al.*, 2006). ZnO NPs are also known to exhibit antimicrobial activities against *Listeria monocytogenes*, *Salmonella enteritidis* and *E. coli* (Russell and Hugo, 1994). The formation of hydrogen peroxide from the surface of ZnO is considered to be mainly responsible for its antimicrobial property (Rai *et al.*, 2009) Thus from this study it can be concluded that green tea leaf extracts can be effectively used for synthesizing Zn nanoparticles. This study also suggests that green synthesised Zn nanoparticles can be used as an alternative to existing antimicrobial agents.

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