Green synthesis and characterization of zinc oxide (ZnO) nanoparticles using *Achras sapota Linn* latex and its antimicrobial activity

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Abstract:

Planted mediated synthesis of metal oxide nanoparticles is an increasing commercial demand due to the wide applicability in the various areas such as electronic, catalysis, chemistry, energy, cosmetics and medicine. In this study Zinc Oxide (ZnO) nanoparticles were synthesized using *Achras sapota Linn* latex as a reducing and stabilizing agent. The synthesized nanoparticles were characterized using fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) techniques. The XRD result revealed that ZnO nanoparticles has flower like shape and the particle size was found to be 12.3 nm. It also showed good antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, (bacterial strains), *Candida albicans (*fungal strains).

Introduction

Nanotechnology concerns with the development of experimental processes for the synthesis of nanoparticles of different shape, size and controlled dispersity [1]. Nanoparticles due to their large surface area to volume ratio exhibit remarkable novel properties and methodical application in the field of biotechnology, sensors, medical catalysts, optical devices, DNA labeling, drug delivery [2]. Currently, a large number of physical, chemical, biological, and hybrid methods are available to synthesize different types of nanoparticles [3- 6] using toxic and hazardous chemical. Green synthesis of nanoparticles using plant is of great important due to environmental friendly, non-toxic and safe reagents compared to chemical method [7]. Green synthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. A study by Yamamoto (2001), to evaluate the antibacterial activity of ZnO with different particle sizes showed that ZnO nanoparticles (10-50 nm) exhibit better antimicrobial properties than bulk ZnO (2 µm) [8]. The antibacterial activity of ZnO nanoparticles is due in part to their electrostatic interaction with cell surfaces. Sharma et. al showed that on contact with bacteria, the cytotoxic behaviour of ZnO nanoparticles ruptures the lipid bilayer of bacterium resulting in leakage of cytoplasmic contents [9]. The antibacterial mechanism of ZnO is due to cell membrane damage by the generated reactive species such as superoxide anion and hydroxyl radical. It also inhibits the growth of pathogenic bacteria under visible lighting condition [10]. The plant phytochemicals with anti oxidant are usually responsible for reduction of metal compounds into their respective nanoparticles. *Achras sapota Linn* belongs to the family of *sapotaceae* .The major phytochemical present in the latex of *Achras sapota Linn* are carbohydrate, alkaloid, proteins, ascorbic acid, phenolics (alkaloids and terpinoids) and carotenoids[11-13]. The latex is used as a crude filling for tooth cavities, to make chewing gum and is assigned for various properties in the traditional system of Indian medicine [14-16]. Plant latex refers generically to a stable dispersion (emulsion) of polymer micro particles in an aqueous medium. It is found in nature as a milky sap which coagulates in nature. The phytochemicals present in the latex acts as a reducing as well as stabilizing agent [17]. Vidya et al synthesized ZnO nanoparticles by *Calotropis Gigantea* [18], Gnanasangeetha et al prepared nanoparticles from aqueous leaf extract of *Corriandrum sativum* [19], and Sing et.al used *Calotropis procera* for surface modification of ZnO [20]. Sangeetha et al synthesized ZnO using *Aloe barbadensis* extract [21]. Chandrasekhar et al synthesized color tunable ZnO/Eu^{3+} nanophosphors via *Euphorbia tirucalli* plant latex [22].Vignesh et.al studied the photocatalytic activity of AgI sensitized ZnO nanopatyicles under visible light irradiation [23]. To the best of our knowledge, green synthesis using latex of *Achras sapota Linn* has been used for the first time as a reducing material as well as surface stabilizing agent for the synthesis of ZnO nanoparticles. The prepared nanoparticles were characterized by (FT-IR), (XRD), (SEM) and (EDX). Further, this study aims to explore the proficiency of these nanoparticles as antibacterial and antifungal agent.

2 MATERIALS AND METHOD

2.1 Materials

All the chemicals used were of analytical grade and purchased from 'MERCK CHEMICALS' Ltd, India. Double distilled water was used in the synthesis.

2.2 Preparation of *Achras sapota* linn Latex solution

Latex of *Achras sapota* linn was collected early in the morning because production of latex is high during early morning. Crude Latex was obtained by cutting green stems of *Achras sapota* linn plant. Milky white latex was stored at -4ºC until further use. 1ml crude latex was diluted to 100ml using double distilled water to make it 0.1 % of latex solution. [24]

2.3 Preparation of ZnO Nanoparticles

ZnO nanoparticles were prepared by co-precipitation method. 0.2 M aqueous solution of zinc acetate solution was put into 100 mL of distilled water under vigorous stirring. After 10 min stirring, 10 mL latex of *Achras sapota linn* was added into the above solution. 2 M NaOH aqueous solution was introduced into the above solution to adjust the pH of the solution, which were then placed in magnetic stirring for 2 h. The precipitate was taken out and washed repeatedly with distilled water for the final products. The obtained white precipitate was dryed at 60ºC in hot air oven over night [25].

2.4 Preparation of agar

Antimicrobial activity of ZnO was performed by disc diffusion method using Muller Hinton agar. Fungal strains (*candida albicans* and *Aspergillus niger*) and bacterial strains (*Staphylococcus aureus, Escherichia-coli, and Pseudomonas aeruginnosa)* were used to study the antimicrobial property. ZnO was dissolved in dimethyl sulfoxide (DMSO) and its concentration was fixed at 200 µg/mL (minimum inhibitatory concentration). The disc was prepared from Whatman no. 1 filter paper and the disc (ZnO at 200 µg/mL) was placed on the agar plate. The zone of inhibition was measured after an incubation period of 24 h at 37°C [26].

2.5 Charaterization

The synthesized nanoparticles were characterized by the following methods. The surface structure was characterized using fourier transform infrared spectrum (FT-IR) (Shimadzu) with KBr pellets. The crystalline phase was determined by X – ray powder diffraction (XRD) with Cu Kα radiation at 25º C (XPERT PRO X – RAY) and the structural assignments were made with reference to the standard JCPDS powder diffraction files. Scanning electron microscopes (SEM) were taken in a JM 6701F – 6701 instrument in both secondary and backscattered electron modes. The elemental analysis was detected by an energy dispersive X-ray spectroscopy (EDX) attached to the SEM.

3 Results and discussion

3.1 FT-IR

The FT-IR spectrum of ZnO is shown in the Fig. 1. The peaks observed at 462 cm^{-1} is due to stretching frequency ZnO. The band at 1032 cm^{-1}, 1393 cm^{-1}, 1554 $cm^{-1}$and 3498 cm^{-1} represent the diverse functional groups like tannins, flavonoids, alkaloids and carotenoids which are abundant in latex and are adsorbed on the surface of the ZnO nanoparticles. The absorption peak at around 1032 cm^{-1} corresponds to C-N stretching vibration of amine, peak at 1393 cm^{-1} corresponding to the C–H and O-H bending, the peak at 1554 cm^{-1} can be assigned to carbonyl stretch and 3498 cm^{-1} was related to N–H stretch.

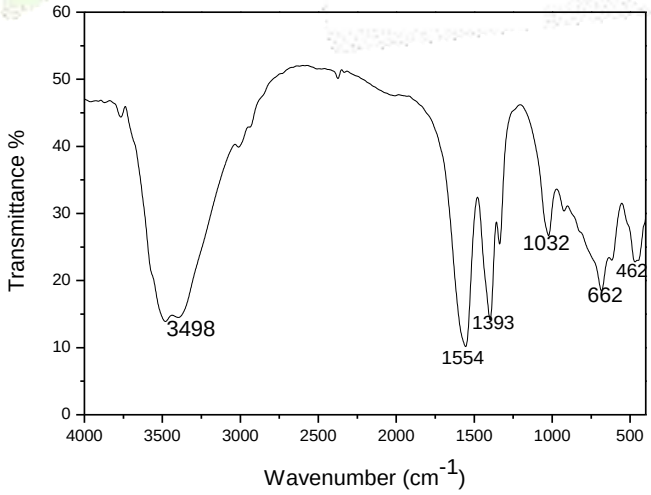


Fig. 1 FTIR spectrum of ZnO

3.2 XRD

XRD patterns of ZnO are shown in Fig .2. The observed diffraction peaks are perfectly indexed (2θ=31.32, 34.76, 36.36, 47.65, 56.85, 62.98, 66.67, 68.13, 69.48) with the standard JCPDS (891397) pattern of ZnO. The average crystallite size was calculated using the Debye's scherrer equation [27].

$$D = \frac{K\lambda}{\beta\cos\theta} \qquad (1)$$

Where, β is the full width half maximum of the most intense 2θ peak, K is the shape factor (0.89). θ and λ are the incident angle and wavelength of X-ray respectively. The average crystallite size of ZnO was found to be 12.3 nm.

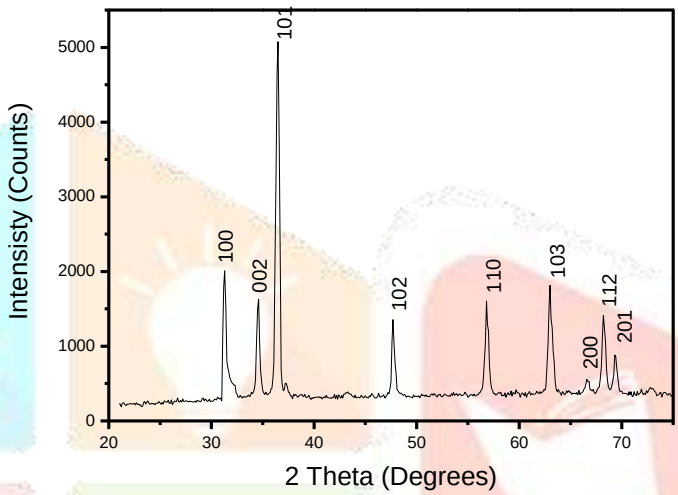


Fig. 2 XRD pattern of ZnO

3.3 SEM and EDX

The SEM micrographs of ZnO are shown in the Fig 3(a).As seen from the figure the nanoparticles have flower like shape and slightly agglomerated. The chemical compositions were confirmed by EDX. The EDX data of ZnO is shown in Fig. 3(b) and Table 3.1. The peaks for Zn and O are clearly observed at their corresponding keV values.

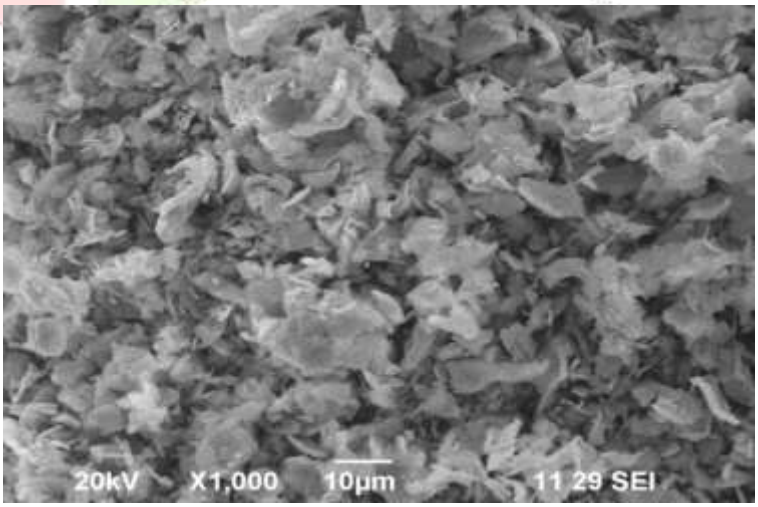


Fig. 3 (a) The SEM micrograph of ZnO

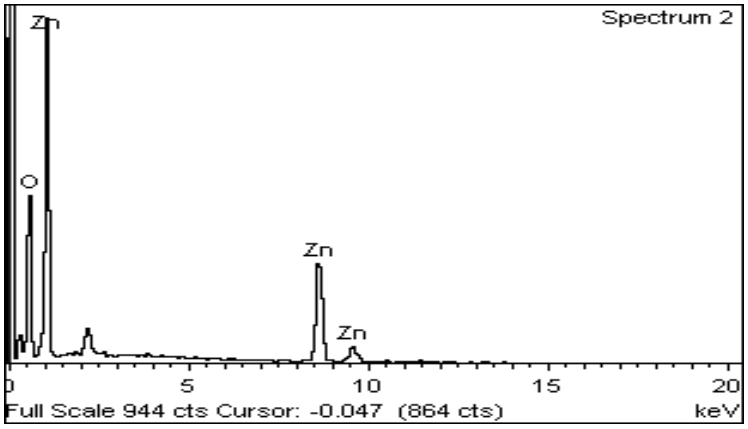


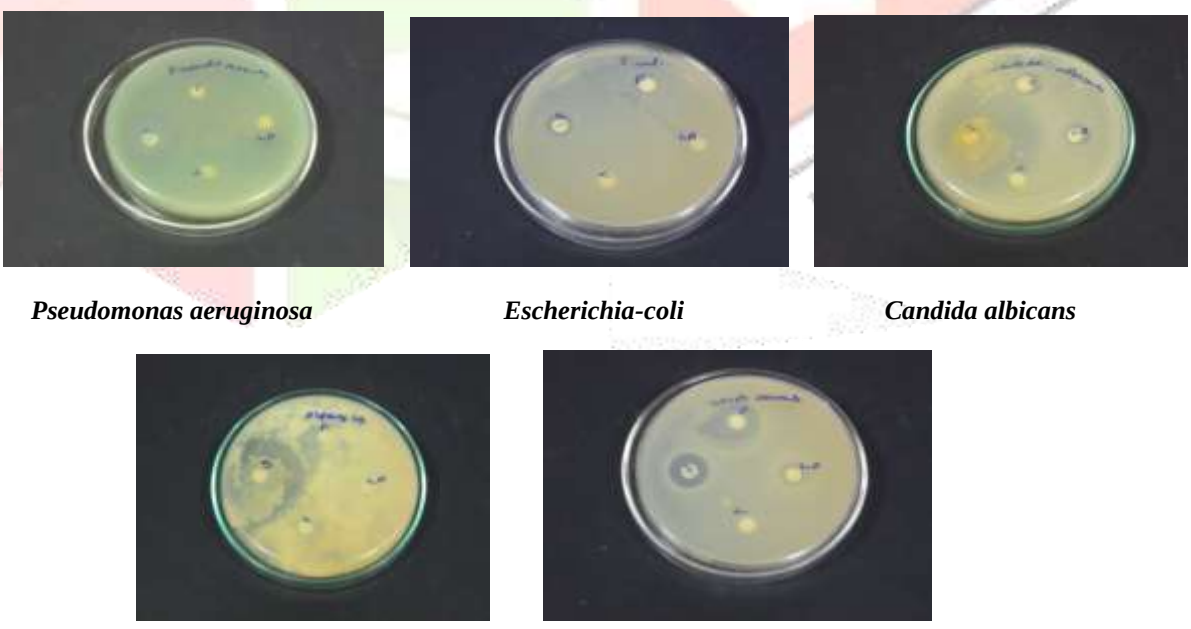
Fig. 3(b) EDX spectrum of ZnO

Table 3.1.EDX data of ZnO

S.No	Element	Weight (%)	Atomic %	keV
1	Zn	65.54	31.76	2.580
2	O	34.46	68.24	0.270

3.4 Antimicrobial activity of ZnO nanoparticle;

The result of the anti-microbial activity of ZnO is shown in Fig. 4 and Table 3.2.It is observed that ZnO showed excellent anti-bacterial activity against *Staphylococcus aureus and Escherchia coli* but it was resistance against *pseudomonas aeruginosa*. This anti-bacterial activity of ZnO is due to the generation of surface oxygen species which leads to the killing of bacteria. ZnO showed remarkable anti-fungal activity against *Candida albicans* and it is resistance against *Aspergillus niger.*



Pseudomonas aeruginosa ***Escherichia-coli*** ***Candida albicans***

Aspergillus niger ***Staphylococcus aureus***

Fig. 4. The photographs of antimicrobial activity of ZnO

Table. 3.2 Anti-microbial activity of ZnO

Type of Pathogen	Name of organism	Zone of inhibition in (mm)		
		Control (DMSO)	Standard (ERYTHROMYCIN)	ZnO
Bacterial strain	*Staphylococcus Aureus*	R	18	10
	Escherichia-coli	R	17	6
	Pseudomonas Aeruginosa	R	17	R
Fungal strain	*Candida albicans*	R	21	7
	Aspergillus niger	R	17	R

4. Conclusion

In conclusion, ZnO nanoparticles have been successfully synthesized through a green method using latex of *Achras sapota* linn. The nanoparticles were characterized by FT-IR, XRD, SEM and EDX techniques. It showed excellent anti-microbial activity against pathogens like *Staphylococcus Aureus*, *Escherchia–coli* and *Candida albicans*. Green synthesizes is a rapid, facile, convenient, time consuming and environmentally safe method.

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