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Characterization and Antibacterial Activity of Zinc Oxide Nanoparticles Synthesized Using *Opuntia ficus indica* Fruit Aqueous Extract

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Zinc Oxide nanoparticles were green synthesized by *Opuntia ficus indica* fruit aqueous extract. The phytochemicals present in the plant extract act as reducing and stabilizing agents. SEM and UV-Vis spectroscopy were used to study the surface morphology and optical properties. Light brown colored Zinc Oxide nanoparticles exhibit an absorption band at 445 nm due to the surface plasmon resonance. The presence of phytochemicals in the synthesized compounds was confirmed by FTIR and EDX. XRD analysis revealed the average particle size as 21.75 nm. It was found that the size of nanoparticle decreased with increase in temperature; also it was smaller in green synthesis than in chemical method. SEM images show aggregated groups of smaller nanoparticles. The sample shows better antibacterial activity.

Keywords: EDX; FTIR; green synthesis; SEM; temperature study; UV.

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1. INTRODUCTION

A number of synthetic routes have been employed to synthesize ZnO nanoparticles such as sol-gel processing, homogeneous precipitation [1], mechanical milling [2], organometallic synthesis [3], microwave method [4], spray pyrolysis [5], thermal evaporation [6] and mechano-chemical synthesis [7]. Organic solvents, synthetic additives and toxic reducing agents used in these methods are reactive and potentially dangerous to the environment. To avoid such implications and for sustainable synthesis of ZnO nanoparticles, biological approach is suitable which involve either bacteria [8], fungi [9], plants [10] or biomass [11-14]. Plant use can be suitably scaled up for large-scale synthesis of nanoparticles. The use of plant extracts has advantages such as easy availability, safe to metabolites, capping and reducing agent for the synthesis of nanoparticles which could be advantageous over chemical methods.

ZnO is used as white pigment in paints, UV filter in sun protection products and for the production of LEDs and TFTs [15]. ZnO is used in ointments because of its antiseptic properties [16]. ZnO is an antimicrobial agent and is effective to inhibit both gram positive and gram negative bacteria [17,18]. Opuntia ficus indica skin contains iron, calcium, magnesium, potassium, manganese, sodium, and selenium. The edible pulp contains biothiols, taurine, flavonols, tocopherols, and carotenoids. The oils from the seeds and peel are good source of polyunsaturated fatty acids. The plant cures Hyperlipedemia, joint diseases, diabetes type 2, adjuvant-induced chronic inflammation. It shows antioxidant activity and enhancement of immune function.

This study is aimed to evaluate the toxicity of biologically and chemically synthesized ZnO nanoparticles along with bulk formulations against plant and human pathogens under laboratory conditions. The prepared ZnO nanoparticles have been characterized by ultraviolet (UV) visible spectrophotometer, powder X-ray diffraction (PXRD), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDAX). The highlight of this work is the antimicrobial activity of green synthesized ZnO Nanoparticles. The study gives large scope for the reliable, eco-friendly green synthesis of ZnO that can show large variety of properties that are useful for human races to make their life to live one step further.

2. MATERIALS AND METHODS

2.1 Preparation of Fruit Extract

Opuntia ficus indica was collected around Theni town. Spine removed 100 g *Opuntia ficus indica* fruit was washed with tap water, rinsed with double distilled water, finely cut into pieces, smashed and heated in 300 mL of double distilled water at 50°C for 30 minutes. The mixture was cooled and filtered through Whatman No. 1 filter paper. The extract was stored at 4°C for further use.

2.2 Green Synthesis of Zinc Oxide Nanoparticles

The ZnO nanoparticles were green synthesized as per the procedure [19]. Two different volumes (10, and 50 mL) of the fruit extract were added separately, dropwise to 50 mL of 1 mM zinc acetate solution under stirring. The contents were continuously stirred for 3 hours for complete conversion of Zinc acetate into zinc hydroxide. The reaction mixture became light brownish and zinc hydroxide precipitate was collected by centrifugation. The precipitate was vacuum dried. Phytochemical concentration plays an important role in the synthesis of nanoparticles. Insufficient amount of bioactive compound present in 10 ml extract lowers the yield, whereas, 50 ml extract yields nearly entire amount of zinc as zinc oxide present in the whole of zinc acetate solution. Thus phytochemical concentration present in 50 ml extract was sufficient to reduce all Zn^{2+} ions present in the 50 mL of 1 mM zinc acetate solution. Samples ZNP60 and ZNP100 were obtained by heating the above precipitate for 4 hours at 60°C and 100°C respectively.

2.3 Chemical Synthesis of Zinc Oxide Nanoparticles

To 50 mL of 1 mM Zinc acetate solution 100 mL of 1 mM NaOH solution was added dropwise under stirring. White Zinc hydroxide precipitate was collected and dried at 100°C for 4 hours.

2.4 Phytochemical Analysis

Phytochemical analysis of the fruit extract shows positive test for alkaloids, terpenoids, glycosides, carbohydrates, saponins, tannins, steroids and aminoacids.

2.5 Characterization of NPs

The electronic spectra was recorded using JASCO 530V UV-Visible Spectrophotometer in the range of 200-1000 nm. The IR spectra was recorded as KBr pellets using JASCO 640 Plus Spectrometers in 4000-400 cm^{-1} region. XRD pattern was analyzed using powder SHIMADZU X-ray diffractometer equipped with a Cukα (λ=1.54Å) source. Surface morphology and elemental analysis was analyzed by SEM coupled with Energy dispersive X-ray spectroscopy (EDX). Microbial strains such as *S. aureus*, *B. subtilis* and *E. coli* are used to study the antibacterial activity.

3. RESULTS AND DISCUSSION

3.1 UV-Vis Absorbance Spectroscopy

Equal volume of reaction mixture was drawn from the reaction vessel and equally diluted to get an appropriate solution for UV analysis. In the Fig. 1, spectra A and B show the UV-Vis absorption spectra of ZnO nanoparticles synthesized with 10 ml and 50 ml fruit extract respectively. Spectra A reveals the insufficient amount of bioactive compound present in 10 ml, since the peak at 445nm is flat than that in spectra B. The change in particle size shows variation in color of the nanoparticles in colloidal solution [20]. The electron clouds on the surface of nanoparticles interact with electromagnetic radiation of particular wavelength resulting in an absorbance band. The appearance of light brown color in our colloidal solution is due to the formation of smaller size zinc oxide nanoparticles [21]. The present zinc oxide nanoparticles synthsised by *Opuntia ficus indica* fruit extract absorbs visible light wavelength of 445 nm due to surface plasmon resonance. λ_{max} value is in correlation with the previous reports [21,22]. Thus the absorbance spectra confirms the reduction of zinc ion and formation of nanoparticles.

3.2 FTIR Analysis of ZnO Nanoparticles

In the Fig. 2A, B, C are the spectras for chemically prepared ZnO, ZNP100 and ZNP60 respectively. FTIR analysis is useful in identifying the phytochemicals responsible for capping and stabilising metal nanoparticles synthesized by plant extract. The broad and strong band centered at 450 cm^{-1} due to stretching of Zn--O bond evidenced the formation of ZnO in all the three samples.

In ZNP100 and ZNP60 the presence of peak at 810 cm^{-1} is attributed to out of plane (C-H) bending vibrations in aromatic rings ($\gamma CH\phi$) [23]. The inplane bending vibrations $\beta CH\phi$ and C-O stretching in alcohol, ester, ether exhibit spectra in the range 1320–1000 cm^{-1}. Our samples show peak at 1108cm^{-1} attributing the presence of the above said groups. In general N-H and aromatic C-C stretching lies in the region 1450-1650 cm^{-1}. Here the peak at 1535 cm^{-1} is due to stretching of C-C, NH_2, NH bonds revealing the presence of amine, amide and aromatics in the plant extract. The observed peak at 1620 cm^{-1} leads to carbonyl group stretching showing the presence of amide, acid and carbonyl compounds in the sample.

Aromatic and alkene C-H stretching frequencies occur in the range 3000-3100 cm^{-1}, amine, amide N-H stretching in 3200-3500 cm^{-1}, and O-H, COOH stretching in 3300-3600 cm^{-1}. Thus the strong and broad band extending from 3000 to 3700 cm^{-1} may lead to the presence of amine, amide, alcohol, acid, aryl or alkene groups in the sample. The IR bands justified the presence of phytochemicals like alkaloids, terpenoids, flavonoids, carbohydrates, tannins, saponins, glycosides, aminoacids with various functional groups surrounding the green synthesized ZnO nanoparticles. This suggests that biological molecules are capable of reducing metal ions and controlling the size of synthesized nanoparticles. Free NH_2, OH and COOH groups of proteins, alkaloids, phenols and flavonoids present in the plant extract may bind to the surface of zinc ion and stabilize metal nanoparticles [24–26].

3.3 XRD Analysis of ZnO Nanoparticles

The crystallinity and particle size was determined by XRD powder diffraction. XRD patterns of the samples are shown in Fig. 3. XRD patterns of all the three samples were found to agree very well with the standard ZnO hexagonal wurtzite structure with JCPDS Data Card No: 36-1451. The three patterns do not show much deviations from the 2θ values corresponding to various diffraction crystal planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) as per the JCPDS data. This confirmed the presence of wurtzite ZnO in the three samples. The presence of well refined peaks corresponding to all diffraction planes confirmes the crystallinity of the samples. Absence of extra peaks other than the JCPDS data reveals the absence of impurity in the sample.

Diameter D was calculated using Debye-Scherrer formula [27] $D = K\lambda/(\beta\cos\theta)$, where K is the scherrer constant, λ the X-ray wavelength, β the peak width of half maximum, and θ is the Bragg diffraction angle. The average crystalline size was found to be 31.22, 24.44, 21.75 nm for chemical ZnO, ZNP60 and ZNP100 respectively. XRD spectra confirmed that crystallinity of the sample increased and particle size decreased with increase in temperature. This decrease in particle size is due to the stabilization of ZnO nanoparticles by phytochemicals present in the plant extract as evidenced from the IR spectra of samples ZNP100 and ZNP60. Thus ZNP100 is selected for further studies since it has the least particle size and better crystallinity.

3.4 SEM Analysis of ZnO Nanoparticles

EDX spectra of the sample ZNP100 is given in the Fig. 4A. ZnO formation is evidenced from the percentage of zinc and oxygen as present in the required stoichiometric ratio in the EDX spectra. The presence of phytochemicals as stabilizing agents is confirmed from the presence of Carbon, nitrogen and some other elements in the EDX spectra. SEM micrograph (Fig. 4B) showed aggregation of group of smaller spherical ZnO nanoparticles.

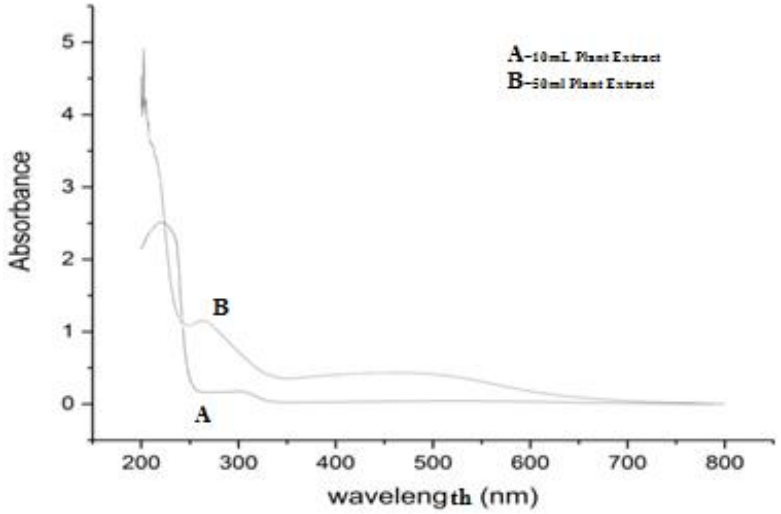

Fig. 1. UV analysis spectrum of ZnO NPs with (A) 10 ml and (B) 50 ml fruit extract

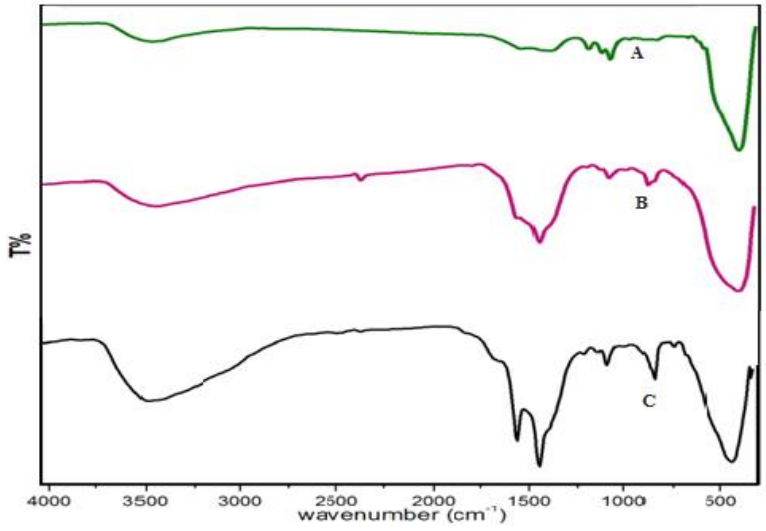

Fig. 2. IR spectrum of ZnONPs (A) ZnO (Chemical), (B) ZNP100 and (C) ZNP60

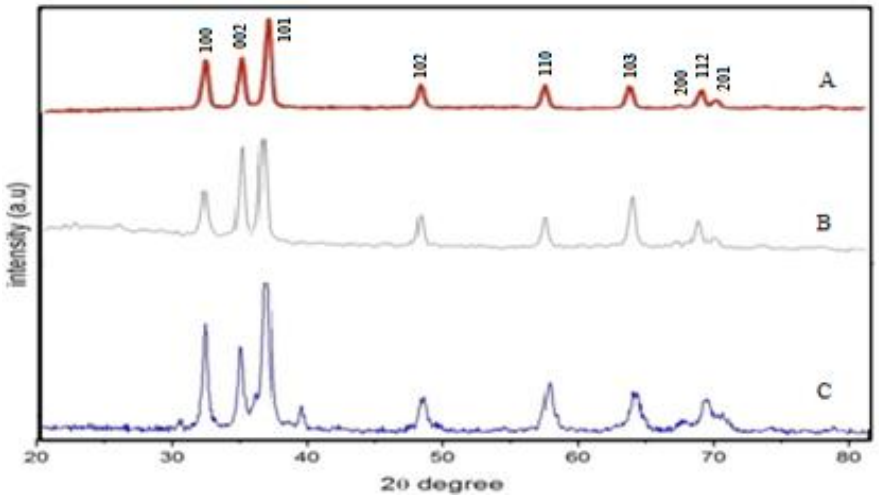

Fig. 3. XRD analysis (A) ZnO (Chemical), (B) ZNP100 and (C) ZNP60

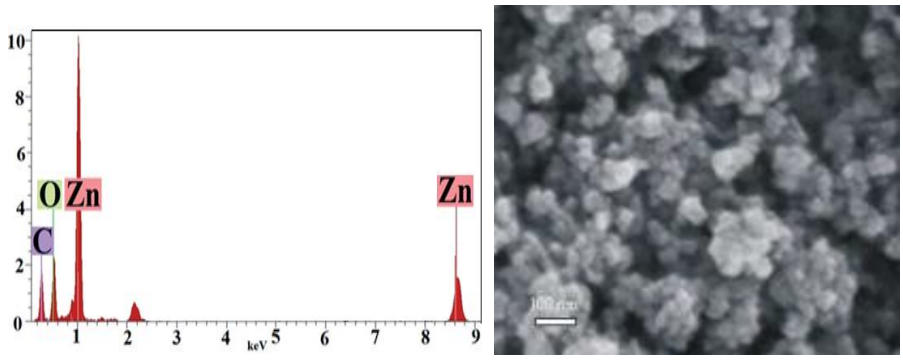

Fig. 4. (A) EDX spectra (B) SEM image of sample ZNP100

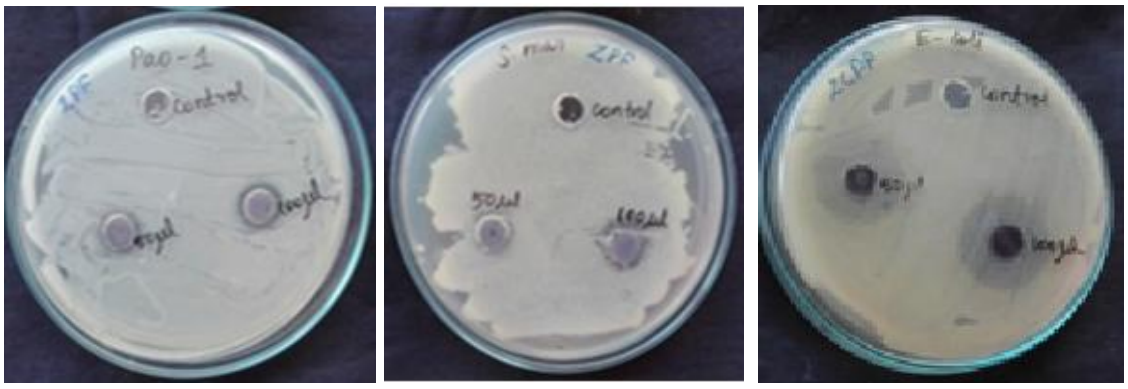

Fig. 5. Antibacterial activity of ZNP100 against Pao-1, *S. mar, E. coli*

3.5 Antibacterial Activity

The antimicrobial activity of green synthesized ZnO nanoparticles was tested by the agar diffusion method against Microbial strains *Escherichia coli, Serratia marcescens* and *Pseudomonas aeuroginosa*. Dimethyl sulfoxide (DMSO) is used to dissolve the sample ZNP100. Disc diffusion method was carried out by using standard protocol and overnight bacterial

cultures (100 mL) was spread over agar medium plates with a sterile glass L-rod. Two different concentrations of 50 and 100 μL extracts were applied to each filter paper disc Whatmann No.1 (5 mm dia) and allowed to dry before being placed on the agar. Each extract was tested to triplicate and the plates were inoculated at 37°C for 24 hours after incubation. The zone of inhibition was measured and expressed as millimeter (Table 1) in diameter. The highest antimicrobial activity was observed against *Escherichia coli* followed by *Serratia marcescens*.

Table 1. Antibacterial activity of ZNP100

Bacterial strains	Zone of inhibition in mm at different conc. of ZNP100		Fruit extract control
	50 μL	100 μL	
Pao-1	5.5±0.5	7.0±0.4	5±0.0
S. mar	6.0±0.4	7.5±0.5	5±0.0
E. coli	11.0±0.7	13.0±0.6	5±0.0

4. CONCLUSION

Zinc Oxide nanoparticles were synthesized using *Opuntia ficus indica* fruit extract. Light brown colored Zinc Oxide nanoparticles exhibit an absorption band at 445 nm due to surface plasmon resonance. The presence of phytochemicals in the synthesized compounds were confirmed by FTIR and EDX. XRD analysis revealed the average particle size as 21.75 nm. It was found that the size of nanoparticle decreased with increase in temperature, also it was smaller in green synthesis than in chemical method. SEM images shows aggregated groups of smaller nanoparticles. The sample shows better antibacterial activity.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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