

Research Article

Carica papaya L. Latex mediated green synthesis of ZnO nanoparticles for its antimicrobial activity

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ABSTRACT

Plant latex is a natural product produced by a number of plant species which are used by different tribal communities in India as a folk medicinal treatment on natural wounds or cuts. Plant latex has a huge demand as herbal products in an aspect of clinical, therapeutical and also in agricultural sectors. Natural latex is composed of different important biomolecules like, tannins, flavonoids, glycosides, sterols, saponins etc. These different active chemical constituents have versatile medicinal activities against different pathogens such as bacteria, fungi, viruses and protozoans etc. Therefore, development of novel biological techniques is significant for the biosynthesis of ZnO nanoparticles using the latex of *Carica papaya* L. This study reports on the biosynthesis of ZnO nanoparticles (Zn NPs) using latex of *C. papaya* as an effective reducing agent. Green synthesis of nanoparticles has advantageous over conventional methods because it does not require the use of toxic chemicals and therefore environmentally sustainable. Zinc oxide nanoparticles have gained potential recognition because of their distinctive characteristics and wide utilization in various fields. Properties of synthesized ZnO NPs were characterized using various methods such as ultra violet visible spectrophotometer (UV-Vis), Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FT-IR). The elemental composition of *C. papaya* latex was also analysed using X-ray Fluorescence (XRF) technique. Green synthesized ZnO nanoparticles also assessed for its antimicrobial activity against selected bacterial species.

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INTRODUCTION

Zinc oxide nanoparticles (ZnO NPs) are essential inorganic material with multiple applications and known for their bactericidal and fungicidal properties [1]. Green synthesis of nanoparticles is considered advantageous over conventional methods as it removes the utilization of toxic chemicals and reduces environmental impact. Among the biological sources, plant latex is one of the important resource for conversion of metal ions to nanoparticles because of potential reducing and stabilizing agents of bioactive compounds. Latex functions well as an antioxidant, anticoagulant, antitumor, and

antimicrobial. Among the latex producing plants, *Carica papaya* L., belonging to the Caricaceae family, is most commonly found throughout Odisha. The plant has extensive biological utilizations, including antibacterial [2], anti-inflammatory, anti-cancer, anti-leprosy, wound-healing, and use against skin conditions. Unripe fruit latex is used in cosmetically for face-lifting operations, reduces blood pressure and whooping cough [2]. Papain has a number of industrial applications in textile, pharmacy, baking, brewing and wine producing industries [3, 4]. Unripe fruit latex contains various bioactive compounds viz., enzymes mostly papain, cyclo-

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transferase, lysozymes and chymopapain [2], polyphenols, alkaloids, tannins, terpenes, sugars, gums [5] and secondary metabolites which contribute to the reduction and stabilization of nanoparticles. This work focuses on antimicrobial activity of crude latex of *C. papaya* L. and synthesis of ZnO nanoparticles using latex as an eco-friendly approach. Various characterization techniques are employed to analyse the nanoparticles, including UV-Vis spectroscopy, FT-IR and SEM. ZnO nanoparticles were evaluated for their potency against selected microorganisms. ZnO nanoparticles (ZnO NPs) were selected for this study because of its stability with a longer lifecycle than other organic-based compounds [6, 7, 8].

EXPERIMENTAL SECTION

Fruit Latex Collection

Latex was obtained in the month of September, 2022, by making longitudinal incisions in the unripe fruit of healthy plant *C. papaya* L. early morning in a sterile container from the medicinal plant garden of Centurion University, Bhubaneswar campus with latitude of 20.1735040 and longitude of 85.7053790 for our study. The plant was identified and authenticated by Dr. Nabin Kumar Dhal, Chief Scientist, CSIR-IMMT, Bhubaneswar, Odisha, India.

ZnO Nanoparticles Synthesis Using Latex of *C. papaya* L.

Precursor zinc acetate ($\text{Zn}(\text{CH}_3\text{COO})_2$) was taken as the precursor in synthesizing Zinc oxide nanoparticles (ZnO NPs) utilising the milky latex of *C. papaya* L. latex. Collected latex was dissolved in 30 ml distilled water and boiled for 15 minutes, then the solution was kept for cooling and filtered using Whatman No.-1 filter-paper. Two millilitre extracted latex was poured drop wise to 20 ml of 0.5 mM (ZCP1), 1 mM (ZCP2), and 2 mM (ZCP3) zinc acetate solution separately and continuously stirred for 4hr. The pH of the mixture was kept slightly acidic (6.5 to 6.7) and no precipitating agent like NaOH was added. A white coloured precipitate was formed, which was filtered, and kept for drying in an oven at 60-80°C overnight. Three samples were calcinated separately at 400°C for 3 hrs to get ZnO nanopowder.

Characterisation of ZnO Nanoparticles

The absorbance of the prepared samples was carried out using UV-Visible light by Ei Make UV-Visible spectrophotometer, and FT-IR by Spectrum Two FT-IR spectrometer.

Morphological analyses of ZnO Nanoparticles

UV-Vis, FT-IR spectroscopy and SEM size and shape of ZnO NPs were characterized using a SEM (JEOL-JSM 6390, Japan).

Antimicrobial activity

Collection of microbial strains

Bacterial strains viz., *Escherichia coli* MTCC 614, *Klebsiella pneumonia* MTCC 109, *Pseudomonas aeruginosa* MTCC

741 and *Proteus mirabilis* MTCC 9242 were collected from Department of Botany, Utkal University, Bhubaneswar, Odisha and maintained on agar slants at 40°C.

Antibacterial activity assay

Potato dextrose agar and Nutrient agar media are taken for culturing the collected fungal and bacterial samples respectively. Antibacterial and antifungal activity was evaluated by agar well diffusion method [9, 10, 11]. Two ml of crude latex was poured to the agar well and incubated at 37°C for 24 hrs for bacterial strains. Sterile double distilled water was poured to the synthesized ZnO NPs and sonicated briefly to make uniform colloidal suspension to carry out antibacterial activity against the collected bacterial strains.

RESULTS AND DISCUSSION

Characterization of synthesized ZnO Nanoparticles

The optical absorption spectra were recorded in the range of 250-800 nm and depicted in Figure 1a. The spectra show a gradual blue shift of absorbance band with increasing the concentration of zinc percentage. As shown in the Figure 1a, the absorbance band for ZCP 1 appears at 360 nm but for ZCP2 and ZCP 3 it shifts to 356 and 346 nm respectively. The absorbance for ZCP2 and ZCP3 also increased with increasing concentration of zinc acetate solution. The blue shift of absorption band can be understood on the basis of quantum confinement effect of ZnO nanoparticles. The blue shift can be due to the smaller crystalline size resulting quantum confinement in the system [12]. Maximum absorption peak of ZnO nanoparticles in the range from 346 to 360 nm range is agreed with the previous findings [12].

FTIR spectra of all the three prepared samples were recorded in the range of 500-4000 cm^{-1} in transmittance mode and presented in Figure 1b.

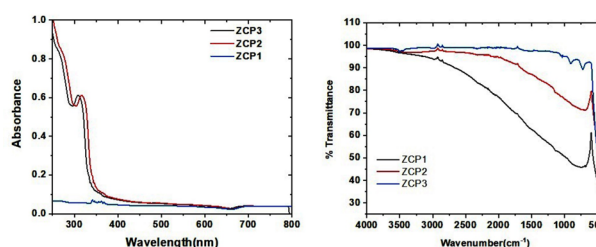


Figure 1. a UV absorption of ZCP1, ZCP2, and ZCP3; Figure 1b. FTIR spectral graph of ZCP1, ZCP2, and ZCP3

The spectra show a broad band at 680-720 cm^{-1} indicating the formation of Zn-O stretching vibration related to metal oxide stretching [13]. As the three samples were calcinated at 400°C, so no corresponding peaks related to OH or H₂O groups were observed. Morphology of ZCP1, ZCP2 and ZCP3 was studied by SEM analyses which revealed the shapes are mostly spherical in shape and size varies in three different samples 25-36 nm, and as observed in Figure 2a, Figure 2b, and Figure 2c.

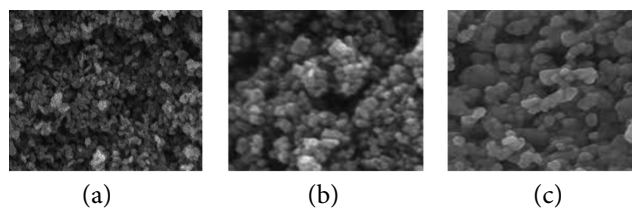


Figure 2. SEM image: (a) ZCP1; (b) ZCP2; (c) ZCP3

The three distinct results (Figure 2a, Figure 2b; Figure 2c) correspond to the concentration ratios (latex : zinc acetate solution) of 2:0.5, 2:1, and 1:1 respectively. ZnO NPs in high latex concentration becomes spherical as observed in Figure 2a, which becomes clustered in increasing concentration of zinc acetate solution.

Antimicrobial activity

The anti-bacterial activity of the raw latex and ZnO NPs was quantitatively assessed against the collected bacteria strains.

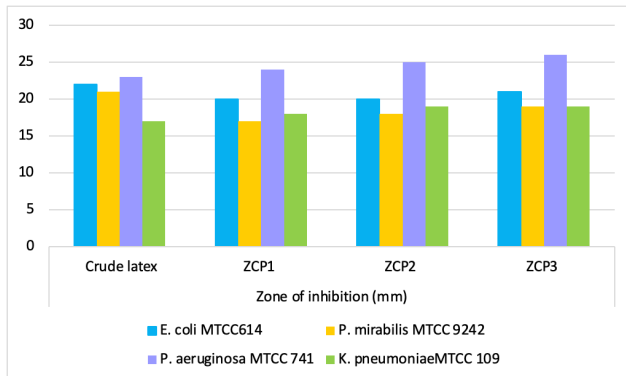


Figure 3. Antibacterial activity of crude latex and ZCP1, ZCP2, and ZCP3 nanoparticles

The antibacterial activity is classified [14] into sensitive, when zone of inhibition is greater than 21mm, intermediate (16-20 mm) and resistant (<15mm). *P. aeruginosa* MTCC 741 showed highest antibacterial activity, followed by *E. coli* in both crude latex and ZnO NPs (Figure 3). Among these samples ZCP3 shows highest antibacterial activity.

C. papaya latex exhibited a zone of inhibition on *Agrobacterium* sp. (20.66 mm) and *E. coli* (16 mm) because of possessing antimicrobial constituents [15, 16]. The protein in the latex with a concentration of 138 mg/mL has been reported to show 100 per cent inhibiting activity against *C. albicans* [17]. Papaya fruit latex had shown to heal the wounds effectively than glycol, and gentamycin in diabetic Wister rats [18, 19]. According to the findings, *C. papaya* latex mediated green synthesised ZnO-NPs demonstrated good antibacterial activity against the tested bacteria. Among the three samples, ZCP3 possessed larger surface area with more numbers of zinc atom, which induce higher level of toxicity to the tested bacterial strains [20, 21, 22] might be because of adhesion of the nanoparticles with bacterial membrane which could be the underlying cause of inhibition of bacterial growth.

CONCLUSION

Plant latex contains different important biomolecules such

as tannins, flavonoids, glycosides, sterols and saponins etc. and have versatile medicinal activities against different microorganisms. Phytochemicals are responsible for rapid reduction of zinc ion to metallic ZnO nanoparticles. In this study, latex of unripe fruit of healthy *C. papaya* L. was used as reducing agent to synthesize ZnO NPs, which is environmentally sustainable as it does not require the use of toxic chemicals. Synthesized ZnO NPs were characterized using UV-Vis, SEM and FT-IR. The spectra show a broad band at 680-720 cm⁻¹ indicating the formation of Zn-O stretching vibration related to metal oxide stretching and these green synthesized ZnO nanoparticles were assessed for its antibacterial activity against selected bacterial strains viz., *E. coli* MTCC 614, *K. pneumoniae* MTCC 109, *P. aeruginosa* MTCC 741 and *Proteus mirabilis* MTCC 9242. The results revealed that *P. aeruginosa* MTCC 741 had the highest antibacterial activity, followed by *E. coli* in both crude latex and ZnO NPs. This results suggest that the ZnO NPs as the promising antibacterial agents which also depends upon the concentration ZnO nanoparticles. This study contributes to eco-friendly synthesis methods and the exploration of effective antimicrobial agents.

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DATA AVAILABILITY STATEMENT

The author confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

USE OF AI FOR WRITING ASSISTANCE

Not declared.

ETHICS

There are no ethical issues with the publication of this manuscript.

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