Biogenic synthesis of ZnO nanoparticles mediated from *Borassus flabellifer* (Linn): antioxidant, antimicrobial activity against clinical pathogens, and photocatalytic degradation activity with molecular modeling

Dharman Kalaimurugan1,2 · Kandhasamy Lalitha3 · Kaliannan Durairaj2,4 · Palaniappan Sivasankar2 · Sungkwon Park5 · Kannan Nithya3 · Muthugoundar Subramanian Shivakumar3 · Wen-Chao Liu6 · Balasubramanian Balamuralikrishnan5 · Srinivasan Venkatesan2

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Abstract

*Borassus flabellifer* leaf extract has been used for rapid biogenic synthesis of zinc oxide nanoparticles (ZnO-NPs) due to rich source of bioactive compounds. The synthesized ZnO-NPs were preliminarily confirmed by UV–visible spectroscopy adsorption peak range at 365 nm. The XRD (X-ray diffraction) confirms purity of ZnO-NPs that were crystalline in nature. The analysis of FT-IR (Fourier-transform infrared spectroscopy) confirms the presence of the following functional group such as alcohol, phenols, carboxylic acids, primary amides, secondary amides, and alkyl halide. The Field Emission Scanning Electron Microscope (FE-SEM) analysis indicated that ZnO-NPs were in spherical shape, followed by EDX analysis which confirmed the presence of Zn-element. Antimicrobial effect of ZnO-NPs was investigated using different clinical pathogens like bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella Pneumonia*, and *Pseudomonas aeruginosa* and fungi *Aspergillus flavus*, *Candida albicans*, and *Penicillium expansum* which confirmed ZnO-NPs efficiency as an antimicrobial agent. ZnO-NP antimicrobial efficiency was observed in higher zone of inhibition at 50 μg/mL concentrations. Antioxidant activity was ascertained to be used for several biomedical applications. The ZnO-NPs efficiently degraded the environmental toxic dyes (methylene blue and crystal violet) under sunlight, and up to 95% higher degradation was achieved in both dyes. In support of photo light degradation, the study was progressed to understand the ZnO-dye interaction stability using molecular mechanism, and it shows efficient bonding features in the NPs environment. Overall, this investigation has great potential for being an effective and eco-friendly material used in environmental applications.

Keywords *Borassus flabellifer* · Zinc oxide nanoparticles · Antimicrobial activity · *In silico* docking · Photocatalytic

**Highlights**
- *Borassus flabellifer* leaf is used for synthesis of zinc oxide nanoparticles (ZnO-NPs).
- The ZnO-NPs synthesized material was characterized by UV spectra, XRD, FE-SEM, and EDX.
- *B. flabellifer* leaf extract and its derived Zn-NPs have been tested for their antibacterial, antifungal, antioxidant, and photocatalytic activity.
- In hacking of photocatalytic study, proceeded to *in silico* docking analysis.
- These simple biological approaches are non-toxic, environmentally safe, and cost efficient.

**Introduction**

Nanotechnology is one of the most fascinating ideas which is to develop particles with nanosize for the use in various fields of science and technology such as increased surface area, catalytic efficiency, profuse reactive sites, and high absorption rates (Dhand et al. 2015; Cittrarasu et al. 2019). There are several types of nanomaterials that have been used like nanocage, nanowire, quantum dot, nanocomposite, and nanoparticles (Kalaimurugan et al. 2019a, b). Green chemistry way for metallic and metallic oxide nanoparticle synthesis was especially intended to condense the ecological noxiousness or remove the conservation effluence. Zinc oxide (ZnO) shows a dynamic part in the day-to-day life of the 3rd uppermost universal making
volume only after SiO2 and TiO2 among the safest metal to use and also has the confirmed biocompatibility summary that “generally recognized as safe” (GRAS) material to the human and animal system by the United States Food and Drug Administration (USFDA) (21CFR182.8991) (Piccinno et al. 2012). Recent studies have shown that zinc oxide nanoparticles (ZnO-NPs) have potent toxicity to bacteria but exhibit minimal effects on human cells (Reddy et al. 2007). The ZnO-NPs are used in textiles for water treatment and sunscreen lotion, because it possesses high photocatalytic degradation (Dhand et al. 2015). The disposals of dyes without any treatment will lead to serious threat to the environment and aquatic system. Furthermore, certain dyes like reactive dyes are calcitrant, non-biodegradable, toxic, and mutagenic (Dalvand et al. 2016). Therefore, there is a need for biological alternatives of producing ecofriendly NPs. The *Borassus flabellifer* (Linn) (Palmyra palm tree) belongs to *Arecales* family and is extensively available in Asian regions such as India, Bangladesh, Thailand, and Sri Lanka. The *B. flabellifer* is used widely for a variety of purposes such as stimulant, anti-laprotic, diuretic, and antiphlogistic. Studies have shown that no biogreen synthesis of ZnO-NPs has been done using *B. flabellifer* leaf extract (BFLE). In this study, using BFLE as reduction agent, aimed at green synthesis of ZnO-NPs characterized by UV–visible spectroscopy, X-ray diffractometer (XRD), field emission scanning electron microscope (FE-SEM), and energy-dispersive X-ray spectroscopy (EDS). Further investigated their antibacterial and antifungal activity against representatives of clinical pathogenic microorganisms, antioxidant activity of radical scavenging activity, and photocatalytic degradation of methylene blue and crystal violet dyes under sunlight along with *in silico* molecular docking analysis to shed more light on the pharmaceutical and industrialized research findings.

**Materials and methods**

**Preparation of BFLE**

The *B. flabellifer* plant leaves were collected (Salem, Tamil Nadu, India), air-dried, powdered, and then dissolved in 100 mL of distilled water. The aqueous part was separated using Whatman no.1 filter paper after boiling at 60°C for 15 min and stored in refrigerator. All the other chemicals used in this experiment were analytical grade reagents.

**Synthesis and characterization of ZnO-NPs**

The ZnO-NPs were prepared by adding 80 mL of 1 mM aqueous ZnSO4 into 20 mL BFLE to form a reaction mixture with continuous stirring at 60 °C for 15 min. Once the ZnO-NPs production was completed, it was subjected to centrifugation at 10,000 rpm for 20 min at 4°C, followed by several washes with nanopure water and ethanol. The obtained material was freeze-dried to make a powder. The freeze-dried powder was used for further characterization and photocatalytic study. Biosynthesis of ZnO-NPs was characterized using UV–visible spectrophotometer to determine the absorption peak. The XRD was measured at the voltage of 40.0 (kV) and the current supplied by 30.0 (kV) with the sample scanning range at 10,000–90,000 for the determination and evaluation of crystalline structure of ZnO conjugated with BFLE. The FT-IR analysis of plant leaf extract and ZnO-NPs was made to identify the presence of functional groups and chemical bonding. The 3-D image of a particle size, elemental composition analysis, surface characterization, and magnification were analyzed using FE-SEM and EDS (Singh and Dutta 2020).

**Antibacterial activity**

The *B. flabellifer* ZnO-NPs were tested for their antibacterial activity against clinical pathogenic bacteria by agar well diffusion methods (Cittrarasu et al. 2019). To the Mueller Hinton agar culture plate, five pathogenic bacteria *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa* were swabbed separately, and wells were punched. The ZnO-NPs dissolved in dimethyl sulfoxide (DMSO) were pipetted out at different concentrations (20, 40, 60, 80, and 100 μL/mL) in the appropriate wells, and as a control antibiotic, tetracycline was used. Petri-dishes were incubated at 37°C for 24h, and the zone of inhibition (mm) was recorded.

**Antifungal activity**

The antifungal activity of synthesized ZnO-NPs along with plant *B. flabellifer* leaf extracts against pathogenic fungus was followed by Jamdagni et al. (2018). To the potato dextrose agar culture plate, three fungi *Aspergillus flavus, Candida albicans,* and *Penicillium expansum* were swabbed separately, and wells were made using gel puncher. Different concentrations (20, 40, 60, 80, and 100 μL/mL) of samples were pipetted out in the appropriate wells, and as a control, fluconazole was used. Petri-dishes were incubated at 25°C for 72h, and the zone inhibition (in mm) was measured and recorded.

**Antioxidant activity**

2-diphenyl-1-picrylhydrazyl assay (DPPH)

The DPPH activity was performed by the modified protocol described previously (Suresh et al. 2015). The constant free radicals of DPPH in oxidized form exist in purple color, and the absorbance was measured at 520 nm. As a positive control, ascorbic acid was used. The stock solution of ascorbic acid was made by adding 3mg of ascorbic acid to the 15mL of deionizer water. Further, different
concentrations (20, 40, 60, 80, 100 μg/mL) were used to plot the standard graph. The scavenging activity of inhibition in percentage for each concentration was calculated using standard graph by the following formula.

DPPH Scavenging effect (%) = \( \frac{A_0 - A_1}{A_0} \times 100 \) (1)

where \( A_0 \) is the absorbance of control and \( A_1 \) is the absorbance of sample.

Photocatalytic activity of ZnO-NPs

The photocatalytic activity of the ZnO-NPs was performed with methylene blue (30mg/L) and crystal violet (30mg/L) dyes at neutral pH and ambient temperature. The method was analyzed as follows: 0.10g of synthesized products was dispensed in 10mL of different dye solution in a container provided with water flow ability. To find the homogeneous solution, the above suspension was continually stirred in the absence of radiation for about 30 min. This is performed to achieve adsorption and desorption equilibrium. The sunlight was used as light source. In a typical photocatalytic activity process, 20 mg sample was added to 10 mL of dye solution, and the adsorption spectrum of the solution was monitored using UV–visible spectrophotometer (Cittrarasu et al. 2019).

Molecular configuration of ZnO with dyes

Structure of methylene blue (CID_6099) and crystal violet (CID_11057) was downloaded from NCBI-PubChem. The 2D-structure was optimized using Chem sketch individually. Both the structures were maintained for universal force field spatial configuration. The ZnO-NPs were drawn and prepared for penta NP layer. The complex of ZnO and NPs was subjected to simulate under vacuum for 100 ns using Gromacs Sunghwan et al. 2016; Duffy and Jorgensen. 2000. The resultant structure was plotted based on root mean square deviation value of the complex interactions.

Statistical analysis

Statistical comparisons were done using Student’s \( t \)-test. \( p < 0.05 \) was considered as a significant value. The inhibition zone of inhibition was estimated in diameter (mm) using two-way variance analysis (ANOVA) and expressed as mean ± standard deviation (GraphPad Prism version 6.0).

Results and discussion

In the present study, BFLE with ZnSO\(_4\) solution showed color change to dark brown that confirms the presence of ZnO-NPs. Similar study on synthesized ZnNPs using \textit{A. calamus} aqueous extract exhibits color change from transparent to reddish dark brown (Duffy and Jorgensen. 2000; Ibrahim and Abdullahi. 2017; Suresh et al. 2015 Abdullahi et al. 2017). Also, ZnO-NP synthesis used two kinds of chemical agents: (i) zinc sulfate that acts as precursor and (ii) phenolic and flavonoid components of BFLE that act as reduction agents. In this study, ZnO-NPs

![Fig. 1](image-url) A UV–vis spectrum of BFLE-mediated ZnO-NPs. B XRD pattern of synthesized ZnO-NPs shows 3 strongest peak values, which is corresponding to the intense counts.
exhibit strong peak from the spectra at the range of 365 nm under UV–visible spectrophotometer (Fig. 1a), whereas previous study of synthesized ZnO-NPs using *Olea europaea* leaf extract showed absorption peak at 374 nm (Awwad et al. 2014). The XRD analysis reveals the phase of ZnO-NPs using aqueous BFLE. The synthesized ZnO-NPs showed three strongest peaks of lattice plane at (100), (101), and (102) (Fig. 1b). This strongly confirms that the ZnO-NPs are in spherical shape. Study on synthesized ZnO-NPs using *J. curcas* latex showed the XRD

![Graph a)](image1)

![Graph b)](image2)

**Fig. 2** FT-IR spectrum analysis in ZnNPs: a plant extracts, b ZnNPs with plant extract
Fig. 3  Field Emission Scanning Electron Microscopic (FE-SEM) images of ZnO-NPs in different magnification ranges: a 1 μm, b 200 nm with EDX analysis (c, d) of BFLE in ZnO-NPs
lattice plane at (111), (200), (220), and (311), and this corroborates the crystalline structure (Safawo et al. 2018).

The FT-IR spectra analysis and functional groups present in synthesized ZnO-NPs were observed with wavelength related to the vibration assignments, as well as corresponds to visible intensity. The FT-IR result shows that the medium peak values at 3418.67, 2926.85, 1625.88, 1384.58, 1317.28, 916.99, 563.71, and 431.39 cm\(^{-1}\) of alcohol, phenols, carboxylic acids, primary amides, secondary amides, and alkyl halide, respectively. These are the functional groups are present in the biosynthesis of nanoparticles and plant leaf extract (Fig. 2). The surface functional groups and capping of synthesized ZnO-NPs were identified under FT-IR spectroscopy. The FT-IR analysis of previous study reported that the band at 3200–3500 cm\(^{-1}\), 1617 cm\(^{-1}\), 890 cm\(^{-1}\), 1534 cm\(^{-1}\), and 1458 cm\(^{-1}\) revealed O–H stretching, bending vibration of H\(_2\)O molecules, one transmission band due to C–O, asymmetry, and symmetry vibration of –COOH group, and it confirms the presence of ZnO-NPs. In the current study, the functional groups at 3418.67 cm\(^{-1}\), 2926.85 cm\(^{-1}\), 1625.88 cm\(^{-1}\), 1384.58 cm\(^{-1}\), 1317.28 cm\(^{-1}\), 916.99 cm\(^{-1}\), 563.71 cm\(^{-1}\), and 431.39 cm\(^{-1}\) were observed. All these bands clearly indicates that the functional groups were involved in the formation of ZnO-NPs (Kalaimurugan et al. 2019a, b).

The FE-SEM analysis was performed to image (at 1–200 μm magnifications) the shape and size of BFLE-capped ZnO-NPs (Fig. 3a, b), and this clearly determines that the ZnO-NPs are spherical in shape with small aggregation (Fig. 3c, d). The purity was further determined with EDX analysis. In addition, the FE-SEM image was used for particle size analysis by ImageJ 1.46r analyzer, and the average particle size is 89 ± 0.5 nm (Fig. 4). In previous study, ZnO-NPs using Z. clinopodioides leaves showed spherical shape with particle size ranging from 5 to 40 nm. In recent study, the synthesized ZnO-NPs showed strong silver signal along with other molecules like weak oxygen, carbon, and aluminum (Suresh et al. 2015). Likewise, in our present study, through the EDX-analysis, the presence of higher concentration of Zn was determined (Kalaimurugan et al. 2019a, b).

The DPPH assay provides an easy and fast antioxidant activity by total estimation method. The color changes from purple to yellow after reduction, which can be quantified at 519 nm due to its reduced absorbance. As the concentration increased (20 μg/mL, 18.47%; 40 μg/mL, 34.66%; 60 μg/mL, 49.01%; 80 μg/mL, 61.03%; and 100 μg/mL, 73.27%), free radical scavenging activity of ZnO-NPs on DPPH was observed (Fig. 5). The antibacterial activity of biosynthesized ZnO-NPs was investigated at different concentrations (20, 40, 60, 80, 100 μg/mL) along with the positive control, tetracycline against clinical pathogen S. aureus, B. subtilis, E. coli, K. pneumonia, and P. aeruginosa (Fig. 6a). We also cross checked the plant extract alone for antibacterial activity, and no zone of inhibition was observed. Only synthesized nanoparticles combined with plant extract (secondary metabolites) showed better result, and this may be due to the sensitivity to zone of inhibition. Antifungal activity of
biosynthesized ZnO-NPs was investigated at various concentrations (20, 40, 60, 80, and 100 μg/mL) against three fungi A. flavus, C. albicans, and P. expansum. The antifungal activity of ZnO-NPs is shown in Fig. 6b. The fungus A. flavus had
a medium sensitivity to 100 μg/mL, and other two fungal species had a strong sensitivity to 100 μg/mL ZnO-NPs. Figure 7 showed the schematic reason for mechanism of microbes cell damage by ZnO-NPs.

An agent’s antibacterial activity is due primarily to two mechanisms, which involve chemically interfering with the synthesis or function of essential components of bacteria and circumventing traditional mechanisms of antibacterial resistance (Mirza et al. 2019; Saravanan et al. 2018). Elumalai et al. (2010) reported that the antifungal activity of synthesized nanoparticles using *E. hirta* against 7 clinically isolated funguses (*C. albicans*, *C. kefyr*, *A. niger*, *C. tropicalis*, *C. kruzei*, *A. flavus*, and *A. fumigatus*) was tested. Among them, *C. albicans*, *C. kefyr*, and *A. niger* exhibit higher zone of inhibition at 50 μg/mL. We have successfully demonstrated strong effects of antifungal activity of ZnO-NPs on fungus strains (*C. albicans*, *A. niger*, and *P. expansum*). There was an increase in the inhibition zone, while ZnO-NPs increased. A probable inhibitory mechanism can be clarified by oxygen species released on the surface of ZnO, which binds the bacterial surfaces and kills the bacteria through electro-static forces. However, it can be assumed that the free Zn²⁺ partially contributes to the antimicrobial effect through the mechanical contact between the bacteria and ZnO rods surface. Likely, ZnO-NPs acted as needles that penetrate the bacterial cell wall which associates with the greater antimicrobial efficacy of the nanosize over their microcounterparts (Saravanan et al. 2018; Elumalai et al. 2010). Previous study reported that the antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *C. albicans*, *A. niger*, and *P. expansum* was tested, and higher zone of inhibition was observed as well as antifungal activity against *C. albicans* by using plant extract-derived ZnO-NPs (Mahdavi et al. 2019; Xie et al. 2011). The ZnO-NPs have prominent antimicrobial activities (<100 nm) because large surface volume ratio facilitates the better dissolution and penetration of the bacteria (Piccinno et al. 2012). Recently, Jamdagni et al. (2019) reported that the ZnO nanosuspension shows potential antifungal and antioxidant activity. Thus, the results of our experiment revealed the excellent antifungal and antibacterial effects of ZnO-NPs against all of the tested bacteria and fungi.
The photocatalytic operation is strongly dependent on the crystallographic structure, morphology and size of the metal oxide NPs, and color degradation with the aid of sunlight (Citrarasu et al. 2019; Duffy and Jorgensen. 2000). The dye degradation mechanisms were schematically presented in Fig. 8. Increased concentrations of methylene blue and crystal violet were tested with fixed amount of ZnO-NPs. The effect of dye concentration on ZnO-NPs photocatalytic function was accessed (Fig. 9). In our present study, the maximum absorption rate was observed at 350 nm for methylene blue and 635 nm for crystal violet. After 1 h, the dye slowly decreases with increased visible light exposure time, and this shows the plot between time and dye concentration at different times. The complex of NPs and dyes was examined using molecular mechanics to understand the behavior of the interacted complex. The ZnO-NPs layers were maintained to have 120° for each Zn and oxide group interactions; meanwhile, the position of individual NPs also properly placed the dynamic environment. All atoms in the systems are intentionally maintained to have non-bonded interaction types. The ZnO and one molecule of methylene blue and crystal violet were subjected to interact and simulated in the vacuum environment for 100 ns (Fig. 10 a, b). The resultant structure was proportionally stable in the proposed environment. The trajectory of each ZnO-NP layer placed propagated the equal environmental projections. It exhibits that the energy containment to carry the dye within the layer was stable and steady in the system. The RMSD shows (Fig. 10c, d) equal distribution of the distance deviation from each native structure.
Conclusion

In this study, BFLE-conjugated ZnO-NPs were synthesized and characterized. The results showed that the average absorption was obtained at 365 nm and the scale of approximately 10–200 nm by FE-SEM and EDAX analysis in atoms element composition presented in synthesis of NPs conformed bind to the Zn present. The XRD analysis indicated the peak in crystalline nature. The biosynthesized ZnO-NPs were verified to have excellent antibacterial and antifungal activity against clinical pathogens with potential antioxidant capacity. Further, methylene blue and crystal violet dyes were effectively degraded in the presence of ZnO-NPs. In silico analysis supports the functional features of ZnO-dye interactions in the various environment under photocatalytic activity. Therefore, eco-friendly BFLE-mediated ZnO-NPs opens a new-fangled path of methods for production and could be potentially utilized for biomedical and industrial applications.
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Availability of data and materials The data presented in this study are available on request from the corresponding author.

Declarations

Ethics approval Not applicable.

Consent to participate All authors agree to participate in this study.

Consent for publication All authors allow the publication of the paper.

Competing interests The authors declare no competing interests.

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Authors and Affiliations

Dharman Kalaimurugan\textsuperscript{1,2} · Kandhasamy Lalitha\textsuperscript{3} · Kaliannan Durairaj\textsuperscript{2,4} · Palaniappan Sivasankar\textsuperscript{2} · Sungkwon Park\textsuperscript{5} · Kannan Nithya\textsuperscript{3} · Muthugoundar Subramanian Shivakumar\textsuperscript{3} · Wen-Chao Liu\textsuperscript{6} · Balasubramanian Balamuralikrishnan\textsuperscript{5} · Srinivasan Venkatesan\textsuperscript{2}

1 Division of Biotechnology, School of Agro-Industry, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand
2 Department of Environmental Science, School of Life Sciences, Periyar University, Salem 636011, India
3 Department of Biotechnology, School of Bio-Sciences, Periyar University, Salem 636011, India
4 Department of Infection Biology, School of Medicine, Wonkwang University, Iksan 54538, Republic of Korea
5 Department of Food Science and Biotechnology, College of Life Science, Sejong University, Seoul 05006, South Korea
6 Department of Animal Science, College of Coastal Agricultural Sciences, Guangdong Ocean University, Zhanjiang 524088, P.R. China