Boswellia sacra leaf extract mediated biosynthesis of ZnO nanoparticles: Characterization, photocatalytic and antibacterial activity

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Abstract

The *Boswellia sacra* or Olibanum tree has unique herbal properties in the *Burseraceae* family and has already been considered a versatile material in traditional Arabic medicine. Zinc oxide nanoparticles (ZnO NPs) were synthesized using *B. sacra* aqueous leaf extract as a mediator in the present study. As a critical reducing and stabilizing agent for ZnO NPs, *B. sacra* aqueous extract with reducing polysaccharides and phytochemicals have been used. UV-visible spectroscopy has preliminarily confirmed the formation of ZnO NPs. FTIR, XRD, and SEM analyses were used to classify the photosynthesized nanoparticles. The functional group present in the nanoparticles was analyzed using FTIR. X-ray diffraction has been used to validate the particles' crystalline existence. SEM technique determined the nanoparticles' morphology and crystalline phase of the nanoparticles. The enhanced photocatalytic activity for methylene blue as model pollutant dye under solar irradiation was 84% in 100 minutes. The antibacterial activity of ZnO NPs was tested using the agar diffusion technique against *Staphylococcus aureus*, *Bacillus subtilis* (gram-positive), and *Escherichia coli Pseudomonas aeruginosa* (gram-negative) species. ZnO NPs synthesized using *B. sacra* leaf extract exhibited promising results against Gram-positive and Gram-negative bacterial strains with a maximum inhibition zone of 15 mm and 14 mm, respectively. In conclusion, the results indicated that the protocol is quick, fast, one-step, eco-friendly, non-toxic, and alternative to physical/chemical traditional methods.

Keywords: *Boswellia sacra*, Zinc oxide nanoparticles, biosynthesis, antibacterial activity, photocatalyst

1. Introduction

Nanotechnology is widely considered an innovative and thriving field of research that deals with nanoparticles (NPs) and nanomaterials' synthesis for many of their applications [1]. These nano-sized materials are controlled and manipulated at the atomic scale (1-100nm) [2, 3]. Based on this scale, the distribution and morphology of these materials are expected to possess superior properties [4-6]. Owing to the enhanced properties, the nanoparticles (NPs) have transformed all major industrial domains, from drug delivery to agriculture and the food industry [7, 8]. In recent years, metal and metal oxide nanoparticles have gained enormous attention from researchers due to their unique features such as optical, catalytic, magnetic, and electrical properties [9]. However, Zinc oxide nanoparticles as a semiconductor in group II-VI exhibit a broad energy band of 3.3 eV and a high energy band of 60meV. In addition, zinc oxide nanoparticles feature unique properties such as superior physiochemical stability, enhanced catalytic activity, multi-wavelength

radiation absorption, and non-toxic [10]. The properties mentioned earlier make Zinc oxide highly applicable in chemical sensors, solar cells, and photocatalysis [11].

Furthermore, Zinc oxide nanoparticles have gained a lot of attention due to their antibacterial, antifungal, and also for possessing high catalytic and photochemical activity. In addition, most of the pathological bacteria are efficiently prevented by ZnO nanoparticles, and some studies also indicate substantial antibacterial activity exhibited by ZnO, MgO, and CaO nanoparticles [12]. Nanoparticles (NPs) are typically synthesized by various physical or chemical methods, such as sputtering, milling, the lithographic nanosphere technique, and chemical reduction. These traditional methods used to synthesize metal NPs are costly and harmful to the environment due to their synthesis of various hazardous chemicals responsible for different health risks [13-15]. To start the reaction, most chemical methods require high temperature and high pressure. However, some reactions also need a safe and inert atmosphere and or the use of toxic substances such as H2S, toxic templates and stabilizers, and metallic precursors [16]. The study demonstrates that most of the harmful chemicals we use in physical and chemical methods may lie in the formulated NPs that may prove dangerous in the field of their medical application [17]. Increased knowledge of green chemistry and other biological processes has now contributed to creating an environmentally sustainable approach to synthesize NPs [18]. Recent studies show that plants are ideally suited for large-scale bioproduction of nanomaterials where biosynthesis takes place at a faster pace than in the case of other organisms. Besides, in contrast with those formed by other species such as bacteria, fungi, and algae, nanoparticles produced by plants are more diverse in form and scale11. Many bioactive components of plants, such as flavonoids, terpenoids, alkaloids, glycosides, proteins, enzymes, and vitamins, can also be involved in the bio-reduction, production, and stabilization of nanoparticles metals [19]. Several reports have been published in recent years for the green synthesis of ZnO NPs by plant leaves [18, 20-23].

The Boswellia sacra or Olibanum tree has unique natural properties in the Burseraceae family and has already been considered in traditional Arabic medicine to be a versatile material [24, 25]. In the present study, Zinc oxide nanoparticles (ZnO NPs) are synthesized by a rapid, quick, eco-friendly approach exploring *Boswellia sacra* aqueous leaf extract as a mediator for the first time. In the current research, using Boswellia sacra leaf, extract-based ZnO NPs are synthesized via a simple green pathway. Thus, the biogenic method of synthesis is greener, more cost-effective, safer, and environmentally friendly. FT-IR, XRD, and SEM were used to characterize the ZnO NPs.

Furthermore, the photocatalytic degradation of methyl blue (MB) was investigated using ZnO NPs. Finally, the antibacterial properties of synthesized ZnO NPs were explored and developed as antibacterial agents against a wide range of Gram-positive and Gram-negative bacteria to control and prevent the spreading of bacterial infections. All the results confirm that the ZnO NPs synthesized in the present work are potential candidates for various biological activities. Hence, it can be helpful in the medical industry.

A facile green approach is adopted to synthesize Bs-ZnO NPs using *Boswellia sacra* leaf extract in the present research work. The biogenic method of synthesis is greener, more cost-effective, safer, and environmentally friendly. UV-visible spectroscopy, FT-IR, XRD, and SEM were used to describe the ZnO NPs. In addition, ZnO NPs have been tested for photocatalytic methylene blue degradation (MB).

2. Materials and Methods

B. sacra leaves were collected from Salalah (Oman). Zinc sulfate heptahydrate (ZnSO4.7H2O) was obtained from Fisher scientific (UK) and methylene blue was purchased from Sigma Aldrich (Germany). All the chemicals were of analytical grade and used as received. All solutions were prepared in Millipore water

obtained from Millipore water system (Millipore USA). Nutrient agar and nutrient broth were purchased from HIMEDIA USA. All glassware used for the preparation of ZnONPs were properly washed with distilled water and dried in hot air oven.

3. Plant collection

Fresh leaves were collected from *B. sacra* plants primarily found in the mountainous region of Salalah Oman. Moreover, dust particles were removed by washing leaves several times with distilled water and afterward sun-dried to remove the residual moisture. *B. sacra* leaf extract for bio-reduction of zinc ions was prepared by placing 40g of washed, dried, and powdered leaves in a 500 mL glass beaker along with 200 mL of sterile distilled water. Furthermore, the resulting mixture was boiled for 30 minutes until it changed its color from a colorless to yellow-brown color. Thus, the obtained aqueous solution was filtered using Whatman filter paper no.1 and was cooled to room temperature. The extract was kept in a fridge to be used for further experiments.

4. Green synthesis of ZnO nanoparticles using *Boswellia sacra* plant leaf extract

Green synthesis of ZnO nanoparticles was carried according to a co-precipitation method initially proposed by Singh et al. but with slight modification [1]. Zinc sulfate heptahydrate (ZnSO4.7H2O) and sodium hydroxide (NaOH) were used as the starting material. Briefly, zinc sulfate (100mM) was prepared in a 250mL flask containing 100mL of distilled water under constant stirring. Moreover, after complete dissolution of the mixture, 10 mL of leaf extract and 1M NaOH solution were added dropwise in the flask until pH12 is reached. Thus, a creamy paste of ZnO nanoparticles is observed in the flask. This creamy paste was kept under magnetic stirring for 2 hours. Finally, the creamy paste was filtered and washed repeatedly with distilled water to remove the impurities. An overnight drying of purified paste at 80 C in an oven resulted in a solid white powder. The powder was further subjected to calcination in a muffle furnace at 400 degrees Celsius for 2hours. Thus, the final product was ground to a fine powder for further characterization.

5. Characterization of *Boswellia sacra* based ZnO NPs

Various studies have been made to picture better the physiochemical, optical, thermal, and electrochemical properties of Zinc Oxide nanoparticles [2-6]. Specifically, the appearance of a creamy precipitate during synthesis indicates Zn2+ reduction to Zn^0 and the formation of ZnONPs. However, Employing UV-Vis spectroscopy, FTIR, SEM, and XRD techniques, the ZnONPs were tested and characterized. Initially, the optical analysis of ZnONPs was performed using a UV-Vis spectrophotometer (UV-1900 Shimadzu Japan) with a quartz cuvette as a sample container. SpectrumOne FTIR-ATR infrared spectrometer from PerkinElmer is used to study the zinc oxide nanoparticles (Range 4000-520 cm-1). Microstructure, topography, and size of ZnONPs were measured by electron scanning microscopy (SU3500, Hitachi with spectral imaging system Thermo Scientific NSS (EDS), detector tape (BSE-3D), acceleration voltage (15.0kV), the working distance (11.6 mm), pressure (in the case of variable vacuum conditions) (40 Pa). X-ray diffraction (XRD) analysis was accomplished using an X-ray diffractometer (XRD, Miniflex 600, Rigaku, Tokyo, Japan). The pattern was recorded with Cu Kα radiation in the scanning range from 2° to 100° in 0.05°/s steps.

6. Antibacterial activity of *Boswellia sacra* based ZnO NPs

The agar diffusion method is a rapid, easy, and effective semi-quantitative test for deciding the antibacterial activity of nanoparticles[7, 8]. ZnONPs (50 µg/ml) were tested against both Gram-negative bacteria (E. coli, P. aeruginosa) and gram-positive (S. aureus, B.subtilis) respectively. The suspension of bacteria was grown in a nutrient broth medium. Test organisms were dispersed over the surface of agar plates. A small sample is slightly pushed over the nutrient agar plate canter and inoculated with bacterial cells having close contact with the ZnONPs sample. Further, the plates were incubated at 37 degrees Celsius for 24 hours. The antibacterial activity of ZnO nanoparticles was established by the diameter of the zone of inhibition developed in and around the sample.

7. Photocatalytic activity of *Boswellia sacra* based ZnO NPs

Methylene Blue (MB) dye was used as a model pollutant and subjected to degradation by bio-synthesized ZnONPs to determine its photocatalytic function. Therefore, in a 250 mL reaction solution containing 25 mg mL^{-1} of MB, about 25 mg of ZnONPs photocatalyst was added. As a result, adsorption-desorption equilibrium between the dye and the photocatalyst was achieved by stirring the mixture for one h in the dark. To further, the system was placed in the sunshine after achieving equilibrium and monitored for one hour. Over appropriate time intervals (20 min), 5 ml of the sample was collected and analyzed over a 200-800 nm scan range using a UV-Vis spectrophotometer.

8. Results and discussion

8.1 UV–Vis spectroscopic analysis

UV-Vis spectroscopy is widely accepted as a common technique for the structural characterization of nanoparticles. Therefore, a UV-Vis spectrophotometer (UV-1900 Shimadzu Japan) with a 300-800 nm wavelength range was used with a resolution of 1 nm. Furthermore, 0.5 mL of the reaction sample was put in a 1cm cuvette for examination and diluted with distilled water to 3.5 mL. Moreover, the absorbance of the reaction mixture was supervised for one hour, and absorption measurements were taken every minute, i.e., 10, 20, 30, 40, 50, and 60. The UV-VIS absorption spectrum of a sample of ZnO nanoparticles at different times is shown in Figure 1. Although, according to past findings, ZnO NPs exhibit a representative absorption peak between 330-460 nm [9-13]. However, a single broad peak at 345 nm represents pure ZnO NPs assisted by active biomolecules in the plant extract. Thus, the absorption peak assigned to the intrinsic ZnO absorption may result from surface plasmon resonance [14-16]. Moreover, there is a more excellent absorption in the wavelength range of 340 to 360nm. This blue shift in the absorption edge of the nanoparticles is due to the quantum confinement effect [17-19].

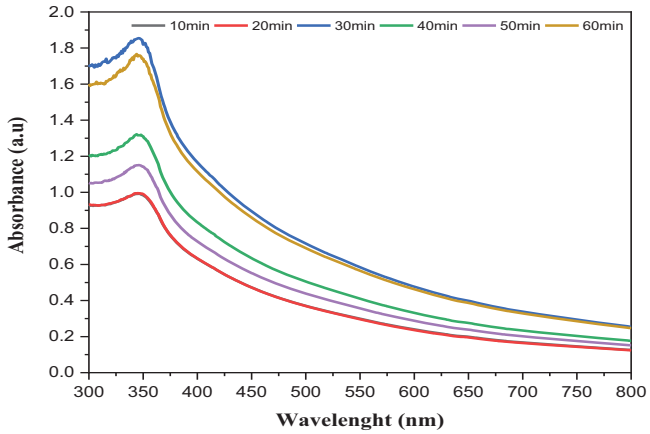

FIG 1. Overlay of UV-Vis spectra of ZnONPs monitored for one hour

8.2 FT-IR study of ZnONPs

The IR technique further confirmed the study outcomes, which showed the changes and variations in the peak areas of the spectrum of Bs-extract and Bs-ZnO NPs. IR spectrum requires the interpretation of the relation between vibrational excitation bands and the chemical species. Moreover, the IR technique is generally employed to identify those phytochemicals responsible for reduction and stabilization during nanoparticle biosynthesis [20-25]. The FTIR spectrum was recorded in the range of 400–4000 cm−1 for Bs-extract and Bs-ZnO NPs. Absorption bands are clearly observed at 3245 cm-1, 1383 cm-1, 1599 cm-1, and 520 cm-1. Bs-ZnO NPS spectrum shows peaks in the region between 400 and 600 cm-1 which strongly confirm Zn-O nanoparticles' presence. The above results imply the importance of various phytochemicals acting as hydrolyzing and stabilizing agents in the green synthesis of metal oxide nanoparticles.

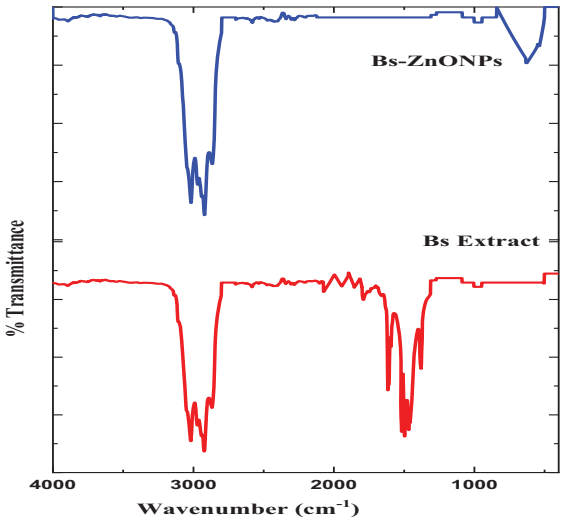

FIG 2. FTIR spectra of (A) Bs- extract and (B) Bs-ZnO NPs

8.3 XRD analysis

For the first time, ZnO NPs synthesis was carried out using B.sacra leaf extract via an eco-friendly biosynthetic approach. Moreover, B.sacra, due to its higher constituents of aromatic hydrocarbons, especially biphenyls, is responsible for ZnO NPs. In biosynthesis, aromatic hydroxyl groups present in B.sacra leaf extract react with Zn2+ to form a reduced complex in strongly basic media of pH12. However, these complexes decompose on annealing at 400 C and lead to the formation of ZnO NPs. To analyze the structural properties of the ZnO NPs and to classify the phase and crystallinity of Bs-ZnO NPs, X-ray powder diffraction (XRD) was used. The diffraction peaks corresponding to the synthesized ZnO NPs correspond well to the stable ZnO's hexagonal wurtzite structure. No additional diffraction peaks were observed corresponding to impurities, suggesting that relatively pure ZnO NPs were obtained. The crystalline peaks located at 2θ of 31.94, 34.66, 36.40, 47.77, 56.72, 62.84, 66.34, 68.09, 69.39, 72.67, 77.15, 81.51 and 89.60 correspond to the indexes (100), (002), (101), (001), (010), (102), (110) and (103). The newly formed ZnO NPs peaks match with the JCPDS NO. 01-080-0074 and confirm the ZnO NPs hexagonal phase (wurtzite structure) without any impurities [26-31].
The average crystallite size of ZnO is found to be 27.83nm which is calculated from the diffraction peak maximum observed at the plane (101), and the crystalline size was calculated by using Scherrer's formula,

$$D = \frac{0.94\lambda}{\beta\cos\theta}$$

D is the crystalline size, λ is the X-ray wavelength, β is the full-width half maximum of the peak **FIG 3.** XRD array pattern of ZnO nanoparticles. The peak position of ZnO in the JCPDS card and in freshly prepared products of ZnO nanoparticles same as shown in the graph.

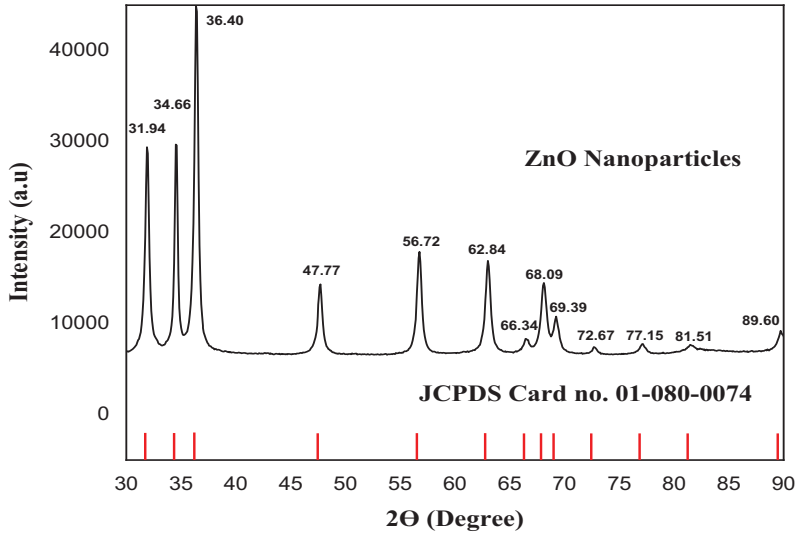

FIG 3. XRD spectrum of ZnO nanoparticles prepared by using B.sacra leaf extract as reducing agent

8.4 SEM analysis

Morphological and structural characterization of prepared zinc oxide nanoparticles using the leaf extract of B.sacra plant were performed using SEM. Typically, ZnO NPs were observed to be transcribed as homogenous, agglomerated, and free of impurities. However, this agglomeration is due to the polarity and electrostatic attraction of ZnO nanoparticles. Moreover, SEM images are explored to anticipate the morphology of zinc oxide nanoparticles. ZnO Nanoparticles SEM images are shown in the Figure under various magnifications. A hexagonal wurtzite structure, characteristic of a ZnO, is confirmed. However, the boundary of the single nanoparticles cannot be regarded by intense observation of the SEM images. Although, the particle size of the synthesized ZnO NPS is in close agreement with the previous findings [32-35]. However, most nanoparticles are spherically shaped, with aggregation, and the average size of approximately 30 nm has been confirmed by intensive observation of the SEM images.

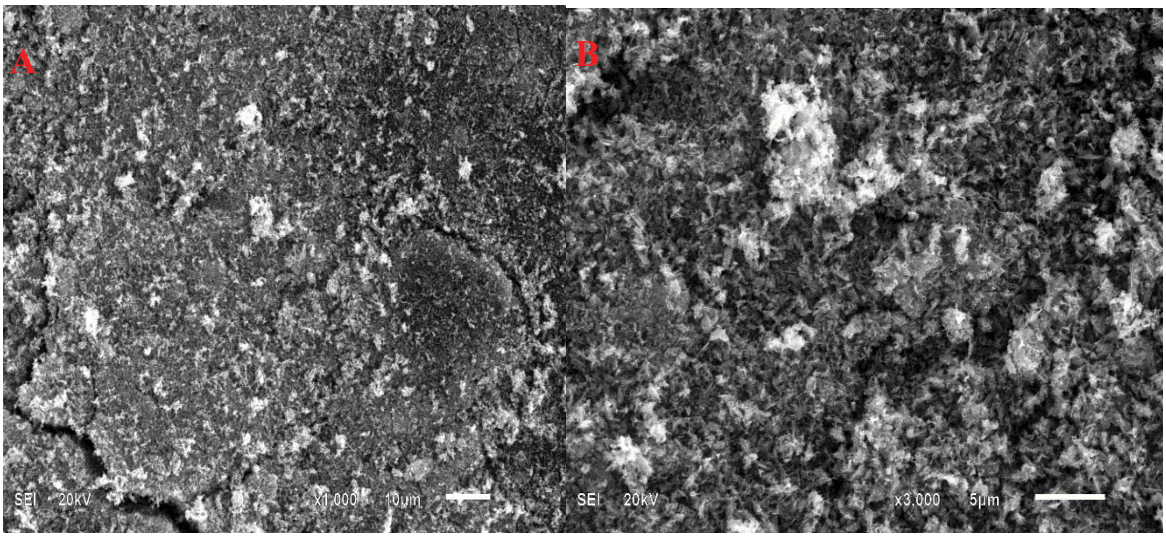

FIG 4. SEM images of ZnO nanoparticles at different magnifications (A) at 10µm (B) at 5µm

8.5 Antibacterial activity

The *Boswellia sacra* leaf extract synthesized ZnO was further studied for its antibacterial activity. Moreover, the agar diffusion method is applied to determine the antibacterial activity and its robustness, ease, and efficacy. Bs-ZnONPs with varying concentrations were tested against gram-positive and gram-negative bacteria, namely Bacillus subtilus (B. subtilus) [+], Staphylococcus aureus (S. aureus) [+], Escherichia coli (E. coli) [-], Pseudomonas aeruginosa (P. aeruginosa) [-] respectively. The sizes of the zones of growth inhibition are presented in Table 1.

In the current investigation, the inhibition zone size was different amongst pathogens. It was further investigated that on increasing the concentration of BS-ZnO NPs, the inhibition zone size also increased, and this was in agreement with Sangeetha et al., who stated that by increasing the concentration of ZnO nanoparticles in wells and discs, the growth inhibition has also been increased consistently because of proper diffusion of nanoparticles in the agar medium. Premanathan et al. reported that Gram-positive bacterium such as S. aureus is more susceptible to ZnO NP toxicity than Gram-negative bacteria such as E. coli and Pseudomonas aeruginosa, which may be attributed to the difference in bacteria's cell membrane structure

that controls the toxicant's access to sites of action. According to Divya et al., 2013, ZnO nanoparticles disrupt bacterial membranes probably by producing reactive oxygen species (ROS), such as superoxide and hydroxyl radicals. Also, ZnO nanoparticles could be attributed to the bacterial cell membrane's damage and the cytoplasmic contents extrusion, resulting in the bacterium's death. Moreover, ZnO nanoparticles have positive zeta potential at their surface. Based on the research, one can conclude that the inhibition of bacterial growth by ZnO nanoparticles could be attributed to the damage of the bacterial cell membrane and the extrusion of the cytoplasmic contents, thereby resulting in killing inhibition of cell growth of bacteria.

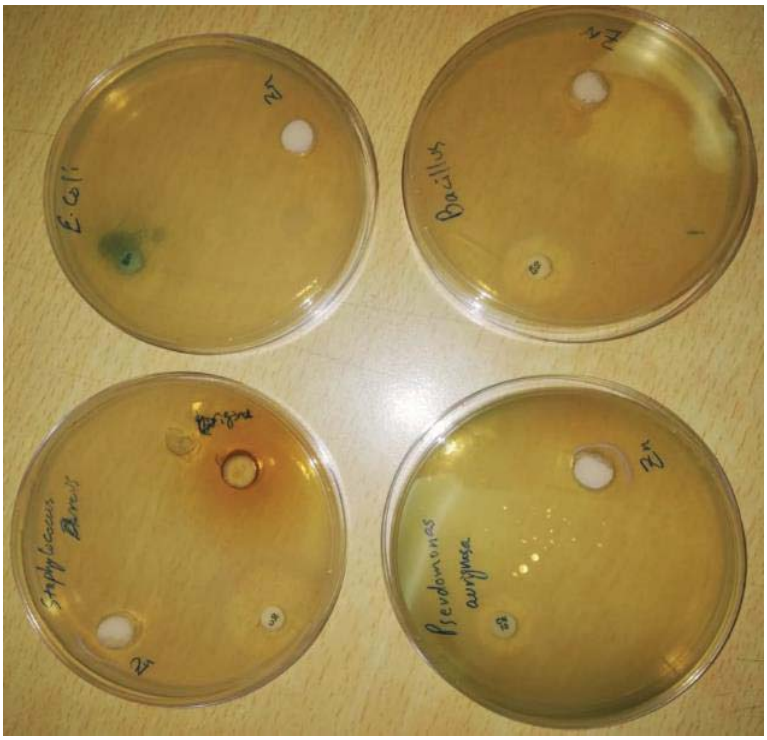

FIG 5. Inhibition zone (mm) study of different pathogenic microorganism against various antibacterial agents and ZnO NPs.

Table 1. Antibacterial activity of ZnO NPs synthesized from B.sacra against Gram-negative and Gram-positive bacteria.

Pathogens	Control	Inhibition zone(mm) at various concentration(µg/mL)			
		20	40	60	100
B.subtilus	28	14	17	19	25
S.aurous	29	15	16	21	27
E.coli	29	13	17	25	31
P.auregonosa	25	11	14	17	24

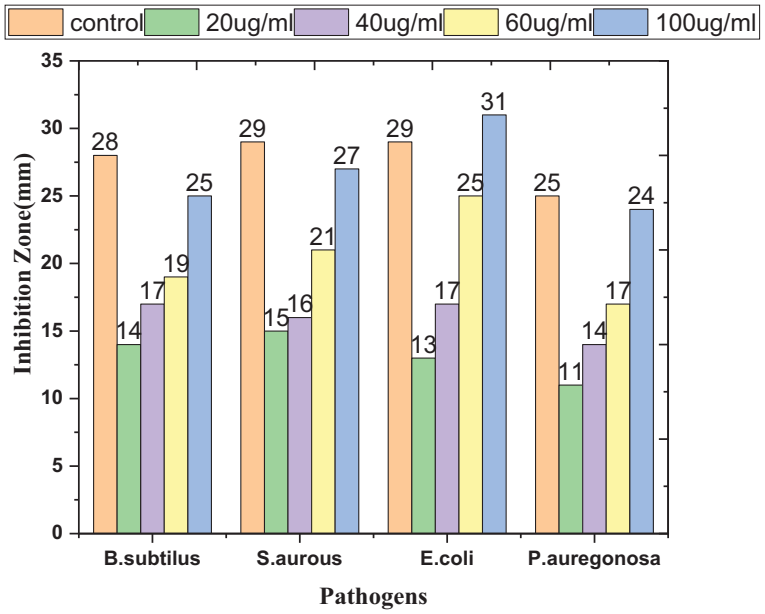

FIG 6. Bar chart representation of antibacterial activity of ZnONPs with varying concentration

8.6 Photocatalytic activity of ZnONPs

The photocatalytic activity of the ZnONPs was substantiated towards photodegradation of Methylene blue (MB) dye in an aqueous solution. The optimum conditions reported by Vidya and co-worker were rigorously followed[60]. Moreover, Methylene blue (MB) dye being a heterocyclic aromatic compound is selected as a model pollutant. Fig. depicts the UV–Visible absorption spectrum of MB dye shows two absorption bands at 612 and 663nm characteristic to phenothiazine derivatives[61]. The degradation process was monitored every 20min for 100 minutes for a 1.5g/L MB dye solution containing 50mg of ZnO nanoparticles as a photocatalyst. Furthermore, an indication of MB dye degradation, the characteristic absorption peak at 663nm contracted stepwise without shifting in the maximum absorption wavelength. Although, no visible change in the peak intensity is observed even after 100 min. The reduction efficiency of MB dye remained well above 84%. This improved degradation of MB by ZnONPs can be attributed to the efficient size control and stability provided by B.sacra leaf extract, which provides the sizeable catalytic surface area. Moreover, ZnONPs proved to be an efficient photocatalyst for Methylene blue degradation.

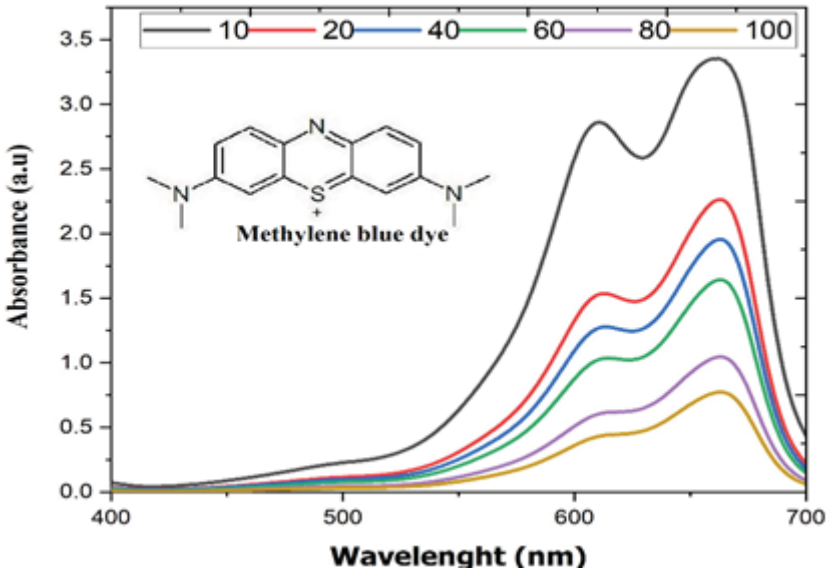

FIG 7. UV-Vis spectra of methylene blue degradation with respect to time

The methylene blue degradation (%) was calculated by following formula

$$\% \text{ Degradation} = \frac{(Co - Ct)}{\text{Co}} \; x \; 100$$

Where C_o is initial concentration and C_t is final concentration after sunshine exposure.

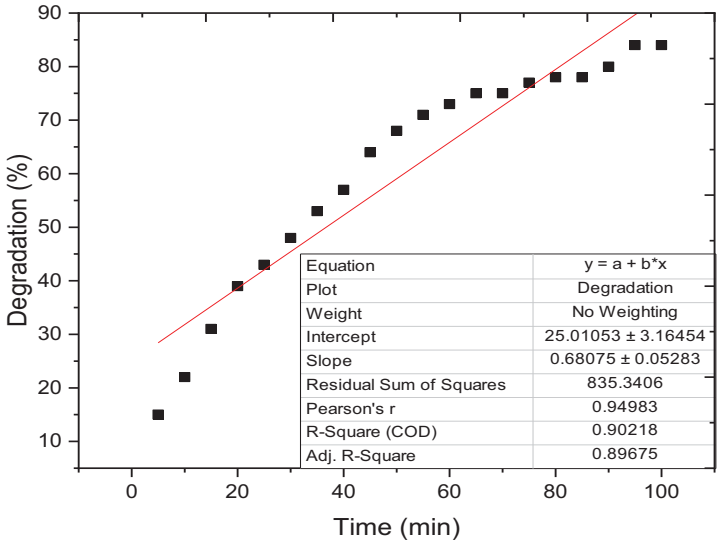

FIG 8. Methylene blue degradation % with time

9. Conclusion

The present research work accomplishes that ZnO NPs can be rapidly biosynthesized using B.sacra leaf extract and are economical, non-toxic, and eco-friendly with an average size of 27.83 nm exhibiting wurtzite structures. The synthesized ZnO nanoparticles were characterized by UV-Vis absorption spectroscopy, Fourier transforms infrared spectroscopy (FT-IR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). These methods confirmed the presence of the synthesized ZnO nanoparticles in the range of 25–30 nm. Furthermore, the photodegradation efficiency of MB was 84% within 100 min of sunlight exposure, and biogenic ZnO NPs obtain such an excellent activity. It was also confirmed that ZnONPs are excellent photocatalysts for the degradation of Methylene blue dye. Furthermore, the synthesized ZnO nanoparticles exhibited high activity against B.subtilis, S.aureus, E. coli, P. aeruginosa; the green synthesis of ZnO nanoparticles using B.sacra leaf extract can be an excellent substitute to chemical methods.

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