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Ocimum sanctum Derived Zinc Oxide Nanoparticles, Physico-chemical Optimization and Mechanism of Antimicrobial Property

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ABSTRACT

The findings were firstly focused on the biomimetic synthesis of zinc oxide nanoparticles through aqueous leaf extract of *Ocimum sanctum*. The physico-chemical stability was achieved by optimizing various parameters. The absorption spectra at 400 nm were observed for zinc oxide nanoparticles. The atomic force microscopy analyzed the morphology of the nanocomposite, which was further confirmed through TEM micrograph. AFM analysis concluded that nanoparticles were non-agglomerated and polydispersed, as confirmed by two and three dimensional distribution views. The electron micrograph image disclosed that zinc oxide nanoparticles were polydispersed and dominantly as spherical with a size around 106 nm. The chemical reductions were further confirmed through FTIR analysis. The biogenic zinc oxide nanoparticles and their drug formulation showed profound antibacterial activity against pathogenic bacteria. The rich binding of zinc oxide nanoparticles showed potential medicinal effects that can be used to treat several harmful infectious diseases. Hence, plant based metal oxide nanoparticles met the demand for less toxic formulations during drug development and delivery.

Key words: Nanoparticles, antibacterial activity, biomimetic, electron microscope, optimization

INTRODUCTION

The development of green nanotechnology is generating interest in researchers towards the eco-friendly biosynthesis of nanoparticles. ZnO nanoparticles have diameters less than 100 nanometers and a large surface are relative to their size and high catalytic activity (Jayachandran *et al.,* 2021). Metal nanoparticles are of great scientific interest as they are, in effect, a bridge between bulk materials and atomic or molecular structures (Chopra *et al.,* 2022). Nanoparticles are tiny materials classified into different classes based on their properties, shapes, or sizes (Labulo *et al.,* 2022). Based on their physical and chemical characteristics, some well-known classes of NPs are: Carbon-based NPs, metal NPs, ceramic NPs, semi conductor NPs, polymeric NPs and lipid-based NPs. The nanoparticles may be produced naturally, incidentally, or can be engineered. Various physico-chemical properties such as large surface area, mechanically strong, optically active and chemically reactive make NPs unique and suitable for various applications (Joudeh and Linke, 2022). Synthesis of NPs using plants is very cost-effective, and thus can be used as an economical and valuable alternative for the large-scale production of NPs (Ahmed *et al.,* 2023). Employing plants the synthesis of nanoparticles is emerging as advantageous because of the broad variability of bio-molecules in plants that act as capping and reducing agents and thus increase the rate of reduction and stabilization of

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nanoparticles (Ronavari *et al.,* 2021). Biomimetics is an interdisciplinary field in which, chemistry and biology principles are applied to the synthesis of materials and synthetic systems with functions that biomimetic biological processes. Zinc oxide is an inorganic compound with the molecular formula ZnO. It appears as a white powder and is nearly in soluble in water. These nanoparticles are relatively biocompatible, which supports their biomedical applications (Salih *et al.,* 2021). Metallic ion nanoparticles are used in increasing industrial products such as rubber, paint, coating and cosmetics (Ohara *et al.,* 2020). In the past two decades, zinc oxide nanoparticles have become one of the most popular metal oxide nanoparticles in biological applications due to their excellent biocompatibility, economics and low toxicity (Faisal *et al.,* 2021). ZnO-NPs have emerged a promising potential in biomedicine, especially in anticancer and anti-bacterial fields, which are involved with their potent ability to trigger excess reactive oxygen species (ROS) production, release zinc ions and induce cell apoptosis (Singh *et al.,* 2020). The current study focused on optimizing the physico-chemical parameters for stable synthesis of zinc oxide nanoparticles using *Ocimum sanctum* aqueous leaf extract.

MATERIALS AND METHODS

The zinc acetate salt ($Zn(CH_3COO)_2 \cdot 2H_2O$) was procured from Sigma-Aldrich (Bangalore, India). The nano synthesis was achieved using 0.4 M salt dissolved in 25 ml of double distilled water under continuous stirring at 80°C with 4000 rpm for 30 min. The extracellular approach was applied to prepare an aqueous leaf extract solution of *O. sanctum*. The freshly collected leaves of *O. sanctum* were washed several times with distilled water before being cut and chopped into appropriate size (1×1 cm). The 10 g leaves were added to 100 ml of distilled water in a 250 ml conical flask. The boiling was done to achieve the extract at 80°C for 20 min. The obtained crude extract was filtered through Whatman filter paper no.1, and the supernatant was stored at 4°C for further use (Singh *et al.,* 2020).

An eco-friendly approach was followed to synthesize zinc oxide (ZnO) nanoparticles. For the chemical reduction process of Zn^{2+} ions, 5 ml filtrate was added to 30 ml of $10^{"3}$ M $ZnNO_3$ solution in a 250 ml flask and kept on a rotary shaker (120 rpm) at 30°C. The plant filtrate was used as both a reducing and stabilizing agent. The physico-chemical optimization was studied for maximum synthesis of metal nanoparticles. The different parameters selected were mixing ratio (1:6, 1:7, 1:8 and 1:9), reaction temperature (50, 60, 70 and 80°C and reaction time (0, 1, 2, 4, 5, 6 and 24 h) (Sharma *et al.,* 2021). To achieve the maximal recovery of zinc oxide (ZnO) nanoparticles, a centrifugation process was performed at 8,000 rpm for 8 min to remove the plant debris and unwanted components in the solution. This process was performed thrice to get a pure form of nanoparticles. The supernatant was collected and frozen at "70 °C for 45 min for two days using Lyophillizer (Micro Modulyo 230) freeze dryer. The absorbance pattern of silver nanoparticles was analyzed using a UV-visible spectrophotometer (UV-Vis), and the readings were recorded at solution of 1 nm between 300 and 700 nm in a 10 mm path-length quartz cuvette. Atomic Force Microscopy (APEresearch:AI00SGS;USA) was analyzed to image the topography of soft biological materials in their native environments. The nanomaterial's particle size analysis and distribution were analyzed using a zeta sizer PALS (Phase Analysis Light Scattering) zeta potential analyzer ver.3.54. The topographic information and surface chemistry of the sample were assessed through TEM. Samples were prepared for the analysis according to the previously reported protocol (Singh *et al.,* 2020). Antibacterial assay was performed to study the effect of ZnO nanoparticles, an amino glycoside antibiotic i. e. gentamicin, and its nanoconjugation against *E. coli* and *P. aeruginosa* using a disc diffusion assay. To determine the combined effect of standard drugs (gentamicin), the paper disc was further impregnated with 20 µl of the freshly prepared ZnO nanoparticles. The agar plates were left for an hour at 25°C to allow diffusion to reduce the effects of variation in time between the applications of different solutions. The culture plates were incubated at 37°C for 24 h to determine the zone of inhibition. Three parallel tests were run with the standard to avoid any errors (Zhang *et al.,* 2023).

RESULTS AND DISCUSSION

The change in colour from white to light yellow was the confirmatory feature for ZnO nanoparticles formation due to the excitation of surface plasmon vibrations (Fig. 1). The spectra showed a well-defined surface plasmon band between 300-450 nm, which was characteristic of ZnO nanoparticles and indicated the formation of nanoparticles in solution (Fig. 2). In the case of zinc ions reduction, the band corresponding to the surface plasmon resonance (SPR) for *O. sanctum* occurred at 400 nm. It was also observed that the maximum absorbance occurred at 400 nm and steadily decreased in intensity as a function of reaction time (Fig. 3). The ZnO nanoparticles solution was found to be extremely stable for *O. sanctum,* with little evidence off locculation of the particles even a month after the reaction. The resonance was sharp and indicated little aggregation of ZnO nanoparticles in solution (Kumar *et al.,* 2020). The findings were completely supported by Singh *et al.* (2021), who also reported that poly dispersed nanoparticles absorbed the wavelength in the NIR region of the electromagnetic spectrum, which corresponded to the longitudinal surface plasmon absorption.

The final absorption intensity at 400 nm steadily increased with time duration, and the final absorption was 1.856 a.u. after 24 h of synthesis. The absorption spectra decreased sharply with a change in wavelength.

The effect of different mixing ratios was studied in terms of intensity. At different mixing ratio concentrations the sharpness in the absorbance intensity of the plasmon peak changed rapidly by varying mixing ratio concentrations 1:6, 1:7, 1:8 and 1:9. The maximum absorbance was achieved with a 1:6 mixing ratio. The initial absorption intensities at 350 nm for 1:6 concentrations were near 1.5 a.u. after 24 h of synthesis. The maximum absorbance was found to be 3.72 a.u. at 400 nm (Fig. 4a). The absorbance pattern for the 1:7 mixing ratio was near 2.2 a.u. after 24 h of synthesis at 400 nm (Fig. 4b). The maximum absorbance was 2.9 a.u. after 2 h of synthesis, but the synthesis was highly unstable with a change in wavelength from 350 to 500 nm. The sharp and intense peak was seen after 3 h of synthesis with 1:8 mixing ratio, as shown in Fig. 4c. The metal nanoparticles displayed sharp absorption after 1 h of synthesis, the stability gradually decreased with a change in wavelength and time duration with 1:9 mixing ratio in Fig. 4d. The absorption spectra exhibited a gradual decrease in absorbance with a shift in wavelength.

The effect of temperature was continuously monitored for optimal synthesis of zinc nanoparticles for different mixing ratios (Fig. 5). A different absorbance pattern was noticed, but maximum absorbance of 2.79 a.u. was obtained at 60°C with a 1:8 mixing ratio.

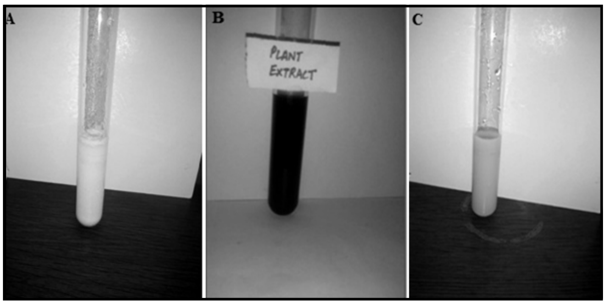

Fig. 1. (A) Milky white colour due to mixing of ZnO, (B) Dark brown colour from aqueous leaf extract of *Ocimum sanctum* and (C) Cream colour indicating the formation of ZnO-NPs.

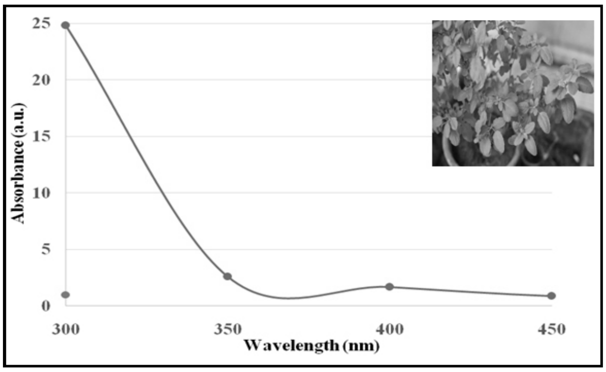

Fig. 2. Absorbance spectra of ZnO-NPs prepared using aqueous leaf extract solutions with 0.4 M zinc acetate precursor.

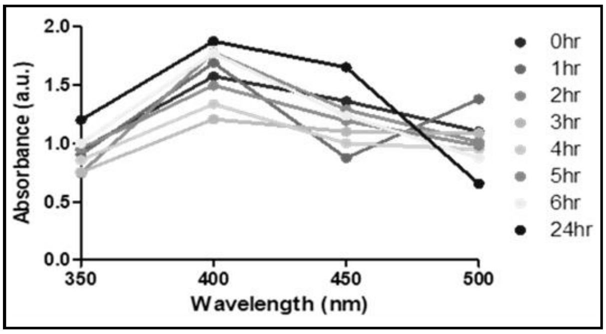

Fig. 3. UV-Vis spectra recorded for various time intervals.

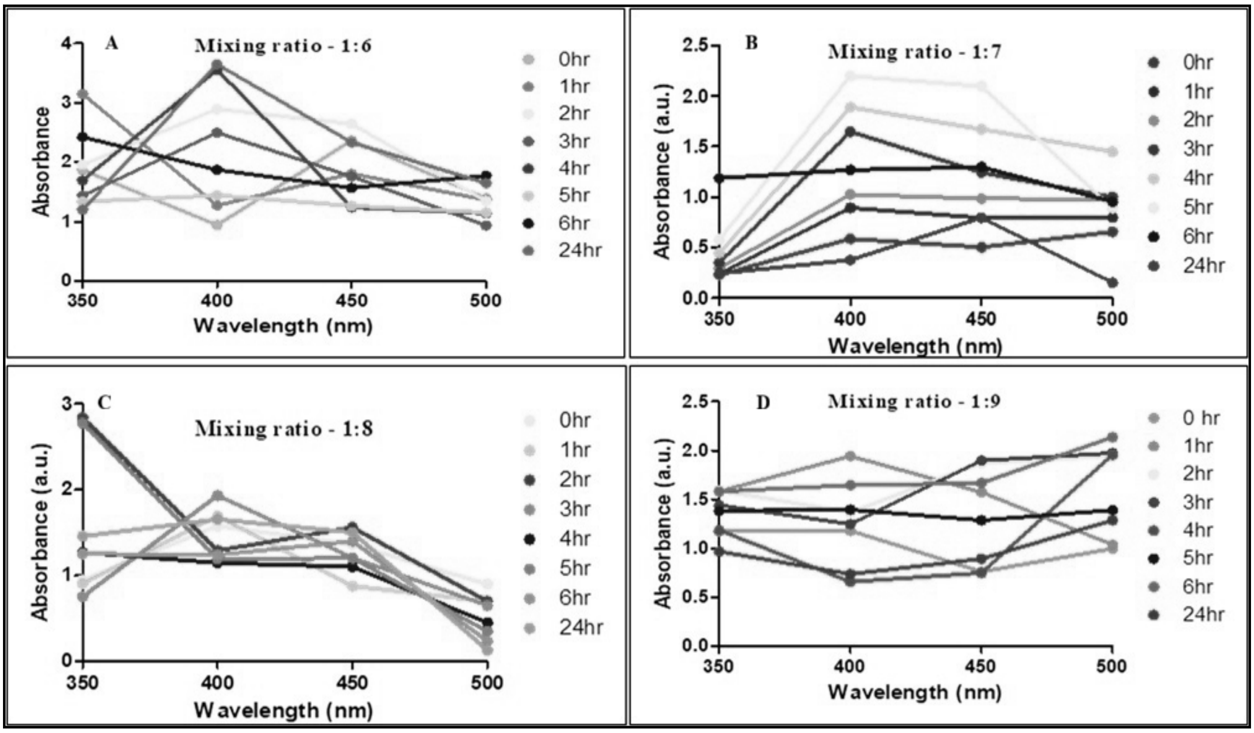

Fig. 4. Effect of mixing ratio (A) 1:6 ratio on synthesis of ZnO NPs, (B) 1:7 ratio on synthesis of ZnO-NPs, (C) 1:8 ratio on synthesis of ZnO-NPs and (D) 1:9 ratio on synthesis of ZnO NPs.

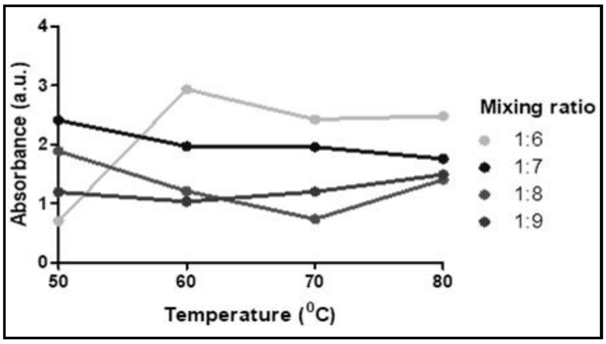

Fig. 5. Effect of temperature on ZnO NPs synthesis under various mixing concentrations.

The effect of mixing concentration of salt and plant extract solution confirmed that at a 1:8 mixing ratio, the maximum synthesis was achieved (Baran *et al.,* 2023). The synthesis rate concerning temperature was maximum at 60 °C for Zinc oxide nanoparticles.The change in the absorbance with the reaction temperature and mixing ratio was also supported by (Manikandan *et al.,* 2020).

The biofunctionalized zinc nanoparticles formed from the aqueous solution of 0.4 M (Zn $(CH_3COO)_2{\bullet}2H_2O$) with 5% aqueous leaves extract of *O. sanctum* at 60° C temperature was characterized for particle size distribution. The peak number and peak amplitude obtained explained the size and size distribution of zinc oxide nanoparticles. The calculated particle size distribution by intensity was observed in the 10-100 nm range (Fig. 6). It can be seen that the mean particle size was *ca.*49.63 nm, with some particles having diameters 100 to 1000 nm. The poly dispersity index (pdI) was 0.418.

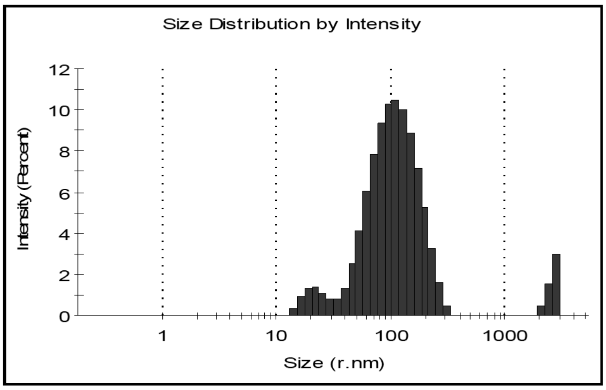

Fig. 6. DLS size distribution of zinc oxide nanoparticles obtained from reduction of 0.4 M zinc nitrate at reaction temperature 60°C.

A mixture of the plate (spherical and hexagons) and spheres was obtained at 60°C (Fig. 7). Representative TEM images revealed that the size distribution of ZnO-NPs was in the range of 40 to 100 nm. The high-resolution TEM

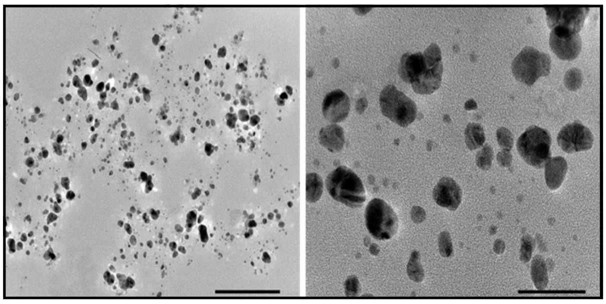

Fig. 7. TEM micrographs showing particle distribution 0.4 M $ZnNO_3$ and 5% *O. sanctum* leaf broth at reaction temperatures at 60ºC.

displayed clear lattice fringes on the particle surfaces.

The AFM images of zinc oxide nanoparticles at 60°C were viewed for two and three-dimensionals. The AFM measurements were made using the tapping mode developed especially for studying bio-functionalized samples. Fig. 8 shows an AFM image of one of the regions that consisted of a rich concentration of organic moieties at the surface. From the topographical view, it was evident nanoparticles were highly poly dispersed with maximum size distribution between 0 to77 nm.

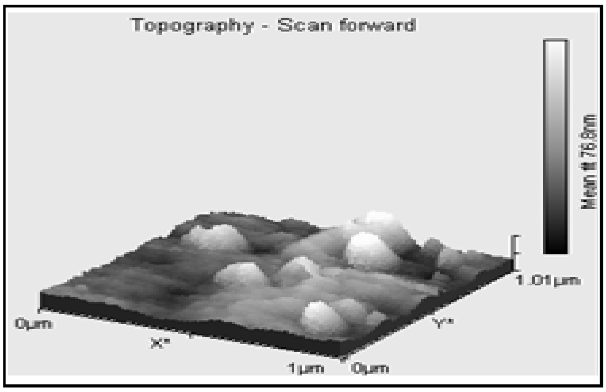

Fig. 8. AFM image of zinc oxide nanoparticles synthesized at 60°C from reaction mixture of 5% aqueous leaves extract by *Ocimum sanctum* and 0.4 M $ZnNO_3$ solution.

FTIR analysis was used for the functional group characterization of the silver nanoparticles optimized at 60°C (Fig. 9). The absorbance bands were observed in the region of 500-4500/cm. The strong, intense peaks were at 3445, 2077 and 1633/cm, respectively. The maximum intense peak of 3445/cm corresponded to the stretching mode of vibration of the amine group. The intense peak at 2077/cm corresponded to the stretching mode of vibration of aldehydes, and the intense peak at 1633/cm corresponded to the carbonyl group, flavonoids and steroids group. The bio-organic like sterols, hydro carbons, calcium compounds, flavonoids, lignin glycosides, lyoniside, tannins, quercetin, alkaloids, alcohol, and beta-sitosterol of the leaves worked as the capping for nanoparticles. These pigments had reductive properties and get released into solution by diffusion (Kaur *et al.,* 2023).

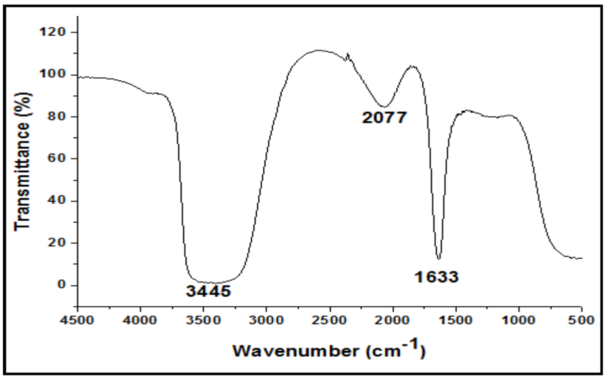

Fig. 9. FTIR spectra of silver nanoparticles.

Next, evaluated the antibacterial susceptibility of rapid biologically synthesized *O. sanctum* zinc oxide nanoparticles and commercial drugs against Gram-negative (*E. coli)* and Gram-positive (*S. aureus)* bacteria. The wells diffusion method evaluated the zone of inhibition (Sana *et al.,* 2020). The surface chemistry and size pattern of metallic ions of the nanometer range helped to investigate silver nanoparticles efficacy as drug carriers. To understand the mechanisms, a comparative analysis of drugs formulated with nanoparticles was performed. The *in vitro* antibacterial activity of gentamicin, along with their nanoformulation, was tested against bacterial strains. The zone of inhibition for gentamicin formulated zinc oxide nanoparticles for *E. coli* was more significant. Whereas, the zone of inhibition for nano formulated drug against *E. coli* (<“28 mm) and *S. aureus* (<“18 mm) was highly significant than its pure form (Fig. 10). The composition of the substance in the cell wall was used to explain the underlying mechanism against bacterial membranes. Thus, in the case of gram-negative bacteria, easier permeability could be achieved (Al-Mohaimeed *et al.,* 2022). When metal nanoparticles and drug formulations binded to bacterial cells, they caused structural changes

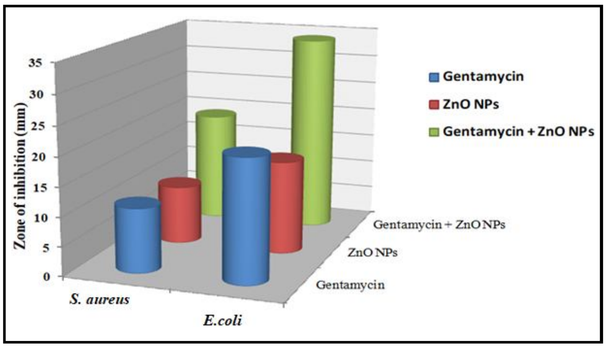

Fig. 10. Graphical representation of zone of inhibition for ampicillin drug mixed with AgNPs against the test bacteria.

in the cell membrane and blocked transport channels (Sharma *et al.,* 2024). The most likely mechanism of inhibition was the loss of the ability to copy genetic material and the immobility of specific cellular proteins and enzymes essential for ATP generation. Once the zinc nanoparticles were firmly bound to the cell surface, they accumulated in the microbial cell wall, causing the degradation and disintegration of the cell membrane. Due to their nano scale size, they easily penetrated the cell membrane, leading to the disturbance of cellular organelles and cell lysis. Moreover, they affected the bacterial transduction process by interfering with the phosphorylation of protein substrates, resulting in cell death and growth. Gram-negative bacteria were more vulnerable to the effects of AgNPs because they had thinner cell walls compared to Gram-positive bacteria (Singh *et al.,* 2022).

CONCLUSION

Seeing the increasing demand for these highly unpredictable and amoebic types of zinc oxide nanoparticles and the best possible medical application, successfully biosynthesized the zinc oxide nanoparticle using *O. sanctum*. Highly poly disperse zinc oxide nanoparticles were synthesized using leaf extract of a well-known medicinal herb, *O. sanctum*. The synthesis was found to be efficient in terms of reaction time and the synthesized nanoparticles stability. The overall results obtained during this work showed that the metal nanoparticles had promising results in growth kinetics against Gram-negative bacteria. This exploration created a new avenue to the standard where the different carotenoids and alkaloids-functionalized metal nanoparticles can be a powerful weapon in the field of nanomedicine. Thus, developing a biosynthetic approach and biofunctionalized targeted metal nanoparticles as therapeutic agents will generate great interest in both academy and industry.

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