

Antimicrobial Potential of Phytosynthesized Zinc Oxide Nanoparticle from *Ocimum Basilicum* Var *Thyrsiflora*

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Recent developments in nanotechnology and nanoscience have radically changed the way how we recognize, control, and prevent an array of diseases in all aspects of human life. Nanoparticles frequently synthesized chemically and used as reducing agent subsequently become responsible for a variety of biological hazards due to their overall toxicity. To resolve this problem biological ways are emerging to fill the void left by chemical approaches. The present study deals with the biosynthesis of zinc oxide nanoparticles using the *O. basilicum* var *thyrsiflora* extract for their biomedical application. Biosynthesized ZnO-NPs were characterized by UV-vis, X-ray diffraction (XRD), Field Emission Scanning Electron Microscope (FE-SEM), Energy-dispersive X-ray (EDX) spectroscopy, and Fourier transform infrared (FT-IR) spectroscopy. The ZnO-NPs are well-crystallized and nanosized, as seen by the sharp and narrow diffraction peaks across the spectrum. The biosynthesized ZnO-NPs morphology was confirmed by FE-SEM analysis to have a uniformly distributed rod-like (cylindrical) shape (mean diameter of around 20.06 nm). The formation of highly pure ZnO-NPs was verified by EDX analysis. The biogenic ZnO-NPs showed strong antimicrobial action against gram-positive & gram-negative bacterial and fungal strains. The results show as compared to bacterial strain fungal strain is more effective.

Keywords: Nanoparticles, Zinc oxide NP, Antimicrobial activity, green synthesis.

1. Introduction

Nanotechnology opens up a new field utilized with phyto therapeutics for the development of innovative drug delivery methods¹. In the past few years, herbal remedies have become known for their effectiveness in treating a range of medical ailments. Exploration and use of nature have increased in the search for novel and improved medicines, especially for the development of antibacterial, anti-diabetic, and anticancer agents². Green synthesis is a safe, eco-friendly, and biocompatible method of synthesizing nanoparticles for a variety of uses, including the biomedical field. Phyto-based nanoparticle green synthesis is now regarded as the gold standard across sustainable biological techniques because of its versatility and ease of use³. The metal and metal oxide nanoparticles are also good nanocarriers for the therapies thus the risk of the pharmaceuticals degrading too soon is reduced. The benefits of utilizing metallic &

metal oxide nanoparticles in drug delivery systems include enhancing drug carrier stability and half-life⁴ in circulation, essential biodistribution, and passive or active targeting into the target region⁴. metal and metal oxide (MO) NPs are thought to be anticipated substances based on their properties such as solubility, chemically stable, adhesion, and surface plasmon resonance⁵⁻⁷. ZnO, CuO, Ag₂O, Fe₂O₃, CaO, NiO, and MgO NPs have garnered significant interest in the literature recently for their antibacterial properties⁸. Among them, ZnO-NPs are the fascinating inorganic substance. ZnO-NPs are applicable to a number of industries, including chemical sensing, textiles, electronics, medical treatment, catalytic activity, and skincare⁹⁻¹³. Additionally, ZnO is viewed as a potential competitor, especially for applications related to antimicrobial and antioxidant α -amylase inhibitory action, biosensors, cell imaging, anti-inflammatory, anti-cancer, and anti-diabetes¹⁴⁻¹⁹. A number of species, including *Parthenium hysterophorus*²⁰, *Aloe barbadensis*²¹, *Ocimum basilicum*²², Trifoliate orange²³ (*Poncirus trifoliata*) *Aeromonas hydrophila*²⁴, *Borassus flabellifer* fruit²⁵, *Solanum nigrum*²⁶, *Tamarindus indica*²⁷, *Plectranthus ambonicus*²⁸, *Caltropis procera*²⁹, *Moringa oleifera*³⁰, *Passiflora caerulea*³¹, and *Garcinia mangostana*³² have all been reported to biosynthetic zinc oxide nanoparticles. Here, we describe the synthesis of zinc oxide nanoparticles from plants using *Ocimum basilicum* var. *thyrsoflora* also known as siam queen (Thai basil) stem extracts. Green ZnO-NP synthesis offers several biomedical uses as well as environmental-friendly features. The metabolites present in *O.basilicum* var *thyrsoflora* extract function as capping, reducing, and oxidizing agents during the generation of biogenic ZnO-NPs. Environmentally friendly synthesized nanoparticles will be characterized with innovative techniques including Fourier transform infrared (FTIR), ultraviolet (UV), scanning electron microscopy (SEM), Energy-dispersive X-ray analysis (EDX), X-ray diffraction (XRD). The NPs will be examined for potential biological uses, such as antimicrobial properties.

2. Materials and methods

2.1 Siam queen stem extract preparation

The plant *Ocimum basilicum* var *thyrsoflora* (Thai basil) was collected from different places of Dhamtari Chhattisgarh India. 10 g of *o.basilicum* var.*thyrsoflora* stem was washed thoroughly and ground in a pestle and mortar. The stems were then dissolved in 100 mL of de-ionized water. The solution was then consistently stirred, and the stem extract was decanted through Whatmann No.1 filter paper. The obtained extract was used as a reducing agent in the nanomaterial preparation process.

2.2 Biogenic Synthesis of Zinc Oxide nanoparticle

Zinc nitrate Zn (NO₃)₂ and *O.basilicum* var *thyrsoflora* stem extract were taken as precursors. The Zinc oxide nanoparticles were prepared by the hot plat combustion method. In a typical process, 0.1 M of Zinc nitrate was dissolved in 90 mL of water and stirred for 30 min. 10 mL of the stem extract was added dropwise to the above solution under vigorous stirring for 1 h. The obtained homogeneous solution was heated by placing it on a hot plate heater set to 60 °C. The resultant powder was dried in a hot air oven at 120 °C. Finally, the resulting Zinc oxide was annealed at 600 °C for 4 h.

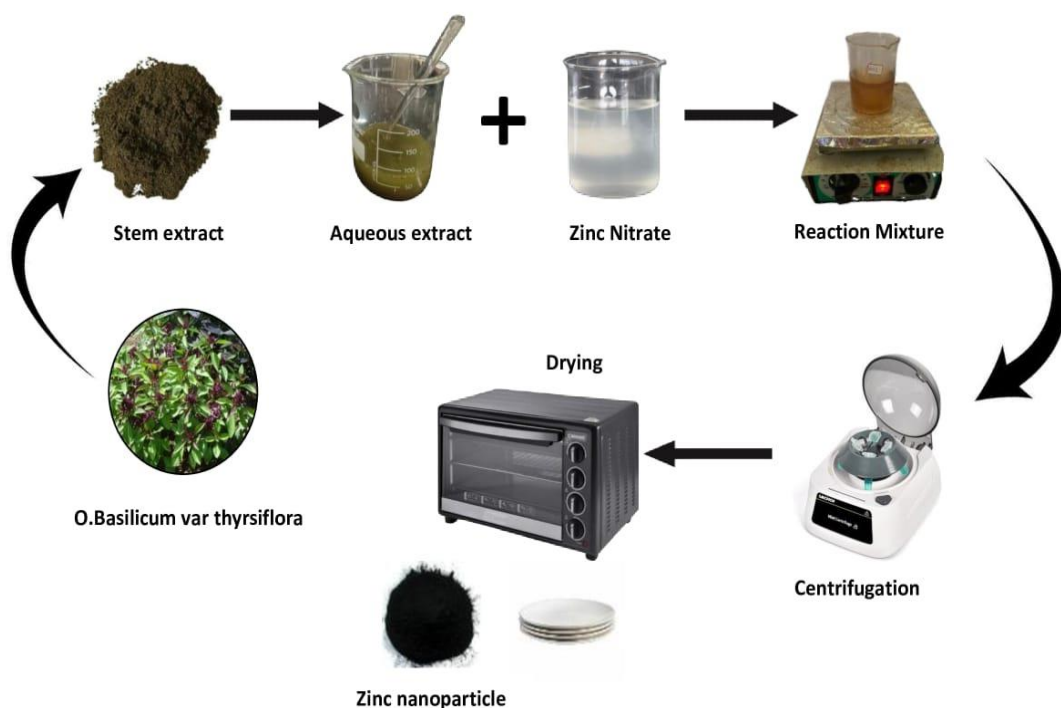


Fig 1 Systematic representation of siam queen synthesized zinc oxide nanoparticle.

2.3 Tested microorganisms

The antimicrobial activity of prepared nanomaterial was employed by using the agar well diffusion method and tested against gram-positive *S. aureus* and gram-negative *Klebsiella pneumoniae* bacterial strain and for one fungal strain *Aspergillus Niger*.

2.4 Kirby Bauer agar well diffusion assay

The agar medium was prepared and sterilized by autoclaving at 121°C 15 lbs pressure for 15 minutes then aseptically poured the medium into the sterile petriplates and allowed to solidify the Bacterial broth culture was swabbed on each petriplates using sterile buds. Then wells were made by well cutters. The organic solvent extracts of leaves were added to each well aseptically. This procedure was repeated for each Petri plate then the Petri plates were incubated at 37°C for 24 hrs. After incubation, the plates were observed for the zone of inhibition.

3 Result & Discussion

3.1 Physiochemical Characterization

The ZnO NPs were characterized by an X-ray diffractometer (model: X'PERT PRO PANalytical). The diffraction patterns were recorded in the range of 20° - 80° for the ZnO samples where the monochromatic wavelength of 1.54 Å was used. The samples were analyzed by Field Emission Scanning Electron Microscopy (Carl Zeiss Ultra 55 FESEM) with EDAX (model: Inca). FT-IR spectra were recorded using a Perkin-Elmer spectrometer. The UV-Vis-NIR spectrum was recorded in the wavelength range 200-1100 nm by using Lambda 35.

3.2 Optical Characterization of ZnO NPs

3.2.1 UV-Visible Spectroscopy

The synthesis of the NPs was further verified using the UV-vis spectrometer. Figure 2 demonstrates the UV-visible spectra of ZnO nanoparticles. The formation of zinc oxide nanoparticles utilizing secondary metabolites of Siam queen was investigated by UV-visible spectra in the range of 280 nm–450 nm. The synthesized zinc oxide nanoparticle from the stem extract of siam queen showed a significant absorption peak at 377 nm because of their plasmon resonance exhibited by Zinc oxide NPs.

3.3 Structural characterization of ZnO NPs

3.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

FT-IR spectrophotometer was used to identify the functional groups involved in the production of nanoparticle constructions. The FTIR spectra of the synthesized ZnO NPs mediated by siam queen extract were obtained between the wavenumber range of 500–4000 cm⁻¹ and are displayed in Figure 3. Phytoconstituents present in the extract of siam queen can be involved in the reduction & capping of the Zn²⁺ ions. FTIR spectrum of ZnO nanoparticles showed peaks at 3427, 2916, 2854, 1639, 1384, 1113, 1050, 873, 517 and 463 cm⁻¹. A broad absorption peak associated with ZnO noted at 3427 cm⁻¹ shows the O–H stretching vibrations of carboxylic acid and hydroxyl stretch vibrations. & peak at 2916 & 2854 cm⁻¹ denotes the stretching vibration of the aromatic C–H³³. Both symmetric and asymmetric bending modes of the respective amino acids' and esters' C=O links may be observed in the medium band appearance seen at around 1639 cm⁻¹ can be the consequence of stem extracts. The absorption peaks of alkenes at 1384 and 1050 cm⁻¹ may have resulted from the vibration of (–C=O) groups while the band at 1113 cm⁻¹ is also attributed to C–O stretching in ethers. The band at 517 cm⁻¹ is linked to metal-oxygen vibrations, in this case Zn–O indicates that the interaction between the zinc nanoparticles and the phenolic components tannins and flavonoids found in the siam queen extract.

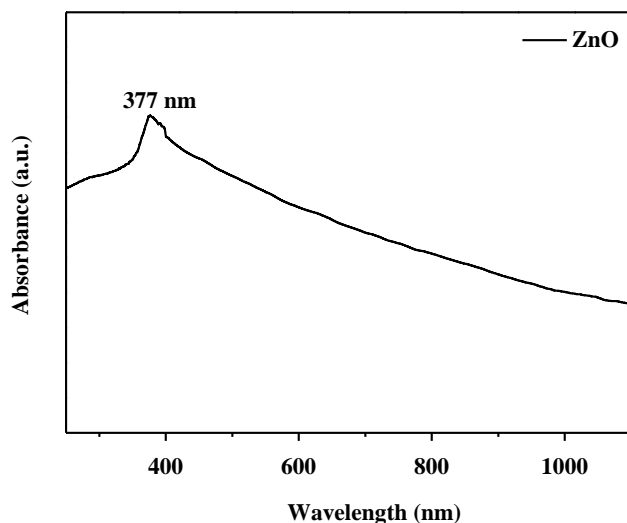


Fig 2 Shows the UV-Vis absorbance spectrum of ZnO nanoparticles

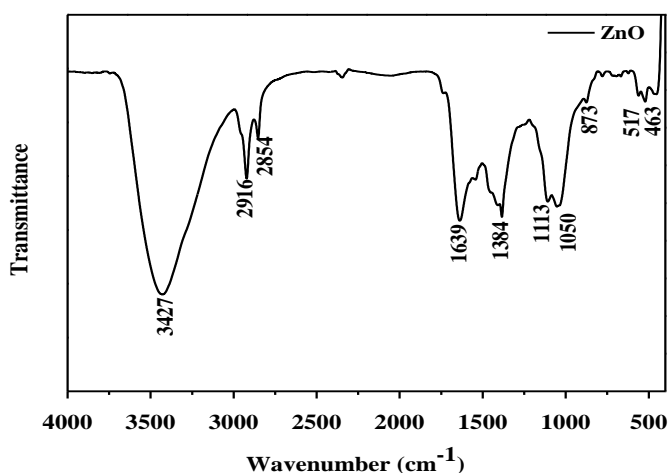


Fig 3 Shows the FTIR spectrum of ZnO nanoparticles

3.4 XRD Analysis

A method for analytically determining the crystalline size of nanoparticle and performing microstructural characterization is X-ray diffraction. The synthesized nanoparticles' XRD patterns are shown in Figure 4. The major peaks of XRD are indexed with 2θ values of 32.72° , 34.48° , 36.72° , 48.55° , 56.84° , 63.88° , 67.96° , 68.24° , and 69.09° , respectively, correspond to the 100, 002, 101, 1102, 110, 103 crystal plane that represents the ZnO phase. The X-ray diffraction pattern of ZnO NPs demonstrate distinct line broadening of the X-ray diffraction peaks as shown in Figure 5 shows that the synthesized nanoparticles were hexagonal & in the

nanoscale range.

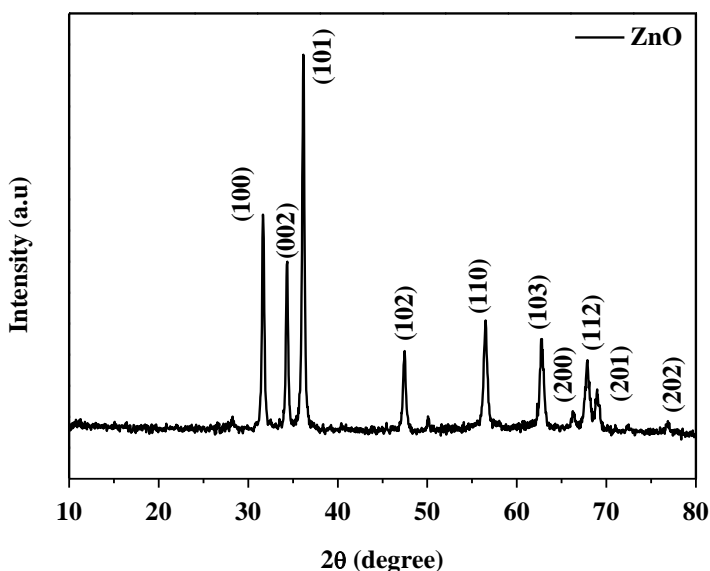


Fig 4 shows the XRD pattern of ZnO nanoparticles

3.5 Surface morphology and composition analysis of ZnO NPs

3.5.1 Field Emission Scanning Electron Microscopy (FE-SEM)

The synthesized ZnO-NP's morphological analysis was carried out using a FE-SEM scanned at various magnifications. A wide range of ZnO nanostructures have been identified including nanospheres and nanorods and many more. Figure 5 shows FESEM images of phyto synthesized ZnO nanoparticles. From this image, it is clearly showed that the particle is in nanostructure form & they are uniformly distributed rod shaped, and in the nanoscale range (20.06 nm).

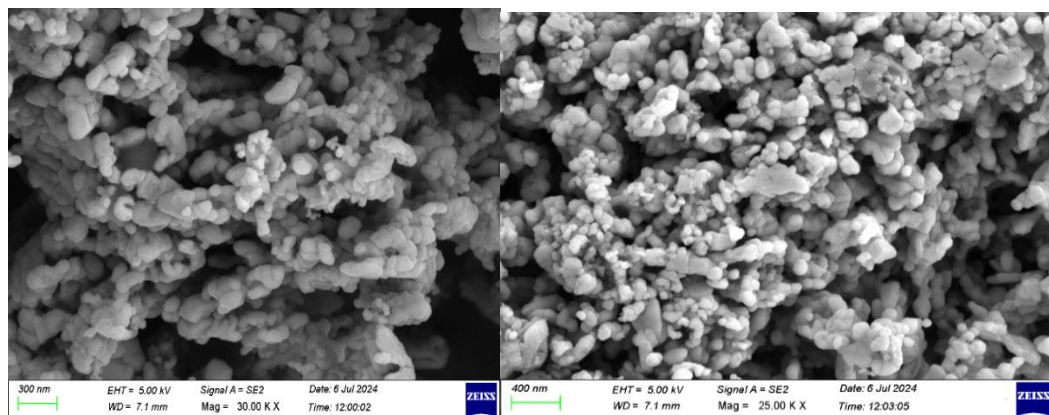


Fig 5 SEM images of Siam queen synthesized ZnO nanoparticles.

3.5.2 Energy Dispersive X-ray analysis (EDX)

Technically, elemental analysis or chemical characterization of a sample can be performed via Energy-dispersive X-ray spectroscopy (EDX). The graph generated by the EDX analysis revealed confirmation of the elemental zinc nanoparticles produced by the leaf extract of siam queen. The EDX analysis's results showed two peaks that were recognized as zinc and oxygen. (Fig. 6) which confirmed the presence of ZnO NPs. The weight percent values of Zn, O observed are 84.77, 15.23 respectively.

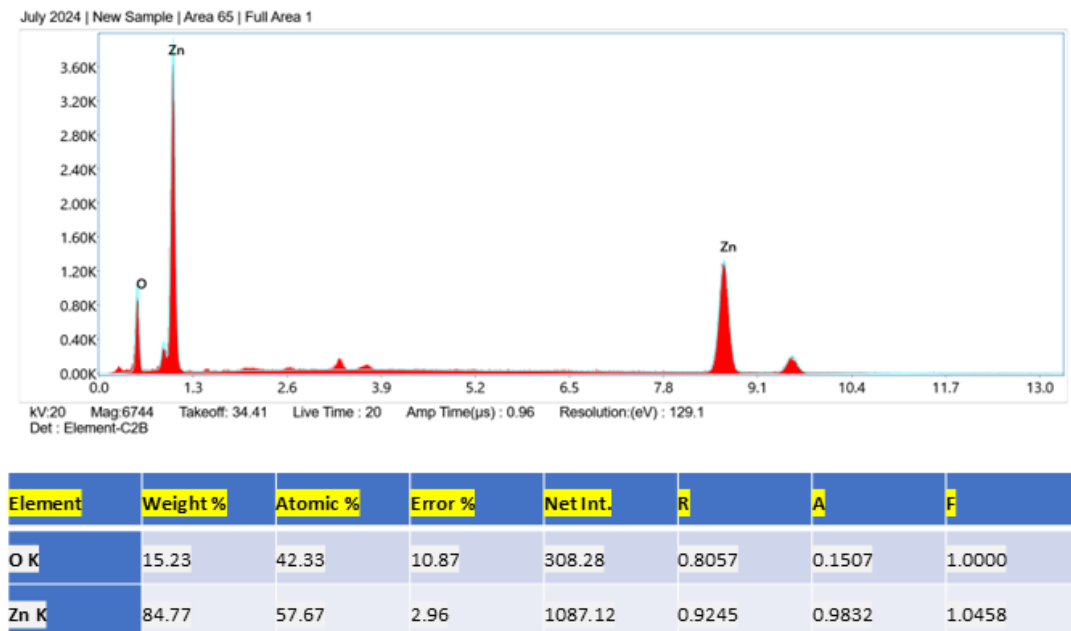


Fig 6 EDX spectra of Siam queen synthesized ZnO nanoparticles.

4 Antimicrobial Activity of Metal & Metal oxide NPs

Many attempts have been made to explore the possible antimicrobial abilities of metal oxides. Addition to being less harmful to human cells, they have shown many other benefits over organic antimicrobial substances including greater effectiveness at lower concentrations, extended shelf life, and significantly more stability in challenging processes. Naturally produced ZnO-NPs have been shown to have potent antimicrobial properties against a range of harmful bacteria and fungi. Similarly, we reported that *Ocimum basilicum* var *thyrsoflora* produced biosynthesized ZnO-NPs with strong antibacterial activity. The microbial interaction to zinc oxide nanoparticles (ZnO-NPs) is a multifaceted process involving many factors, and the combined effect of different simultaneous methods modifies the activity to yield notable results³⁶. Some parameters such as the particles' size, shape, and surface charge, influence Zinc oxide-NPs' antimicrobial activity³⁴⁻³⁵. ZnO-NPs may readily penetrate an organism's nuclear content since they are smaller than surface microbial pores, especially in Gram-negative strains. Also, smaller particles have stronger antibacterial interactions due to their larger surface area. However, a number of studies indicate that various shapes of NPs with the same

surface area might exhibit different microbicide activity. Additional important factors include the microorganism strain chosen, the biogenesis technique used, the concentration of zinc oxide used, treatment time that was administered. Different microbial strain development patterns could lead to variations in ZnO-NPs' antibacterial efficacy. Certain strains are more likely than others to grow strongly on the plate surface, which increases their exposure to ZnO-NPs³⁵. The antimicrobial activity of Zinc Oxide NPs probably activated via electrostatic attraction between the positively charged nanoparticle and the negatively charged cell membranes of bacteria³⁷. Though more research is necessary to determine the exact mechanisms of action of ZnO nanoparticles

4.1 Antimicrobial Susceptibility Testing

Tables 1 and 2 display the zone of inhibition surrounding ZnO-NPs for particular bacterial and fungal cultures as well as the typical synthetic medicines that were utilized. The antibacterial test of ZnO-NPs synthesized in different dilutions (1 mg/ml–2 mg/ml) against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella pneumoniae*) strains of bacteria is displayed in Table 1. ZnO-NPs' antibacterial efficacy was evaluated in comparison to the conventional antibacterial treatment using streptomycin (positive control). Although streptomycin is highly effective in inhibiting the growth of *K. pneumoniae* (22 mm) and *S. aureus* (25 mm). As compared to strains of Gram-negative bacteria, streptomycin was noticeably more efficient against Gram-positive bacteria. Furthermore, the ability to inhibit bacterial growth was greater than ZnO nanoparticles. ZnO-NPs demonstrated a remarkable inhibitory effect on the investigated bacterial strains, exhibiting significant differences in their sensitivity to ZnO-NPs in a dose-dependent pattern. After increasing the solution's concentration from 1 mg/ml to 2 µg/ml, ZnO-NPs' effectiveness was seen to be improved in every examined sample. The zone of inhibition around zinc oxide-NPs for each bacterial cultures ranged from 11 mm, 10 mm in *S. aureus* & *K. pneumoniae* respectively at 1 mg/ml to 15.9 mm, 12mm (2 mg/ml). *S. aureus* and *K. pneumoniae* were the two bacterial strains that showed the greatest and lowest sensitivity to ZnO-NPs, with inhibition zones of 11, 13.5, 15.9 mm and 10, 10.5, and 12 mm as treated to 1, 1.5 and 2 mg/ml of ZnO-NPs, respectively. *S. aureus*, *K. pneumoniae* exhibited an intermediary susceptibility to the nanomedicine. In general, compared to Gram-negative bacteria, the Gram-positive strains exhibited a slightly greater resistance to ZnO-NPs.

Additionally, the zone of inhibition of different ZnO-NP doses (1-2 mg/ml) against fungal strains was also reported (Table 2). The conventional synthetic antibiotic streptomycin (positive control) was also tested against fungi strains. In comparison to ZnO-NPs, streptomycin was generally more effective against the infections. According to antifungal assessments, ZnO-NPs effectively and dose-dependently inhibited the growth of a fungus strain. The average growth of strains was consistently suppressed throughout incubation when the concentration of ZnO-NPs increased from 1 to 2 mg/ml (Fig. 7). *Aspergillus niger* fungal strains that showed sensitivity to ZnO-NPs, with inhibition zones of 9, 11, and 17 mm as treated to 1, 1.5 and 2 mg/ml of ZnO-NPs. In summary, compared to the bacterial strains tested, the fungal strain (*A. niger*) showed a notable sensitivity to the nanomedicine.

Pathogen		Zone of inhibition (mm)			
		1.0 mg/ml	1.5 mg/ml	2.0 mg/ml	Streptomycin
Gram Positive	S.aureus	11	13.9	15.9	25
Gram Negative	K.pneumoniae	10	10.9	12	22

Table 1. Antibacterial activity of ZnO-NPs against S. aureus & K. pneumoniae

Pathogen		Zone of inhibition (mm)			
		1.0 mg/ml	1.5 mg/ml	2.0 mg/ml	Streptomycin
Aspergillus niger		9	11	17	20

Table 2. Antifungal activity of ZnO-NPs against A. niger

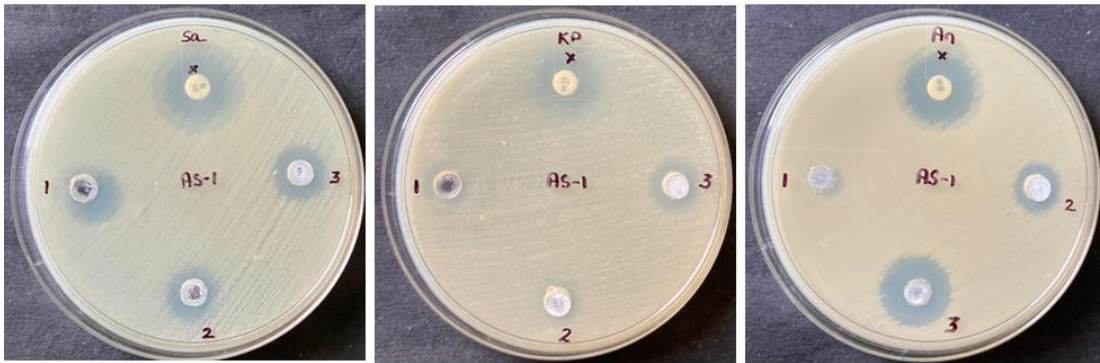


Fig 7 Testing Microbial strain (Bacterial & Fungal strains)

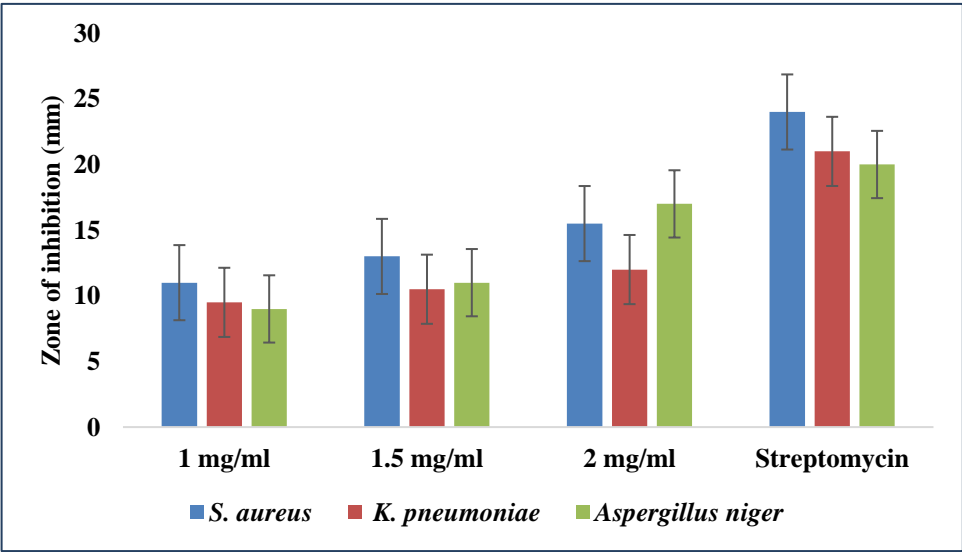


Figure 8 Shows the antimicrobial activity of ZnO nanoparticles

5 Conclusion

Nature has elegant & effective approaches in search of the most viable miniaturized functional materials. The growing interest in sustainable science and using green methods to synthesize metal and metal oxide nanoparticles lead to a desire to create environmentally acceptable methods³⁸. One such medicinal plant *Ocimum basilicum* var. *thyrsoflora* belongs to Lamiaceae family and is frequently applied for treating anxiety, coughs, the common cold, headaches, fevers, diabetes, migraines, neuropathy relief, heart disease, gastrointestinal disorders, insect stings, cramps, and sinus infection & variety of neurological conditions As an anti-inflammatory and antidepressant. (Bora et al 2011). Furthermore, the plant's leaves and flowering branches are known to have anti-inflammatory, carminative, stomachic, and galactagogue properties in traditional medicines (Sajjadi 2006). Extracts of basil, including those made from roots, stems, flowers, leaves, and seeds have been used in therapeutic applications.

The increasing prevalence of dangerous susceptible bacterial and fungal infections is an important issue. Therefore, crucial to identify novel forms of natural substances that may be effective against fungi and bacteria. Whether it comes to developing new and improved treatment plans for many different kinds of diseases, including cancer and infectious disorders, nanomedicines can play an important tool³⁸. A simple method was adapted for the phytochemical-assisted synthesis of ZnO-NPs using aqueous stem extracts of *Ocimum basilicum* var *thyrsoflora*. The synthesized NPs' shape was shown by FE-SEM analysis to be non-smooth rod-shaped cylindrical structures with a size range of 20.06 nm. The XRD outcome justifies the crystalline form and nanoscale particle size. The synthesis of very pure ZnO-NPs was validated by EDX analysis. Additionally, the biogenic zinc oxide nanoparticles exhibited strong antimicrobial action against certain selected pathogenic bacteria and fungi.

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