Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 27, No. (3–4) : 2025 : 217-224
© EM International
ISSN-0972-3005

DOI No.: http://doi.org/10.53550/AJMBES.2025.v27i03-04.013
Journal Home Page: www.envirobiotechjournals.com

GREEN SYNTHESIS AND CHARACTERIZATION OF ZNO NANOPARTICLES USING *OCIMUM TENUIFLORUM* AND ITS ANTIBACTERIAL ASSAY

GOPINATH B.[1]*, JANANI AISWARYA M.[2], PRANEESH S[3], SIVAPRIYA S.[4] AND HEMAREKHA S.[5]

Department of Biotechnology, Sri Shakthi Institute of Engineering and Technology,
Coimbatore 641 062, T.N., India

(Received 23 February, 2025; Accepted 26 April, 2025)

Key words: *Zinc oxide nanoparticle, Ocimum tenuiflorum, including UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS), and ZETA Potential*

Abstract– The growing demand for eco-friendly and biocompatible nanomaterials has led to the exploration of green synthesis routes for nanoparticle production. In this study, zinc oxide (ZnO) nanoparticles were synthesized using the aqueous extract of *Ocimum tenuiflorum* (Holy Basil), a medicinal plant known for its rich phytochemical content. The green synthesis approach leverages the natural reducing and capping agents present in plant extracts, offering a sustainable and non-toxic alternative to conventional chemical methods. The synthesized ZnO nanoparticles were characterized using various analytical techniques, including UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and ZETA Potential. These analyses confirmed the formation of well-dispersed, crystalline ZnO nanoparticles with desirable size and morphological features. The biological efficacy of the green synthesized ZnO NPs was evaluated through antimicrobial assays and cancer cell lines. The enhanced therapeutic performance is attributed to the synergistic effects of ZnO and the bioactive compounds present in *O.tenuiflorum*, such as eugenol, flavonoids, and phenolic acids. This research emphasizes the potential of plant-mediated green synthesis as a sustainable method for developing multifunctional nanomaterials with promising applications in antimicrobial therapy and cancer treatment. The findings support the continued exploration of phytochemical-based nanoparticle synthesis for future biomedical and pharmaceutical innovations.

INTRODUCTION

Nanotechnology has emerged as one of the most dynamic and transformative areas of research in modern science, with broad-ranging applications in electronics, energy, agriculture, and medicine. Among the various products of nanotechnology, nanoparticles materials with dimensions less than 100 nanometres have drawn significant attention due to their unique physicochemical properties. These include a high surface-area-to-volume ratio, enhanced reactivity, tuneable optical properties, and the ability to penetrate biological barriers. In particular, metal oxide nanoparticles such as zinc oxide (ZnO) have found numerous applications in biomedical sciences owing to their notable antimicrobial, antioxidant, and anticancer activities.

Zinc oxide nanoparticles are regarded as generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration (FDA). ZnO NPs possess semiconducting, piezoelectric, and photocatalytic properties, making them valuable in sensors, drug delivery systems, cosmetics, and environmental purification. More importantly, ZnO nanoparticles have demonstrated strong antimicrobial activity against a wide spectrum of pathogens and have shown cytotoxic effects against various cancer cell lines. The antimicrobial mechanism of ZnO is multifaceted, involving the generation of reactive oxygen species (ROS),

([1]*Assistant Professor)

disruption of microbial membranes, leakage of intracellular contents, and interference with cellular metabolism. Similarly, their anticancer action is primarily associated with ROS-induced apoptosis, oxidative stress, mitochondrial dysfunction, and DNA damage in cancer cells.

Despite the promising applications of ZnO nanoparticles, the method of synthesis plays a crucial role in determining their size, morphology, stability, and biological compatibility. Conventional synthesis techniques such as sol-gel methods, though effective, often require high temperatures, toxic chemicals, and energy-intensive processes. These not only pose environmental and safety concerns but also limit their applicability in biomedical fields due to residual toxicity. In contrast, green synthesis approaches, particularly those utilizing plant extracts, offer an eco-friendly, sustainable, and cost-effective alternative. These methods avoid the use of harsh chemicals and rely on naturally occurring phytochemicals in plant extracts as reducing and stabilizing agents.

Plant-mediated synthesis of nanoparticles has gained increasing popularity in recent years due to its simplicity, scalability, and biocompatibility. Phytochemicals such as flavonoids, alkaloids, terpenoids, phenolic compounds, and proteins present in plant extracts facilitate the reduction of metal ions to nanoparticles and also act as capping agents to enhance nanoparticle stability. Among the wide variety of medicinal plants investigated, *Ocimum tenuiflorum* commonly known as Tulsi or Holy Basilhas emerged as a potent candidate for green synthesis due to its rich pharmacological profile.

Ocimum tenuiflorum, (Family:*Lamiaceae)* is commonly cultivated and used in traditional Indian medicine as therapeutic agent. Tulsi has been used as traditional medicine to treat respiratory disorders, skin diseases, digestive problems, and even cancers. The leaves of *O. tenuiflorum* are rich in biologically active compounds such as eugenol, carvacrol, rosmarinic acid, apigenin, linalool, and ursolic acid, many of which exhibit antimicrobial, antioxidant, and anticancer properties. These compounds not only contribute to the health benefits of the plant but also make it a suitable bio reductant in nanoparticle synthesis.

The use of *O. tenuiflorum* extract in the biosynthesis of ZnO nanoparticles offers dual benefits: it provides a green and sustainable route of synthesis and imparts additional bioactivity to the nanoparticles through the phytochemical corona formed on their surface. Recent studies have shown that such biofunctionalized nanoparticles can exhibit enhanced biological activities, including increased antimicrobial efficacy and greater selectivity in targeting cancer cells. However, despite its promising potential, literature on the synthesis of ZnO nanoparticles using *O. tenuiflorum* remains limited, particularly in the context of their comparative evaluation for antimicrobial and anticancer properties.

The emergence of antimicrobial resistance (AMR) and the limitations of conventional cancer therapies underscore the urgent need for new therapeutic agents. Because of the misuse and overuse of antibiotics, AMR has now become a serious public health threat. Multidrug-resistant bacterial and fungal strains are becoming increasingly prevalent, rendering standard treatments ineffective. Similarly, current cancer therapies often suffer from lack of specificity, severe side effects, and resistance development. In this context, ZnO nanoparticles synthesized using medicinal plant extracts such as Tulsi may offer a new generation of nano medicines with broad-spectrum antimicrobial and selective anticancer properties.

This study aims to develop a green synthesis method for ZnO nanoparticles using aqueous leaf extract of *Ocimum tenuiflorum*, characterize the synthesized nanoparticles using advanced analytical techniques, and evaluate their antimicrobial and anticancer properties. The synthesis process is designed to be eco-friendly, avoiding the use of toxic chemicals and utilizing the natural phyto chemicals in Tulsi for nano particle formation. The antimicrobial activity of the synthesized ZnO nanoparticles will be assessed against selected bacterial and fungal strains using standard microbiological assays. In addition, the anticancer potential will be evaluated using in vitro studies on human cancer cell lines, focusing on cytotoxicity and apoptosis-inducing capabilities.

By integrating nanotechnology, green chemistry, microbiology, and oncology, this study not only advances the understanding of plant-based nano particle synthesis but also contributes to the development of alternative therapeutic agents. The outcomes of this research could pave the way for scalable production of biocompatible ZnO nanoparticles for biomedical applications, particularly in combating infectious diseases and cancer.

MATERIALS AND METHOD

Plant material

The plants in the *Ocimum* belong to the Lamiaceae family and are widespread in the world's tropical, subtropical and warm mild regions Mail. These plants are known to produce essential oils consisting of a series of aromatic compounds, and for this reason, Tulsi is known as " Queen of Herbes". In India, these plants are mainly grown at home for worship and grown by the suggestion of the temple. Among the plants with medicine, those in *Ocimum* are very important fragrant herbs or shrubs (Gandhimathi, 2015).

Ocimum sanctum L. (also called Tulsi) has been used for thousands of years inthe field of linguistic academic fields for traditional Indian medicine, Ayurveda and various healing properties. This plant is considered a sacred sanctuary in the sanctuary throughout the traditional Hindu temple, sacred forests and homes, and thus classified synonyms *O. sanctuary* L. is more popular in Indian science. It relieves people of stress, restore and improve body immunity and digestion. The chemical constituents of *Ocimum tenuiflorum* are linalool, alkaloids, ursolic acid, glycosides, carvacrol, tannins, rosmarinic acid, aromatic compound (Yosatha, 2020).

Preparation aqueous plant extract

The plant extract was prepared by taking 50g of *O. tenuiflorum* leaves. The leaves were washed with distilled water to remove impurities and dust particle present in them. Then the leaves were crushed using mortar pestle to grind it into fine paste. Later the paste is filtered using Whatman filter paper to obtain clean and pure aqueous extract of *O. tenuiflorum.*

Fig. 2. Crushing of leaves

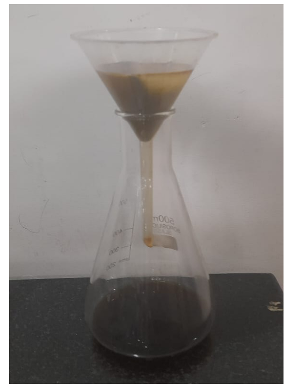

Fig. 3. Aqueous extraction using Whatman filter paper

Synthesis of ZnO Nanoparticles

To synthesis the ZnO nanoparticle, 30ml of aqueous plant extract and 470ml 0.1M of zinc nitrate dihydrate solution was taken. The leaf extract was added to the zinc nitrate dihydrate solution and this mixture was placed on magnetic stirrer at 450-500 rpm for 1 hour. During this 2M NaOH solution was added drop wise (maintain pH at level 12) until it leads to formation white precipitate of ZnO nano particle. After precipitation the beaker was kept undisturbed in dark room overnight for sedimentation of the precipitate.

After sedimentation of precipitate the clear water solution is removed carefully using a pipette and the precipitate is centrifuged at 7500 rpm for 20 mins to obtain the pellet. The pellet is transferred to clean petri dish. The petri dish is placed in hot air oven at 60! overnight to get dry pellet. Then the dried pellet is grinded into fine powder using mortar and pestle. Calcination of the powder at 500! for 1 hour was done to obtain pure form ZnO nano particle.

Fig. 4. Synthesis of ZnO nanoparticle from zinc nitrate dihydrate using *O. tenuiflorum* extract

Fig. 5. Precipitation of ZnO nanoparticle

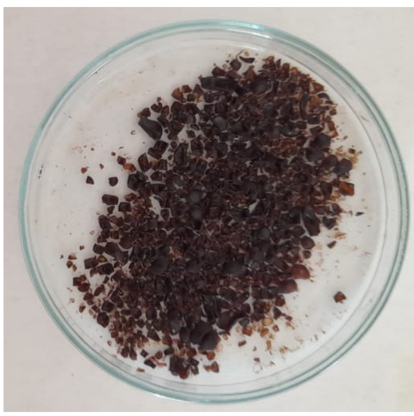

Fig. 6. Dried pellet of ZnO nano particle

Fig. 7. Calcinated ZnO nano particle powder.

Characterization methods

X-ray powder diffraction (XRD)

X-ray diffraction is an important characterization technique used for investigating about the crystalline structure of nano particle. It helps to determine if sample powder is crystalline or amorphous in nature. The crystalline structure of the sample was determined using the formula,

$$D=k\ \lambda/\mathrm{Cos}\theta$$

where, D denotes the average crystalline size, k represents broadening constant, λ denotes wavelength of Cu Kλ radiation source, denotes full width at half maximum and θ denotes the angle of diffraction.

FT-IR Spectroscopy

The FT-IR analysis is done to identify the functional group present on the surface of the synthesized nano particle. Using FT-IR we can determine the functional groups such as -OH, -NH, -COOH and -C=O which are originated from precursor material or reducing agent used. Here the IR spectrum was analysed at the range between 4000-400 cm^{-1}.

Scanning Electron Microscope (SEM)

The surface morphology of ZnO nano particle was analysed using SEM. It can help us to analyse the shape, size and aggregation behaviour of the sample nano particle was observed.

Dynamic Light Scattering (DLS) and ZETA potential Analysis

Dynamic Light Scattering (DLS) analysis was done to determine the size distribution of the sample nano particle. Based on Brownian motion of the particle in suspension and Strokes-Einstein equation the particle size distribution the nano particle is found. The particle size ranging from 1-100 nm has high potential in nano scale application and 100-300 nm size particle can be used in drug delivery application depending on their chemical and biological characteristics.

ZETA potential analysis is done to find the surface charge of the nano particle. Depending on the charge, the stability of the nano particle can be determined. The charge is between ±30 mV. If charge is and above ±10 mV then the particle is strongly stable.

The DLS and ZETA potential is mainly used in industrial sector (pharmaceuticals) to analyse the particle size and charge to identify their potential application.

Biological sample

In order to find the anti-bacterial potential of sample nano particle, *Escherichia coli* (*E. coli)* is used here.

Preparation of *E. coli* culture

10 ml of nutrient agar broth was prepared and autoclaved at 121 ^{o}C for 15 mins to prevent the contamination. Then 100 µl of *E. coli* from mother culture was taken, added to 10 ml broth and placed in incubator overnight. Fresh culture of sample is prepared.

Anti-bacterial assay (Well diffusion method)

100 ml of nutrient agar was prepared and autoclaved. It was then poured into petri dish and allowed to cool down. Then the agar plate was steaked with *E.coli* using inoculation loop. Four wells were created in the petri plate. Here two different suspensions with different concentration of ZnO nanoparticle is taken.

The solvent and ZnO concentration are:

i. Ethanolic solution (5 ml)-100 µg/ml, 50 µg/ml, 20µg/ml, 10µg/ml.
ii. Water solution (5 ml) - 100 µg/ml, 50 µg/ml, 20 µg/ml, 10µg/ml.

The wells were loaded with different concentration of ZnO solution and allowed to incubate at 37 ^{o}C for 24 hours. The zone inhibition is observed the next day to determine the potential anti-bacterial property.

RESULTS

XRD Analysis

The presence of nano particle and their crystalline structure property was confirmed using XRD. Here

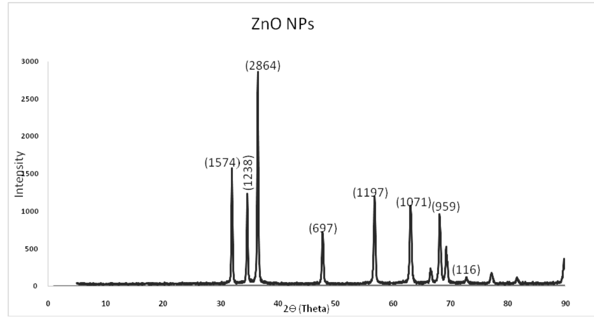

Fig. 8(b). XRD graph plot of ZnO nanoparticle

ZnO nano particle synthesized using *O. tenuiflorum* shows peaks with 2˜ value identified at 31.9°, 34.6°, 36.4°, 47.7°, 56.7°, 63.1°, 68.1°, 72.8°, 77.1° which are indexed as (1574), (1238), (2864), (697), (1197), (1071), (959), (116), (180). Here the intensity of the nanoparticle shows it purity. Highter the index greater the purity and crystallinity of the nanoparticle.

FT-IR

Here the FTIR spectrum of the sample was recorded in the range of 4000–400 cm^{-1} using a MIRacle10

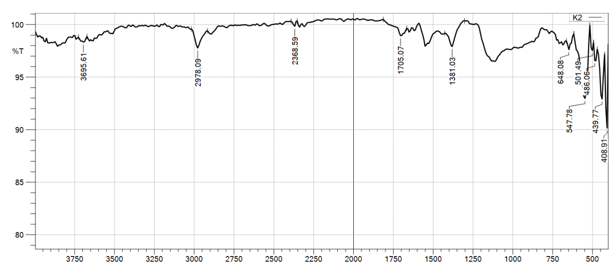

Fig. 9. FT-IR graph of ZnO nanoparticle

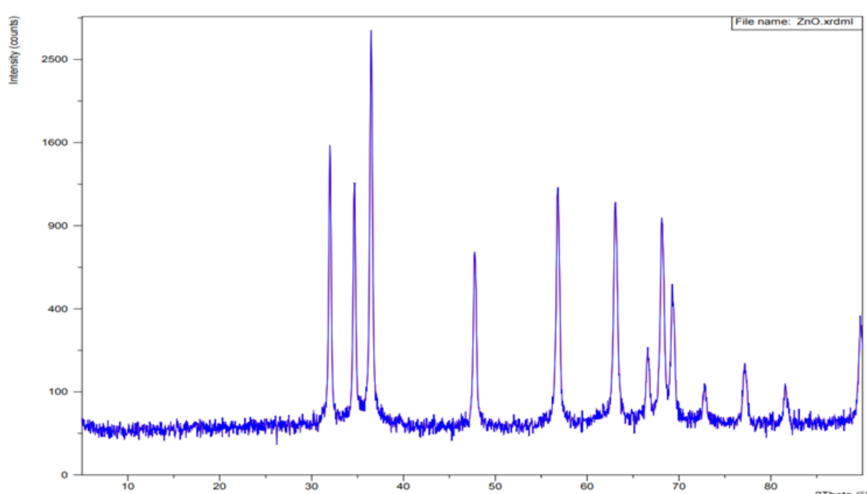

Fig. 8(a). XRD of ZnO nanoparticle

(ZnSe) ATR accessory. The spectrum reveals several characteristic peaks, indicating the presence of specific functional groups in the sample. The peaks at 486 cm^{-1}, 439 cm^{-1}, and 408 cm^{-1} are strong indicators of Zn–O vibrations, confirming the presence of ZnO in the sample. The broad peak at 3365 cm^{-1} shows the O–H stretching vibration, indicating the presence of alcohols or phenols. The peak at 2978 cm^{-1} shows C–H stretching which confirms the presence of alkanes. At 2886 cm^{-1} an another C–H stretching band, supporting the presence of aliphatic hydrocarbons. At 1765 cm^{-1} a sharp peak attributed to C=O stretching, suggesting the presence of carbonyl groups (e.g esters, carboxylic acids, or ketones). And at 1381 cm^{-1} shows C–H bending vibrations, possibly from methyl groups. So this confirms that the sample contain other function group excluding ZnO which might be due to the reducer *O.tenuiflorum* or the precursor zinc nitrate dihydrate we used for this study.

SEM

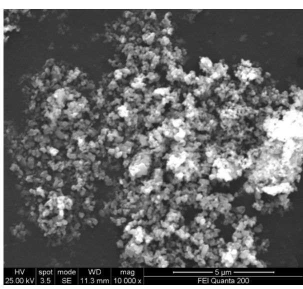

Fig. 10(a). SEM of ZnO NPs captured at 10000×

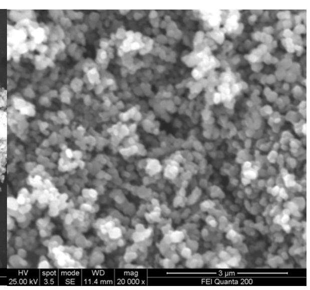

Fig. 10(b). SEM of ZnO NPs captured at 20000×

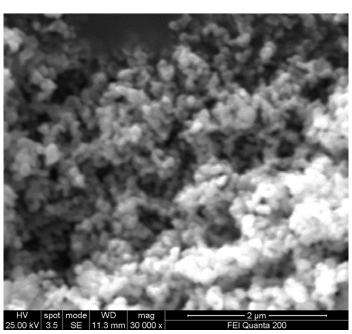

Fig. 10(c). SEM of ZnO NPs captured at 30000×

The above images show the morphological structure of ZnO nano particle by scanning electron microscopy (SEM) at different magnification. Here the images were recorded at magnification of 5µm to 1µm. The topographical (surface) structure image of the ZnO nano particlesare more or less spherical clusters and aggregates have a very less smooth surface. The

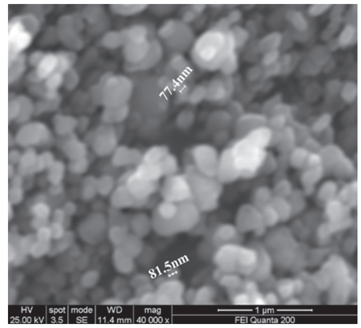

Fig. 10(d). SEM of ZnO NPs captured at 40000×

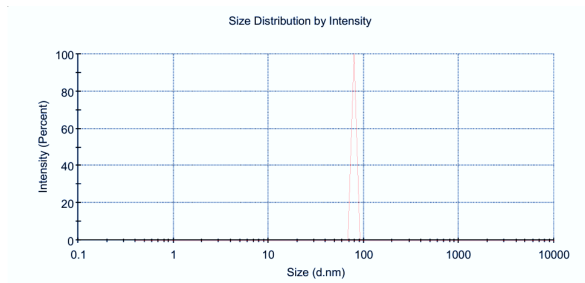

Fig. 11(a). Size distribution of the ZnO nanoparticle

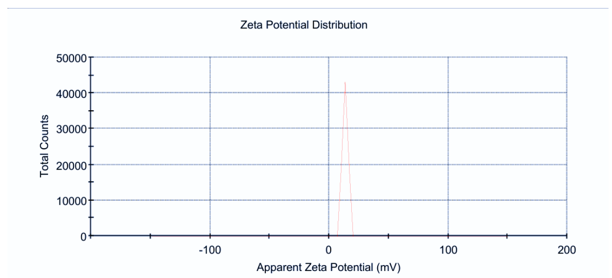

Fig. 11(b). ZETA potential of ZnO nano particle

SEM results shows that green synthesized ZnO NPs are pure and nano scale size. The shape plays crucial role during it application properties and hence the SEM images of the sample here prove that ZnO synthesised using *O. tenuiflorum* is pure and have effective activity against pathogens and can be also used in drug delivery application.

DLS – Zeta Potential

Dynamic Light Scattering (DLS) analysis shows the average diameter of the *O. tenuiflorum* mediated ZnO nano particle. Here the average diameter is 78.9nm. the result shows that sample is of ultrafine in size i.e less than 100nm. Which also means that ZnO NPs has higher surface. Higher the surface area greater the antimicrobial property of the nanoparticle.

ZETA potential measurement shows that ZnO particle is of +13.6mV. It represents the charge of the nano particle. And it confirms that ZnO nano particle is stability

Through DLS – ZETA Potential analysis it is confirmed that *O. tenuiflorum* mediated ZnO nanoparticle is strongly stable and highly applicable for antimicrobial as well as other application like drug delivery and anti-cancer application.

An antibacterial assay was conducted to evaluate the antimicrobial efficacy of ZnO at different concentrations against *Escherichia coli* (*E. coli*). So agar well diffusion method was employed here. The image above shows the zone of inhibition by ZnO NPs against *E. coli*. the ethanolic solvent of concentration 100 μg (A) and 50 μg (C) and water solvent of concentration 100 μg (B) and 50 μg (D) shows strong antibacterial property. Whereas ethanolic and water solvent of concentration 20 μg (E & F) shows very small inhibition rate and as for concentration of 10μg (G & H) of ethanolic and water solvent no zone of inhibition is found.

CONCLUSION

This research confirms that zinc oxide nanoparticles can be effectively synthesized using an eco-friendly method involving *Ocimum tenuiflorum* leaf extract. The green synthesis approach eliminates the need for harmful chemicals and supports a sustainable production route. Analytical techniques verified that the resulting nanoparticles are crystalline, stable, and within the nanoscale range. Their notable antibacterial effect against E. coli, especially at higher concentrations, showcases their practical potential in antimicrobial applications. Additionally, the phytochemicals from *O. tenuiflorum* appear to enhance the biological functionality of the ZnO nanoparticles. These findings suggest that plant-based synthesis not only supports environmental safety but also opens new pathways for developing effective materials for healthcare and pharmaceutical use.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Head, Department of Biotechnology, Sri Shakthi Institute of Engineering and Technology, Coimbatore for support rendered to completing the research work.

Conflicts of Interest: The authors declare that they have no conûict of interest.

REFERENCES

Arya, V. 2010. Living systems: Eco-friendly nanofactories. *Dig. J. Nanomater Bios.* 5: 9-21.

Ahmed, S., Ahmad, M., Swami, B.L. and Ikram, S. 2016. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *J. Adv. Res*. 7: 17-28.

Awwad, A., Albiss, B. and Ahmad, A.L. 2014. Green synthesis, characterization and optical properties of zinc oxide nanosheets using *Olea europea* leaf extract. *Adv. Mater. Lett.* 5: 520-524.

Al-Dhabi, N.A. and Arasu, M.V. 2018. Environmentally friendly green approach for the production of Zinc oxide nanoparticles and their anti-fungal, ovicidal, and larvicidal properties. *Nanomaterials.* 8: 500.

Bahorun, T., Neergheen, V.S. and Aruoma, O.I. 2005,. Phytochemical constituents of Cassia fstula. *Afri. J. Biotechnol*. 4: 1530-1540.

Divya, M.J., Sowmia, C., Joona, K. and Dhanya, K.P. 2013. Synthesis of zinc oxide nanoparticle from hibiscus rosa-sinensis leaf extract and investigation of its antimicrobial activity. *Res. J. Pharm. Biol. Chem. Sci.* 4: 1137-1142.

Fan, Z. Y. and Lu, J.G. 2005 Zinc oxide nanostructures: Synthesis and properties. *J. Nanosci. Nanotechnol*. 5: 1561-1573.

Gunalan, S., Sivaraj, R. and Rajendran, V. 2012. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Progress in Natural Science: Materials International.* 22: 693-700.

Kavitha, K.S. 2013. Plants as green source towards synthesis of nanoparticles. *Int. Res. J. Biol. Sci*. 2: 66-76.

Kalpana, V. N. and Rajeswari, V. D. A review on green synthesis, biomedical applications, and toxicity studies of ZnO NPs. Bioinorganic chemistry and application, 2018, Article 3569758. https://doi.org/10.1155/2018/3569758

Malik, P., Shankar, R., Malik, V., Sharma, N. and Mukherjee, T.K. Green chemistry based benign routes for nanoparticle synthesis. https://doi.org/10.1155/2014/302429

Makarov, V. V. 2014. "Green" nanotechnologies: synthesis of metal nanoparticles using plants. *Acta Naturae.* 6: 35-44.

Mittal, A.K., Chisti, Y. and Banerjee, U.C. 2013. Synthesis of metallic nanoparticles using plant extratcs. *Biotechnol. Adv*. http://dx.doi.org/10.1016/.

Rajan, R., Chandran, K., Harper, S. L., Yun, S. I. and Kalaichelvan, P. T. Plant extract synthesized silver nanoparticles: An ongoing source of novel biocompatible materials. Ind. Crop Prod. 70, 356–373 (2015).

Ramesh, P., Rajendran, A. and Sundaram, M. 2014. Green syntheis of zinc oxide nanoparticles using fower extract *Cassia auriculata. J. Nanosci. Nanotechnol*. 2: 41-45.

Sushma, N.J., Mahitha, B., Mallikarjuna, K. and Deva, P.R.B. 2016. Bio-inspired ZnO nanoparticles from *Ocimumtenuiforum* and their in vitro antioxidant activity. *Appl. Phys.* A 122: 544.

Santoshkumar, J., Kumar, S.V. and Rajeshkumar, S. 2017. Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen. *Resource-Efcient Technologies.* 3: 459-465.

Shah, R.K., Boruah, F. and Parween, N. 2019. Synthesis and characterization of ZnO nanoparticles using leaf extract of Camelia sinensis and evaluation of their antimicrobial efcacy. *Int. J. Curr. Microbiol. Appl. Res*. 4: 444-450.

Veerachari, U. and Bopaiah, A.K. 2011. Preliminary phyto-chemical evaluation of the leaf extract of five cassia species. *J. Chem. Pharm. Res*. 3: 574-584.