Asian Journal of Pharmacognosy

Research paper

Green synthesis of zinc oxide nanoparticles using leaves extract of *Mangifera indica* L. and evaluation of its antibacterial activity

Sushma Khatri, Dharma Prasad Khanal

Manmohan Memorial Institute of Health Sciences, Kathmandu-13, Soalteemode, GPO Box: 15201, Kathmandu, Nepal.

*Corresponding author: sushmakc97@gmail.com
(Accepted October 24, 2024)

ABSTRACT

***Objectives*:** The aim of the research study was the synthesis of zinc oxide nanoparticles using leaf extract of *Mangifera indica* L and the evaluation of its antibacterial activity. ***Methods*:** The zinc nanoparticles were synthesized using leaves extract of *Mangifera indica* L. mixed with zinc acetate. The characterization was done using UV-visible spectroscopy, FTIR, and XRD analysis. Anti-bacterial activity of both the extract and the synthesized nanoparticles was evaluated using the Agar well diffusion method. Thecharacterization of the synthesized zinc nanoparticles was done by using UV-visible spectroscopy which exhibited an optical property as indicated by the surface plasmon resonance at 350nm. FTIR analysis shows the essential functional groups as phenols, carbonyl, alcohol, and alkane. The XRDanalysis was used to calculate the size of zinc nanoparticles, and the average size was found to be 11.68nm. ***Results*:** The antibacterial activity of synthesized zinc nanoparticles showed improved sensitivity towards both the gram-positive and gram-negative bacteria in comparison to plain extract. ***Conclusion*:** The study shows that zinc nanoparticles possess more promising anti-bacterial activity than plain extract. The synthesis of nanoparticles using the plant source is environment-friendly, cost-effective, and safer.

***Keywords*:** antibacterial activity, Fourier TransformInfrared Spectroscopy (FTIR), *Mangifera indica* Linn, UV-visible spectroscopy, zinc nanoparticles, X-ray diffraction (XRD)

eISSN-0128-1119

INTRODUCTION

Among the various aspects of modern science, nanotechnology is gaining greater importance all around the globe. "Nano "means very small and indicates one billionth of a meter and the particle size ranges between 1 and 100nm. Nanotechnology is the science and use of matter at a tiny scale and at this size, the atom and the molecule work differently providing interesting and new uses. Various types of nanoparticles are there and among them, metallic nanoparticles such as silver, copper, zinc, and iron nanoparticles have gained greater attention due to their wide application in science such as medicine, chemistry, agriculture, and biotechnology (Sarsar et al., 2017). Among the various methods for the synthesis of nanoparticles, the chemical reduction method is highly implied among all themethod but its application is limited due to higher toxicity (Andrews, 2019). Hence, Green synthesis of nanoparticles has been evolving as an important branch as it deals with environmentally friendly, cost-effective, and safer methods for the synthesis of nanoparticles. It is gaining importance as it eliminates the use of harmful reagents and synthesizes the expected product in an economical manner (Sorbiun et al., 2018).

Nanoparticles are commonly employed for imaging, sensing, and drug delivery vehicles to target specific sites such as lungtissue as well as cancer therapy and vaccinations (Wang & Wang, 2014). *Mangifera indica* L. belonging to the family Anacardiaceae is commonly called mango, is a large evergreen tree, long living 10-45m high, and is found all over the tropical regions of the world where it is used in horticulture and medicinal plant (Shah et al., 2010). Various parts of the plant are used as dentifrice,astringent, antiseptic, diaphoretic, stomachic, vermifuge, tonic, laxative, and diuretic and to treat diarrhea, dysentery, anemia, asthma, hemorrhage, and piles. The seeds are used in asthma and act as astringent (Wang & Wang, 2014). The major nutritional antioxidants, vitamin E, vitamin C, and β-carotene, may be beneficial in preventing several chronic disorders (Shah et al., 2010). The main active components found are mangiferin and chinonin. Among all the components, mangiferin shows strong antioxidant activity. It has a number of therapeutic actions and many health benefits such as antidiabetic, antifungal, antimicrobial, anti-inflammatory, antiviral, hypoglycemic, anti-allergic, and anticancer activity, etc. (Ojha et al., 2013).

METHODS

Plant material: The leaves of plant *Mangifera indica* L. were collected from Kirtipur, Kathmandu, Nepal. The collected material was identified at the National Herbarium and Plant TissueLaboratory in Godawori, Lalitpur. The Herbarium entry number was 114. The leaves of *Mangifera indica* L. were thoroughly washed and shade-dried for a few days. After complete drying, the leaves were reduced to powder form with the electric grinder.

Chemicals and equipment: All the solvents and chemicals used during the experimental process suchas extraction and synthesis of zinc nanoparticles are of laboratory and analytical grade. Distilled water, methanol, and ethanol are used as solvents. All chemicals and reagents used were manufactured by Fisher Scientific (India) and Merck India. The following equipment and glass wares were used: Electric Grinder (Panasonic, Japan), Electric balance (Ohaus, China), Rotary evaporator (Accumax, India), Hot air Oven

eISSN-0128-1119

(Memmate, Germany), Autoclave (Accumax, India), Hot air Oven (Memmate, Germany), UV-Visible Spectrophotometer (Thermo Spectronics), IR spectrophotometer (Shimadzu 8400 S), D2 Phaser Bruker XRD, PH meter (made by Labtronics) and all glassware of Borosil India were used.

Preparation of zinc nanoparticles: Zinc Acetate dihydrate Zn $(CH_3COO)_2.2H_2O$ and Sodium hydroxide (NaOH)were used as a precursor for the formation of zinc nanoparticles. Distilled water was used for dilution. The extraction was done using distilled water as a solvent.10gm of powderedleaves were taken in a 250ml beaker containing 100ml of distilled water. The mixture was then boiledfor 15 minutes and then filtered using Whatman no. 41 filter paper. The resulting filtrate was used for the preparation of zinc nanoparticles.

Preparation of zinc oxide nanopowder: For the synthesis of zinc nanoparticles, 0.02M of zinc acetate solution was prepared. 50 ml of zinc acetate solution was taken and stirred for 10 minutes and1 ml of the extract of the mango plant was dripped to the same. The mixture was stirred for 20 minutesthe pH was maintained at pH12 by adding 1M NaOH dropwise which resulted in a pale white aqueous solution. This was then agitated for 2 hrs. After that pale white precipitate was taken out and washedwith distilled water 2-3 times followed by ethanol. Then the powder was dried at 40°C in an oven overnightand zinc oxide nanoparticles were obtained (Janjal et al., 2017). The characterization of zinc oxide nanoparticles is essential to confirm their presence which is done by methods like:

- UV-visible spectra analysis
- X-ray diffraction analysis
- FTIR analysis

Evaluation of antibacterial activity: Antibacterial activity was evaluated by agar well diffusion method. In the method test organisms were collected, pure culture of the organism was isolated and standardized with reference to 0.5M Mac-Farland standard. All ATCC subcultures of bacteria wereprovided by the Natural Product Research Laboratory, Thapathali, Kathmandu. The fresh culture bacterial isolates were used. *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus,* and *Bacillus subtilis* were inoculated into a 5ml peptone water solution. The mixture was incubated at 37°C for 4 hrs. to produce standard inoculum i.e. 1.5×10^8 CFU/ml. inoculum standardization was done by comparing it to 0.5 Mac Farland standard. An 8mm standard well cutter was used to bore well into the media. Then, extracts were placed inside a cup with a micropipette. The plate was then incubatedovernight, after which the zones of inhibition were observed. The antimicrobial activity of zinc oxide NPs and the extract was done against gram-positive (*Bacillus subtilis* ATCC 6051, *Staphylococcus aureus*ATCC 6538P) and gram-negative (*Klebsiella pneumoniae* ATCC700603, *Escherichia coli* ATCC2091) using bore well diffusion method (Balouiri et al., 2016; Sumon & Tamalika, 2018).

eISSN-0128-1119

RESULTS AND DISCUSSION

UV-visible spectroscopy analysis: UV-visible spectroscopy is mostly used for the characterization of nanoparticles which allows the identification, characterization, and analysis of metallic nanoparticles (Khandel et al., 2018). For UV visible analysis the plant extract was mixed with zinc acetate solution inthe ratio of 1:50 and the solution was further diluted to 1:10 ratio by addition of distilled water. The solution of *Mangifera indica* L. and zinc acetate when observed in the UV-visible spectroscopy confirmed the formation of zinc nanoparticles showing a peak at 350nm. In the current study, the UV spectroscopy analysis showed an absorbance maximum for zinc nanoparticles at 350nm, and in the previous study by Shah, Rajesh Kumar in 2015 it wasfound to be 330nm (Shah et al.).

FTIR analysis: The functional groups present in *Mangifera indica* L. Leaf extract were predicted by the FTIR analysis. FTIR analysis of nanoparticles was also carried out. The IR spectra thus obtainedare as in Figures 2 and 3. The FTIR analysis of the synthesized zinc oxide nanoparticles from the leaves extract was done to find out the functional group present in the particles ranging from 4000-400 $cm^{-1.}$ The peak in the area of 3200-3600 $cm^{-1.}$ represents the –OH stretching vibration that comes from some phenolic compounds in the extract. the peak in the area of 325 $cm^{-1,}$ 1791 cm^{-1} ,1380 cm^{-1} indicates C=O stretching whereas the peak at 2162 cm^{-1} indicates C=C stretching. The bandwidth of 1500-600 cm^{-1} exhibited the fingerprint region of zinc oxide nanoparticles. After the Zinc nanoparticle formation, there are some shifts of valuable peaks such as the O-H vibration, and C=O vibration to higher wave numbers. The higher wavenumber means the higher vibration energy required and it can suggest the higher energy vibration caused by the presence of zinc nanoparticles.

XRD Analysis: X-ray Diffraction was carried out by using X-ray diffractometer to determine the structure and size of the crystals. The figure below illustrates the XRD spectrum of the zinc oxide nanopowder synthesized by the aqueous chemical method. The XRD spectrum indicates that the zincoxide powder has a hexagonal structure. The graph obtained from the XRD analysis is shown in Figure 4. The FWHM (Full Width at Half Maximum) of the highest peak thus obtained from the graph is used in the Debye equation to estimate the size of the crystals. Debye-Scherrer equation: $D=K\lambda/ (\beta \cos \theta)$ where D is the mean size of the crystal, K is the crystalliteshape factor i.e.0.89, B is the structural broadening (FWHM), and θ is the Braggs angle.

Calculation:
Full Width of Half Maxima =
0.71142radianWavelength
of X-ray = 0.154060nm
Shape constant for spherical
hexagonal = 0.89 Angle of the
obtained maxima (2θ) =
36.16412 Diameter of particle
(D) = $K\lambda/ \beta\cos\theta$ = 11.68nm

Therefore, the approximate average crystal size of zinc nanoparticles is 11.68nm. In the

eISSN-0128-1119

previous research performed by Narayan, Aswath in 2018. The size of zinc oxide nanoparticles was 26nm which were nearly spherical and hexagonal in shape (Narayana et al., 2018).

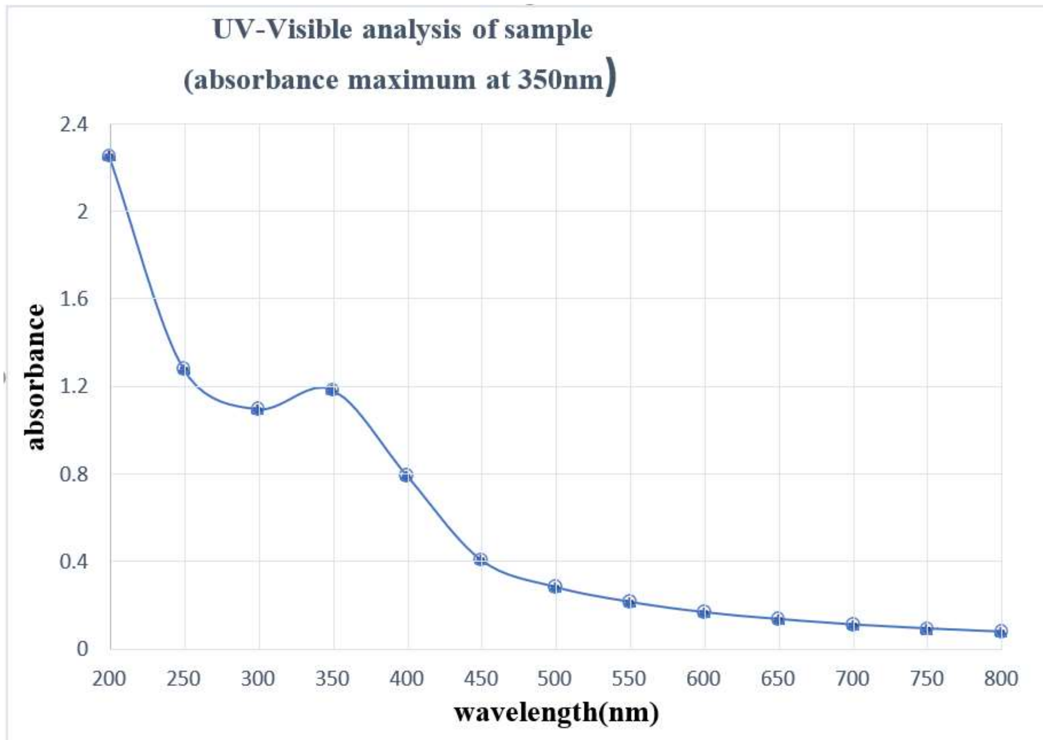

Figure 1:. Graphical representation of zinc nanoparticle showing a maximum peak at 350nm

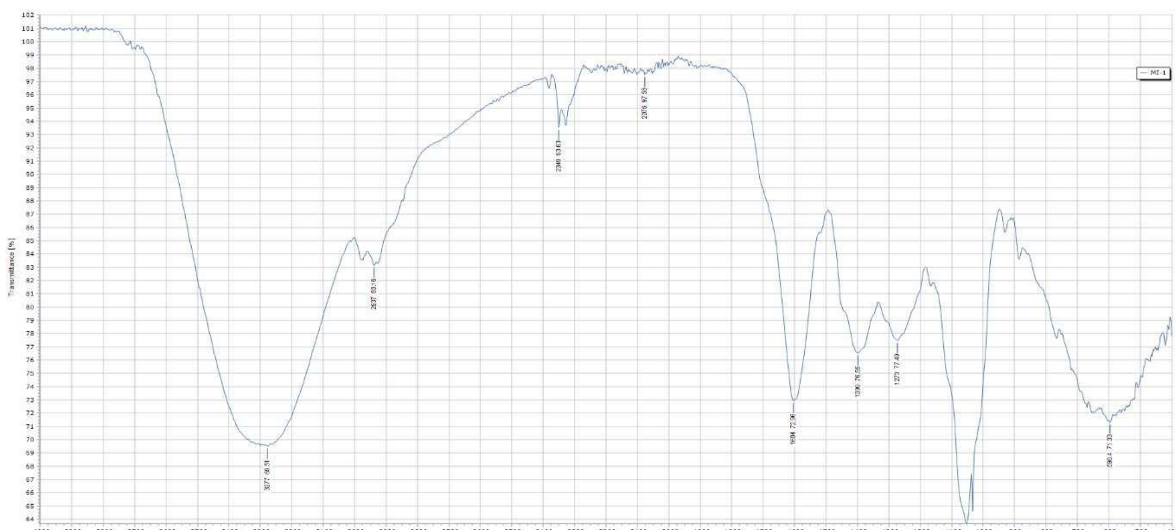

Figure 2: FTIR analysis of extract of leaves of *Mangifera indica* L.

eISSN-0128-1119

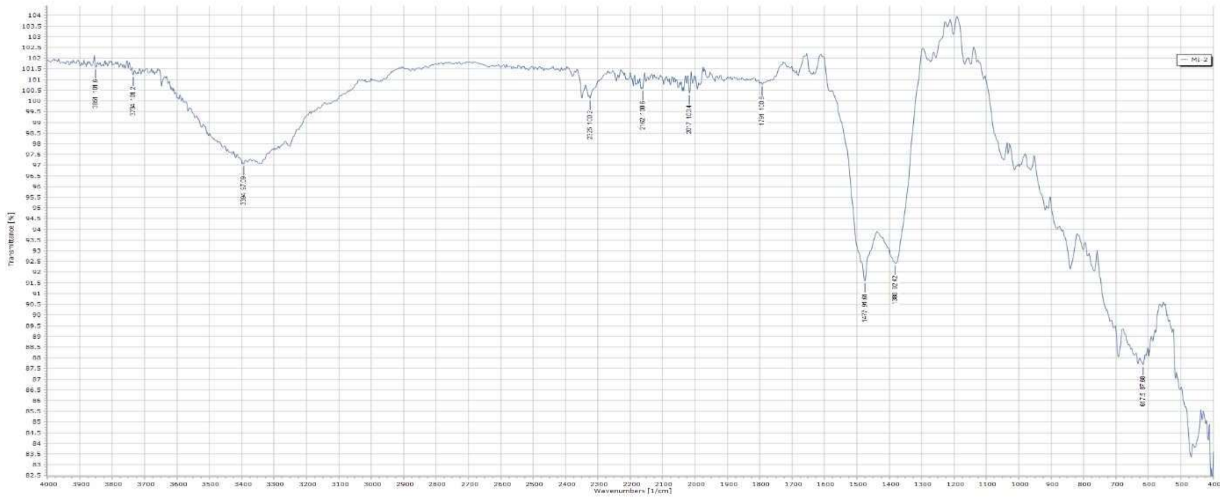

Figure 3: FTIR analysis of zinc nanoparticles

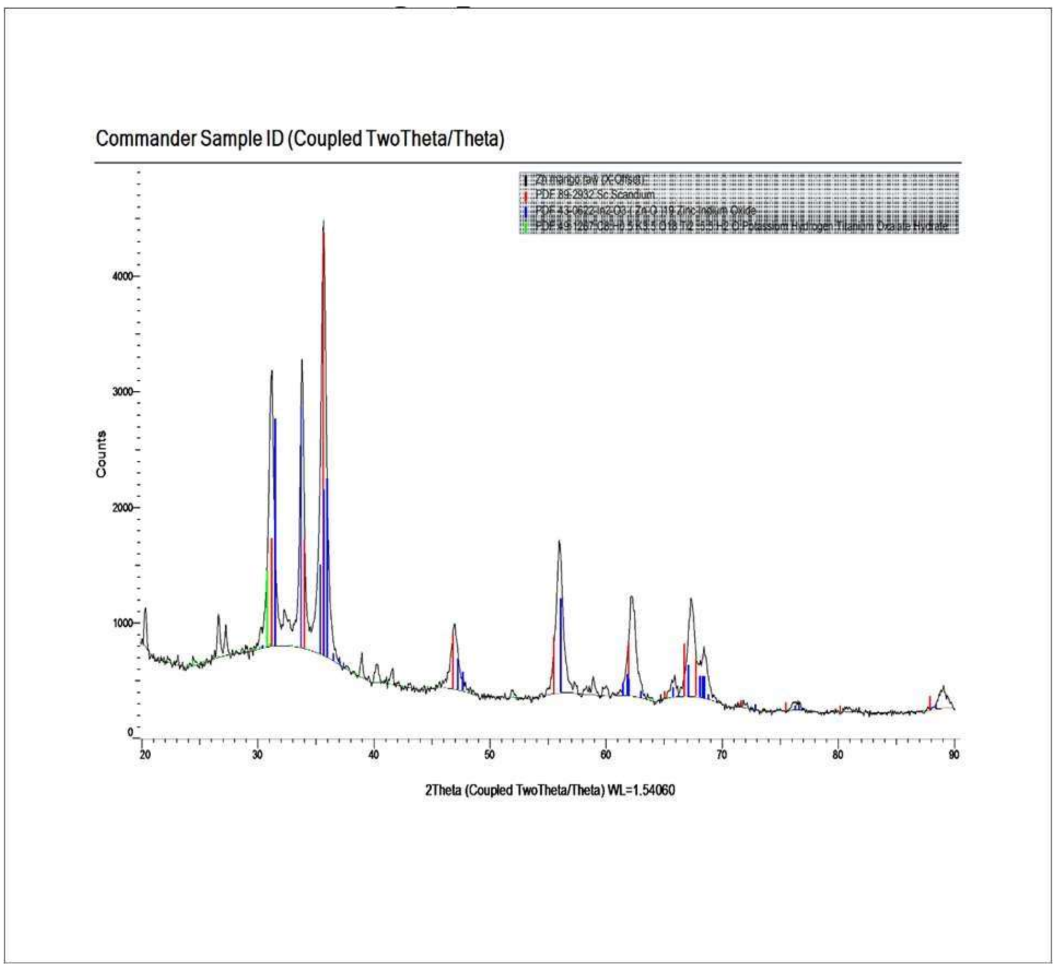

Fig 4: X-ray diffraction analysis of zinc nanoparticles

eISSN-0128-1119

Antibacterial activity: Antibacterial screening was performed by an agar well diffusion method. The organisms were collected from the Natural Product Research Laboratory, Thapathali, Kathmandu, and MMIHS lab. Anti-bacterial screening of extracts was performed at 40 µg/ml, 80µg/ml, 160 µg/ml and 320 µg/ml.
All the ATCC microorganisms were provided by the Natural Product Research Laboratory.

- Gram- positive: *S. aureus, B.s subtilis*
- Gram – negative: *E. coli, K. pneumoniae*
- Control- DMSO
- Standard –Azithromycin and Gentamycin

The aqueous extract of *Mangifera indica* Linn showed antibacterial activity against *K. pneumoniae*, *B. subtilis,* and *S. aureus* in comparison with Gentamicin and Azithromycin as standards whereas it was ineffective against *E. coli* in the current research. The zinc nano solution was effective against all four strains of bacteria (Table 1). In the previous study conducted by Jiang Jinhuan et.al in 2018, similar results were obtained. The difference in the activity of the extract may be due to the different geographical locations, time of collection of leaves, and method of extraction (Jiang et al., 2018).

CONCLUSION

The zinc nanoparticle of average size 11.68 nm was synthesized successfully at low temperature by using zinc acetate as a source of zinc and sodium hydroxide. The method used is simple and cheap which does not require the use of harmful reagents or calcination after drying or sophisticated equipment. The synthesized zinc nanoparticle has potent antibacterial activity as compared to that of the extract. The nanoparticle can be used as an antibacterial agent in both gram-positive and gram-negative bacteria. Recommendations: The effect of geographical variation on the phytoconstituents can be performed to identify the commercial viability of the plant resources. Seasonal variation study is important to study the medicinal value of this plant.

DECLARATION OF CONFLICT OF INTEREST

No conflict of interest to declare.

DECLARATION OF HONOUR

We declare in our honor that our results are not fake and made up.

ACKNOWLEDGMENTS

The authors are thankful to the Institutional Review Committee (IRC) for permitting us to carry out the research (Letter IRC No:360/ dated: 2076/01/25 (May 07/2019) and management of ManmohanMemorial Institute of Health Sciences for providing the lab facility.

Table 1: Antibacterial activity of extract and zinc nanoparticle

Sample	Organisms	Zone of inhibition at different concentration (mm)			
Extract		40(µg/ml)	80(µg/ml)	160(µg/ml)	320(µg/ml)
	K. pneumoniae	3mm	4mm	4mm	4mm
	E. coli	0mm	0mm	0mm	0mm
	B. subtilis	2mm	3mm	3mm	3mm
	S. aureus	0mm	0mm	3mm	4mm
Gentamycin	*K. pneumoniae*	*3mm*	*3mm*	*4mm*	*7mm*
	E. coli	*8mm*	*9mm*	*13mm*	*15mm*
Azithromycin	*B. subtilis*	*10mm*	*12mm*	*19mm*	*21mm*
	S. aureus	*5m*	*7mm*	*12mm*	*14mm*
Zn-NPs	*K. pneumoniae*	4mm	4mm	6mm	7mm
	E. coli	4mm	5mm	6mm	6mm
	B. subtilis	4mm	4mm	6mm	7mm
	S. aureus	2mm	3mm	4mm	6mm

REFERENCES

Andrews, A. (2019). PHYSICAL AND CHEMICAL CHARACTERIZATION OF GREEN SYNTHESIZED SILVER NANOPARTICLES USING STEM OF HIBISCUS VITIFOLIUS L. AND ITS ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL. *Asian Journal of Pharmaceutical and Clinical Research*, *12*, 46-53.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71-79. https://doi.org/https://doi.org/10.1016/j.jpha.2015.11.005

Janjal, S., Agale, A., Rajbhoj, A., & Gaikwad, S. (2017). Synthesis and electrochemical characterization of zinc oxide nanoparticles using green method. *Int. J. Appl. Res*, *10*, 2394-7500.

Jiang, J., Pi, J., & Cai, J. (2018). The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications. *Bioinorg Chem Appl*, *2018*, 1062562. https://doi.org/10.1155/2018/1062562

Khandel, P., Yadaw, R. K., Soni, D. K., Kanwar, L., Shahi, S. K. (2018). Biogenesis of metal nanoparticles and their pharmacological applications: present status and application prospects. *Journal of Nanostructure in Chemistry*, *8*(3), 217-254. https://doi.org/10.1007/s40097-018-0267-4

Narayana, A., Azmi, N., Tejashwini, M., Shrestha, U., & Lokesh, S. (2018). Synthesis and characterization of zinc oxide (zno) nanoparticles using mango (Mangifera indica) leaves. *IJRAR-International Journal of Research and Analytical Reviews (IJRAR)*, *5*(3), 432-439-432-439.

Ojha, A. K., Rout, J., Behera, S., & Nayak, P. (2013). Green synthesis and characterization of zero valent silver nanoparticles from the leaf extract of Datura metel. *Int. J. Pharm. Res. Allied Sci*, *2*, 31-35.

eISSN-0128-1119

Sarsar, V., Selwal, K. K., & Selwal, M. K. (2017). Green synthesis of silver nanoparticles using leaf extract of Mangifera indica and evaluation of their antimicrobial activity. *Journal of Microbiology and Biotechnology Research*, *3*, 27-32.

Shah, K. A., Patel, M. B., Patel, R. J., & Parmar, P. K. (2010). Mangifera indica (mango). *Pharmacogn Rev*, *4*(7), 42-48. https://doi.org/10.4103/0973-7847.65325

Shah, R., Forishmeeta Boruah, F. B., & Nikahat Parween, N. P. (2015). Synthesis and characterization of ZnO nanoparticles using leaf extract of Camellia sinesis and evaluation of their antimicrobial efficacy. *International Journal of Current Microbiology and Applied Sciences*, *4*(8), 444–450.

Sorbiun, M., Shayegan Mehr, E., Ramazani, A., & Mashhadi Malekzadeh, A. (2018). Biosynthesis of metallic nanoparticles using plant extracts and evaluation of their antibacterial properties. *Nanochemistry Research*, *3*(1), 1-16. https://doi.org/10.22036/ncr.2018.01.001

Sumon, D., & Tamalika, C. (2018). A review on green synthesis of silver nanoparticle and zinc oxide nanoparticle from different plants extract and their antibacterial activity against multi-drug resistant bacteria. *Journal of Innovations in Pharmaceutical and Biological Sciences*, *5*(4), 63-73. https://jipbs.com/index.php/journal/article/view/342

Wang, E. C., & Wang, A. Z. (2014). Nanoparticles and their applications in cell and molecular biology. *Integr Biol (Camb)*, *6*(1), 9-26. https://doi.org/10.1039/c3ib40165k

eISSN-0128-1119