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

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Abstract: The aim of the research study was synthesis of zinc oxide nanoparticles using leaves extract of *Mangifera indica* L and evaluation of its antibacterial activity. 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 extract and the synthesized nanoparticles were evaluated using Agar well diffusion method. The characterization 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 XRD analysis was used to calculate the size of zinc nanoparticle and the average size was found to be 11.68nm. The antibacterial activity of synthesized zinc nanoparticle showed improved sensitivity towards both the gram positive and gram-negative bacteria in comparison to plain extract. The study shows that the zinc nanoparticles of leaves extract of *Mangifera indica* Linn is worth to synthesize. The synthesized nanoparticle possesses promising anti-bacterial activity than plain extract. The synthesis of nanoparticle using the plant source is environment friendly, cost-effective and safer.

***Keywords**: Mangifera indica* Linn, zinc nanoparticles, UV-Visible spectroscopy, X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), antibacterial activity.

INTRODUCTION

Among the various aspects of the 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,

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copper, zinc and iron nanoparticles have gained a greater attention due to their wide application in science such as medicine, chemistry, agriculture and biotechnology. [1] Among the various method for the synthesis of nanoparticles, the chemical reduction method is highly implied among all the method but its application is limited due to higher toxicity.[2] Hence, Green synthesis of nanoparticle has been evolving as an important branch as it deals with environmentally friendly, cost effective and safer method for the synthesis of nanoparticles. It is gaining importance as it eliminates the use of harmful reagents and synthesize the expected product in an economical manner. [3] Nanoparticles are

commonly employed for imaging, sensing, drug delivery vehicles to target specific sites such as lung tissue as well as cancer therapy and vaccinations. [4]

Mangifera indica L. belonging to the family Anacardiaceae is commonly called as 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 as horticulture and medicinal plant. [5] Various part of the plant is 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 acts as astringent. [4] The major nutritional antioxidants, vitamin E, vitamin C and β-carotene, may be beneficial to prevent several chronic disorders. [5] The main active components found are Mangiferin and chinonin. Among all the components, Mangiferin shows strong antioxidant activity. It has a number of therapeutic action and many health benefits such as antidiabetic, antifungal, antimicrobial, anti- inflammatory, antiviral, hypoglycemic, anti- allergic and anticancer activity, etc. [6]

MATERIALS AND METHOD

Plant material: The leaves of plant *Mangifera indica* L. were collected from Kirtipur, Kathmandu, Nepal.The collected material was identified at National Herbarium and Plant Tissue Laboratory Godawori, Lalitpur. The Herbarium entry number was114.The leaves of *Mangifera indica* Linn were thoroughly washed and was shade dried for 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 such as extraction, synthesis of zinc nanoparticles is of laboratory and analytical grade. Distilled water, methanol, ethanol is 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 (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 powdered leaves were taken in a 250ml beaker containing 100ml of distilled water. The mixture was then boiled for 15 minutes and then filtered using whatman no. 41 filter paper. the resulting filtrate was used for the preparation of zinc nanoparticles.

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Preparation of zinc oxide nano powder: 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 and 1 ml of the extract of the mango plant was dripped to the same. The mixture was stirred for 20 minutes the pH was maintained at pH12 by adding 1M NaOH dropwise which resulted in pale white aqueous solution. This was then agitated for 2 hrs. after that pale white precipitate was taken out and washed with distill water 2-3 times followed by ethanol. Then the powder was dried at 40°C in oven overnight and zinc oxide nanoparticles was obtained.[7]The characterization of zinc oxide nanoparticles is essential to confirm its presence which is done by methods like:

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

Evaluation of anti-bacterial activity: Antibacterial activity was evaluated by agar well diffusion method. In the method test organisms were collected, pure culture of organism was isolated and was standardized with reference to 0.5M Mac-Farland standard. All ATCC subculture of bacteria were provided from Natural Product Research Laboratory, Thapathali, Kathmandu. The fresh culture bacterial isolates were used. *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus subtilis* were inoculated into5ml 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 to 0.5 Mac Farland standard. A 8mm standard well cutter was used to bore a well into the media. Then, extracts were placed inside a cup with a micropipette. The plate was then incubated overnight, 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. [8,9]

RESULT 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.[10] For UV visible analysis the plant extract was mixed with zinc acetate solution in the ratio of 1:50 and the solution was further diluted to 1:10 ratio by addition of distill water.The solution of *Mangifera indica* L. and zinc acetate when observed in the UV-Visible spectroscopy confirmed the formation of zinc nanoparticle showing peak at 350nm.

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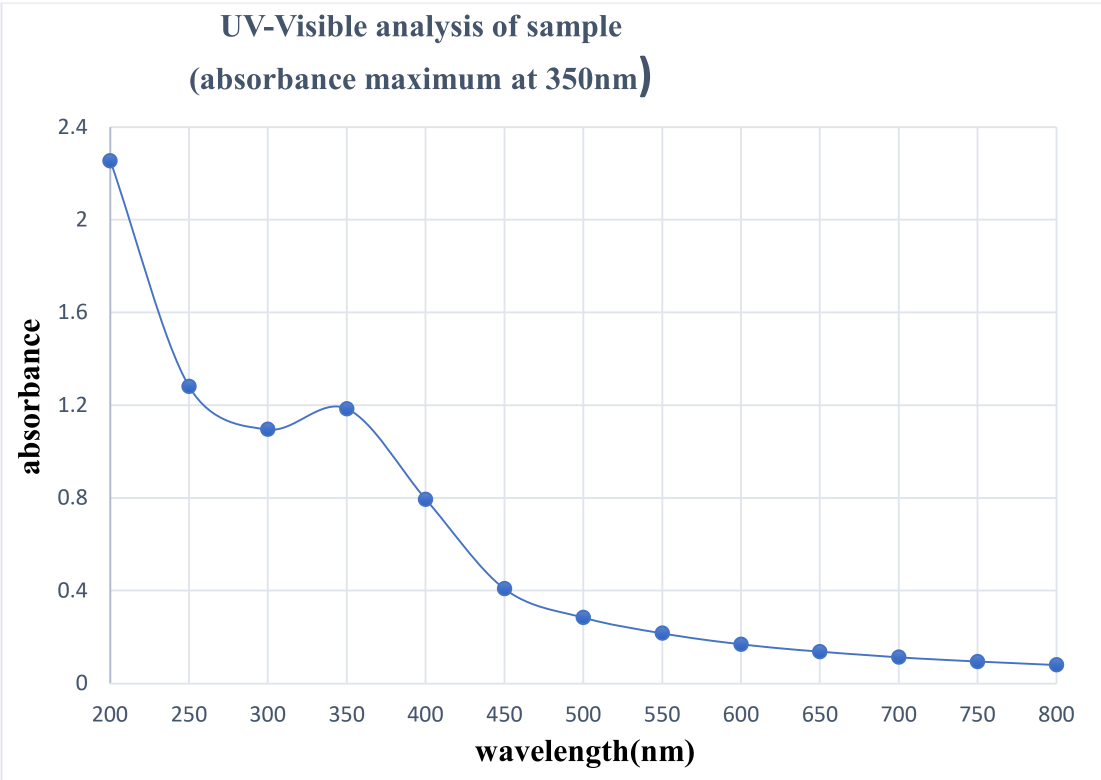

Fig 1: graphical representation of zinc nanoparticle showing maximum peak at 350nm

In the current study the UV spectroscopy analysis showed absorbance maximum for zinc nanoparticles at 350nm and in the previous study by Shah, Rajesh Kumar in 2015 it was found to be 330nm.[11]

FTIR analysis: The functional groups present in *Mangifera indica* L. Leaf extract was predicted by the FTIR analysis. FTIR analysis of nanoparticle was also carried out. The IR spectra thus obtained are as in fig 2 and 3 below:

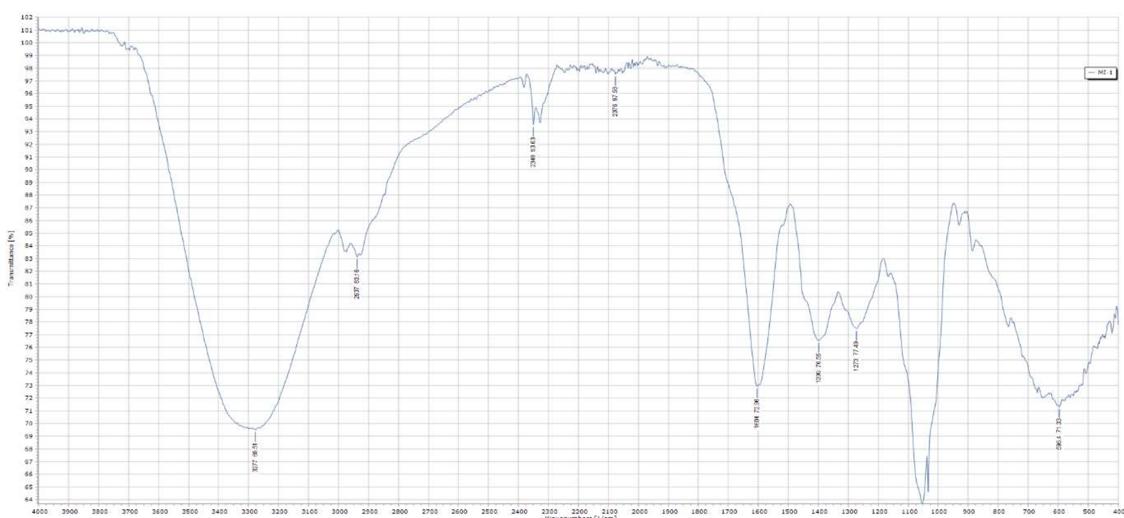

Fig 2: FTIR analysis of extract of leaves of *Mangifera indica*

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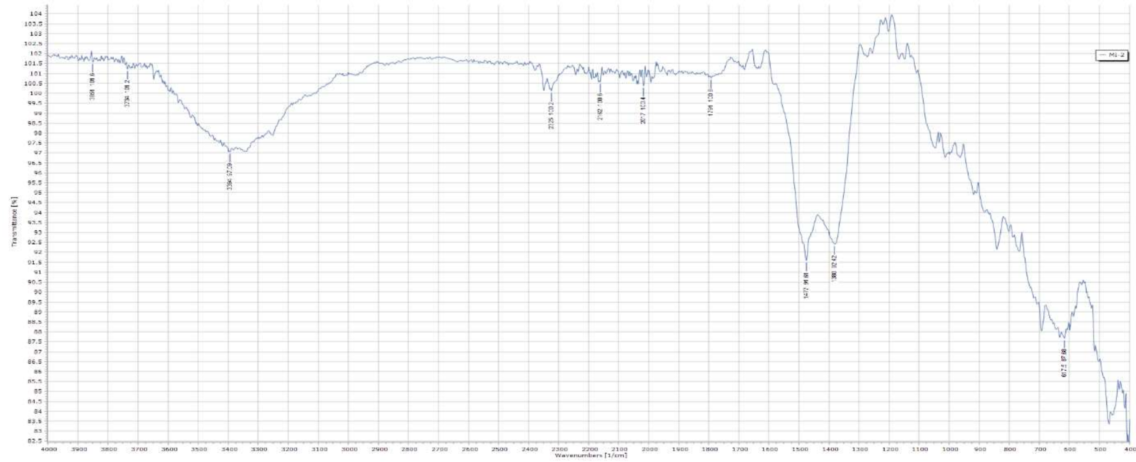

Fig 3: FTIR analysis of zinc nanoparticles

The FTIR analysis of the synthesized zinc oxide nanoparticles from the leaves extract were done in order to find out the functional group present in the particles ranging from4000-400 $cm^{-1.}$ The peak in the area of 3200-3600 $cm^{-1.}$ represents the –OH stretching vibration come 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, C=O vibration to higher wave number. 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 nano powder synthesized by aqueous chemical method. The XRD spectrum indicates that the zinc oxide powder has hexagonal structure. The graph obtained from the XRD analysis is shown below:

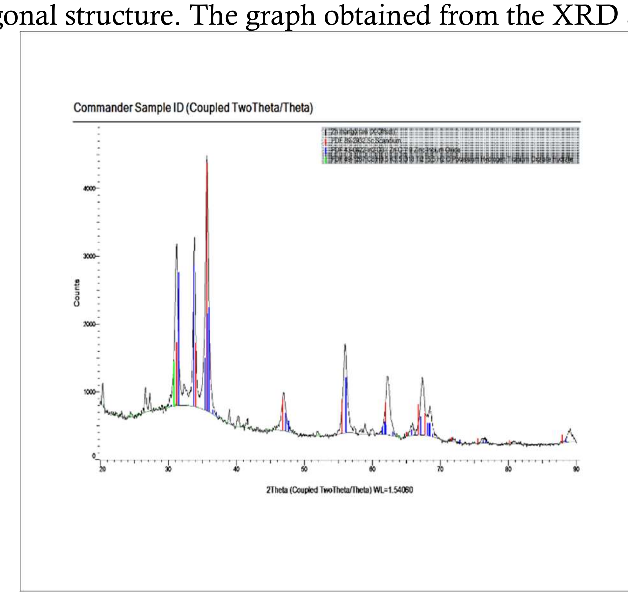

Fig 4: X-Ray diffraction analysis of zinc nanoparticles

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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 crystallite shape factor i.e.0.89, B is the structural broadening (FWHM), and θ is the Braggs angle.

Calculation:
Full Width of Half Maxima = 0.71142radian
Wavelength 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 nanoparticle is 11.68nm.
In the previous research performed by Narayan, Aswath in 2018. The size of zinc oxide nanoparticles was 26nm which were nearly spherical and hexagonal in shape.[12]

*Anti-bacterial activity:*Anti-bacterial screening was performed by 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 Natural Product Research Laboratory.

- Gram- positive: *S. aureus, Bacillus subtilis*
- Gram – negative: *E. coli, Klebsiella 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. 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 location, time of collection of leaves and method of extraction. [13]

CONCLUSION

The zinc nanoparticle of average size 11.68 nm was synthesized successfully at low temperature by using the zinc acetate as 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 nano particle has potent antibacterial activity as compared to that of the extract. The nanoparticle can be used as antibacterial agent in both the gram positive and gram-negative bacteria. Recommendations: 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. Suggested to estimate the IC50 value before proceeding further research. Biological studies study in animal model is suggested.

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Table no.1: Anti-bacterial activity of extract and zinc nanoparticle

Sample	Organisms	Zone of inhibition at different concentration (mm)			
		40(μg/ml)	80(μg/ml)	160(μg/ml)	320(μg/ml)
Extract	*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

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DECLARATION OF CONFLICT OF INTEREST

No conflict of interest to declare.

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