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Utilizing Aqueous Extracted Fraction Of Mangifera Indica For The Green Synthesis Of Zinc Oxide Nanoparticles

Mradul Mishra[1], Surya Prakash Gupta[2*]

[1]Rajiv Gandhi Institute of Pharmacy, Faculty of Pharmaceutical Science & Technology, AKS University, Satna, India
[2*]Professor & Director, Rajiv Gandhi Institute of Pharmacy Faculty of Pharmaceutical Science & Technology, AKS University, Satna, India – 485001, suryatony@yahoo.co.in

***Corresponding author:** Dr. Surya Prakash Gupta
*Professor & Director, Rajiv Gandhi Institute of Pharmacy Faculty of Pharmaceutical Science & Technology, AKS University, Satna, India – 485001, suryatony@yahoo.co.in

ABSTRACT
The present study was commenced with an objective to produce zinc oxide nanoparticles aided with *Mangifera indica* leaf extract and study the antibacterial activity of the nanoparticles. The leaves of Mangifera indica were extracted using deionized water using cold maceration method. The zinc oxide nanoparticles were produced utilizing the plant extract with zinc nitrate in alkaline condition. The synthesized ZNPs were characterized using spectrometry, XRD, particle size and SEM. The lowest particles size was obtained with 30% extract concentration in relation to the zinc nitrate solution. The particle size was between 44.2 nm to 27.4 nm. The ZNPs were found to be spherical structures with smooth surface. The reduction and thermal decomposition were carried out to conclude a reduction in particles size with an initial increase in concentration of the extract but after increasing the concentration to higher than 30% the particle size remained unaffected. The UV absorption spectra revealed broad absorption band at around 367 nm, characteristic of zinc oxide. The ZNP displayed antibacterial action in gram positive and gram-negative bacteria. It could be concluded from the results that the aqueous extract of Mangifera indica was effective for biosynthesizing zinc oxide nanoparticles with good antibacterial activity.

Keywords: Zinc oxide, nanoparticles, antibacterial, Mangifera indica, aqueous extract

INTRODUCTION
The production of nanostructures and nanoparticles (NPs) and their application in a variety of industries, including pharmaceutics, biomedical technologies, cosmetics, nutrition and health care, and other disciplines, are the topics of the emerging new research field known as nanotechnology (Ahmed et al., 2017). Polymeric nanoparticles as drug delivery vehicles offer a multitude of advantages, including increased oral bioavailability of drugs and reduced dosage frequency. A variety of anticancer, antimicrobial, antituberculosis, peptide and protein-based drug formulations with nano drug carriers have been researched for their therapeutic effectiveness. In spite of the wide spread research, sustained release formulations with nanopolymeric carriers could be found only in small numbers in the market, with most of them in the form of ointments or wound healing bandages.

NPs are frequently referred to be the "wonder of modern medicine" because of their unique qualities. For the creation of NPs, a number of physical and biological methods are introduced, using a number of hazardous and extremely toxic substances (Siddiqi et al., 2018). Therefore, a suitable replacement is necessary and might be obtained by greener synthesis methods (GS). The use of plant extract in GS of NPs is a usual environmentally friendly approach, and it has many benefits, such as superior products and NPs produced, easiness of processing and controlling, safety, the richness of resources, and cost-effectiveness (Matussin et al., 2020; Rahman et al., 2021; Ettadili et al., 2022; Urge et al., 2023; Habeeb et al., 2022; Tirumala et al., 2022; Furtat et al., 2020; Abel et al., 2021). The primary aim of this investigation is to perform the green synthesis of Zinc Oxide nanoparticles using Mangifera indica leaf aqueous extract.

MATERIALS AND METHODS
Materials
The chemical and reagents used for the study were procured from various suppliers. *Mangifera indica* leaves were collected from the plants around the local region and were authenticated before use.

Methods
(1) Preparation of plant material
The leaves were rinsed a number of times with distilled water for removal of debris followed by drying at room temperature in dark to remove the moisture content. The dried leaves were crushed to coarse powder using a slow driven grinder.

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(2) Extraction of plant material
The powdered leaf was weighed (42 g) and filled in the extractor of a Soxhlet extraction apparatus. Petroleum ether (95 mL) was flown down the extractor and the temperature of the solvent was raised to 80°C for 2.5 h. The solvent was separated from the marc, the marc was dried. The dried marc was macerated with 500 mL of cold water for 24 h by intermittent shaking for first 6 hours followed by standing for 18 hours. The menstruum was filtered using muslin cloth and was stored in refrigerator till further use (Kaksen et al., 2021).

(3) Preparation of Zinc oxide nanoparticles
The zinc oxide nanoparticles were prepared using the plant extract and zinc nitrate in alkaline condition. Various ratio of extract and zinc nitrate were used for preparing the nanoparticles (Table 1). Accurately weighed quantity of zinc nitrate was mixed to 50 mL of distilled water to attain final strength of zinc nitrate (8% & 10% w/v). The required milliliters of Mangifera indica leaf extract were slowly mixed in the zinc nitrate solution. 1 M solution of NaOH was added dropwise to this solution for controlling the pH at 12 (Shaheen et al., 2022; Rashid et al., 2020). After eight to ten hours of stirring the mixture at room temperature, the greenish liquid gradually began to disappear, giving way to a yellow suspension and the creation of a pale-yellow precipitate. To get rid of insoluble zinc nitrate and other contaminants, the precipitate was collected using filter paper and washed with tap water and ethanol. The precipitate was first dried in an oven at 80 °C for about 12 hours, and then it was calcined in a muffle furnace for 2 hours at 350 °C.

Table 1: Formulation variables for ZnO nanoparticles

Formulation	***Mangifera indica* extract (%)**	**Zinc nitrate (g)**
ZNP1	10	4
ZNP2	20	4
ZNP3	30	4
ZNP4	40	4
ZNP5	50	4
ZNP6	30	5

(4) Characterization of ZNPs
(a) UV-Visible spectroscopic study
The ZNPs synthesized were dissolved in 0.1N HCl solution and the UV-visible absorption spectra of the solution was obtained between 700-200 nm. The spectra were studied for the observed band gap (Cheena et al., 2011).

(b) Morphology and Size
The particle size of the ZNPs was determined by a light scattering particle size analyzer. The ZNPs were suspended in deionized water and the sample was placed in the sample holder of the analyzer. The particle size and the polydispersity index were obtained. The morphology of the ZNPs was studied with the help of scanning electron microscopy. The particles were coated with gold sputter on a metal stub and was scanned with an electron beam to obtain magnified image of the surface. The surface characteristics were observed from the image.

(c) X-ray diffraction study
X-ray diffraction pattern of the prepared ZNPs was studied for obtaining the information about the crystal structure of the particles.

(d) FT-IR spectral study
The stretching vibrations characteristic of the zinc oxide nanoparticles was studied by obtaining the FT-IR spectra of ZNPs.

Antibacterial activity of ZNPs
The bacteria (*Escherichia coli* (MTCC 40), and *Staphylococcus aureus* (MTCC 3160) were used for the antibacterial evaluation of the produced ZNPs. A few drops of the bacterial solution were added to pre-poured, 3 mm thick nutrient agar plates by swabbing the agar's surface. The disc diffusion method was used to screen for antibacterial activity. Using a cork borer (10 mm), 15 wells were drilled into the agar plate at equal intervals. 200 µL of ZNPs (50, 75, 100, and 150 µg/mL) were added to each well. The plates were allowed to grow microbiologically by being incubated for 24 hours at 37 ± 0.1°C. Each plate's zone of inhibition was measured in millimeters (Mishra et al., 2013).

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RESULTS AND DISCUSSION
The clean and dried leaves were powdered and defatted with petroleum ether. The defatted material was macerated using deionized water to obtain the extract solution. The extract solution was dark green in color, with a characteristic odor.

Synthesis of ZNPs
The schematic illustration for the synthesis of ZNPs is presented in Figure 1. The precursor used in the synthesis was zinc nitrate in alkaline medium (1 M NaOH). The mixtures containing the precursor and plant extract were stirred and was observed for color change from dark green to light brown through the course of stirring. The change in color of the solution indicates the conversion of zinc nitrate to zinc oxide. The presence of sodium hydroxide causes formation of zinc hydroxide through reduction as the initial intermediate which on thermal decomposition leads to the formation of zinc oxide (da Silva et al., 2020). Calcination at 400°C was done in muffle furnace to obtain particles with lower particle size. Previous studies have suggested that a temperature higher than 350°C for thermal decomposition of the zinc hydroxide leads to particles with higher particles size (Baharaudin et al., 2018; Ashraf et al., 2015).

Figure 1: Schematic representation of ZNP preparation

Effect of extract ratio on particles size of ZNP
The influence of extract concentration on the size of ZNP has been observed by varying the concentration of the extract and using fixed amount of zinc nitrate (8 %w/v). The reduction and thermal decomposition were carried out and it was observed that the size of the ZNP reduced with an initial increase in concentration of the extract but after increasing the concentration to higher than 30% the particle size remained unaffected (Figure 2). The lowest particles size was obtained with 30% extract concentration in relation to the zinc nitrate solution.

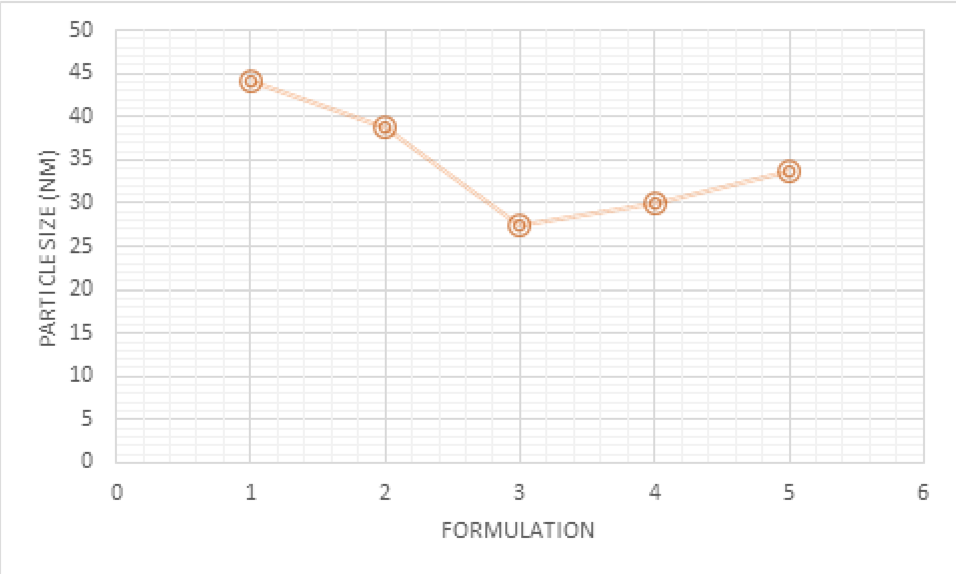

Figure 2: Effect of extract concentration on ZNP particles size

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Effect of zinc nitrate on particle size of ZNP
With the intention of assessing the effect of zinc nitrate ratio on the size of the particles synthesized, nanoparticles were synthesized using 30% extract and 10%w/v zinc nitrate. The particles size was found to remain unaffected by the amount of zinc nitrate in the solution. The particles size of the formulation was obtained to be 28.1 nm, almost equal to the particle size obtained with 30% extract and 8% w/v zinc nitrate.

Characterization of ZNPs
The synthesized ZNPs were characterized with respect to their size, surface morphology, crystallinity, FT-IR spectral study and UV absorption spectra.

(1) Particle size and morphology
The particle size of the ZNPs was measured using dynamic light scattering principle with the aid of particle size analyzer. The size of the synthesized ZNPs occurred between 44.2 nm to 27.4 nm (Table 2). The particles size was found to be affected by the concentration of the extract whereas the concentration of zinc nitrate did not affect the particle size. The particle size data obtained for ZNP3 is presented in Figure 3.

Table 2: Particle size of synthesized ZNPs

Formulation	Particle size (nm)
ZNP1	44.2
ZNP2	38.9
ZNP3	27.4
ZNP4	30.1
ZNP5	33.6

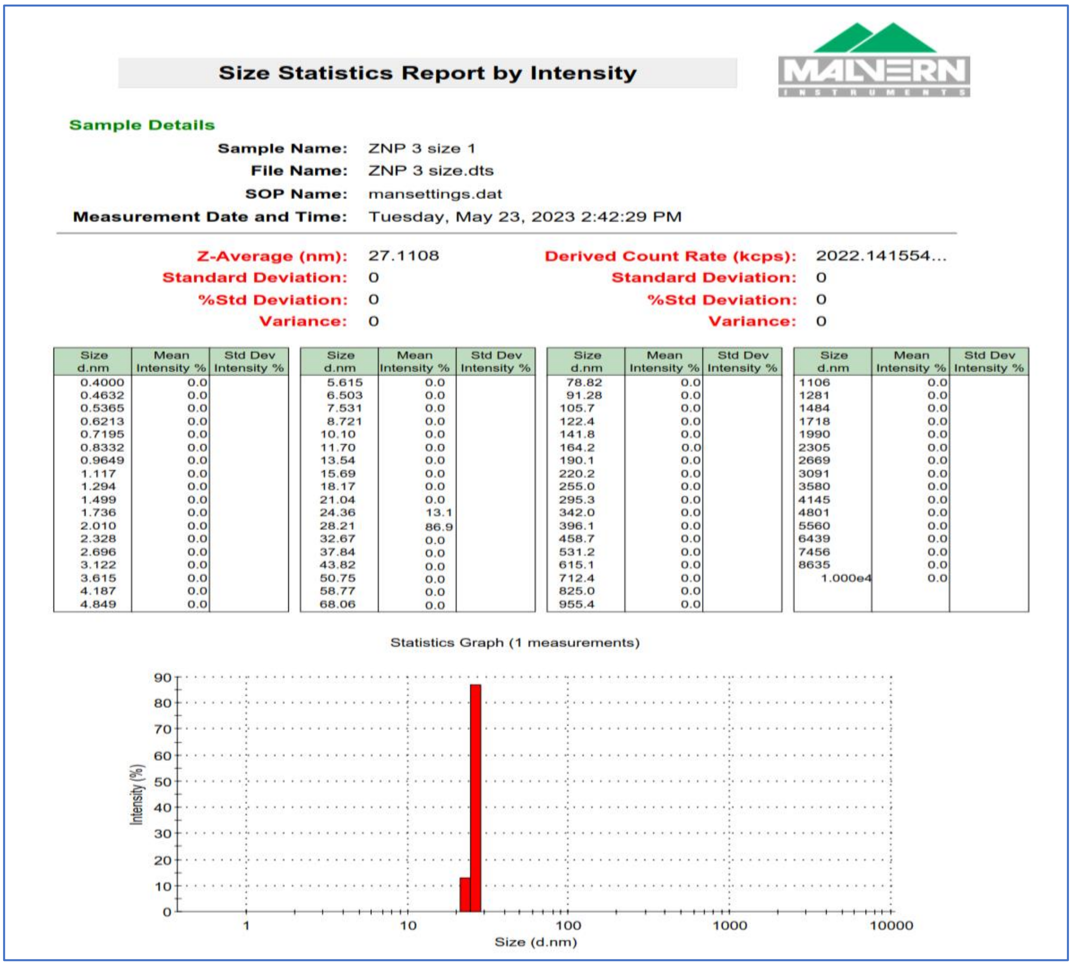

Figure 3: Particle size by intensity for ZNP3

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The external morphology of the synthesized ZNP was studied using scanning electron microscopy (SEM). The gold sputter coated particles were scanned under beam of electron and the image obtained was used to study the surface characteristics of the ZNPs. The ZNPs were found to be spherical structures with smooth surface. Moreover, the clusters of particles were also observed in the images (Figure 4).

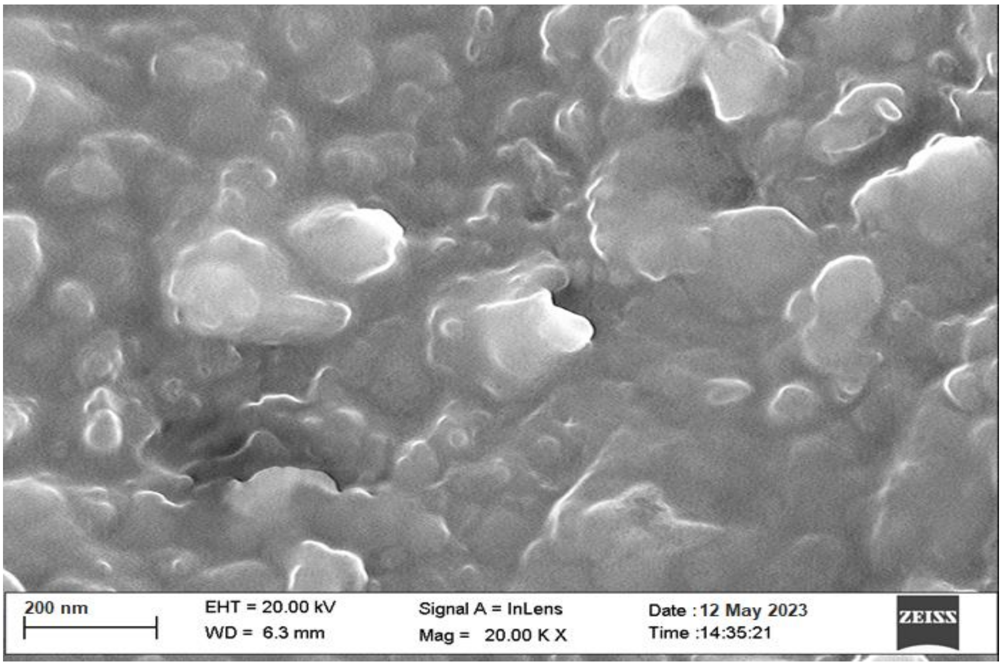

Figure 4: SEM image of ZNP3

(2) X-ray diffraction study
The crystallinity of the synthesized ZNPs was studied using XRD. Diffraction peaks was observed at 36°, 41°, 43°, 47.5° and 50° indicating the pattern of pure Zinc oxide with a hexagonal wurtzite polycrystalline structure with lattice planes (Figure 5).

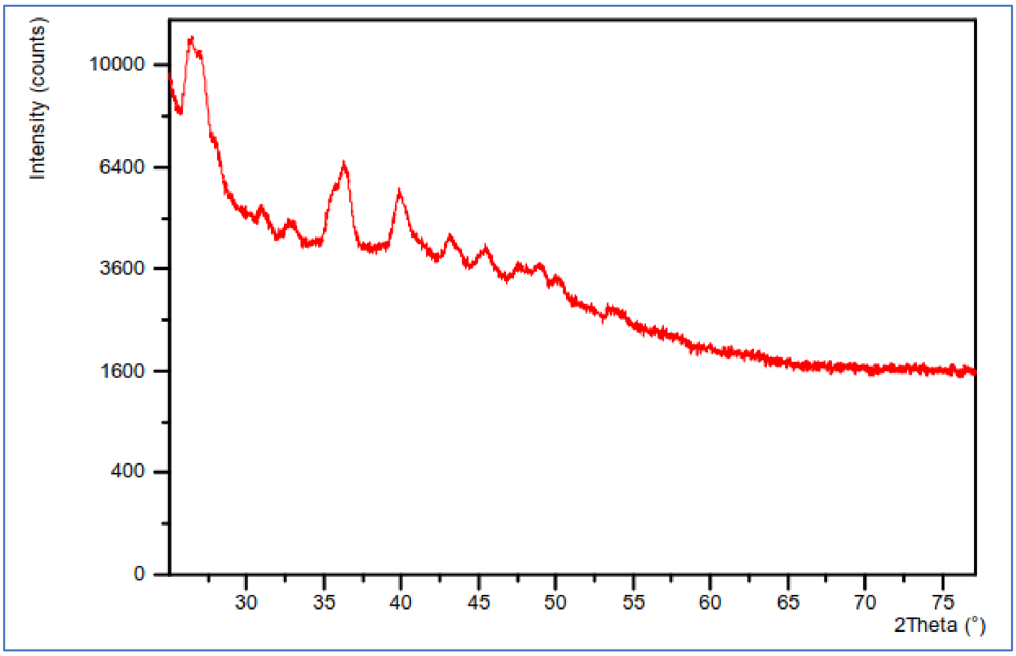

Figure 5: XRD pattern exhibited by ZNP3

(3) FT-IR spectral study
The FT-IR of the solid zinc oxide nanoparticles was obtained and observed for the occurrence of stretching of the characteristic groups. The occurrence of transmittance peaks at 510 and 490 cm-1 are typical of metal-oxygen (ZnO

stretching vibrations) (Figure 6). The broader peak at around 3500 cm-1 could be ascribed to the O-H stretching of flavonoids and polyphenols of the extract.

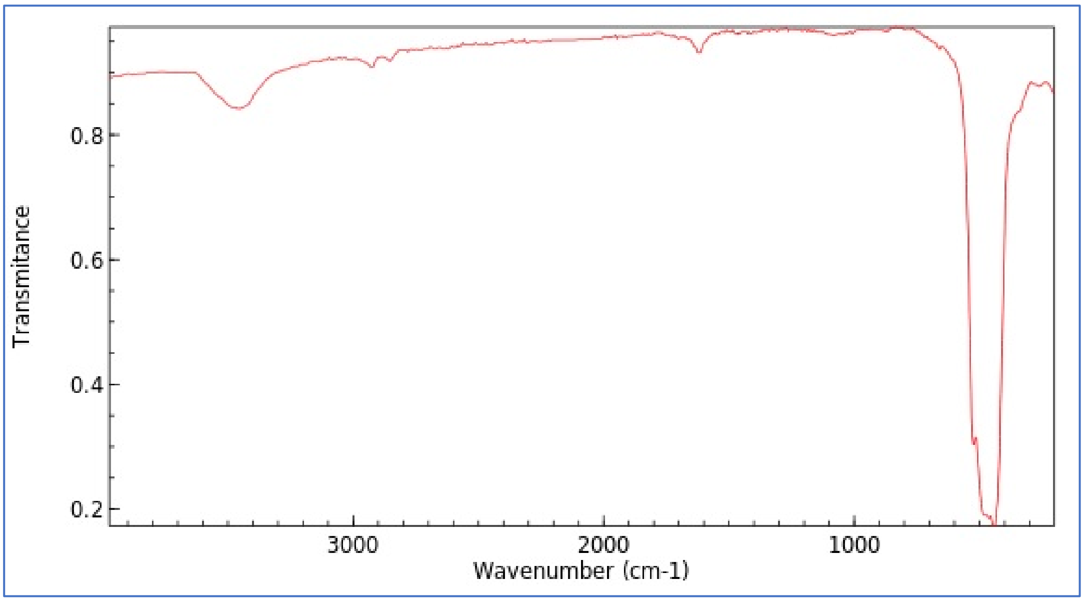

Figure 6: FT-IR spectrum of ZNP3

(4) UV-Visible spectrophotometric study
The solid white zinc oxide nanoparticles were dissolved in 0.1N HCl and UV absorption was studied. Typically, the temperature, size, and morphologies of the manufactured nanostructures affect the spectrophotometric absorptive pattern. There is a relationship between the ZNPs' size and UV–visible absorption. In deionized water, the ZnO-HPNs' UV–Vis spectrum was measured. At 367 nm, broadband was detected, which was comparable to the "1s–1s electron transition" bandgap of zinc oxide.

Antibacterial activity
The antibacterial action of ZNP3 was studied using disc diffusion method. The zone of inhibition obtained was taken as a measure of antibacterial activity. The ZNP was found to show activity against both gram positive and gram-negative bacteria (Figure 7).

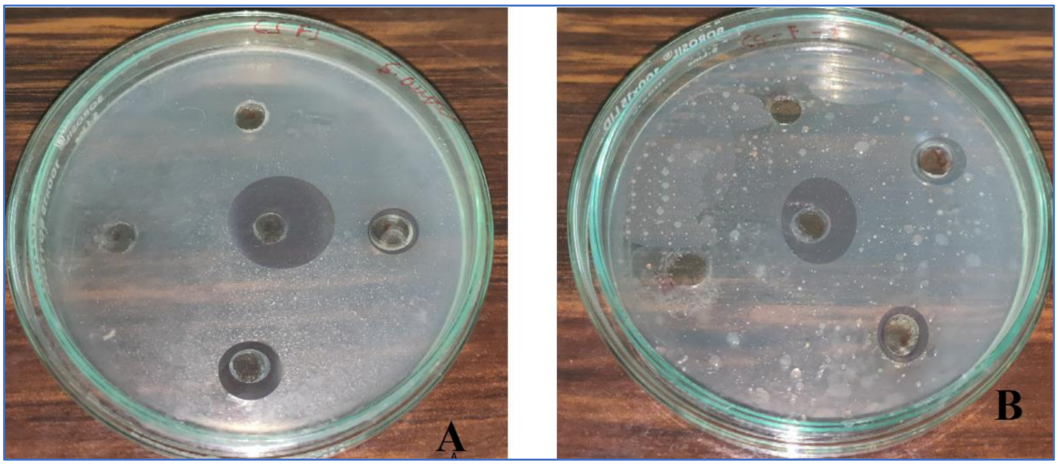

Figure 7: Antibacterial action of ZNP (A) S. aureus (B) E. coli

CONCLUSION
The aim of the present study was to synthesize zinc oxide nanoparticles using Mangifera indica leaf extract and study the antibacterial action of the produced nanoparticles. The results suggest that *Mangifera indica* aqueous extract is potential source of reducing agent for synthesize of zinc oxide nanoparticles. The ZNPs were evaluated for particle size, surface morphology, XRD and UV absorption. The smallest particles were of smaller in size when 30% extract was used with 8%w/v solution of zinc nitrite in alkaline medium and was considered as the best formulation (ZNP3).

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