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Formulation and Characterization of zinc oxide nanoparticles by green synthesis method

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Abstract
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, anti-tuberculosis, peptide and protein based drug formulations with Nano drug carriers have been researched for their therapeutic effectiveness. Inspite of the wide spread research, sustained release formulations with nano-polymeric carriers could be found only in small numbers in the market, with most of them in the form of ointments or wound healing bandages. Green synthesis of nanoparticles is a common eco-friendly method using plants extract is due to numerous advantages, including better products and worth of NPs obtained, easiness of process and control, safety, the richness of resources, and cost-effective. The objective of the present investigation is to perform the green synthesis of Zinc Oxide nanoparticles using *Psidium guajava* leaf aqueous extract. The ZNPs were found to exhibit antibacterial activity against gram positive and gram negative bacteria.

Keywords: Nanoparticles, *Psidium guajava*, green synthesis

1. Introduction
Drug delivery has always been an evolving component of biomedical research, ever since the advent of synthetic drugs and surgical medicine. Numerous techniques and materials have been explored for the delivery of drugs through intravenous, intramural, intramuscular, oral and transdermal administrations in animals and humans. Conventional delivery of drugs, though very simple and widely followed even today to treat common ailments, often requires frequent and higher dosage of medicines and produces a spectrum of side effects. Paradigm shift in the treatment of diseases such as cancer, tuberculosis, autoimmune disorders and cardiovascular malfunctions have become possible due to the development of targeted and sustained delivery of the drugs using delivery vehicles.

Nanotechnology
The term "nanotechnology", first used by Taniguchi in 1974 refers to the development and study of materials with at least one dimension on the order of 1 - 100 nm. Nanotechnology is a rapidly growing and diverse field that impacts many areas of science and engineering, and has the potential to revolutionize a vast array of technologies, from power generation and electronics to disease detection and treatment. A nanoparticle is the most fundamental component in the fabrication of a nanostructure, and is far smaller than the world of everyday objects that are described by Newton's laws of motion, but bigger than an atom or a simple molecule that are governed by quantum mechanics. The human use of nanoscale materials is not new: For example, colloidal gold has been deliberately studied for over a century (Cao, 2004) [3] and has been applied in medical treatments since the late 1940s by (Swanson, 1949) [4] but examples of its use as a pigment for glass and ceramics date back as far as the Roman era, such as in the Lycurgus Cup (Freestone *et al*., 2007) [5]. Many of the ideas and possibilities for miniaturization to the atomic and nanoscale were famously outlined by Feynman, in his 1959 [6] lecture "There's Plenty of Room at the Bottom" (Feynman, 1959) [6] however, the most significant surge in nanomaterials research has occurred only in the last twenty-five years, driven by vast improvements in scientists "ability to image and manipulate matter on the atomic scale, and by the realization of the unique, size-dependent properties of materials on length scales of a few nanometers (Rogers *et al*., 2008; Cao, 2004 and Marshall, 2023) [2, 3, 7].

Types of Nanoparticles

Nanoparticles can be broadly grouped into two, namely, organic nanoparticles which include carbon nanoparticles (fullerenes) while, some of the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semi-conductor nanoparticles (like titanium oxide and zinc oxide). There has been a growing interest in inorganic nanoparticles i.e. of noble metal nanoparticles (Gold and silver) as they provide superior material properties with functional versatility. Due to their size features and advantages over available chemical imaging drug agents and drugs, inorganic particles have been examined as potential tools for medical imaging as well as for treating diseases. Inorganic nonmaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good compatibility, and capability of targeted drug delivery and controlled release of drugs (Xu *et al*., 2006) [8].

2. Material and Methods

2.1 Collection of plant material

The leaves of *Psidium gujava* were collected from the local surroundings of Bhopal and were authenticated at Minor Forest Produce Processing & Research Centre (MFP PARC), Bhopal.

2.2 Preparation of plant material

The leaves were washed several times with water to remove the dust particles and allowed to dry at room temperature in dark to remove the moisture content. The dried leaves were crushed to coarse powder using a slow driven grinder. The powder was stored in airtight container till use.

2.3 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 solvent was heated at 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 menstrum was filtered using muslin cloth and was stored in refrigerator till further use (Ruksar and Chaurey, 2021) [9].

2.4 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).

Table 1: Formulation variables for ZnO nanoparticles

Formulation	*Psidium gujava* extract (%)	Zinc nitrate (g)
F1	10	4
F2	20	4
F3	30	4
F4	40	4
F5	50	4
F6	30	5

Accurately weighed quantity of zinc nitrate was added into 50 mL of deionized water to get final concentration of zinc nitrate (8% & 10% w/v). The required milliliters of *psidium gujava* leaf extract were slowly added to zinc nitrate solution in the beaker. 1 M of NaOH was added drop-by-drop to the solution to control the pH of the solution at 12 (Shaheen *et al*., 2022 and Rashid *et al*., 2020) [10, 11]. The mixture was stirred at room temperature for eight to ten hours until the greenish liquid slowly started to fade with the appearance of yellow-colored suspension followed by the formation of a pale-yellow precipitate. The precipitate was collected with filter paper and cleaned with tap water and ethanol to eliminate insoluble zinc nitrate and other impurities. The precipitate was dried in an oven for about 12 hour at 80 °C, and lastly calcining for 2 hour at 350 °C in a muffle furnace.

2.5 Characterization of ZNPs

2.5.1 UV-Visible spectroscopic study

The ZNPs synthesized (F1-F6) were dissolved in 0.1N HCl solution and the UV-visible absorption spectra of the solution was obtained between 700-200 nm. The spectra was studied for the observed band gap (Chena *et al*., 2011) [12].

2.5.2 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.

2.5.3 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.

2.5.4 FT-IR spectral study

The stretching vibrations characteristic of the zinc oxide nanoparticles was studied by obtaining the FT-IR spectra of ZNPs.

3. Results and Discussion

The present investigation was undertaken with an objective to synthesize zinc oxide nanoparticles using *Psidium gujava* leaf extract and study the antibacterial action of the produced nanoparticles.

3.1 Extraction of the leaf

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 (Figure 1 & 2).

Fig 1: (A) Leaves of *Murraya koenigii* (B) dried leaf powder

Fig 2: Aqueous extract solution of *Psidium gujava* leaves

3.2 Synthesis of ZNPs

The schematic illustration for the synthesis of ZnPs is presented in Figure 3. 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) [13]. 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 (Baharudin *et al*., 2018 and Ashraf *et al*., 2015) [14, 15].

Fig 3: Schematic representation of ZNP preparation

3.3 Effect of extract ratio on particles size of ZNPs

The effect of extract concentration on the particle size of ZNPs was 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 found that the particle size of the ZNP decreased with an initial increase in concentration of the extract but after increasing the concentration to higher than 30% the particle size remained unaffected (Figure 4).

The lowest particles size was obtained with 30% extract concentration in relation to the zinc nitrate solution.

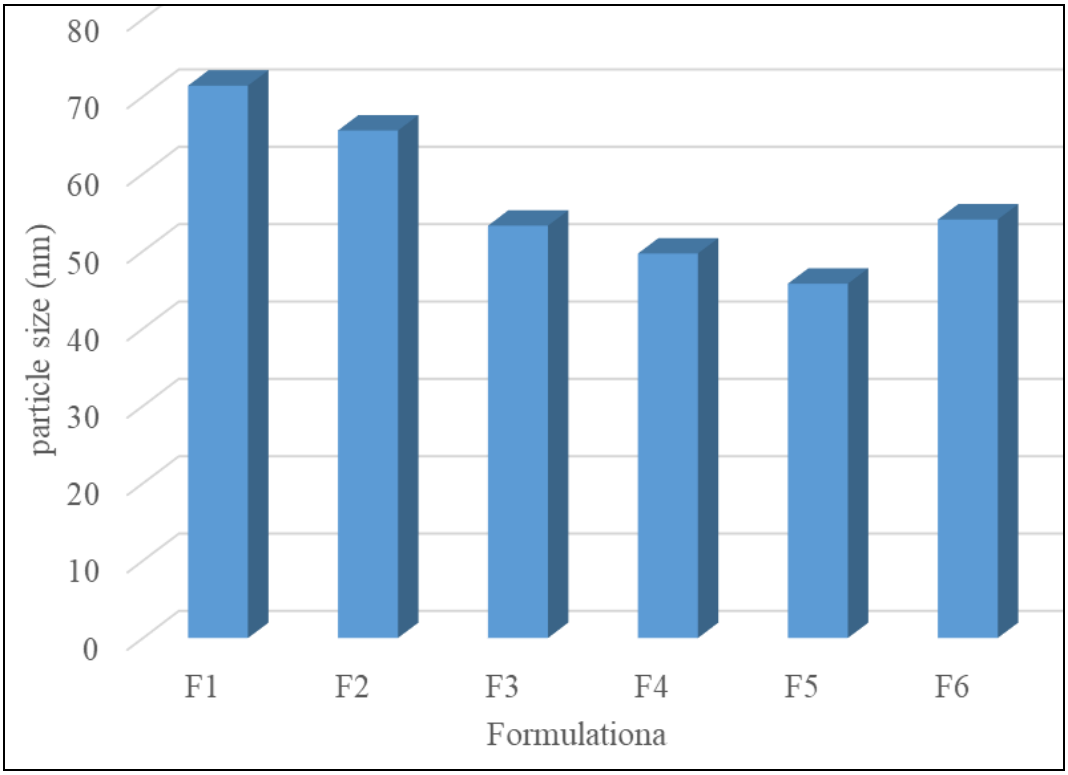


Fig 4: Effect of extract concentration on ZNP particles size

3.4 Effect of zinc nitrate on particle size of ZNP

In order to assess the effect of zinc nitrate concentration on the size of the particles synthesized, nanoparticles were synthesized using 30% extract and 10%w/v zinc nitrate (F6). 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 54.1 nm, almost equal to the particle size obtained with 30% extract and 8% w/v zinc nitrate.

3.5 Characterization of ZNPs

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

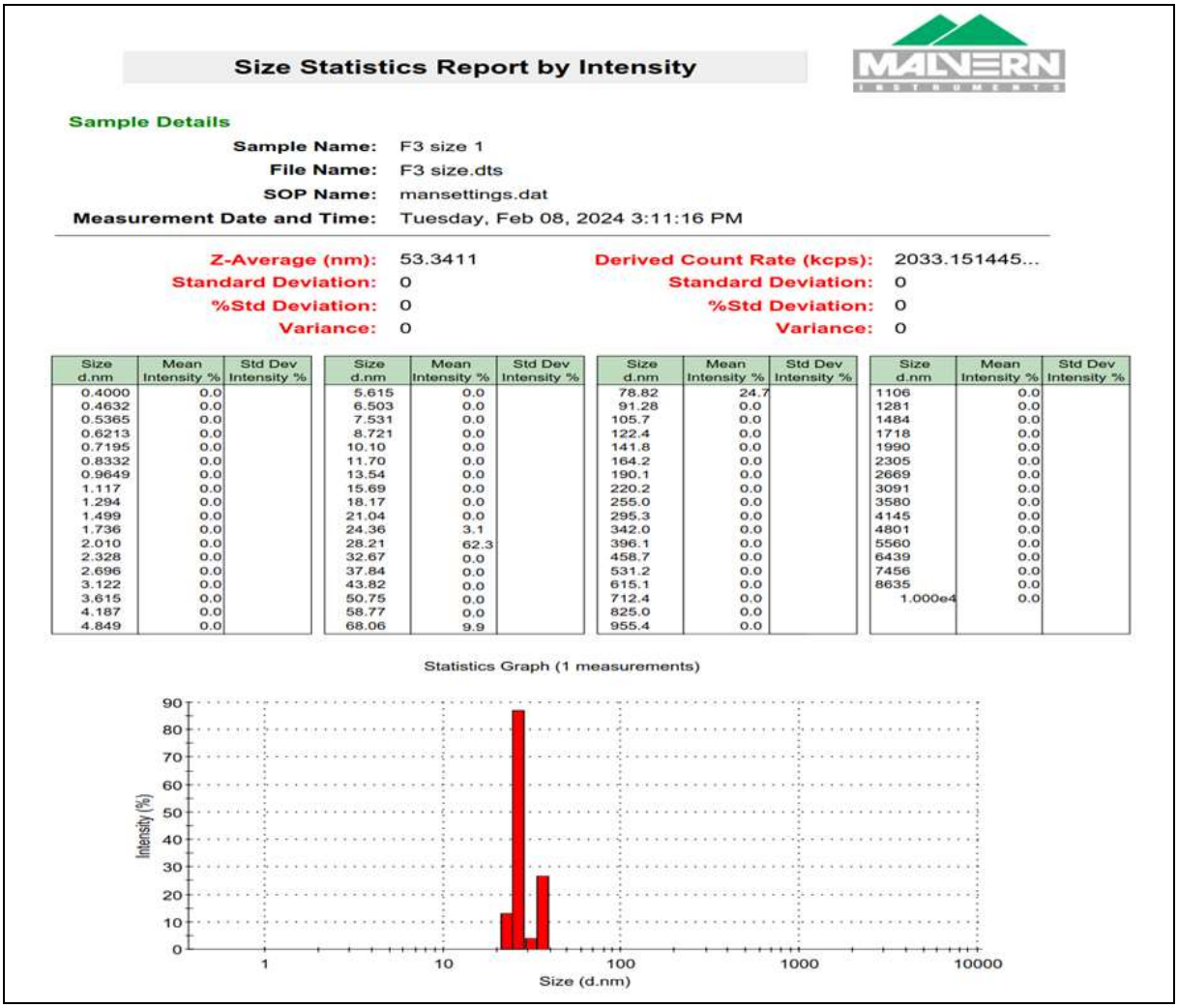
3.6 Particle size and morphology

The particle size of the ZNPs was measured using dynamic light scattering principle with the aid of particle size analyzer. The particle size of the synthesized ZNPs ranged from 71.4 nm to 45.8 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 F3 is presented in Figure-5.

Table 2: Particle size of synthesized ZNPs

Formulation	Particle size (nm)
F1	71.4
F2	65.6
F3	53.3
F4	49.7
F5	45.8
F6	54.1

The surface morphology of the synthesized ZNPs 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-6).



Size Statistics Report by Intensity

Sample Details

Sample Name: F3 size 1
File Name: F3 size.dts
SOP Name: mansettings.dat
Measurement Date and Time: Tuesday, Feb 08, 2024 3:11:16 PM

Z-Average (nm): 53.3411
Standard Deviation: 0
%Std Deviation: 0
Variance: 0

Derived Count Rate (kcps): 2033.151445...
Standard Deviation: 0
%Std Deviation: 0
Variance: 0

Size d.nm	Mean Intensity %	Std Dev Intensity %
0.4000	0.0	
0.4632	0.0	
0.5365	0.0	
0.6213	0.0	
0.7195	0.0	
0.8332	0.0	
0.9649	0.0	
1.117	0.0	
1.294	0.0	
1.499	0.0	
1.736	0.0	
2.010	0.0	
2.328	0.0	
2.696	0.0	
3.122	0.0	
3.615	0.0	
4.187	0.0	
4.849	0.0	

Size d.nm	Mean Intensity %	Std Dev Intensity %
5.615	0.0	
6.503	0.0	
7.531	0.0	
8.721	0.0	
10.10	0.0	
11.70	0.0	
13.54	0.0	
15.69	0.0	
18.17	0.0	
21.04	0.0	
24.36	3.1	
28.21	62.3	
32.67	0.0	
37.84	0.0	
43.82	0.0	
50.75	0.0	
58.77	0.0	
68.06	9.9	

Size d.nm	Mean Intensity %	Std Dev Intensity %
78.82	24.7	
91.28	0.0	
105.7	0.0	
122.4	0.0	
141.8	0.0	
164.2	0.0	
190.1	0.0	
220.2	0.0	
255.0	0.0	
295.3	0.0	
342.0	0.0	
396.1	0.0	
458.7	0.0	
531.2	0.0	
615.1	0.0	
712.4	0.0	
825.0	0.0	
955.4	0.0	

Size d.nm	Mean Intensity %	Std Dev Intensity %
1106	0.0	
1281	0.0	
1484	0.0	
1718	0.0	
1990	0.0	
2305	0.0	
2669	0.0	
3091	0.0	
3580	0.0	
4145	0.0	
4801	0.0	
5560	0.0	
6439	0.0	
7456	0.0	
8635	0.0	
1.000e4	0.0	

Statistics Graph (1 measurements)

Fig 5: Particle size by intensity for F3

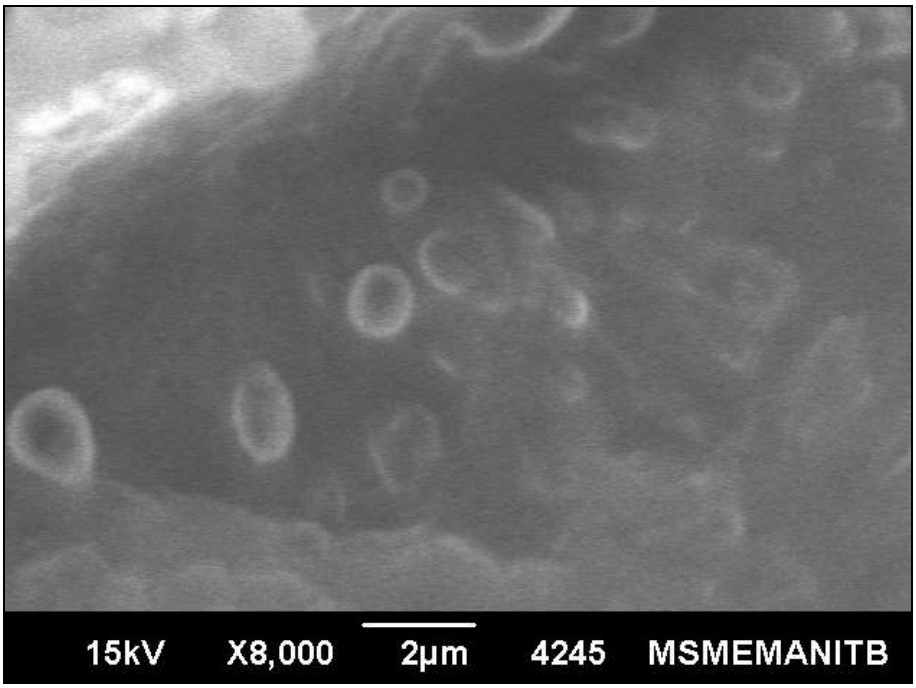


Fig 6: SEM image of F3

3.7 X-ray diffraction study

The crystallinity of the synthesized ZNPs was studied using XRD. Diffraction peaks was observed at 27.5°, 37.5°, 40.8°, 43°, 46.5° and 50° indicating the pattern of pure Zinc oxide with a hexagonal wurtzite polycrystalline structure with lattice planes (Figure-7).

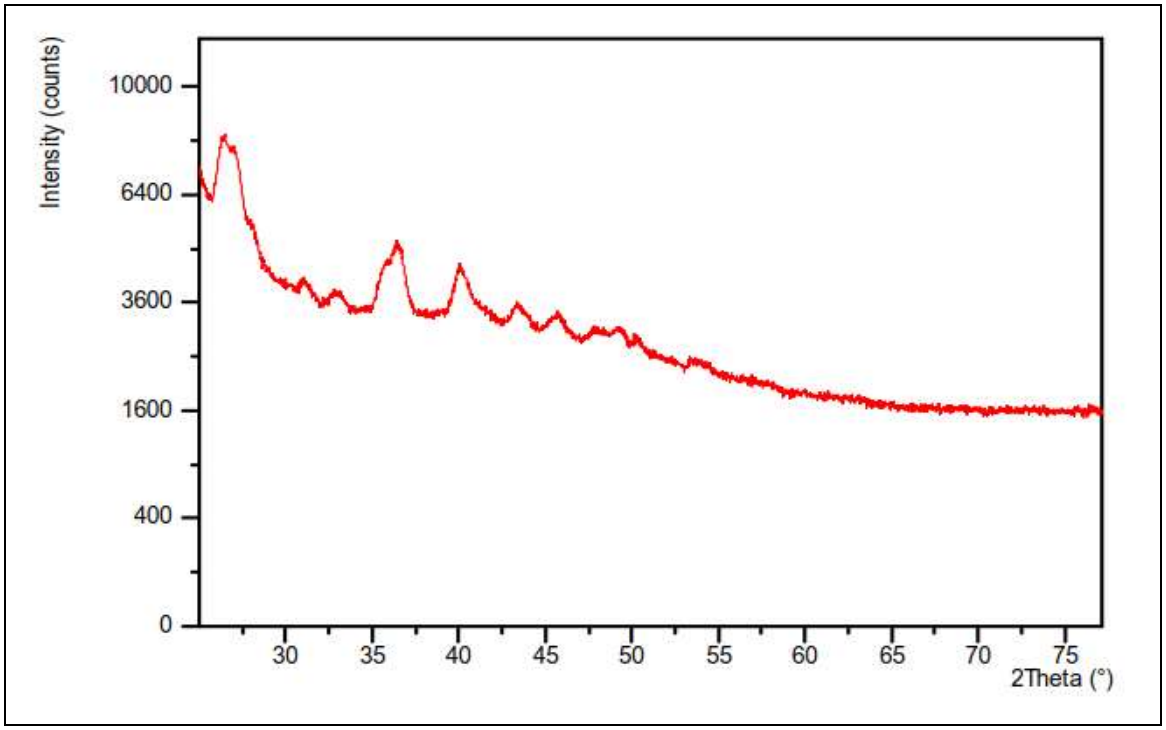


Fig 7: XRD pattern exhibited by F3

3.8 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 514 cm^{-1} and below are characteristic of metal-oxygen (ZnO stretching vibrations) (Figure-8). The broad peak at around 3400 cm^{-1} could be attributed to the O-H stretching of flavonoids and polyphenols of the extract.

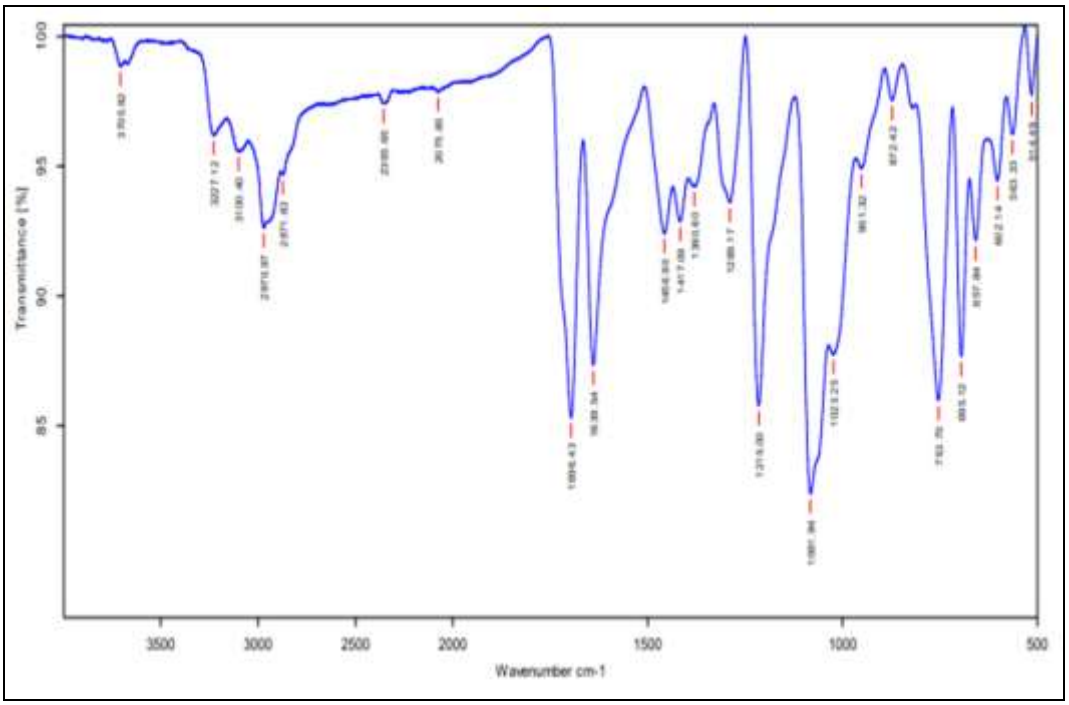


Fig 8: FT-IR spectrum of F3

3.9 UV-Visible spectrophotometric study

The solid white zinc oxide nanoparticles were dissolved in 0.1N HCl and UV absorption was studied. The spectrophotometric absorptive pattern normally depends on the variables like the temperature, size, and shapes of the synthesized nanostructures. The UV–Visible absorption of the ZNPs is correlated with their size. The UV–Vis spectrum of the ZNPs was measured in deionized water. Broadband can be observed at 360 nm, which was similar to the bandgap of zinc oxide "1s–1s electron transition" (Figure-9).

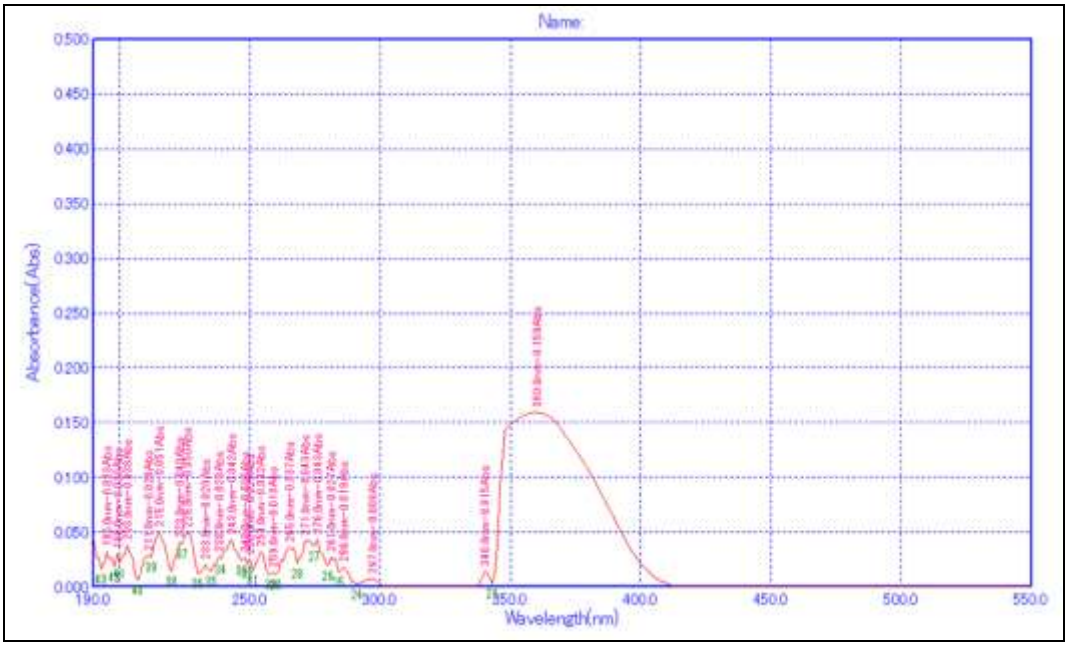


Fig 9: UV spectra of F3

3.10 Antibacterial activity

The antibacterial action of NP3 was studied by obtaining zone of inhibition using cup and plate method. The zone of inhibition obtained was taken as a measure of antibacterial activity. The NP was found to show activity against both gram positive and gram negative bacteria (Figure-10).

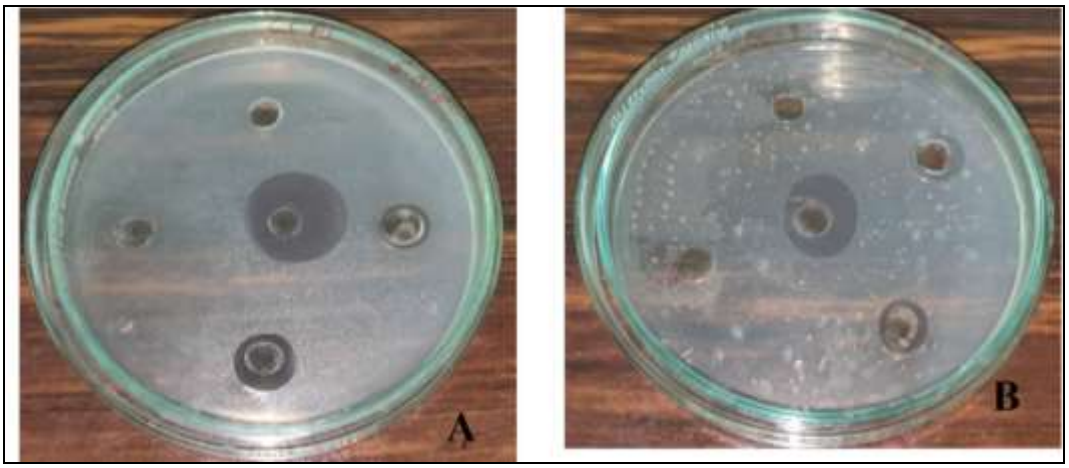


Fig 10: Antibacterial action of ZNP (A) *S. aureus* (B) *E. coli*

Conclusion

The present investigation was undertaken with an objective to synthesize zinc oxide nanoparticles using *Psidium gujava* leaf extract and study the antibacterial action of the produced nanoparticles.

The aim of the present study was to synthesize zinc oxide nanoparticles using *Psidium gujava* leaf extract and study the antibacterial action of the produced nanoparticles. The results suggest that *Psidium gujava* 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 (F3).

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