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GREEN SYNTHESIS, SPECTROSCOPIC ANALYSIS AND STABILISATION ENERGY OF IRON OXIDE NANOPARTICLES FROM AQUEOUS *Ziziphus* LEAF EXTRACT

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ABSTRACT

Ziziphus mauritiana (ZM) leaf extract was used to synthesize FeO nanoparticles (NPs) and characterised by UV-visible, FTIR, XRD, and SEM. From this study the phytochemical constituents present in the aqueous *Ziziphus* extract were identified to include phenols, saponins, flavonoids and tannins which both serve as reducing, capping and stabilising agents. The presence of these phytochemicals was confirmed from laboratory tests and infrared spectroscopy. A wavelength of 400 nm was found to correspond to the surface plasmon resonance and used to determine the stabilisation energy of the FeO NPs and found to be 299 kJ/mol. More so, a broad peak was observed at 3426 cm^{-1} for the leave extract and 3386 cm^{-1} for the FeO NPs corresponding to O-H stretching vibration probably from alcohols, flavonoids, and phenols. A significant IR peak observed at 536 cm^{-1} in the FeO NPs spectrum but absent from that of the plant extract, was assigned to Fe-O stretching vibration and serves as a strong evidence for the formation of the nanoparticles. The XRD analysis revealed face-centred cubic morphology with an average crystal size of 36.3 nm calculated by Debye – Scherrer equation and the SEM analysis showed the nanoparticles to be spherical in shape. The synthesized NPs were found to possess significant antimicrobial activity against *S. typhi* (18 µg/L), *E. coli* (15 µg/L) *S. aureus* (16 µg/L0 and *S. pneumonia* (16 µg/L) by well-diffusion method. The inhibiting activity of the synthesized NPs increased with increase in concentration of the nanoparticles.

Keywords: Green Synthesis, Iron oxide Nanoparticles, Spectroscopic Analysis, *Ziziphus Mauritiania*

INTRODUCTION

Nanotechnology in recent years has attracted enormous interest from researchers worldwide due to its enormous applications in the various fields of science and technology such as biomedical, electronic and pharmaceutical industry etc (Govindappa *et al*., 2016). Green approach for the syntheses of metal oxides from plant extracts in particular has ignited significant interest from researchers by virtue of its ease of preparation, non-toxicity, low-cost economy and its environmental friendliness (Govindappa *et al*., 2016). Of the numerous nanoparticles synthesized from plant extracts, evaluation of their antimicrobial activities stand out distinctively. Such studies abound in literature but few of the recent studies are cited. Jayadran *et al.* (2015) synthesized manganese nanoparticles using plant extracts, evaluated its antimicrobial activity and found it to display higher antimicrobial activity than standard drug against *S. aureus, C. lunatus* and *T. simii*. Kumari *et al.* (2019) synthesized silver nanoparticles from leaf extract of *Menta piperita* and evaluated the anthelminthic activity on cestode and nematode parasites of country fowl. In another separate study, Amaliyah *et al.* (2020) also reported the green synthesis and characterisation of copper nanoparticles using *Piper retrofractum Vahl* extract as a bio-reductor and capping agent.

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The role of phytochemicals in the syntheses of NPs goes beyond acting as a capping agent. It is widely reported that phytochemicals play major roles such as in surface plasmon resonance phenomenon, reduction, and stabilisation of the nanoparticles (Ezealiji *et al*., 2019; and Karu *et al*., 2020). Phytochemicals available in abundance in plants include but not limited to, are amino acids, carbohydrates, flavonoids, proteins, saponins, terpenoids etc. Components of the plant extracts known to be responsible for the afore-mentioned functions are polyphenols flavonoids, terpenoids and phenolic acids (Izadiyan *et al.* 2020). The mechanism of the roles played by the phytochemicals in the formation of the nanoparticles should be a matter of interest. Hence some authors (Ezealiji *et al*., 2019; Bhuiyan *et al*., 2020 and Devi *et al*., 2019) have gone a step further in their studies to provide a mechanism for the preparation of the nanoparticles and proposed that the initial step involves reduction of the metal precursor to the metal oxide by the phytochemical that aggregates to form the nanoparticles followed by capping and stabilisation of the NPs. However, documentation on the stabilisation and its quantisation as far as we could find, has not been adequately reported.

Ziziphus mauritiana (ZM) commonly known as Indian jujube, Indian plum, Chinese date, Chinese apple, and dunks belongs to the family *Rhamnaceae*, is a fruit tree that is grown in the tropics and sub-tropical regions of the world. Different parts of the plant are known to be used for traditional medicine and other purposes as reported by Preedy *et al*. (2011). In addition ZM is well known for anticancer and anti-inflammatory activity (Asimuddin *et al*., 2020) that prompted researchers to explore its applications extending to nanomaterials. From literature we could find several nanomaterials that have been obtained from the plant extract for various purposes but few are cited here. In a recent publication Sameen and co-workers (20220) reported the biosynthesis of silver nanoparticles (Ag NPs) from plant extract and found that the Ag NPs showed greater activity against tumours and reduced inflammation in hepatic carcinoma. Pansambal *et al*., (2017) used the plant extract from leaves to prepare copper oxide nanoparticles (CuO NPs) for screening and identification of phytochemicals (coumaris, tannins, flavonoids and glycosides) in the plant and the synthesis of MgO NPs from the leaves extract as a catalyst for the production of biodiesel from the same plant was studied (Saman *et al*., 2021). Memon and his colleagues (2020) synthesized AG NPs from *Ziziphus* Leaf extract (ZL) and successfully used it for the colorimetric determination of Hg^{2+} in aqueous medium.

Iron is a highly reactive transition metal and has many properties that make it incredibly useful in many industrial applications. It forms the oxides FeO, Fe_2O_3 and Fe_3O_4. The latter oxide is widely studied because it is the most stable iron oxide and has wide range of applications. Biosynthesis of the iron oxides from several plant extracts have been investigated. For example, Makarov *et al*. (2014) obtained stable FeO nanoparticles from aqueous extracts of Hordeum vulgare and Rumex acetosa plants while Rufus *et al.* (2016) reported the synthesis of the haematite (α-Fe_2O_3) nanoparticles from P. guajava leaves extract and ascertained its antibacterial and nano fluid applications. Similar studies have been reported on the antimicrobial and catalytic activities (Bhuiyan *et al*., 2020; Devi *et al*., 2019; and Vasantharaj *et al*., 2019). But the first biosynthesis of Ag/Fe_3O_4 nanocomposite was reported by Sajjadi and co-workers in 2017 using *Euphorbia peplus* L. leaf extract as a stabilising and reducing agent. In a recent study, Droepenu *et al*. (2022) evaluated the antioxidant, anti-inflammatory, and antimicrobial activities of the ZnO nanostructure extracts of the leaf, fruit, and seed of Chrysophyllum albidum.

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The aim of the present study was to prepare iron oxide nanoparticles using ZM leaves extract, characterise the nano material and explore its antibacterial activity against four bacterial strains. More so, not much research has been undertaken on the quantisation of the stabilisation energy of the FeO NPs and thus the results of this work are unique, and to date have not yet been reported.

MATERIALS AND METHODS

Preparation of Aqueous Extract

20 gram of the dried leaves of ZM was pulverized using pestle and mortar and stirred with 100cm^3 of deionized water in 250 cm^3 of beaker, heated at 80 ºC for one hour and then filtered with a Whattman No.1 filter paper (Shehu *et al*., 2020, 2021).

Preparation of Powdered Extract

100 cm^3 of the sample leaf extract was measured with 50 cm^3 measuring cylinder into a 250 ml of beaker and placed in a laboratory oven. The extract was heated at 100 ^{o}C for one and half hours to dryness. A red brown residue was obtained and weighed 1.53 g. The procedure was repeated three times and the product was stored for further analysis.

Preparation of FeO NPs

The FeO NPs were prepared by a method reported by Padalia and Chanda (2017) for the synthesis of ZnO NPs except that $FeSO_4.7H_2O$ salt was used instead of $ZnNO_3$. 1.0g (0.359 mol) $FeSO_4.7H_2O$ was weighed and dissolved in 100 cm^3 of distilled water. 50 cm^3 of the leaf extract was measured with 100 cm^3 measuring cylinder, placed into a 250 cm^3 beaker and heated on a hot plate with stirring for 30 minutes at 80 °C. Then 20 cm^3 of the iron sulphate solution was measured and added slowly to it with stirring. The colour of the mixture changed from pale yellow to dark brown. The colour changed to dark brown indicates the air oxidation of Fe^{2+} to Fe^{3+} and the formation of Fe(III) oxide particles. Finally, a brown precipitate was formed indicating the formation of FeO NPs. The precipitate (FeO NPs) was allowed to cool, and decanted. The supernatant was placed in the oven at 100°C for 2hours to dry. The dried FeO NPs was then collected and dried in the desiccator for 24 hours and then weighed. The procedure was repeated 3 times with a yield of 1.6 grams. The product was stored in a sample bottle for further analysis.

Phytochemical Analysis

The ZM leaves extract was subjected to preliminary phytochemical screening following methods reported by Madike*et al*., (2017), Jaradat *et al*., (2015) and Deshmukh *et al*. (2018) for detection of the following constituents.

Saponins

One (1) cm^3 of extract was added to 2 cm^3 distilled water and shake vigorously. The formations of stable persistent bubbles were taken as a positive for saponins constituent (Ezealiji *et al*., 2019; Bhuiyan *et al*., 2020 and.Izadiyan *et al*., 2020).

Flavonoids

Alkaline Reagent Test:3 cm^3 of the extract of ZM was taken into a test tube and treated with 1 cm^3 of 10 % NaOH solution. The formation of an intense yellow colour was an indication of the presence of flavonoids (Ezealiji *et al*., 2019; Bhuiyan *et al*., 2020 and.Izadiyan *et al*., 2020).

Phenols and Tannins

Lead acetate test:10 mg of extract was taken and 0.5 cm^3 of 1 % lead acetate solution was added and the formation of precipitate indicates the presence of Tannins and Phenolic compounds (Ezealiji *et al*., 2019; Bhuiyan *et al*., 2020 and.Izadiyan *et al*., 2020).

Characterisation of FeO NPs

The synthesized FeO NPs were characterized by UV–Visible, IR and X-Ray diffraction studies (XRD). The particle size and morphology were determined by

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scanning electron microscopy (SEM). UV spectrum was recorded on a Perkin Elmer UV-Visible spectrophotometer model 725 in the wavelength range 200 – 800 nm to determine the wavelength of maximum absorbance. The UV-visible spectrum provided the wavelength of maximum absorbance corresponding to the surface plasmon resonance (SPR) phenomenon of the nanoparticles. The IR spectra were acquired by the KBr pellet technique using a Perkin Elmer spectrophotometer model 10.03.09.

Antibacterial Sensitivity Test

Preparation of Media

3.8 g of nutrient agar was weighed and dissolved in 100 cm^3 of distilled water in a conical flask and autoclaved for 45mins to sterilize the media. The media was removed and allowed to cool, then poured into a petri dish on a flat surface to a uniform depth of 4mm and allowed to solidify further dried at 30 ºC in an incubator for 30 mins until the surface moisture evaporated.

Antibacterial Susceptibility Assay

The antibacterial activity was tested using a method adapted by Kaviya *et al*., (2011). The nanoparticles synthesized using ZM leaves extract was tested for antibacterial activity by agar well diffusion method against pathogenic bacteria *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S.aureus*), *Streptococcus pneumonia (S.pneumonia) and Salmonella typhi (S. typhi)*. The pure cultures of bacteria were sub-cultured on nutrient agar medium. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 10 mm diameter was made on nutrient agar plates using gel puncture. Using a micropipette, a 50 µL of nanoparticles solution was poured onto each well on all plates. After incubation at 37 ºC for 24 hrs, the zone of inhibition was measured. Antibacterial sensitivity test was carried out using Agar diffusion technique. The surfaces of Muller Hinton's potato dextrose Agar into petri dishes were inoculated uniformly with 0.3$cm^3$for 20 hours old test bacteria culture. 1-4 mg/cm^3 solution of iron nanoparticles in hot DMSO/Benzene and that of the extract of ZM leaves were added to 6 mm bore hole into the Agar. The plates were allowed to stand on the bench under aseptic condition for about 30 minutes after inoculation before incubating at 37 ^oC for 24hours. After incubation, the inoculated plates were observed for Inhibitory Zones (ZI) (in mm diameter). The result was taken by considering the zone of growth and inhibition of the organisms as done by Osowole *et al*.; (2015). The sensitivity test was conducted in triplicates and the activity and inactivity were observed in accordance with the standard method.

RESULTS AND DISCUSSION

Phytochemical Analysis and verification of Beer's Law

The results for the phytochemical tests are presented in Table 1.

Table 1: Phytochemical analysis of ZM

S/N	Phytochemical test	Remark
1	Flavonoids	+
2	Tannins	+
3	Saponnins	+
4	Phenol	+

UV-visible spectroscopy for FeO NPs

synthesized FeO NPs. This is in agreement with a maxima observed at 405 nm by Marakov *et al*. (2014) of the FeO NPs obtained from aqueous leaf extracts of *Hordeum vulgare and Rumex acetosa* plants. The stabilisation energy at the wavelength of SPR was calculated from Plank's

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Quantisation energy (E = hν) and found to be 299kJ/mol at 400 nm.

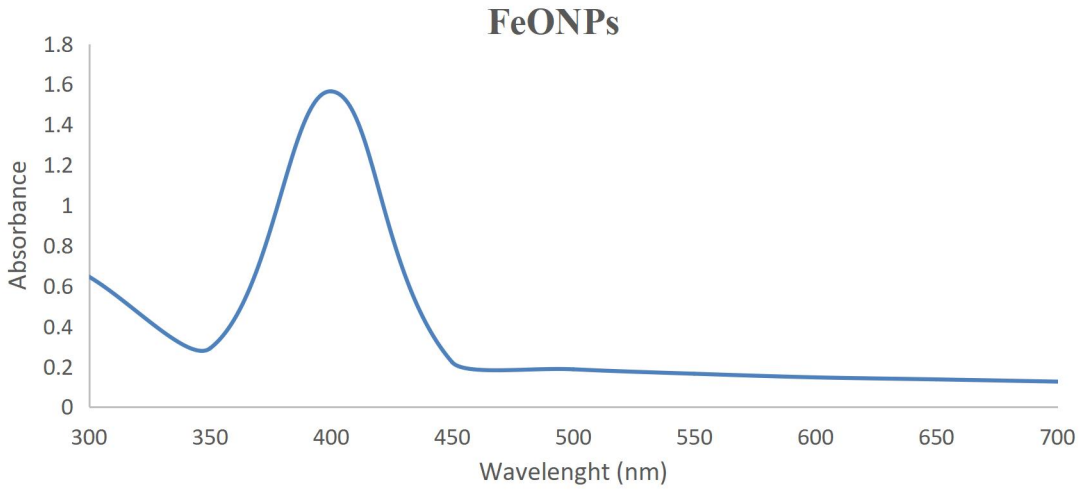

Figure 1: UV-spectrum of FeO NPs supernatant acquired on a PerkinElmer 725 UV-visible spectrophotometer in 1-cm pathlength quartz cuvette against deionized water in the reference beam.

FTIR Analysis

The IR spectra were obtained by using the KBr pellet technique on FTIR Perkin Elmer spectrophotometer model 10.03.09 that was operated through a scan range 4000 – 400 cm^{-1} (Figure 2) to ascertain the phytochemicals responsible for the reduction, capping and stabilisation of the nanoparticles. The infrared absorption frequencies for the nanoparticles and plant extract are tabulated in Table 2.

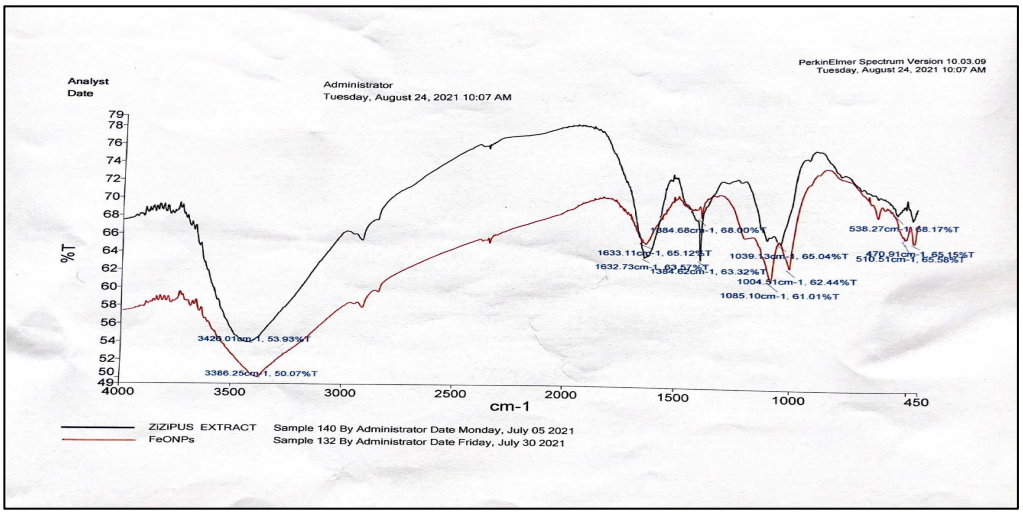

Figure 2: FTIR spectrum of plant extract and FeO NPs acquired on a PerkinElmer spectrum 10.03.09.

Table 2: Assignment of Infrared absorption frequencies (cm^{-1}) of extract, FeO NPs

	ν(O-H)	ν (C=O)	ν (C-O)	ν (Fe-O)
Plant Extract	3426br	1632s	1039m	n.o
FeO NPs	3386br	1633s	1085m	536m
	Alcohol, phenols,	carboxylic	ether	

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Flavonoid and water functional group

Key n.o = not observed, ν = stretching vibration, br = broad, s = strong, m = medium

A broad peak around 3426-3386 cm^{-1} for the extract and FeO NPs corresponds to O-H stretching vibration which is probably from alcohols, flavonoids, and phenols. The medium peaks at around 1632-1633 cm^{-1} for both the extract and FeO NPs corresponds to C=O stretching vibrations and the medium peak at around 1039-1085 and 1004 cm^{-1} in the extract and the FeO NPs correspond to C-O stretching or probably the bending vibration in biomolecule. These findings are in agreement with the previous studies reported by Devi *et al.* (2019). Lakshminarayanan *et al*. (2021) in a study of green synthesis of iron oxide nanoparticles from *Bauhinia tomentosa* reported the iron-oxygen vibration to be at 555.7 cm^{-1}. In our work a weak peak occurs at 536 cm^{-1} was observed in the FeO NPs spectra but absent from that of the plant extract, could be assigned to Fe-O stretching vibration. This finding confirms the existence of the FeO NPs.

X-ray diffraction (XRD) and scanned electron microscopy (SEM) for FeO NPs

The XRD of the FeO NPs is presented in Figure 3. A strong diffraction peak characteristic for FeO NPs were observed at 2 θ value 32.71° corresponding to the hkl value (111) which shows Face Centered Cubic (FCC) cell. The average crystal size was determined by Debye-Scherrer equation. $D = K\lambda\beta\cos\theta$ (1)

where D = particle in nm, K is a constant (Sherrer constant = 0.9), λ is the wavelength of the X–ray radiation, θ is Bragg's angle and β is Full Width Half Maximum (FWHM). The calculated mean crystal size was found to be 36.3nm. The broadening of the peak suggested that the resultant FeO NPs is crystalline in nature (Niraimathee*et al.,* 2016). The particles size is in agreement with other studies whereby an average crystalline size of 24.1 nm was reported by Rufus *et al* (2016). However, an average crystalline particle size as low as 4.50 nm was reported by Bhuiyan *et al*. (2020).

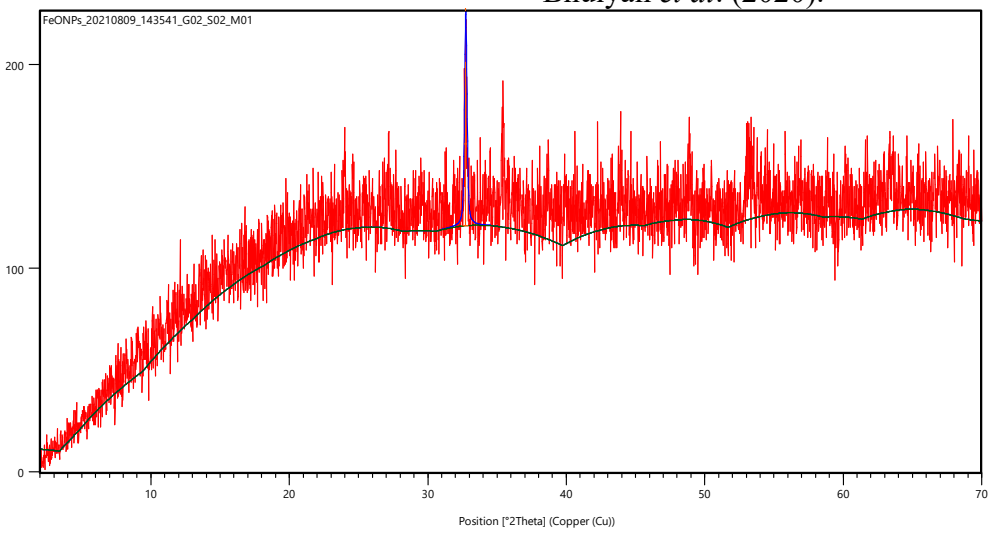

Figure 3: X-ray diffraction spectra of the synthesized Iron Nanoparticles

Scanned electron microscopy was undertaken to confirm the morphology of the synthesized iron oxide nanoparticles. The images of the SEM analysis is presented under Figure 4.

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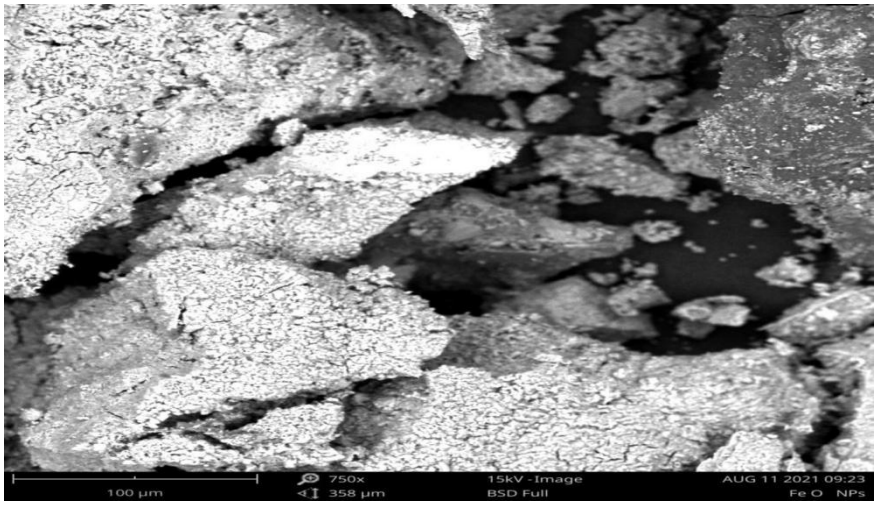

Figure 4: SEM micrographs of FeO NPs synthesized with *Ziziphus M.* extract

The SEM analysis shows from Figure 4 that the FeO nanoparticles possessed a spherical shape having rough surface and well dispersed with close compact arrangement. The spherical shape is akin to that reported by Prodan *et al*. (2013) for the synthesized FeO NPs.

Antibacterial Activity

Table 3 shows the zone of inhibition (mm) of FeO NPs against bacterial isolate. FeO NPs exhibit significant anti-bacterial activity against *E. coli* and *S. aureus*, but exhibit a moderate activity against *S. pneumonia* and *S. typhi*. The data obtained shows that the anti-bacterial activity increases with increase in concentration of nanoparticles.

Table 3: Anti-bacterial activity FeO NPs synthesized with ZM extract

Tested organism	Concentration µg/L mm					Control drug (5 mg)
	500	250	125	62.5	control	
S. typhi	18	10	9	7	23	Ofloxacine
E. coli	15	11	8	8	20	Ofloxacine
S. aureus	16	11	8	7	23	Ofloxacine
S. pneumonia	16	10	10	7	19	Ofloxacine

CONCLUSION

FeO NPs were successfully synthesized from *Ziziphus mauritiana* (ZM) leaf extract and characterised by UV-visible, FTIR, XRD, SEM. Its antimicrobial activity was also evaluated.

From this study the phytochemical constituents present in the aqueous *Ziziphus* extract were identified to include phenols, saponins, flavonoids and tannins which both serve as reducing, capping and stabilising agents. The presence of these phytochemicals was confirmed from laboratory tests and infrared spectroscopy. The wavelengths of maximum absorbance in the uv-visible spectra attributed to the Surface Plasmon Resonance phenomenon for the FeO NPs were found to be at 400 nm. This was in agreement with the previous literature reports cited. The synthesized FeO NPs was found to obey Beer's Law for the range 10 – 100 ppm and with absorptivity coefficients of 100 ml/ug/cm. The corresponding stabilization energies at the wavelengths of maximum absorbance for the NPs obtained from the plant extract was calculated to be 299 kJ/mol at 400 nm. The synthesized NPs were found to possess

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significant antibacterial activity against the tested bacteria by well diffusion method. The inhibiting activity of the synthesized NPs increased with increase in concentration of the nanoparticles.

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