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Assessment of the Anti-microbial Action of Zero Valent Iron Nanoparticle Synthesized by *Aspilia pluriseta* Extracts

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Authors' contributions

This work was carried out in collaboration between all the authors. Authors AON and PGK designed the study, wrote the protocol and wrote the first draft of the manuscript. Author ESM reviewed the experimental design and all drafts of the manuscript. Authors AON and ISW managed analyses of the study. Author EGM performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Antimicrobial resistance has proved to be a great burden to the current health care system as more potent drugs are required to combat this global challenge. Due to this problem there was need to explore new ways that would eradicate drug resistance hence the need to utilize the potential of metallic nanoparticles as a new alternative to combat resistance. In this study, focus was on the synthesis of Fe^{o} NPs using *Aspilia pluriseta* aqueous extracts, its characterization and antimicrobial activities against gram (+) and gram (-) microorganisms. Preliminary phytochemical screening was carried out to test for presence of secondary metabolites; phenol, flavonoid, phytosterol, carbohydrate, tannin, saponin, glycoside and terpenoid, the results tested positive test for all the metabolites. Folin-Ciocalteu method and aluminium chloride method respectively, were used to quantify amount of phenolic 31.45 ±0.017 milligram per gram and flavonoid 7.223 ±0.081 milligram

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per gram. Characterization of zero valent iron oxide NPs was achieved using UV-visible spectrophotometer, FT-IR, XRD and XRF. UV-Vis spectrophotometer displayed a peak at 346 nm. Fourier-transform infrared spectra displayed existence of functional groups such as OH, C-O and C-C that aids in the formation of NPs. XRD indicated the presence of peaks at 16.06° and 43.73°.XRF data displayed that NPs contained Fe 31.58%, MgO 12.02%, Al_2O_3 1.883%, SiO_2 13.84%, P_2O_5 11.14%, K_2O 4.699% and CaO 1.522% of respective oxides. This therefore confirmed the presence of secondary metabolites in *Aspilia pluriseta* aqueous extracts which aids formation of iron NPs. Finally, the antimicrobial activity was determined against *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* which exhibited significant zones of inhibition.

Keywords: Aspilia pluriseta; NPs; environmental friendly; characterization; XRF.

1. INTRODUCTION

Communicable infections remain the world's foremost source of untimely demises, killing nearly fifty thousand persons each day due to resilience of infectious microorganism because of constant usage of antibiotics, [1]. Many developing countries more so in Africa, mortality and morbidity rates are still high as a result of diarrhea, continues to be a major challenge, especially amongst children [2]. On top of this challenge, antibiotics are occasionally linked to adversarial effects on human beings that comprise immuno-suppressant, allergies and hypersensitivity. This has brought greater medical problems in dealing with communicable ailments [1].

According to 2008 research on antibiotic synthesis comprising smaller and larger drug making firms exposed that out of one hundred and sixty seven, only fifteen antibiotics under development possessed fresh mode of action [3]. Continuity in the pattern could result to a challenge in the treatment of people having a serious illness.

Nanotechnology a modern field of science dealing in production, manipulation and the use of very small particles with sizes measured in nanometers has found its application in the field of medicine [4]. Nanoparticles are majorly obtained using chemical and physical process. Production of NPs using plant materials results for low cost of production, short production time, it is relatively safe and its ability to up production. On industrial scale, efficient extraction, isolation and purification are a challenge. Plant materials have varying concentration of bio-active components. Size and morphology depends on localization in plant material that depends on differences in content of metal tissues [5]. Various researches have been done using different metal NPs contributing towards the production of alternative nano-therapeutics, in treating infections emanating from drugs which are resistant [6]. Owing to various physiochemical characteristics; great surface area, mechanical strength, optical activity and their reactiveness [7], iron nanoparticles has also found application in water treatment [8]. Borohydrate reduction of Fe (III) ions in aqueous media to zero valent ions is usually carried out in inert conditions to keep iron in its zero valent form that is unsteady forming Fe_3O_4, Fe_2O_3 and FeOOH [9]. Extraction and isolation of natural products [10]. Biosynthesis possess extra reimbursement in relation to other conventional synthetic methods owing to the presence of other biological entities and environmental friendly processes, relatively cheap, bulk synthesis is enabled, low pressure energy and non-toxic chemicals are used [11,12].

Various reports from the medicinal plant analysis have indicated secondary metabolites to be mainly accountable for bio-reduction of ionic iron resulting to bulk metallic NPs. Iron nanoparticles in our current study were obtained by employing quick and single step approach [13].

2. MATERIALS AND METHODOLOGY

2.1 Sampling

Fresh samples of *Aspilia pluriseta* were obtained, kept in a labelled polythene bag and taken to the laboratory. Thoroughly washed plant material were then air dried in a shade for four days, thereafter crushed into powder form with an in-house mechanical grinder and stored to await chemical analysis [14].

2.2 Extraction of *Aspilia pluriseta* Using Deionized Water

With slight changes from work done by Vélez et al. [15], 5 g of *Aspilia pluriseta* leaves powder was weighed into a 250 ml conical flask, thereafter 100 ml of deionized water added followed by boiling in a water bath for one hour maintaining the temperature at 80°C. Having obtained *Aspilia pluriseta* aqueous extract, filtration was carried out thereafter. Filtrate obtained was then kept in the refrigerator ready for analysis [16].

2.3 Qualitative Screening of Secondary Metabolites

The following standard protocols were used for qualitative analysis to check for the presence of phenols, flavonoids, carbohydrates, glycosides, tannins, phytosterol, terpenoids & saponins [17], [18,19].

2.4 Quantity of Reducing Agents

2.4.1 Phenolics content

The phenolics were quantified using protocols developed by Baba & Malik, [20] and Alara, et al. [21], and absorbance measured at 769 nm using UV spectrophotometer.Phenolics concentration was obtained via a calibration curve using gallic acid as the standard.

2.4.2 Flavonoids content

With slight modifications from protocol presented by Baba & Malik, [20] and Spiridon, et al. [22], amount of flavonoid was quantified with absorbance being measured at 511 nm using UV spectrometer. Flavonoids concentration was then obtained via a calibration curve using rutin as standard.

2.5 Preparation of Iron Salt and Synthesis of Zero Valent Iron Oxide Nanoparticle

Preparation and synthesis of iron NPs were carried out with slight modification of procedures, from work done by *Ksv,* et al. [12]. 0.1M $FeCl_3.6H_2O$ solution salt was prepared by adding 2.703g of solid $FeCl_3.6H_2O$ into 100ml of deionized H_2O followed by 5 minutes of shaking to obtain a homogenous mixture. Thereafter, the NPs was synthesized by addition of 0.1M$FeCl_3$.6H2O into the plant sample in the ratio of 2:5 in which a black precipitate was observed, indicating presence of NPs [23]. Formed nanoparticle was then retrieved from the mixture by centrifuging (350 rpm for ten minutes) and washing severally using deionized H_2O .Finally, it was oven dried for characterization [24].

2.6 Characterization of Zero Valent Iron NPs

Functional groups which necessitated the development of nanoparticles were characterized by the use of FT-IR Spectrophotometer, Make FTS-8000 and analysis run using the KBr pellet technique [25]. Optical properties of Fe^o NPs were determined by use of the Perkin Elmer Spectrometer [26]. Crystal phase for Fe^o NPs was identified by use of STOE STADIP P XRD machine with slight modifications from work done by Gondwal, [27]. Elemental composition of the prepared powder sample was then determined using X-ray fluorescence spectrometry [28].

2.7 Antibacterial Activity

Evaluation of the antimicrobial activity against selected microorganisms for green-synthesized zero valent iron nanoparticle was carried out using standard disc diffusion assays with slight modifications from work done previously by Mostafa et al. [29] and Groiss et al. [26]. Dimethyl sulphoxide was used to dissolve zero valent iron nanoparticles. The concentrations used were 10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} µg/ml. DMSO without nanoparticles was the negative control, while Nitrofurantoin (200 mg) was the positive control.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening of *Aspilia pluriseta* Leaves Extract

Phytochemicals constitute primary and secondary metabolites. Primary metabolites are basically carbohydrate while secondary constituents consist of; glycosides, flavonoids, terpenes, terpenoids, saponins, phenols and tannins [30]. *Aspilia pluriseta* aqueous extract gave a positive test for phytochemical screening of all the secondary metabolites under study [31]. Phytochemical constituents of *Aspilia pluriseta* are involved in Fe^o NPs synthesis hence the leaves were boiled with the aim of rupturing and releasing intracellular materials into the solution [32].

3.2 Quantitative Phytochemical Screening

The total phenolic and flavonoid contents determined from standard curves (y= 0.0043x+0.0464, R^2=0.9919) and (y=0.064x+0.0061, R^2=0.9955) respectively is presented in Table 1 below.

Table 1. Concentration of phenolic and flavonoid

Metabolite	Quantity
Phenol	31.45±0.017 mg GE/g DW
Flavonoid	7.223± 0.081 mg RE/g DW

Results shown in Table 1 above are presented as the mean ± standard deviation. The polarity of extracting solvent, isolation procedure and compounds present constitutes natural extracts activity. The phenolic components contained in aqueous extract was 31.45±0.017 mg/g gallic acid equivalents/g, while flavonoid was found to be 7.223±0.081 mg/g rutin equivalents/g. Flavonoids and phenolic contents are major contributors of plants antioxidant action [33]. Flavonoids are naturally occurring phenolics comprising; flavones, flavonone, flavanonol, and isoflavone derivatives. Number of O-H functional groups and structures in flavonoids play a significant role in metal-binding action. Iron chelates have shown to have pro-oxidant potential [34].

3.3 Observations and UV-vis Analysis of Iron Nanoparticle

Fig. 1 shows the UV-Visible spectrum of zero valent iron nanoparticle and iron (iii) chloride solution.

Optical characterization of synthesized zero valent iron nanoparticle was achieved by studying absorption spectra of green synthesized Fe-NPs and aqueous solution of iron (iii) chloride (Fig. 1). From preliminary characterization of Fe^{3+} ions bio-reduction using UV-Visible absorption spectrum, a peak was recorded at 209nm as shown in (Fig. 1) which is almost similar from work done by Chaki, et al. [35]. Aqueous solution of iron (iii) chloride gave two peaks at 214nm and 286nm. Thus the absence of a peak at 214nm and 286nm in the NPs spectrum could signify the formation of zero valent iron NPs. Greater change in absorption spectra, indicates *Aspilia pluriseta* alone acts as a better stabilizer, this is in agreement from work done by *Jain & Mehata, [36].* Also presence of a single peak in the NPs developed indicates, particles formed are of uniform size and shape [37]. Upon addition of *Aspilia pluriseta* leaf extract into $FeCl_3$ solutions in the ratio of 5:2 at room temperature a visible color change was observed as the yellow aqueous solution of $FeCl_3$ turned to black [38]. Color change is the easiest and commonly used indicator of nanoparticles formation [39]. $FeCl_3$ salt greater reduction capability is as a result of;

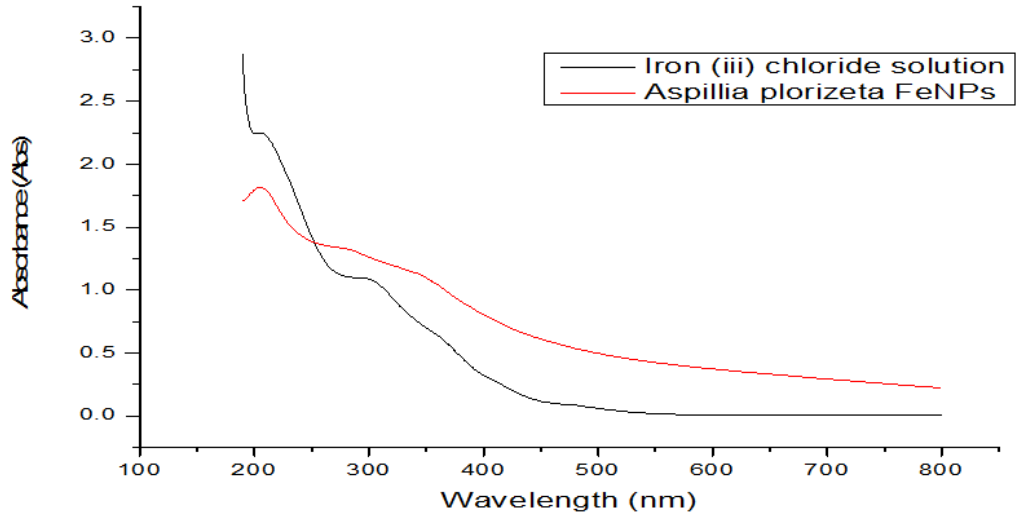

Fig. 1. UV-vis spectrum of *Aspilia pluriseta* Fe^{o} NPs and $FeCl_3$ solution

its attachment on a chloride part and also a greater tendency to give out electrons. Dissolution in de-ionized water leads to the formation of an ionic solution thus making Fe^{3+} and Cl^- mobile. As a result, Fe^{3+} undergo reduction tending to their stable existence by the help of *Aspilia pluriseta* extract. Existence of metabolites identified during screening, absorbs electrons from Fe^{3+} species and in turn reduces them to Fe^o. Thereafter, we have got stabilization, growth and even capping [5].

3.4 Fourier Transform-Infrared Characterization

Fig. 2 indicates Fourier transform infrared spectrum of *Aspilia pluriseta* iron oxide nanoparticle.

Peak 3145.7cm^{-1} is as a result of OH stretching vibration arising from hydroxyl groups from the phenolics on nanoparticles, it also denotes reduction of the iron (iii) chloride. The absorption peaks 700.1cm^{-1} and 619.1cm^{-1} corresponds to the Fe-O bond vibration of the formed nanoparticle this is almost similar from research carried out by (Ahmad et al., 2013, Chaki et al., [35] and Silvia et al., 2016), absorption peak 1400.2cm^{-1} corresponds to the aromatic stretch of C=C while the peak at 1596.9 cm^{-1} is a carbon-carbon stretch in aromatic. Peaks at 1000-1300 cm^{-1} is for carbon-oxygen stretch [40]. Other remaining peaks corresponds to small amount of organic acids responsible for low pH of the sample helping in the synthesis of NPs [41]. From the FT-IR analysis in (Fig. 2) presence of hydroxyl groups of phenolic in plant extract acts as a bio-reduction agents and are directly responsible for reduction of Fe^{3+} ions to zero valent iron NPs [42].

3.5 X-Ray Diffraction (XRD)

Fig. 3 is showing XRD peaks for zero valent iron nanoparticles.

Crystallinity of the Fe^o NPs was determined by analysis of XRD patterns shown in Fig. 3.Peak at 16.06° confirms the presence of polyphenols that aids in reduction of iron (iii) salts while peak at 43.73° indicates formation of zero valent iron nanoparticles [9]. Other remaining peaks 21.47° could be as a result of iron oxohydroxide (FeOOH),36.44° is due to the presence of magnetite [26] ,32.71° is almost similar to work done by Jain & Mehata, [36] and 58.34° was as a result of the bioorganic crystallization which occurred on NPs surface [43]. Formation of the various crystal planes emanated from crystallite growth of iron metal with oxygen species [34]. Presence of distinctive diffraction peaks indicates formed NPs are not amorphous [44]. From the XRD spectrum it is evident that as intensity increases the peaks also increases this is due to capping [45]. Developed nanoparticles exhibited a crystallite size of 2.382 nm as generated from Scherrer's formula.

3.6 X-Ray Fluorescence Spectro-photometric Analysis

Results depicted in Fig. 4 represents % of various elements constituting the black precipitate after carrying out XRF analysis.

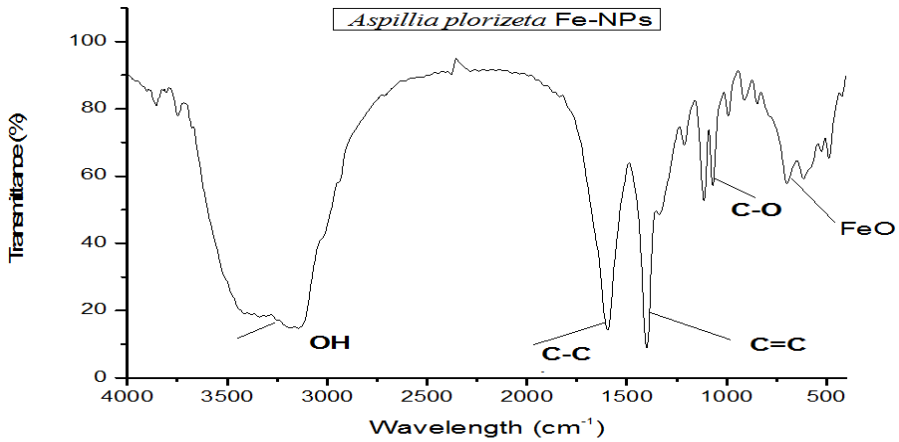

Fig. 2. Fourier Transform-Infrared analysis of Zero Valent Iron nanoparticle

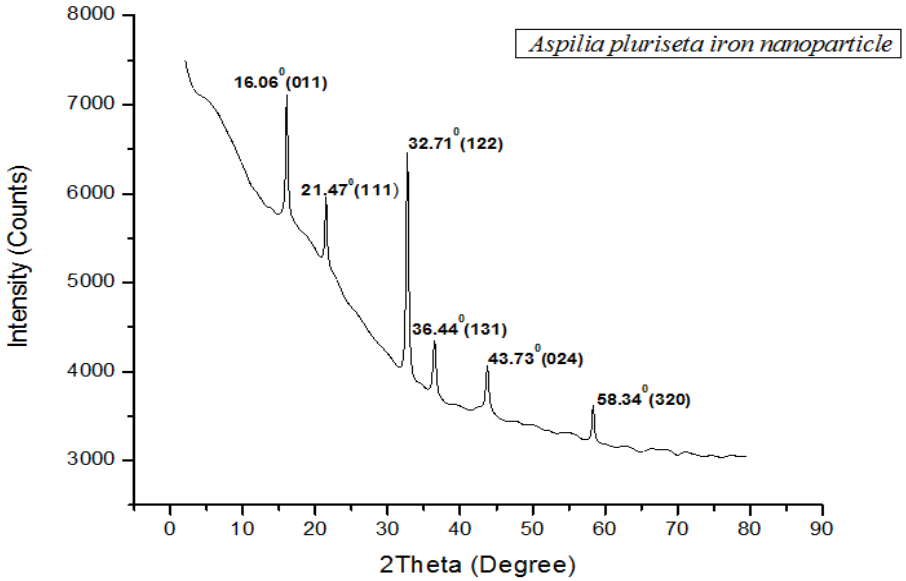

Fig. 3. XRD analysis of Fe^{o} NPs

X-RF spectrophotometer was used to determine elemental composition, results obtained in Fig. 4 above confirmed the presence of iron in the developed nanoparticle. A relatively higher percentage of FeO was occasioned by reaction between *Aspilia pluriseta* aqueous extract and Fe^{3+} resulting to Fe^{o}. SiO_2 (13.84%) in the developed nanoparticle provides the following merits; it attaches different biological/ligands on the nanoparticles surface for different applications, helps nanoparticles to possess good biocompatibility and avoids interparticle interaction [46].

3.7 Antimicrobial Activity

Fig. 5 shows observed zones of inhibition of the five selected microorganisms at various concentrations of the developed NPs.

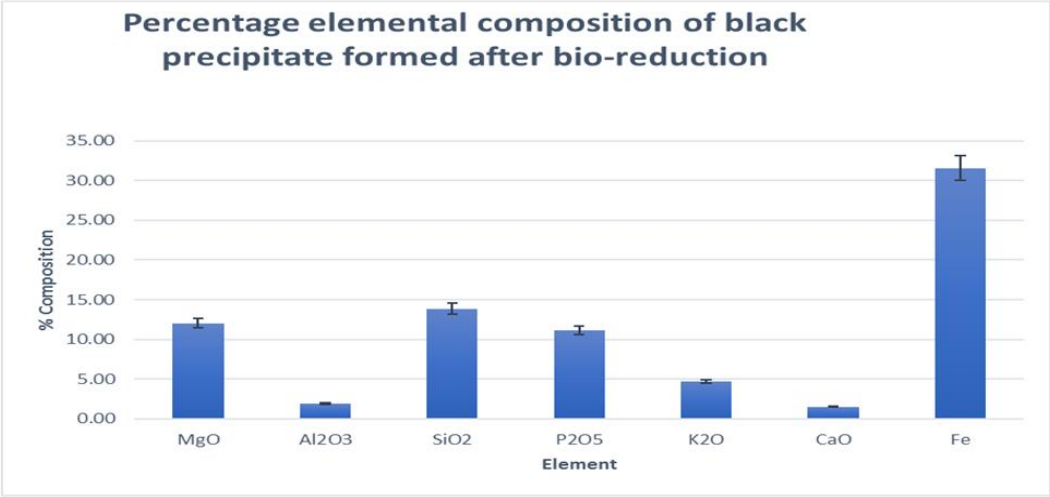

Fig. 4. Percentage elemental composition of black precipitate formed after bio-reduction

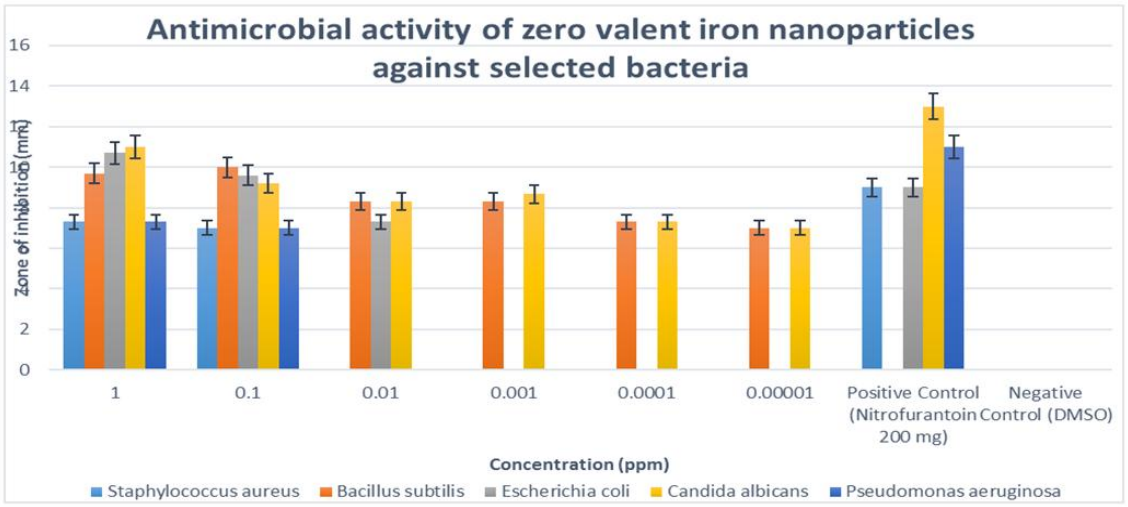

Fig. 5. Antimicrobial activity of zero valent iron nanoparticles against selected microorganisms

The zones of inhibition (mm) exhibited by the various concentrations of synthesized nanoparticle in relation to the standard drug (Nitrofurantoin 200 mg) are presented in Fig. 5 above. Of the five selected microorganism, the developed NPs are more effective in *Escherichia coli* at concentrations above (0.1 ppm) in comparison to the standard drug had an inhibition zone of (9.000 mm). *Pseudomonas aeruginosa, Candida albicans* and *Staphylococcus aureus* were the most resistant strains. DMSO did not exhibit any zone of inhibition. *Bacillus subtillis* was more effective at even very lower concentrations (0.00005 ppm). Phenolics present in *Aspilia pluriseta* NPs played a greater role as they are known for their antimicrobial activities [8]. Polyphenols attached on Fe^{o} NPs play an important role in the prevention of oxidative stress caused by generation of ROS species. Antimicrobial activity of the iron nanoparticle can be summarized in three steps; antibiotic enters the cell, thereafter it must accumulate to a minimum concentration within the cell and finally it acts on its target. The antimicrobial potential of Fe^{o} NPs is similar to original aqueous plant extract [41,47,48].

4. CONCLUSION AND RECOMMENDATIONS

Formation of a black precipitate, absence of a peak at 214 nm and 286 nm, respectively from UV analysis, crystallite size of 2.382 nm which is lesser than 100nm and orthorhombic nature as depicted from XRD analysis, confirmed Fe^{o} NPs formation. NPs effectiveness in relation to the standard drug selected in antimicrobial analysis could provide a solution towards drug resistance on various ailments. Even though nanoparticles have been used extensively for applications such drug discovery, drug conveyance and disease diagnostics, availability of different plant species which have not been explored fully, provides scientists with another great opportunity to discover other new therapeutic agents which could act efficiently against target bacteria, thus quelling the challenge of drug resistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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