Assessment of the Anti-Microbial Action of Zero valent iron nanoparticle synthesized by *Aspillia plorizeta* extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NAO and KPG designed the study, wrote the protocol and wrote the first draft of the manuscript. Author MES reviewed the experimental design and all drafts of the manuscript. Authors NAO and WIS managed the analyses of the study. Authors MEG performed the statistical analysis. All authors read and approved the final script.

ABSTRACT

Antimicrobial resistance poses a great burden to existing health care system as more potent drugs are required to combat this global problem. As such there is need to explore new ways in which we can be able to combat antimicrobial resistance hence the need to utilize the potential of metallic nanoparticles as a new alternative to combat resistance. In this study focus was on synthesization of Fe^{o} NPs using *Aspillia plorizeta* aqueous extracts its characterization and antimicrobial activities against gram (+) and gram (-) microorganisms. Preliminary phytochemical screening was carried out in test for presence of secondary metabolites; phenol, flavonoid, phytosterol, carbohydrate, tannin, saponin, glycoside and terpenoid resulting in a 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 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 showed existence of functional groups such as OH, C-O and C-C that aid in formation of NPs. XRD indicated the presence of peaks of peaks at 16.06° and 43.73°.XRF data showed the NPs containing 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. Thus presence of secondary metabolites in *Aspillia plorizeta* aqueous extracts aids in 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: Aspillia plorizeta; 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 and through constant usage of antibiotics, microorganism have become resilient (Alavijeh, *et al.,* 2012).Many developing countries more so in Africa, mortality and morbidity rates as a result of diarrhea, which continues to be a major challenge especially amongst children (Hills, *et al*., 2014). Of late medicine resistibility towards pathogenic microorganisms has been usually conveyed (Frieri,*et al.*, 2017). On top of this this challenge, antibiotics are occasionally linked with adversarial effects on human beings that comprise immunosuppressant, allergies and hypersensitivity. This has brought greater medical problems in dealing with communicable ailments (Alavijeh *et al*., 2012).

According to 2008 research on antibiotic synthesis comprising smaller companies and larger drug making firms exposed that out of one hundred and sixty seven, only fifteen antibiotics under development possessed fresh mode of action (Leung *et al.,* 2011). Continuity in the pattern could result to a challenge in treatment of people having serious illness.

Nanotechnology a modern field of science dealing in production, manipulation and use of very small particles with sizes measured in nanometers has found its application in the field of medicine (Heera & Shanmugam, 2015). Nanoparticles are majorly obtained using chemical and physical process. Production of NPs using plant materials results to low cost of production, short production time, it is relatively safer and its ability to up production. On industrial scale efficient extraction, isolation and purification is 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 (Makarov, *et al*., 2014) .Various research work has been done using different metal NPs contributing towards production of alternative nano-therapeutics, in treating infections emanating from drugs which are resistant (Enrique, *et al.,* 2018). Owing to various physiochemical characteristics; great surface area, mechanical strength, optical activity and their reactiveness(Khan, *et al.,* 2017), iron nanoparticles has also found application in water treatment (Devatha, *et al*., 2018). 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 (Yuvakkumar, *et al.,* 2011).Extraction and isolation of natural products (Xiao, *et al.*, 2018). Biosynthesis possess extra reimbursement in relation to other conventional synthetic methods owing to presence of other biological entities and environmental friendly processes, relatively cheap, bulk synthesis is enabled ,low pressure energy and non-toxic chemicals is used (Kuppusamy, *et al.,* 2016; Ksv, *et al.*, 2017).

Various reports from medicinal plants 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 (Saranya, 2017).

2. MATERIALS AND METHODOLOGY

2.1 *Aspillia plorizeta* Sampling

Fresh samples of *Aspillia plorizeta* 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 (Ahmed, *et al*., 2016)

2.2 Extraction of *Aspillia plorizeta* using water

With slight changes from work done by (Vélez *et al.*, 2018) ,5g of *Aspillia plorizeta* leaves powder was weighed into a 250 conical flask, thereafter 100ml of distilled water added followed by boiling in a water bath for one hour maintaining the temperature at 80°C. Having obtained *Aspillia plorizeta* aqueous extract, filtration was carried out thereafter .Filtrate obtained was then kept in the refrigerator ready for analysis (Logeswari, *et al.*, 2015).

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 (Prabhavathi, *et al.*, 2016,Khalid, *et al*., 2018 and Gupta & Gupta, 2014).

2.4 Quantity of reducing agents

2.4.1 Phenolics Content

The phenolics were quantified using protocols developed by (*Baba & Malik, 2015* and *Alara, et al., 2017*) ,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 represented by (*Baba & Malik, 2015 and Spiridon, et al., 2011*), 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 was carried out with slight modification of procedure, from previous work done by (Ksv, *et al.*, 2017). 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 5 minutes shaking to obtain a homogenous mixture. Thereafter NPs was synthesized by addition of 0.1MFeCl3.6H2O into the plant sample in the ratio of 2:5 in which a black precipitate was observed indicating presence of NPs (Silveira, *et al*., 2017). Formed nanoparticle was then retrieved from the mixture by centrifuging (350rpm for ten minutes) and washing severally using deionized H_2O .Finally it was oven dried for characterization(Fierascu, *et al.*, 2014).

2.6 Characterization of zero valent iron NPs

Functional groups which necessitated development of nanoparticles was characterized by use of FT-IR Spectrophotometer, Make FTS-8000 and analysis run using the KBr pellet technique (*Madivoli, et al., 2012)*. Optical properties of Fe^{o} NPs was determined by use of Perkin Elmer Spectrometer (*Groiss, et al., 2017)*. Crystal phase for Fe^{o} NPs was identified by use of STOE STADIP P XRD machine with slight modifications from work done by (*Gondwal, 2018).*Elemental composition of prepared powder sample was then determined using X-ray fluorescence spectrometry *(Santos, et al., 2017).*

2.7 Antibacterial Activity

Evaluation of the antimicrobial activities against selected microorganisms for green-synthesized zero valent iron nanoparticle was carried out using standard disc diffusion assay with slight modifications from work done previously by (*Mostafa et al., 2017 and Groiss et al., 2017*). Dimethyl sulphoxide was used to dissolve zero valent iron nanoparticles. Concentrations used were 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} µg/ml. DMSO without nanoparticle acted as negative control, while Nitrofurantoin (200 mcg) acted as positive control.

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening of *Aspillia plorizeta* leaves extract

Phytochemicals constitutes primary and secondary metabolites. Primary metabolites are basically carbohydrate while secondary constituents consists; glycosides, flavonoids, terpenes, terpenoids, saponins, phenols and tannins (Ranjitha & Suganthi, 2017). *Aspillia plorizeta* aqueous extract gave a positive test for phytochemical screening of all the secondary metabolites under study (Martínez-cabanas, *et al*., 2016). Phytochemical constituents of *Aspillia plorizeta* are involved in Fe^{o} NPs synthesis hence the leaves were boiled with the aim of rupturing and releasing intracellular materials into the solution (*Ganesan, 2015*).

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. Polarity of extracting solvent, isolation procedure and compounds present constitutes natural extracts activity. 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 (Jing, *et al.*, 2015).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 (Marslin *et al*., 2018).

3.3 Observations and UV-vis analysis of Iron nanoparticle

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

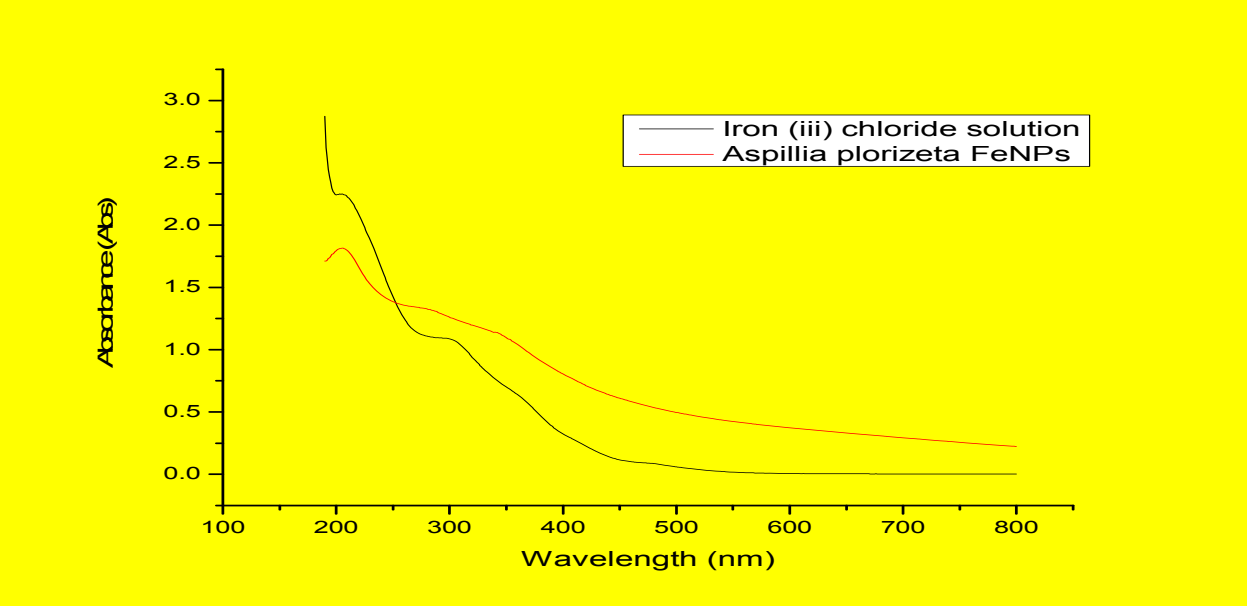

Figure 1: UV-vis spectrum of *Aspillia plorizeta* Fe^{o} NPs and $FeCl_3$ solution

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 previous work done by (Chaki, *et al.*, 2015).

Aqueous solution of iron (iii) chloride gave two peaks at 214nm and 286nm. Thus absence of a peak at 214nm and 286nm in the NPs spectrum could signify formation of zero valent iron NPs. Greater change in absorption spectra, indicates that *Aspillia plorizeta* alone acts as a better stabilizer, this is in agreement from work done by (Jain & Mehata, 2017) . Presence of a single peak in the NPs developed indicates ,particles formed are of uniform size and shape (Joe, *et al*., 2011).Upon addition of *Aspillia plorizeta* 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 (Balamurugan, *et al*., 2014). Color change is the most easy and commonly used indicator of metal nanoparticles formation (Atarod, *et al.,* 2016). $FeCl_3$ salt greater reduction capability is as a result of; its attachment on a chloride part and also 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 *Aspillia plorizeta* 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 (*Makarov, et al., 2014*).

3.4 Fourier Transform-Infrared characterization

Figure 2 indicates Fourier transform infrared spectrum of *Aspillia plorizeta* iron oxide nanoparticle.

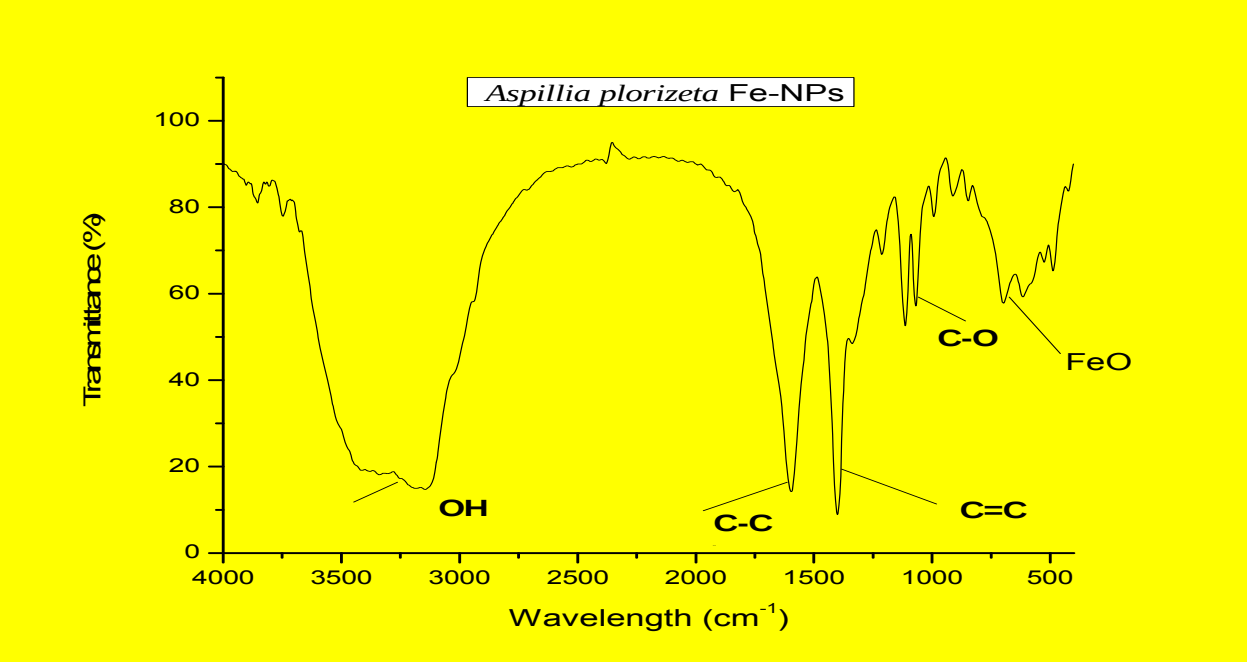

Figure 2: Fourier Transform-Infrared analysis of Zero Valent Iron nanoparticle

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., 2015* and *Silvia et al., 2016*), absorption peak 1400.2cm^{-1} corresponds to aromatic stretch of C=C while the peak at 1596.9cm^{-1} is carbon-carbon stretch in aromatics. Peaks at 1000-1300 cm^{-1} is for carbon-oxygen stretch (Khodadadi,*et al.*, 2017). Other remaining peaks corresponds to small amount of organic acids responsible for low pH of the sample helping in synthesis of NPs (Kanagasubbulakshmi*, et al.,* 2017). From the FT-IR analysis in (fig. 2) presence of hydroxyl groups of phenolic in plant extract acts as bio-reductant agents and are directly responsible for reduction of Fe^{3+} ions to zero valent iron NPs (Samaneh, *et al.*, 2017).

3.5 X-Ray Diffraction (XRD)

Figure 3 is showing XRD peaks for zero valent iron nanoparticles

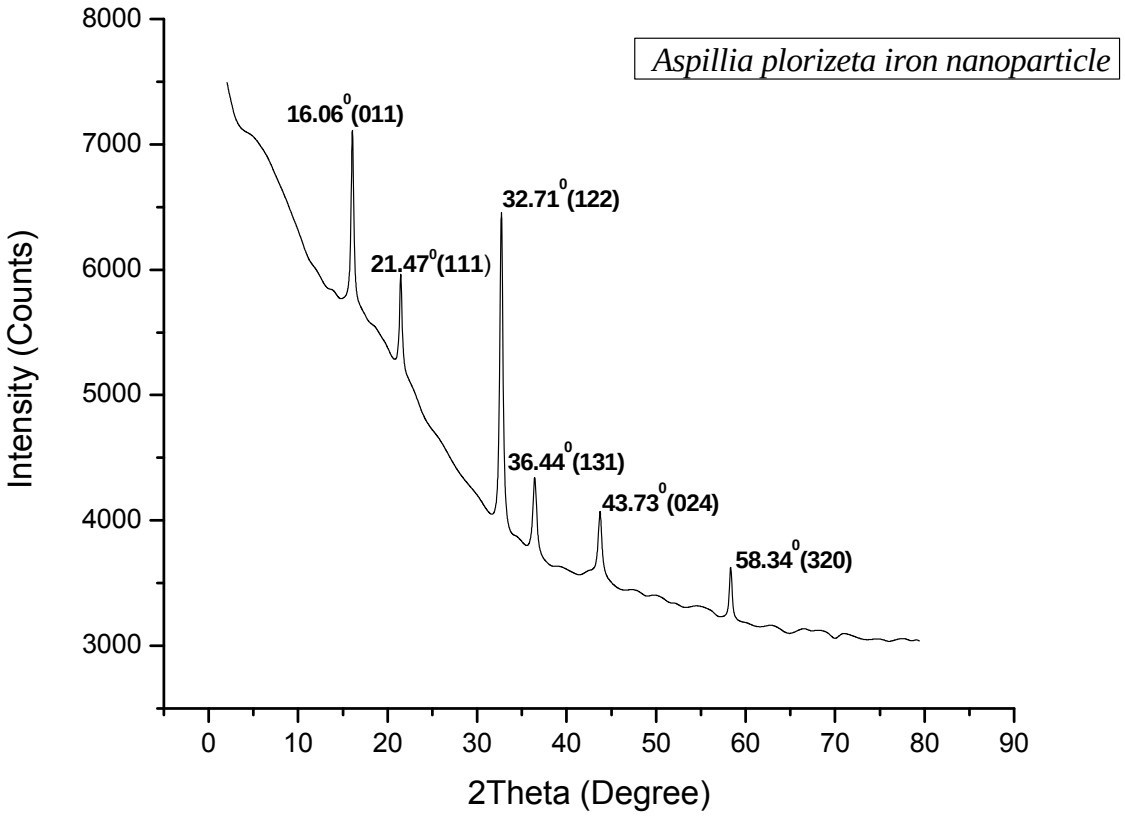

Figure 3: XRD analysis of Fe^{o} NPs

Crystallinity of the Fe^{o} NPs was determined by analysis of XRD patterns shown in Fig. 3.Peak at 16.06° indicates polyphenols present in plant that aids in reduction of iron (iii) salts while peak at 43.73° indicates formation of zero valent iron nanoparticles(Yuvakkumar et al., 2011).Other remaining peaks 21.47° could be as a result of iron oxohydroxide (FeOOH),36.44° is due to presence of magnetite (*Groiss et al*., *2017*) ,32.71° is almost similar to work done by (*Jain & Mehata, 2017*) and 58.34° was as a result of the bioorganic crystallization which occurred on NPs surface (*Rafi et al., 2018*). Formation of the various crystal planes emanated from crystallite growth of iron metal with oxygen species (*Marslin et al., 2018*).Presence of distinctive diffraction peaks indicates formed NPs are not amorphous*(Wang, Fang, & Megharaj, 2014*). From the XRD spectrum it is evident that as intensity increases the peaks also increases this is due to capping (*Ullah, et al., 2018*). Developed nanoparticles exhibited a crystallite size of 2.382 nm as generated from Scherrer's formula.

3.6 X-Ray Fluorescence Spectrophotometric analysis

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

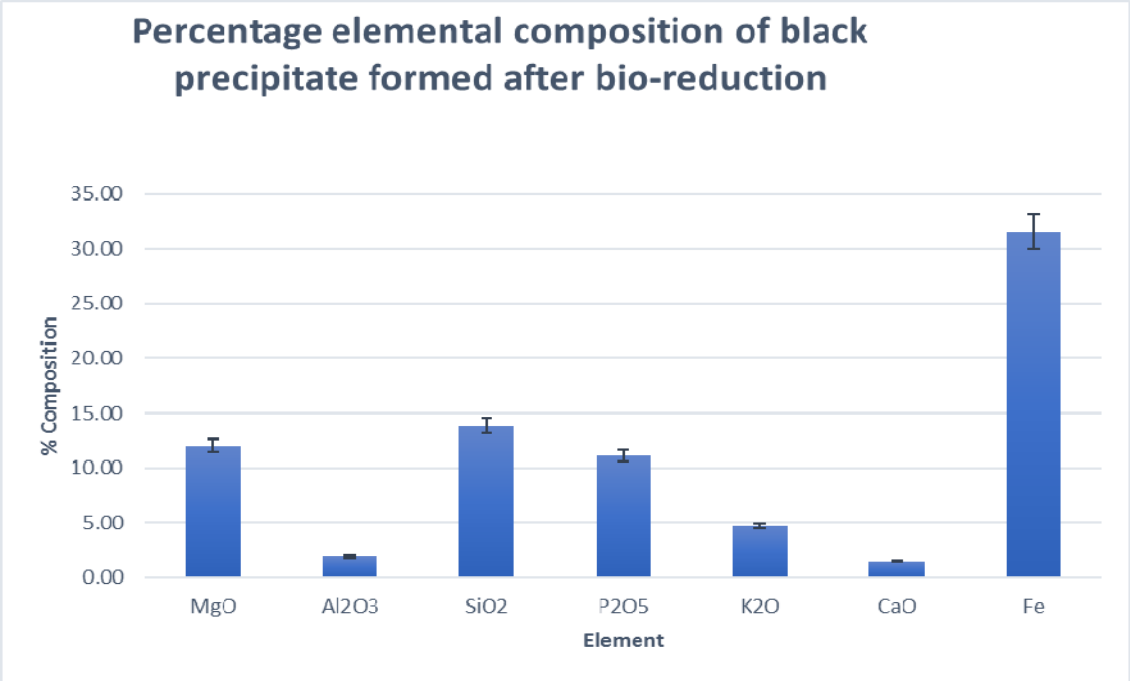

Figure 4: Percentage elemental composition of black precipitate formed after bio-reduction

X-RF spectrophotometer was used to determine elemental composition, results obtained confirmed presence of 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% .Thus from data in (figure. 4) ,presence of iron in developed nanoparticle was confirmed. Relatively higher percentage of FeO was occasioned by reaction between *Aspillia plorizeta* aqueous extract and Fe^{3+} resulting to Fe^{o}. SiO_2 (13.84%) in the developed nanoparticle provides the following merits; attaching different biological/ligands on nanoparticles surface for different application, helps nanoparticles to possess good biocompatibility and avoids interparticle interaction (Wu, He, & Jiang, 2008).

3.7 Antimicrobial activity

Figure. 5 shows the observed zone of inhibition of the five selected microorganisms and various concentrations of developed NPs obtained through serial dilutions.

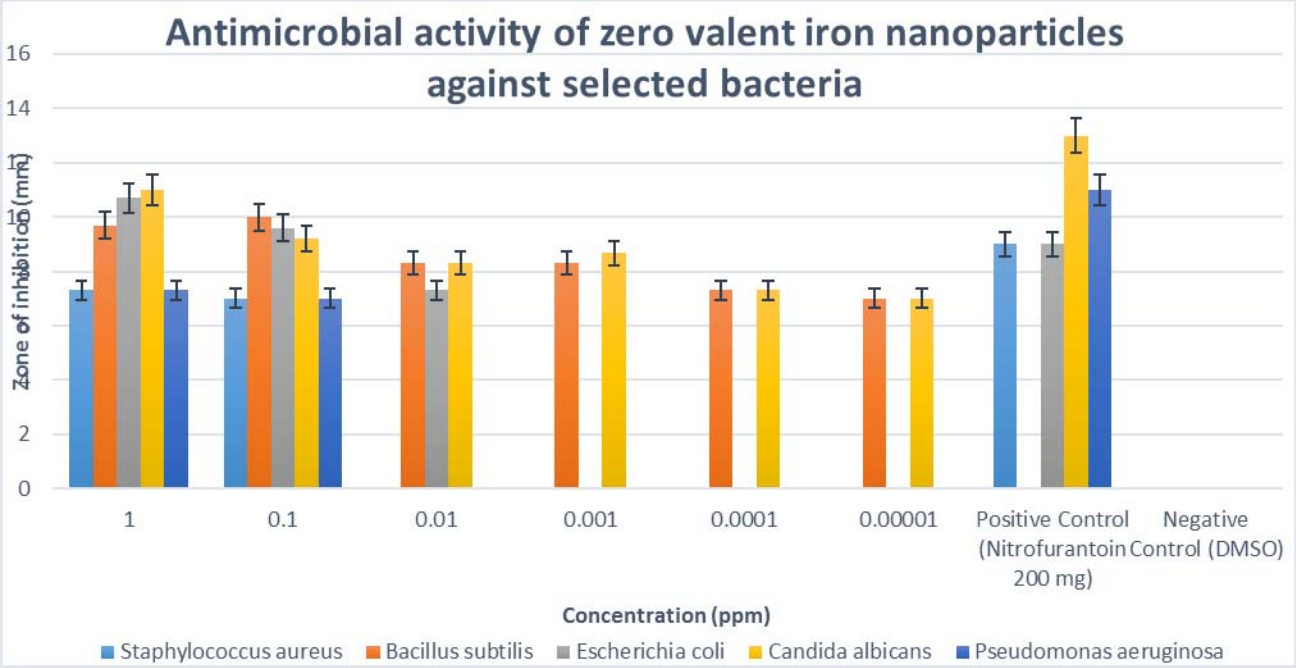

Figure: 5 Antimicrobial activity of zero valent iron nanoparticles against selected bacteria

Antibiotic susceptibility test applied in our present study was the standard disk diffusion assay on the following microorganism *Candida albicans, Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. The zones of inhibition (mm) exhibited by the various concentrations of synthesized nanoparticle in relation to the standard drug (Nitrofurantoin 200 mg) is presented in figure 5 above. Of the five selected microorganism, the developed NPs is more effective in *Escherichia coli* at

concentrations above (0.1ppm) in comparison to the standard drug which had a zone of inhibition (9.000 mm). *Pseudomonas aeruginosa, Candida albicans* and *Staphylococcus aureus* were the most resistant strains. The negative control in our experiment was (DMSO) and it did not exhibit any zone of inhibition. *Bacillus subtillis* was more effective at even very lower concentrations (0.00005ppm) Phenolics present in *Aspillia plorizeta* NPs played a greater role as they are known for their antimicrobial activities (Devatha *et al.*, 2018).Polyphenols attached on Fe^{o} NPs play an important role in 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 *(Kanagasubbulakshmi & Kadirvelu, 2017 , Abdel-Rahman et al., 2017*and *Abdel-Rahman et al., 2013)*.

CONCLUSION AND RECOMMENDATIONS

Optical properties where a black precipitate was formed, absence of a peak at 214nm and 286 nm from UV analysis and smaller 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. Incorporating green synthesis approach in our studies provides a clean, cheap and safer method. 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, 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.

REFERENCES

Abdel-Rahman, L.H.; El-Khatib, R. M.; Nassr, L.A.E.; Abu-Dief, A.M.; Lashin, F.E.; Design, characterization, tetraogenicity testing, antibacterial, antifungal and DNA interaction of few high spin Fe (II) Schiff base amino acid complexes, spectrochim. Acta part A, 111, 2013, 266-276

Abdel-Rahman, L.H.; El-Khatib, R. M.; Nassr, L.A.E.; Abu-Dief, A.M.; Lashin, F.E.; Some new nano-sized Fe(II), Cd(II) and Zn(II) Schiff base complexes as precursor for metal oxides: Sonochemical synthesis, characterization, DNA interaction, in vitro antimicrobial and anticancer activities Biorganic Chemistry 69 (2016) 140-152

Ahmed, S., Saifullah, Ahmad, M., Swami, B. L., & Ikram, S. (2016). Green synthesis of silver

nanoparticles using Azadirachta indica aqueous leaf extract. Journal of Radiation Research and Applied Sciences, 9(1), 1–7.

Alara, O. R., Abdurahman, N. H., & Olalere, O. A. (2017). Journal of King Saud University – Science Ethanolic extraction of flavonoids , phenolics and antioxidants from Vernonia amygdalina leaf using two-level factorial design. *Journal of King Saud University - Science*, 1–10.

Alavijeh, P. K., Alavijeh, P. K., Sharma, D., & Pharmacy, C. (2012). A study of antimicrobial activity of few medicinal herbs, *2*(4), 496–502.

Atarod, M., Nasrollahzadeh, M., & Sajadi, S. M. (2016). Journal of Colloid and Interface Science Green synthesis of Pd / RGO / Fe 3 O 4 nanocomposite using Withania coagulans leaf extract and its application as magnetically separable and reusable catalyst for the reduction of 4-nitrophenol. *JOURNAL OF COLLOID AND INTERFACE SCIENCE*, *465*, 249–258.

Baba, S. A., & Malik, S. A. (2015). Determination of total phenolic and flavonoid content , antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. *Integrative Medicine Research*, *9*(4), 449–454.

Balamurugan, M., Saravanan, S., & Soga, T. (2014). Synthesis of Iron Oxide Nanoparticles by Using Eucalyptus Globulus Plant Extract. *E-Journal of Surface Science and Nanotechnology*, *12*(0), 363–367.

Chaki, S. H., Malek, T. J., Chaudhary, M. D., Tailor, J. P., & Deshpande, M. P. (n.d.). Magnetite Fe 3 O 4 nanoparticles synthesis by wet chemical reduction and their characterization.

Devatha, C. P., Jagadeesh, K., & Patil, M. (2018). Environmental Nanotechnology , Monitoring & Management E ff ect of Green synthesized iron nanoparticles by Azardirachta Indica in di ff erent proportions on antibacterial activity. *Environmental Nanotechnology, Monitoring & Management*, *9*(November 2017), 85–94.

Enrique, C., Castro, B., Saucedo-, E. M., Morales, R. M. C., Soto, D. I. R., Treviño, F. M., & Carrazco, J. L. (2018). In vivo antimicrobial activity of silver nanoparticles produced via a green chemistry synthesis using Acacia rigidula as a reducing and capping agent, 2349–2363.

Fierascu, R. C., Bunghez, I. R., Somoghi, R., Fierascu, I., & Ion, R. M. (2014). Characterization of Silver Nanoparticles Obtained By Using Rosmarinus Officinalis Extract and their antioxidant activity. *Rev. Roum. Chim.*, *59*(March), 213–218.

Frieri, M., Kumar, K., & Boutin, A. (2017). Antibiotic resistance. *Journal of Infection and Public Health*, *10*(4), 369–378.

Ganesan, V. (2015). ECO-FRIENDLY SYNTHESIS AND CHARACTERIZATION OF SILVER

NANOPARTICLES SYNTHESIZED AT DIFFERENT P H USING LEAF BROTH OF HYPTIS SUAVEOLENS (L .) POIT ECO-FRIENDLY SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES SYNTHESIZED AT DIFFERENT P H USING LEAF, (September).

Gondwal, M. (2018). Synthesis and Catalytic and Biological Activities of Silver and Copper Nanoparticles Using Cassia occidentalis, *2018*.

Groiss, S., Selvaraj, R., Varadavenkatesan, T., & Vinayagam, R. (2017). Structural characterization, antibacterial and catalytic effect of iron oxide nanoparticles synthesised using the leaf extract of Cynometra ramiflora. *Journal of Molecular Structure*, *1128*(September), 572–578.

Gupta, M., & Gupta, S. (2014). Qualitative and Quantitative Analysis of Phytochemicals and Pharmacological Value of Some Dye Yielding Medicinal Plants Qualitative and Quantitative Analysis of Phytochemicals and Pharmacological Value of Some, (May).

Heera, P., & Shanmugam, S. (2015). Review Article Nanoparticle Characterization and Application : An Overview, *4*(8), 379–386.

Hills, M., Khan, A. M., Qureshi, R. A., Gillani, S. A., & Ullah, F. (2014). Antimicrobial activity of selected medicinal plants of, (March 2011).

Jain, S., & Mehata, M. S. (2017). Medicinal Plant Leaf Extract and Pure Flavonoid Mediated Green Synthesis of Silver Nanoparticles and their Enhanced Antibacterial Property. *Scientific Reports*, *7*(1), 1–13.

Jing, L., Ma, H., Fan, P., Gao, R., & Jia, Z. (2015). Antioxidant potential, total phenolic and total flavonoid contents of Rhododendron anthopogonoides and its protective effect on hypoxia-induced injury in PC12 cells. *BMC Complementary and Alternative Medicine*, *15*(1), 1–12.

Joe, J., Sivalingam, P., Siva, D., Kamalakkannan, S., Anbarasu, K., Sukirtha, R., … Achiraman, S. (2011). Colloids and Surfaces B : Biointerfaces Comparative evaluation of antibacterial activity of silver nanoparticles synthesized using Rhizophora apiculata and glucose. *Colloids and Surfaces B: Biointerfaces*, *88*(1), 134–140.

Kanagasubbulakshmi, S., & Kadirvelu, K. (2017). Green Synthesis of Iron Oxide Nanoparticles using Lagenaria Siceraria and Evaluation of its Antimicrobial Activity, *2*(4), 422–427.

Khalid, S., Shahzad, A., Basharat, N., Abubakar, M., & Anwar, P. (2018). Phytochemical Screening and Analysis of Selected Medicinal Plants in Phytochemistry & Biochemistry, *2*(1), 2–4.

Khan, I., Saeed, K., & Khan, I. (2017). Nanoparticles : Properties , applications and toxicities. *Arabian Journal of Chemistry*.

Khodadadi, B., Bordbar, M., & Nasrollahzadeh, M. (2017). Journal of Colloid and Interface Science

Achillea millefolium L . extract mediated green synthesis of waste peach kernel shell supported silver nanoparticles : Application of the nanoparticles for catalytic reduction of a variety of dyes in water. *Journal of Colloid And Interface Science*, *493*, 85–93.

Ksv, G., P, H. R., & Zamare, D. (2017). Biotherapeutic Discovery Green Synthesis of Iron Nanoparticles Using Green Tea leaves Extract, *7*(1), 1–4.

Kuppusamy, P., Yusoff, M. M., & Maniam, G. P. (2016). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report. *Saudi Pharmaceutical Journal*, *24*(4), 473–484.

Leung, E., Weil, D. E., Raviglione, M., & Nakatani, H. (2011). The WHO policy package to combat antimicrobial resistance. *Bulletin of the World Health Organization*, *89*(5), 390–392.

Logeswari, P., Silambarasan, S., & Abraham, J. (2015). Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. *Journal of Saudi Chemical Society*, *19*(3), 311–317.

Madivoli, E. S., Maina, E. G., Kairigo, P. K., Murigi, M. K., Ogilo, J. K., Nyangau, J. O., … Madivoli, E. (2012). In vitro a ntioxidant and antimicrobial activity of Prunus africana (Hook . f .) Kalkman (bark extracts) and Harrisonia abyssinica Oliv . extracts (bark extracts): A comparative study, 1–9.

Mahdavi, M., Namvar, F., Ahmad, M. Bin, & Mohamad, R. (2013). Green Biosynthesis and Characterization of Magnetic Iron Oxide (Fe3O4) Nanoparticles Using Seaweed (Sargassum muticum) Aqueous Extract, 5954–5964.

Makarov, V. V, Love, A. J., Sinitsyna, O. V, Makarova, S. S., & Yaminsky, I. V. (2014). “ Green ” Nanotechnologies : Synthesis of Metal Nanoparticles Using Plants, *6*(20), 35–44.

Marslin, G., Siram, K., Maqbool, Q., Selvakesavan, R. K., Kruszka, D., Kachlicki, P., & Franklin, G. (2018). Secondary metabolites in the green synthesis of metallic nanoparticles. *Materials*, *11*(6).

Martínez-cabanas, M., López-garcía, M., Barriada, J. L., Herrero, R., & Vicente, M. E. S. De. (2016). Green synthesis of iron oxide nanoparticles. Development of magnetic hybrid materials for efficient As (V) removal. *CHEMICAL ENGINEERING JOURNAL*, (V).

Mostafa, A. A., Al-askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2017). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*.

Prabhavathi, R. M., Prasad, M. P., & Jayaramu, M. (2016). Studies on Qualitative and Quantitative Phytochemical Analysis of Cissus quadrangularis, *7*(4), 11–17.

Rafi, M., Id, S., Khan, M., Id, M. K., Al-warthan, A., Mahmood, A., … Adil, S. F. (n.d.). Plant-Extract-

Assisted Green Synthesis of Silver Nanoparticles Using Origanum vulgare L . Extract and Their Microbicidal Activities, 1–14.

Ranjitha, S., & Suganthi, A. (2017). Preliminary phytochemical analysis of galinsoga parviflora (Cav) leaves and flowers, *2*(3), 18–20.

Samaneh, S., Nasrollahzadeh, M., & Rustaiyan, A. (2017). Journal of Colloid and Interface Science Biosynthesis and application of Ag / bone nanocomposite for the hydration of cyanamides in Myrica gale L . extract as a green solvent. *Journal of Colloid And Interface Science*, *499*, 93–101.

Santos, F. S. Dos, Lago, F. R., Yokoyama, L., & Fonseca, F. V. (2017). Synthesis and characterization of zero-valent iron nanoparticles supported on SBA-15. *Journal of Materials Research and Technology*, *6*(2), 178–183.

Saranya, S. (2017). Green Synthesis of Iron Nanoparticles using Aqueous Extract of Musa ornata Flower Sheath against Pathogenic Bacteria, *79*(January), 688–694.

Silveira, C., Shimabuku, Q. L., & Silva, M. F. (2017). Iron-oxide nanoparticles by the green synthesis method using Moringa oleifera leaf extract for fluoride removal, *3330*.

Spiridon, I., Bodirlau, R., & Teaca, C. (2011). Total phenolic content and antioxidant activity of plants used in traditional Romanian herbal medicine, *6*(3).

Ullah, A., Kareem, A., Nami, S. A. A., Shoeb, M., & Rehman, S. (2018). Journal of Photochemistry & Photobiology , B : Biology Biogenic synthesis of iron oxide nanoparticles using Agrewia optiva and Prunus persica phyto species : Characterization , antibacterial and antioxidant activity. *Journal of Photochemistry & Photobiology, B: Biology*, *185*(April), 262–274.

Vélez, E., Campillo, G., Morales, G., Hincapié, C., Osorio, J., & Arnache, O. (2018). Silver Nanoparticles Obtained by Aqueous or Ethanolic Aloe vera Extracts : An Assessment of the Antibacterial Activity and Mercury Removal Capability, *2018*.

Wang, Z., Fang, C., & Megharaj, M. (2014). Characterization of Iron − Polyphenol Nanoparticles Synthesized by Three Plant Extracts and Their Fenton Oxidation of Azo Dye, 10–13.

Wu, W., He, Q., & Jiang, C. (2008). Magnetic iron oxide nanoparticles: Synthesis and surface functionalization strategies. *Nanoscale Research Letters*, *3*(11), 397–415.

Xiao, J., Chen, G., & Li, N. (2018). Ionic Liquid Solutions as a Green Tool for the Extraction and Isolation of Natural Products. *MDPI*, 23.

Yuvakkumar, R., Elango, V., Rajendran, V., & Kannan, N. (2011). PREPARATION AND CHARACTERIZATION OF ZERO VALENT IRON, *6*(4), 1771–1776.