Colorimetric detection of Hg(II) ions present in industrial wastewater using zinc nanoparticle synthesized biologically with *Rauwolfia vomitoria* leaf extract.

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AbstractThis study highlights the synthesis of zinc nanoparticle using Rauwolfia vomitoria leaf extract for the colorimetric detection of Hg (II) ions in industrial wastewater. Characterization of zinc nanoparticlcles using UV-vis spectroscopy, revealed that maximum absorption was obtained at 219nm which indicated surface plasmon absorption of zinc nanoparticle. The XRD analysis of the zinc nanoparticle indicated that the zinc nanoparticles formed are crystalline in nature with a mixed irregular phase structure (polygonal and spherical) in shape. The average crystallite size of the zinc nanoparticle was found to be 57nm. FTIR analysis was carried out to ascertain the possible functional groups responsible for the reduction of zinc ion to zinc nanoparticle. The reduction was observed by the disappearance of C-O (due to ether) in the FTIR spectrum of the reduce compound, which that this functional group was involved in the reduction of zinc ions to zinc nanoparticle. Scanning electron microscopy (SEM) revealed that the morphology of the zinc nanoparticle possessed polygonal, spherical or faceted shape of various sizes that are agglomerated. Further magnifications revealed that these images possessed rough surfaces. Detection of Hg(II) ions in industrial wastewater using the colloidal zinc nanoparticle was feasible even at very low concentration. However, absorption peak became more intense with increasing concentration. The results and findings of our study provide commendation for the use of economic synthesis of zinc nanoparticle in the detection of Hg (II) ions present in industrial wastewater.

Key Words: *Colorimetric detection, Zinc nanoparticle, Industrial wastewater, Rauwolfia vomitoria.*

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1.0 Introduction

According to Pinon-Segundo*et al.,* (2013), metal nanoparticles are submicron scale entities made of pure metals (example, gold, platinum, silver, titanium, zinc, cerium, iron, and thallium) or their compounds (example, oxides, hydroxides, sulfides, phosphates, fluorides, and chlorides). In recent years, nanotechnology is an escalating field of modern research (Edhaya and Prakash2013; Odiongenyi and Afangide, 2019) in synthesis design, characterization, production, and application of structures, devices, and systems by controlling shape and size at the nanometer scale (that is within the range of 1 to 100 nm) (Madhuri *et al.,* 2012). Nanotechnology has extended to different filed of science and technology but one of the interesting areas of nano technology is in the field of bionanotechnology, which involves the application of nanotechnology tools to solve biological problems in order to create specialized applications (Kathiresan *et al.,* 2009*;* Qi and Wang, 2004; Roduner, 2004). The bio-based protocols for synthesis of nano-metals are significant because the methods are economical and ecofriendly, which fits into green process. This green synthetic route has several advantages because most chemical methods are relatively expensive and some of them use or generate lethal chemicals that are comparatively toxic, non-biodegradable, not easily accessible and not eco-friendly. Therefore, biosynthesis of nanoparticles using biological agents such as microbes or plant extracts is receiving much attention in the field of nanotechnology (Malik *et al.,* 2014). Green synthesis of nano materials is simplified by three major steps which are reaction medium selection, biological reducing agent selection, and selection of non-carcinogenic substances for stability of nanoparticles (El-Shishtawy *et al.,* 2011) yet, plant-mediated preparation of nanoparticles can be advantageous over other bio-based synthesis because the procedure of maintaining cell cultures can be omitted and can be adopted for large scale production under non-aseptic environments (Makarov, 2014).Zinc is a trace element that is necessary for a healthy immune system. Zinc deficiency can lead to some health challenges and possible risk of infection. Zinc nanoparticles (ZnNPs) are important materials that primarily differ from their macroscopic counterparts in size and have been widely used in many different fields such as electronics, dyestuff, coating, medicine, clothing, and cosmetics (Felieu and Fadeel, 2010; Lee *et al.,* 2010;Roco, 2011; Kumar *et al.,* 2012; Lefebvre*et al.,* 2015).ZnNPs are engineered nanomaterials that are commonly used in various human applications such as sunscreens, ceramics, rubber processing, wastewater treatment, and children's products (Ma *et al.,* 2013; Feradez-cruz *et al.,* 2013). It has been reported that a variety of environmental transformations, including aggregation (Bian *et al.,* 2011), dissolution (Mudunkotuwa *et al.,* 2012), sulfidation (Ma *et al.,*) and phosphorylation (Lv *et al.,*2012) are possible for ZnNPs. Applications of zinc nanocrystals include; as an anti-microbial, anti-biotic and anti-fungal (fungicide) agent when incorporated in coatings, bandages, nanofiber, nanowire, plastics, alloy and textiles and further research for their potential electrical, dielectric, magnetic, optical, imaging, catalytic, biomedical and bioscience properties. *Rauwolfia vomitoria* plant had been considered as a source of medicinal agent for the treatment of various diseases such as malaria, leprotic ulcer, skin infections, (James *et al.,* 2008) high blood pressure (Akpanabiatu *et al.,*). *Rauwolfia vomitoria* contains many different phytochemicals, including alcohols, sugars and glycosides, fatty acids, flavonoids, phytosterols, oleoresins, steroids, tannins, alkaloids and mostly the important alkaloids found in the plant are indole alkaloids, with more than 50 of those alkaloids having been isolated in the plant (Verma and Verma, 2010) hence could be could be used as a capping agent in synthesizing metal nanoparticle. This prompted interest to synthesize zinc nanoparticles using extract from the leaves of *Rauwolfia vomitoria* and equally to ascertain its ability in detecting Hg (II) ions from industrial wastewater.

2.0 Materials and Methods

2.1. Sample collection and preparation

Fresh leaves from *Rauwolfia vomitoria* plant were obtained from Michael Okpara University of Agriculture botanical garden and were identified in the Taxonomy unit in the Department of Forestry of the same University. The leaves were washed with distilled water and preserved. The fresh leaves were washed with deionized water and air dried to a constant weight. 25 g of the dried and pulverized sample was added to a 500 ml beaker containing 300 ml of de-ionized water and stirred for 20 minutes before it was heated at 100 °C for 15 minutes. The heated sample was allowed to cold, filtered through Whatman No. 1 filter paper (Springfield Mill. Maidstone. Kent, England). The filtrate was used for the synthesis of zinc nanoparticle.

2.2. Synthesis of zinc nanoparticles

For the synthesis of zinc nanoparticles, 100mL of the aqueous leaves extract was added to 900 mL of 1×10^{-3} M aqueous ($ZnSO_4.7H_2O$) solution in a 1000mL bottle and was stirred for 30 minutes. Within 10 minutes, change in colour was observed from reddish brown to amber yellow indicating the formation of zinc nanoparticles. The zinc nanoparticles solution obtained was purified by repeated centrifugation at 4000 rpm for 15 minutes followed by re-dispersion of the pellet in deionized water. The zinc nanoparticles were dried in an oven at 80 °C and then allowed to cool before storing in an airtight sample container.

2.3. UV-visible spectroscopy analysis

The bio-reduction process of zinc ions in aqueous solution was measured by sampling 1mL aliquot compared with 1 mL of de-ionized water used as blank and subsequently measuring the UV-visible spectrum of the solution. UV-visible spectrum was monitored on Cary Series UV-Visible spectrophotometer Agilent Technology, operated within the wavelength range of 200 to 800 nm.

2.4. FT-IR spectroscopy measurement

FT-IR measurement of the samples and nanomaterials to be analysed was performed using FTIR-Cary 630 Fourier Transform Infrared Spectrophotometer, Agilent Technology, in a transmittance method at a resolution of 8 cm^{-1} in potassium bromide (KBr) pellets in the wave number range of 4000-400cm^{-1}

2.5 Scanning electron microscopy (SEM) analysis

Morphology of the nanoparticles was studied using scanning electron microscope 000x (MODEL-PHENOM ProX Scanning Element Microscope manufactured by Phenom World Eindhoven, Netherlands) operating at magnification of 80- 150.

2.6. X-ray diffraction (XRD) analysis

XRD (PAN analytical, Netherlands) patterns were obtained with a diffractometer (Empyrean Model, Netherlands) operated at a voltage of 45 KV and a current of 40 mA using Cu-K(alpha) radiation in a -2 configuration with a wavelength (λ) of 0.1541. The sample was made smoother and was imparted on a slide which was then charged into the machine after setting the analytical functions in the instrument.

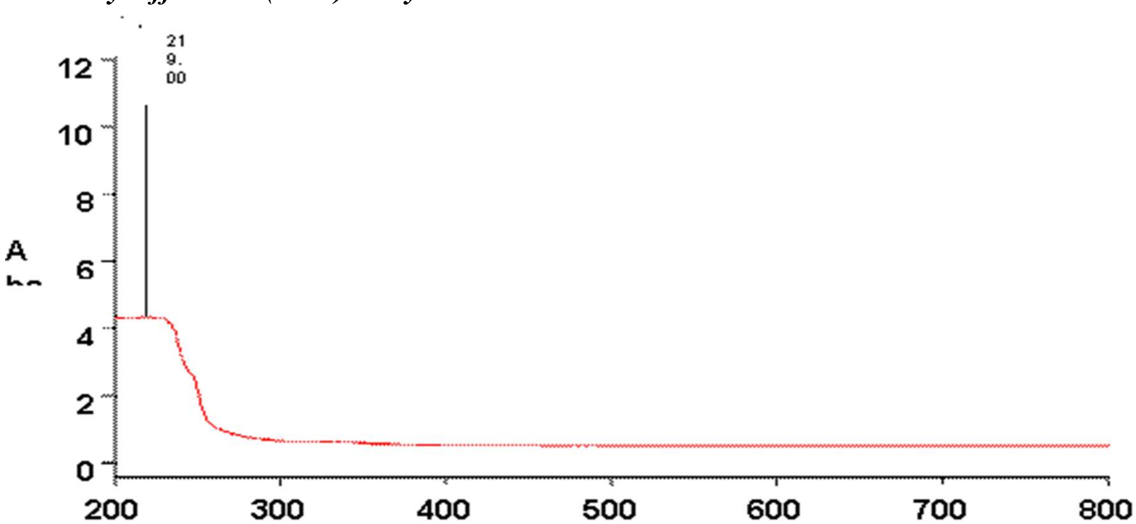
2.7 Colorimetric detection of Hg (II) ions using zinc nanoparticles (ZnNPs)

The procedure used by Sithara *et al.* (2017) was employed for the colorimetric detection of mercury (II) ions with sight modification. This was performed by the addition of 2.5 mL of Hg(II) (5–25 μM) to the colloidal solution of volume 3 mL. This was then placed on a magnetic stirrer at atmospheric pressure and room temperature for 10 min and the color transformation was noted followed by acclaiming the SPR peak. The ZnNPs-Hg^{2+} complex was centrifuged and analyzed with a colorimeter.

3. Results and discussion

3.1 UV- visible spectroscopy

Fig. 1. Shows the UV-vis spectrum of the synthesized zinc nano particle. The spectrum indicates the formation of a peak at 219 nm. *Rauwolfia vomitoria* leaf extract reduced the zinc compound to nano zinc materials at 219nm as shown in Fig. 1.



Wavelength (nm)

Fig 1: UV-Visible spectrum of zinc nanoparticles

3.2 Fourier transform infrared spectroscopy

The FTIR analysis was carried out on *Rauwolfia vomitoria* leaf extract before and after synthesis ofthe zinc nanoparticles in order to identify the capping and the functional groups involved in the stabilization of the metal nanoparticles by biomolecules present in *Rauwolfia vomitoria* leaf extract. Table 1 and Fig. 2 show the functional groups present in the leaf extract before synthesis while Table 2 and Fig. 3 show the functional groups present in the zinc nanoparticles. The absorption band at 3306 cm^{-1} indicates the presence of O-H stretching vibration of alcohol. The band at 2117 cm^{-1} indicates C≡C stretching vibration of unsymmetrical alkynes. 1636 cm^{-1}band indicates the presence of C=C stretching vibration of unsymmetrically conjugated alkenes while the peak at 1084 cm^{-1}which occurs only in the spectrum of the leaf extract corresponds to C-O stretching of ethers. Therefore, from every indication, it could be seen that C-O functional group from ether is responsible for the formation, capping, stabilization and reduction of zinc ions to zinc nanoparticle because such peak did not appear in the spectrum of zinc nanoparticles.

Table 1: Peaks and frequency of infra red absorption by extract of *Rauwolfia vomitoria* leaf

Frequency (cm^{-1})	Assignment
3306	O-H of alcohol
2117	C≡Cof alkyne
1636	**C=C of alkene**
1084	**C-O due to ether**

Table 2: Peaks and frequency of infra red absorption by synthesized zinc nanoparticle

Frequency (cm^{-1})	Assignment
3306	Alcohol OH
2117	Alkyne C≡C
1636	Alkene C=C

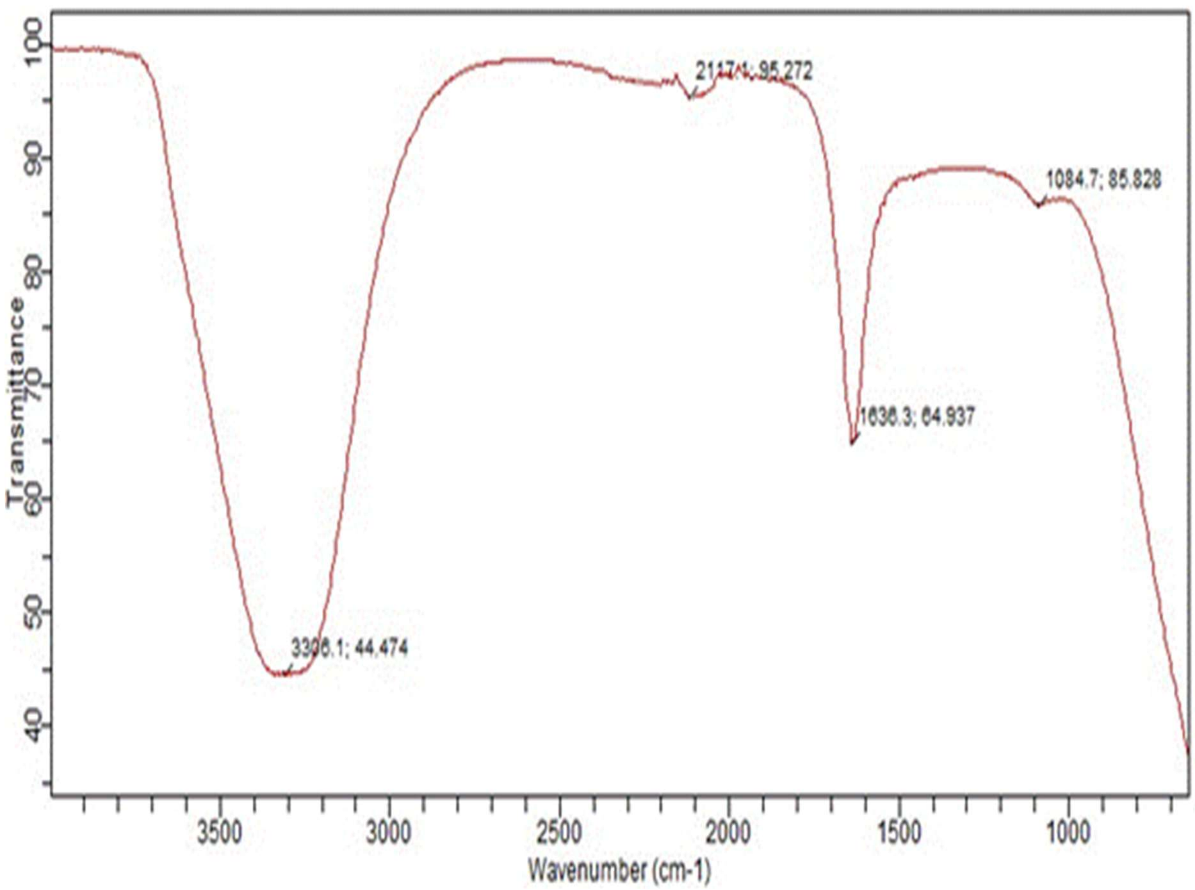


Fig. 2: FTIR Spectrum of *Rauwolfia vomitoria* leaf extract

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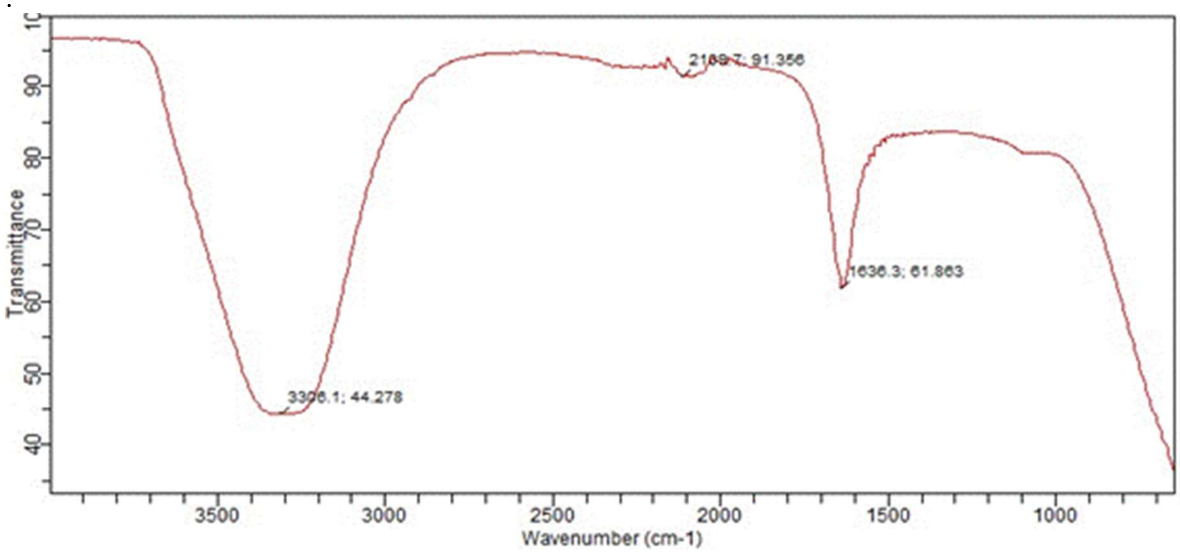

Fig. 3: FTIR Spectrum of the synthesized zinc nanoparticle

3.3 Scanning electron microscopy analysis (SEM)

The scanning electron micrographs of zinc nanoparticles obtained at different magnifications are shown in Figs. 4 to 6.

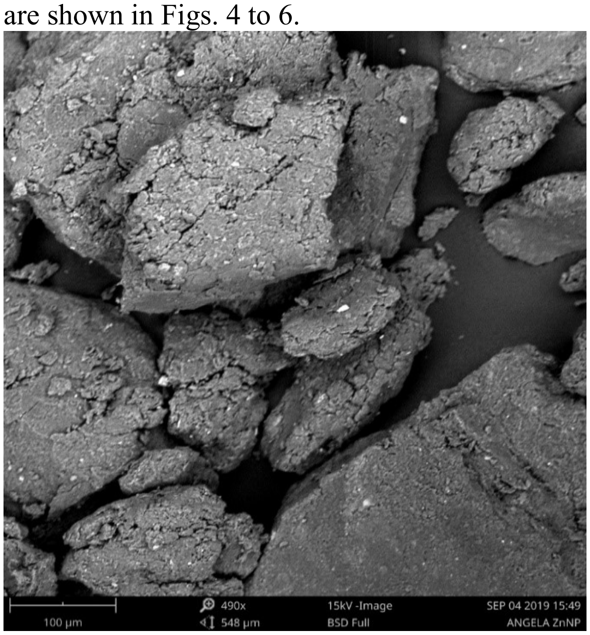

Fig. 4: SEM image of zinc nanoparticles.

The morphology of the nanoparticles indicates polygonal, spherical or faceted shape of various sizes that are agglomerated. Further magnifications revealed that these images possess rough surfaces. Surface morphology of the zinc nanoparticles is the most important one and the adsorption capacity mainly depends on its surface structure and surface porosity.

Fig 5: Zoomed SEM image of zinc nanoparticles.

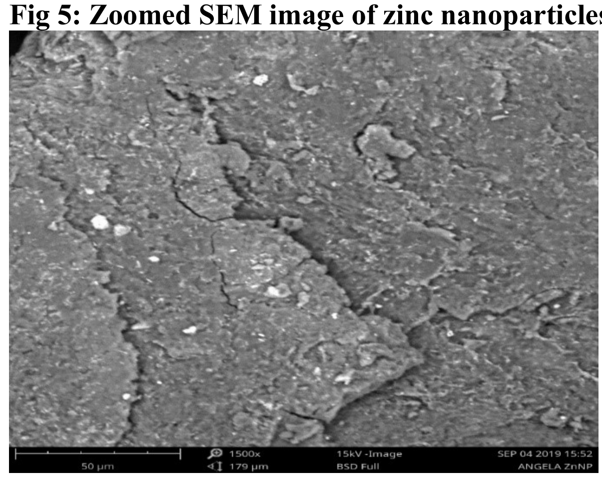

Fig. 6: Zoomed SEM image of zinc nanoparticles.

3.4 X-ray diffraction analysis (XRD)

Fig. 7 shows the XRD pattern of zinc nanoparticles biosynthesized from the leaf extract of *Rauwolfia vomitoria.*A number of Bragg reflections values 22.83, 32.42, 39.96, 46.53, 52.36, 57.84, 67.79, 72.53, 77.16 and 81.69 within the angle range of 10 and 89.98 were observed. The XRD pattern indicates that the zinc nanoparticles formed are crystalline in nature with a mixed irregular phase structure (polygonal and spherical). The average crystallite size of the zinc nanoparticles was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Debye-Scherrer equation (equation 1)

$$D = \frac{k\lambda}{\beta cos\theta} \quad (1)$$

where D is the particle size (in nm), k is a constant equal to 0.9, λ is the wavelength of X-ray radiation (0.541), β is the full-width at half maximum (FWHM) of the peak (in radians) and Θ is the Bragg angle (in degrees). The average crystallite size was found to be 57nm.

3.5 Colorimetric analysis

Different concentrations of Hg (II) ions with the corresponding red shift of the UV-Visible spectra are depicted in Fig. 8. The colloidal zinc nanoparticle was tested with various concentration (5-25μM) of Hg (II) ions. It was observed that absorption of Hg (II) ions were detected even at low concentrations though absorption increases as concentration is increasing (Fig. 8) indicating high absorption Hg (II) ions at high concentrations. This is in agreement with the work of (Okwunodulu *et al.,* 2019)
on the biological synthesis of cobalt nanoparticles from *Mangifera indica* leaf extract and its application in the detection of manganese (II) ions present in industrial wastewater.

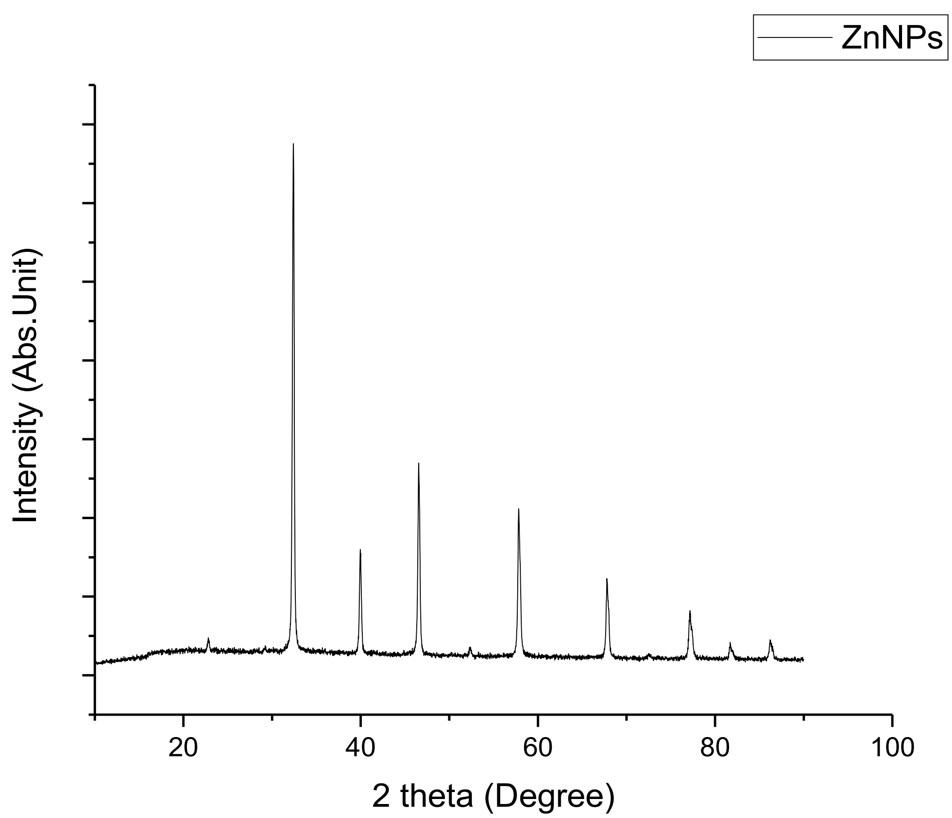

Fig. 7: X-ray diffraction pattern of zinc nanoparticles synthesized from Rauwolfia vomitoria leaf extract.

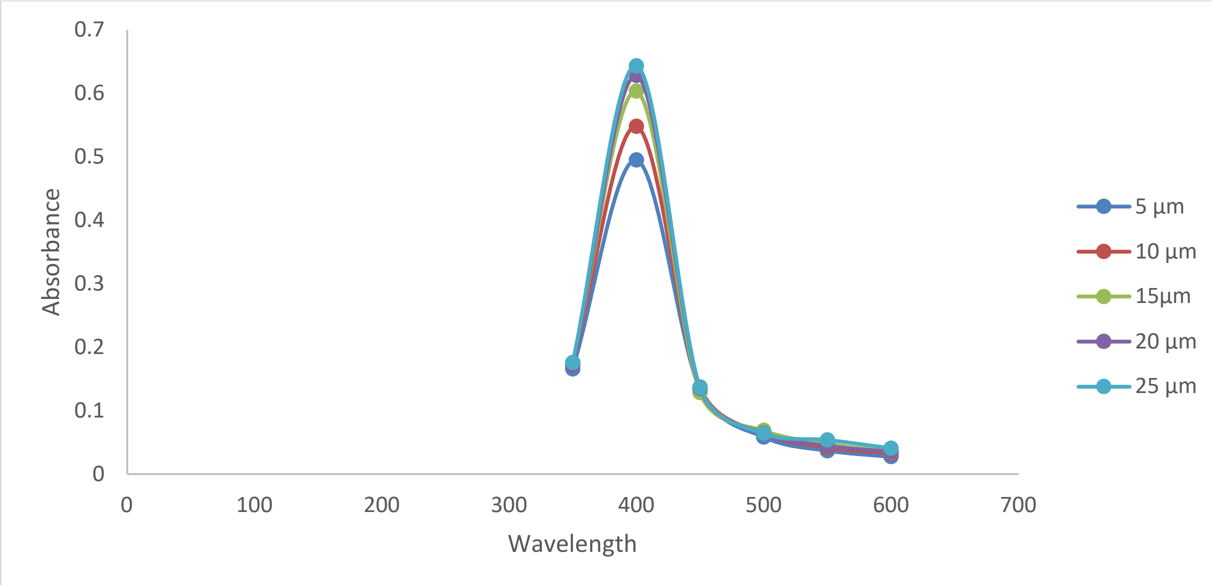

Fig.8: UV-Visible spectra of Hg (II) ions

4. Conclusion

Characterization of the synthesised zinc nanoparticles was carried out using UV, FTIR, SEM and XRD spectroscopic methods. Our results revealed maximum absorption at 219nm which pointed towards surface plasmon absorption for zinc nanoparticles. Similar functional groups were observed in both the leaf extract of *Rauwolfia vomitoria* and zinc nanoparticles synthesized with *Rauwolfia vomitoria* but it was observed that the C-O functional groupat 1084 peak which was native to the spectrum of leaf extract of *Rauwolfia vomitoria* did not appear in thespectrum of zinc nanoparticles indicating their usage in the formation of zinc nanoparticles. The morphology of the zinc nanoparticles indicated irregular, polygonal, spherical or faceted shape of various sizes with rough surfaces. The average crystalline size was found to be 57nm. Application of zinc nanoparticles in detecting Hg (II) ions in industrial wastewater was feasible because Hg (II) ions were detected even at low concentrations.

Zinc nanoparticles synthesized with*Rauwolfia vomitoria* should be used in detoxifying other toxic heavy metals in industrial effluents. Application of green chemistry in nanotechnology that are eco-friendly and cost effective should be recommended for further use. Treatment of industrial wastewater with zinc nanoparticles and other metal nanoparticles should be encouraged.

5.0 References

Pinon-Segundo, E., Mendoza-Munoz, N. &Quintanar-Guerrero, D. (2013). *Nanoparticles as Dental Drug-Delivery Systems.In :Nanobiomaterials in Clinical Dentistry.* Elsevier, Netherlands, pp. 475-495.

Edhaya, N., & S. Prakash, S. (2013). Biological synthesis of gold nanoparticles using marine algae *Gracilaria corticata* and its application as a potent antimicrobial and antioxidant agent. *Asian Journal of Pharmaceutical and Clinical Research*., 6, 2, pp. 179–182.

Madhuri, S.,Maheshwar, S., P. Sunil, P. & Oza, G. (2012). *Nanotechnology: concepts and applications.* 4, *CRC Press,* USA.

Kathiresan, K., Manivannan, S., Nabeel, M. A. &Dhivya, B. (2009). Studies on silver nanoparticles synthesized by a marine fungus, *Penicillium fellutanum* isolated from coastal mangrove sediment,*Colloids Surf B.* 71, 133–137.

Qi, W. H. & Wang, M. P. (2004). Size and shape dependent melting temperature of metallic nanoparticles. *Materials Chemistry and Physics,* ,88, 2-3, pp. 280–284.

Roduner, E. (2006). Size matters: why nanomaterials are different. *Chemical Society Review*, 35, pp. 583–592.

Malik, P., Shankar, R., Malik, V., Sharma, N. &Mukherjee, T. K. (2014). Green chemistry based benign routes for nanoparticle synthesis. *Journal of Nanoparticles*, pp. 1-14.

El-Shishtawy, R. M., Asiri, A. M. &Al-Otaibi, M. M. (2011). Synthesis and spectroscopic studies of stable aqueous dispersion of silver nanoparticles. *Spectrochimica Acta Part A Mol. Biomolecular Spectroscopy*, 79, 5, pp. 1505–1510.

Makarov, V.V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., Yaminsky, I. V. Taliansky, M. E.& Kalinina, O. (2014). Green nanotechnologies: synthesis of metal nanoparticles using plants. *Acta Naturae* ,6, 1, pp.35–44.

Feliu, N. & Fadeel, B. (2010). Nanotoxicology: no small matter. *Nanoscale*,2, 12, pp. 2514–2520. https://doi.org/10.1039/C0NR00535E.

Lee, J., Mahendra, S. & Alvarez, P. J. J. (2010). Nanomaterials in the construction industry: a review of their applications and environmental health and safety considerations. *ACS Nano*, 4, 7, pp. 3580–3590,

Roco, M. C. (2011). The long view of nanotechnology development: the national nanotechnology initiative at 10 years. *Journal of Nanoparticle Research*, 13, pp. 427–445. DOI: https://doi.org/10.1007/s11051-010-0192-z.

Kumar, V., Kumari, A., Guleria, P. & Yadav, S. K. (2012). Evaluating the toxicity of selected types of nanochemicals. *Reviews of Environmental Contamination and Toxicology*. 215, pp. 39–121.**DOI:** https://doi.org/10.1007/978-1-4614-1463-6_2.

Lefebvre, D.E., Venema, K., Gombau, L., Valerio, L. G., Raju, J., Bondy, G. S., Bouweester, H., Singh, R. P., Clippinger, A. J., Collnot, E. M., Mehta, R. & Stone, V. (2015). Utility of models of the gastrointestinal tract for assessment of the digestion and absorption of engineered nanomaterials released from food matrices. *Nanotoxicology*,9, 4, pp.523–542..

Ma, H., Williams, P. L. & Diamond, S. A. (2013). Ecotoxicity of manufactured ZnO nanoparticles—a review. *Environmental Pollution*, 172, pp. 76–85.

Fernández-Cruz, M. L., Lammel, T. & Connolly, M. (2013). Comparative cytotoxicity induced by bulk and nanoparticulatedZnO in the fish and human hepatoma cell lines PLHC-1 and Hep G2. *Nanotoxicology*, 7, 5, pp. 935–952.

Bian, S. W., Mudunkotuwa, A., Rupasinghe, T. &V.H. Grassian, V. H. (2011). Aggregation and dissolution of 4 nm ZnO nanoparticles in aqueous environments: Influence of pH, ionic strength, size, and adsorption of humic acid. *Langmuir,*27, 10, pp. 6059–6068, https://doi.org/10.1021/la200570n.

Mudunkotuwa, I. A., Rupasinghe, T., Wu, C. M. & Grassian, V. H. (2012). Dissolution of ZnO nanoparticles at circumneutral pH: a study of size effects in the presence and absence of citric acid. *Langmuir,* 28, 1, pp. 396–403.

Ma, R., Levard, C., Michel, F. M., Brown, G. E. & G.V. Lowry, G. V. (2013). Sulfidation mechanism for zinc oxide nanoparticles and the effect of sulfidation on their solubility. *Environmental Science and Technology*, 47, 6, pp. 2527–2534. DOI: 10.1021/es3035347.

Lv, J., Zhang, S., Luo, L., Peter, C., Shizhen,Z., Wei, H. & Ke, Y. (2012). Dissolution and microstructural transformation of ZnO nanoparticles under the influence of phosphate. *Environmental Science and Technology,*46, 13, pp. 7215–7221.

James, D. B., Owolabi, A. O., Ibiyeye, H., Magaji, J. &Ikugiyi Y. A. (2008). Assessment of the hepatic effects, haematological effect and some phytochemical constituents of *Ximenia americana* (leaves, stem and root) extracts. *African Journal of Biotechnology*, 7, pp.4274–4278

Akpanabiatu, M. I., Umoh, I. B., Eyong, E. U., Edet, E. E. & Uboh, F. E. (2006). Influence of *Rauwolfia vomitoria* root bark on cardiac enzymes of normal wistar albino rats. *Recent Progress in Medicinal Plant*, 14, pp.273–8.

Verma, K. C. &Verma, S. K. (2010). Alkaloids analysis in root and leaf fractions of sarpaghanda (*Rauwolfia vomitoria*). *Agricultural Science Digest,* 30, pp. 133–135.

Sithara, R., Selvakumar, P., Arun, C., Anandan, S. &Sivashanmugam, P. (2017). Economical synthesis of silver nanoparticles using leaf extract of *Acalyphahispida*and its application in the detection of Mn(II) ion. *Journal of Advanced Research,* 8, pp. 561–568.

Okwunodulu, F. U., Chukwuemeka-Okorie, H. O. & Okorie, F.C. (2019). Biological synthesis of cobalt nanoparticles from *Mangifera indica* leaf extract and application by detection of manganese (II) ions present in industrial wastewater. *Chemical Science International Journal, 27,* pp. 1-8.

Odiongenyi, A. O. & Afangide, U. N. (2020). Adsorption and Thermodynamic Studies on the Removal of Congo Red Dye from Aqueous Solution by Alumina and Nano-alumina. *Communication in Physical Sciences*, 4,1, pp.1-7.

Conflict of interest

Authors declared no conflict of interest.