ECO-FRIENDLY SYNTHESIS OF IRON NANOPARTICLES USING *Kola Acuminata:* CHARACTERISATION AND BIOLOGICAL ACTIVITY

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ABSTRACT

Iron nanoparticles were synthesized by a rapid, cost-effective and environmentally friendly technique using ground leaf extract of *Kola acuminata* as reducing as well as capping agents. Iron nanoparticles (FeNPs) were formed within 20 minutes by the reaction of an aqueous solution of (0.01M) ferric chloride ($FeCl_3$) with leaf extract of *Kola acuminata*. The synthesized nanoparticles were characterized using Uv-visible spectrophotometer which showed absorbance in the region of 280nm due to surface Plasmon resonance, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Transmission electron microscopy (TEM), Scanning electron microscopy (SEM) and Energy Dispersive Spectroscopy (EDS). The SEM and TEM analyses showed that the FeNPs were irregular in shape with narrow size distribution with an average particle size of 40.4nm. The EDS analysis revealed the percentage of iron (19.4%) as well as other elements contained in the sample. The Iron nanoparticles exhibited antimicrobial activity against clinical isolates of *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, and Candida albican.* The leaf extract showed inhibition effect against all the test organisms. The minimum inhibitory concentration (MIC) for all the isolates tested was 0.5mg/mL. The antioxidant activity of both the extract and synthesized FeNPs was analyzed using 1,1- diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant power (FRAP) using vitamin C as control and *kola acuminata* as well as its nanoparticles showed good antioxidant activity with percentage inhibition of 54.05% and 58.90%, respectively at a concentration of 250µg/mL. The mosquito larvicidal bioassay revealed that the FeNPs showed potent larvicidal activity against second instar larvae of *Culex quinquefasciatus* and *Anopheles gambae* especially at high concentrations. These results suggest that the synthesized iron nanoparticles have the potential to be used as an eco-friendly compound for the control of mosquitoes and harmful microorganisms.

INTRODUCTION

Nanoparticles are of great interest due to their extremely small size and large surface to volume ratio which lead to both physical and chemical differences in their properties compared to bulk material of the same chemical composition. Nano materials can be synthesized by different methods including chemical, physical, and biological methods. However, synthesis of nano materials by chemical or physical methods has resulted in environmental contamination, since the chemical procedures involved in the synthesis of nano materials generate large amount of hazardous byproducts (Amato *et al.*, 2011).Thus, there is a need to explore a more sustainable process for the synthesis of nano materials and "green nanotechnology" is a better option, (Pallavicini *et al.*, 2010). The biological methods include synthesis of nano materials from the extracts of plant, bacteria and fungi (Dallas *et al.*, 2011).

Many recent studies have indicated the potential of iron nanoparticles for environmental remediation. The interest in nano scale zero-valent iron (nZVI) in environmental remediation is

increasing due to the reactivity of nano scale iron having a large surface to volume ratio (Lin *et al.*2008; Gui *et al.,* 2012). However, not much has been reported on the potential of iron nanoparticles for antimicrobial and mosquito larvicidal activities. In this paper, green synthesis of iron nanoparticles and their potential as antimicrobial and larvicidal agents are reported.

MATERIALS AND METHODS

The sample preparation and synthesis of iron nanoparticles were carried out according to the method described by Pattanayak and Nayak (2013). Aqueous extract of *Kola acuminata* (5mL) was added to freshly prepared 5mL of 0.01M aqueous solution of $FeCl_3$ and mixed thoroughly by manual shaking, iron nanoparticles were formed within 10 - 15 minutes at room temperature. The bio-reduction of the metal ion (Fe^{3+}) was monitored periodically using UV-Vis spectrophotometer (190- 700nm). The formation of metal nanoparticles was indicated by change in colour of the solution. The metal nanoparticles so formed were purified by centrifugation followed by dispersion of the pellets in demonized water to remove water-soluble biomolecules. The pellets were dried in a desccicator and stored for further analysis. The synthesized iron nanoparticles were characterized using transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffractometer, FT-IR spectroscopy and energy dispersive spectroscopy (EDS).

Figure1. *Kola acuminata (Source-* **https://commons.wikimedia.org/wiki/File)**

Biological Activity of the Synthesized Iron Nanoparticles

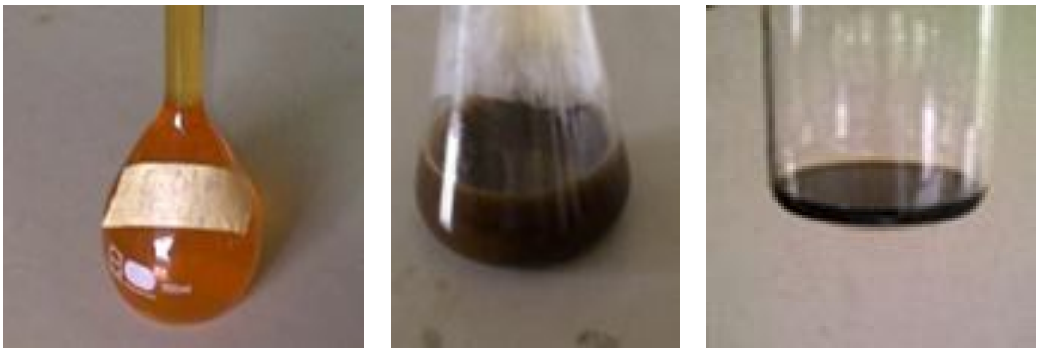
The antimicrobial activity of the synthesized iron nanoparticles and the plant extract were evaluated utilising agar well diffusion assay. Five microorganisms were assayed; the organisms were *Escherichia coli, Pseudomonas aeruginosa, Bacillus substili, Staphylococcus aureus* and *Candida albicans.*

Antioxidant activity of the synthesized nanoparticles was evaluated by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free – radical scavenging method.. Ascorbic acid was used as a reference standard**.** Larvicidal potential of the extract and the synthesized nanoparticles was evaluated using second instar larvae of *Anopheles gambiae* and *Culex quinquefasciatus.*

RESULTS AND DISCUSSION

Characterization

The preliminary detection of iron nanoparticles formation was carried out by visual observation of the change in colour of the solution from faint yellow to dark brown and then to black (Fig. 2). These changes are attributed to the excitation of surface plasmon resonance (SPR) in the metal nanoparticles. This was monitored using UV-Vis spectrophotometer. The absorption spectra of the synthesized iron nanoparticles showed a surface Plasmon resonance (SPR) peak at 280nm (Fig. 3) which is characteristic of iron nanoparticles.



a b c

Figure 2: Vials containing samples of **(a)** ferric chloride, **(b)** *Kola acuminata* extract, **(c)** iron nanoparticles

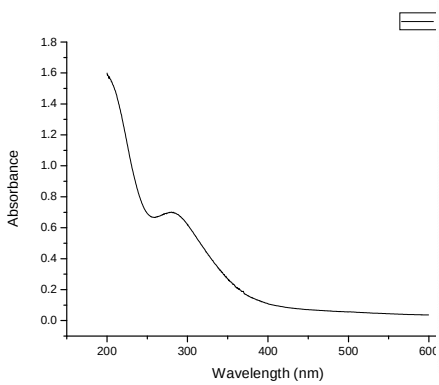


Figure 3: UV-Vis spectrum for the prepared FeNPs

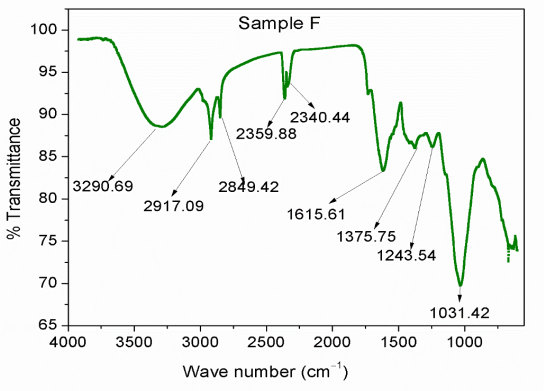


Figure 4: FTIR spectrum of the FeNPs

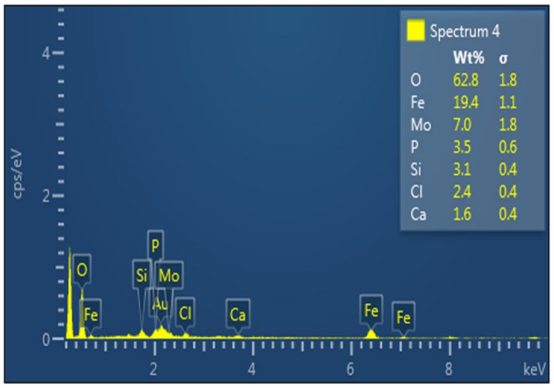


Figure 5. EDS spectrum of the iron nanoparticles

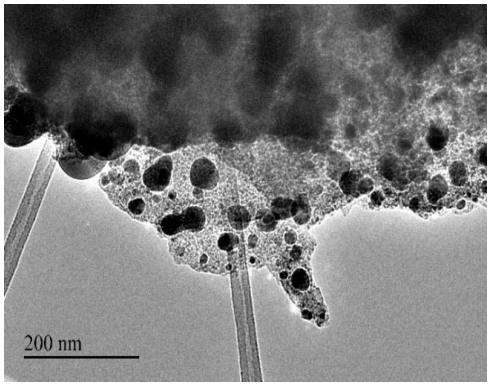
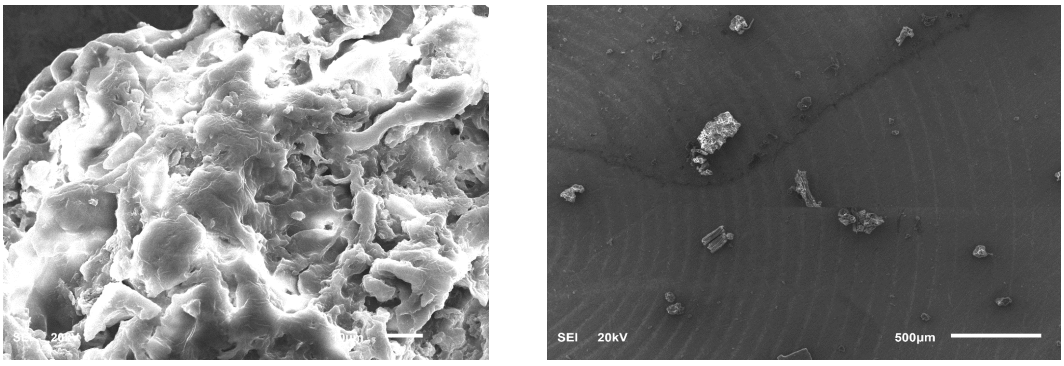


Figure 6: TEM images of the FeNPs

FTIR spectrum of the synthesized iron nanoparticles exhibited prominent peaks at 3290.69, 2917.09, 1615.61, 1375.5, 1031.4cm^{-1} indicating the presence of phenolic OH – stretch, C-H stretch of alkanes, C=C stretch of alkenes, CH_3 deformation and C-N stretching of aliphatic amine.



7a 7b

Figure 7. SEM images of the synthesized iron nanoparticles: a, 10µm; b, 500µm

Energy dispersive analysis spectroscopy (EDS) revealed the presence of elements like oxygen, iron, phosphorous, silicon, chlorine, and calcium for the synthesized FeNPs (Fig.5). The presence of iron (19.4%) indicates the formation of iron nanoparticles. The signal for oxygen is mainly due to the different phytochemicals present in the plant extract. The TEM result revealed an average size of 40.4nm for the synthesized FeNPs (Fig. 6). From the SEM result (Fig. 7a and 7b) it is observed that the synthesized iron nanoparticles are irregular in shape with rough surfaces and are not in direct contact, indicating stabilization of the synthesized nanoparticles.

Table 1: Zones of inhibition of *Kola acuminata* extract and FeNPs

Microorganism	Concentration (mg/mL) / Zones of inhibition (mm)							
	Kola acuminata extract				Iron nanoparticles (FeNPs)			
Escherichia coli	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0
Pseudomonas	6	7	8	9	9	10	15	18
aeruginosa	7	9	10	11	10	10	17	15
Bacillus subtilis	6	8	9	10	11	10	14	14
Staphylococcus aureus	6	9	12	13	12	18	18	17
Candida albican	11	10	12	12	15	16	19	20

Table 2: Results of larvicidal study on *A. gambae and C. quinquefasciatus*

Time	Concentration µg/mL	*Anopheles gambae*		*Culex quinquefasciatus*	
		Extract	FeNPs	Extract	FeNPs
12hrs	0.2	10	10	10	10
	0.4	10	10	10	10
	1.0	10	10	10	10
	1.5	10	10	10	10
	Control	10	10	10	10
24hrs	0.2	7	0	10	0
	0.4	7	0	10	0
	1.0	6	0	8	0
	1.5	5	0	5	0
	Control	10	0	10	10

Biological Activity

The results of antimicrobial activity (Table 1) revealed that both the extract and the synthesized iron nanoparticles showed an inhibitory effect against the tested microorganisms with the nanoparticles having higher inhibition zones. This indicates that the plant extract contains metabolites which are capable of inhibiting the growth of microorganisms (Okwu and Iroabuchi, 2009) and is further enhanced by the presence of the iron metal. The minimum inhibitory concentration (MIC) for all the isolates was 0.5mg/mL. The FeNPs also, showed reasonable radical scavenging activity with percentage inhibition of 58.90 at a concentration of 250µg/mL.

The results of the larvicidal study (Table 2) revealed that FeNPs exhibits good larvicidal effect against the second instar larvae of both *Culex quinquefasciatus* and *Anopheles gambae.* Susceptibility of the larvae to FeNPs increased with an increase in exposure time and concentration. The control, which had no extract or FeNPs had no effect on the larvae.

CONCLUSION

Iron nanoparticles have been synthesized through a green, ecofriendly and cost effective route using *Kola acuminata* leaf extract. The biomolecules in the extract acted both as reducing and capping agents. The FeNPs were irregular in shape with narrow size distribution and showed good antimicrobial, mosquito larvicidal and antioxidant activity.

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