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Full Length Research Article

GREEN SYNTHESIS OF COPPER AND IRON NANOPARTICLES FROM EXTRACTS OF EUCALYPTUS WITH THEIR ANTIMICROBIAL ACTIVITIES

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ABSTRACT
Eco-friendly iron and copper nanoparticles were synthesized using masquerade and eucalyptus leaves extract as a reducing and stabilizing agents. Iron chloride heptahydrate ($FeCl_2.7H_2O$) and Copper sulphatepenta hydrate ($CuSO_4.5H_2O$) as metal precursors. It was characterized using UV visible, FTIR, XRD and SEM analysis. The UV result shows the highest peaks at 500 and 600 nm for iron and copper respectively. This is due to the surface plasma vibration of the phytochemical constituents present in the extract. FTIR shows presence of alkanoids and triterpenes, SEM shows the spherical granular with no well-defined morphology and mono dispersed structures for iron and copper nanoparticles respectively. It shows good antibacterial activity when tested against *Escherichia-coli* and *Pseudomonas auredinosa* (gram-negative), *Staphylococcus aureus* and *Klebsella pneumonia* (gram-positive).

Keywords: Metal nano-particles, Green synthesis, characterization, morphology, antibacterial activity.

INTRODUCTION

The Green Chemistry which aimed to reduce or eliminate substances that are hazardous to human health and environment in the design, development and implementation of chemical and physical process and product is recently becoming more interesting and important. (Tahir *et al* 2016)
The use of nanoparticles is gaining importance in the present century as they possessed definite chemical, optical and mechanical properties. Metal nanoparticles have potential applications in many field of science and engineering. Therefore, nanotechnology has the ability to fight and prevent diseases using atomic scale tailoring of materials (Umesh *et al*., 2011). The term Nano is Greek word which means “dwarf” or “miniature”. It can be explained as the fabrication, characterization, exploration and application of Nano sized materials (1-100 nm) for the development of science. It deals with the study of extremely minute structure (Preeti, 2017).
Nanotechnology was for the first time used by physics Richard Feynman in 1959 that was considered as the origin of modern nanotechnology. It is the field of science that deals with the study of matter at extremely small scale level which is 1-100 nm (Tahir *et al*., 2016). It provides ability to the engineering properties such as surface engineering, Tribology of materials by controlling their size and it has been spread to number of areas including biomedical services, cosmetics, drugs, gene delivery, environmental health care, food, catalysis, mechanics, nonlinear optical devices, optic, photo-elect chemical application, single electron transistors, and space industries (Preeti, 2017).
The development of the technique for the control of synthesis of nanoparticles of well-defined size, shape and composition has become a big challenge that requires a reliable eco-friendly process for synthesis of metallic nanoparticles. The use of biological method for the green synthesis of metallic nanoparticles is an important step and one of the ways to achieve this objective (Vijayaram *et al*., 2023).
There are three different methods of synthesising nanoparticles, the physical, chemical and biological (green synthesis) methods. Both the physical and chemical methods have harmful effect to both human and environment but green method has little or no harm to human and environment (Vijayaram *et al*., 2023). Plant extracts have been tested and used successfully as natural reducing agent which reduces the metal iron into their corresponding metal atom during the green synthesis of nanoparticles. Other natural reducing agent includes yeast, fungus and Algae. (Tahir *et al.,* 2016).

MATERIALS AND METHODS

Sample collection
Fresh matured leaves of eucalyptus were randomly collected by hand plucking within the Bauchi State University, Gadau Campus vicinity and was identified and authenticated by the University Herbarium Section in the Department of Biological Science.

Sample preparation
The fresh eucalyptus leaves were washed with tap water to remove adhered dirt and then rinsed with distilled water, it was then allowed to dry for 2 days (48 hours) under shade, and it was grounded using a wooden mortar and pestle and was sieve size to obtain a fine homogeneous solid sample.

Extract preparation
By using slightly modified method of Igwe and Ekebo (2018). A 30g of each leaf sample was weighed and poured into 200 ml of distilled water in a 500 ml glass beaker and boiled at 60 ^{0}C for 30 minutes. It was cooled, then filtered using vacuum pump and the filtrate was used immediately for the synthesis of nanoparticles.

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Preparation of $FeCl_2.7H_2O$ solution
A 0.05M solution of $FeCl_2.7H_2O$ was prepared by dissolving 13.9 g of $FeCl_2.7H_2O$ salt into 1000ml volumetric flask and distilled water was added up to the mark.

Preparation of $CuSO_4$ $5H_2O$ Solution
A 0.03M solution of $CuSO_4.5H_2O$ was prepared by dissolving 7.49 g of $CuSO_4.5H_2O$ in 1000 ml volumetric flask and the distilled water was added up to the mark.

Synthesis of iron nanoparticles (FeNPS). The method of Igwe and Ekebo (2018) was adapted for the synthesis. The prepared $FeCl_2.7H_2O$ solution was added drop-wise into plant extract (eucalyptus) in a ratio of 1:9 (that is 10 ml extract and 90 ml metal precursor) with constant stirring at 60 ˚C for 60 minutes with magnetic stirrer. Within the 15 minutes the colour changed from reddish brown to black, which indicates the formation of nanoparticles. It was then processed and allowed to settle for 24 hours (one day) after which was decanted and dried up using heating mantle at 100^0C for 2 hours. The collected nanoparticles were stored for further analysis.

Synthesis of Copper Nanoparticles (CuNPS)
The modified method of Dinker *et al*. (2017) was adopted with slight modifications. The prepared CuSO4.5H2O solution was added drop-wise into the plant extract (Eucalyptus) in a ratio of 2:8 (that is 20 ml plant extract and 80 ml metal precursor) with constant stirring at 80 ˚C for 60 minutes using magnetic stirrer. Within the first 10 minutes colour changed was observed from reddish brown to green which shows the formation of nanoparticles. It was then decanted and dried using heating mantle at 100 ^0C for 2 hours. The collected nanoparticles were stored for further analysis.

Antimicrobial Activity Test
Media preparation
The media preparation depends on the manufacturer's specification. A 28 g of nutrient agar was dissolved into 100 ml deionised water and autoclaved at 121 ˚C for 15 minutes to sterile.

Antimicrobial activity tests
The test was carried out using disc diffuse method with the use of punch filter papers, 10 ml of dimethyl Sulphate(IV) oxide (DMSO) was mixed with the samples and the punch of filter papers was mixed and the prepared media of nutrient agar which was already on the plate. The mixed solution of the DMSO and sample was placed on the plate and incubated for two days in the oven.

RESULTS AND DISCUSSION
The result obtained during the green synthesis of iron and copper nanoparticles, the colour change was observed as the first indication of the formation of nanoparticles. The iron nanoparticles changes from reddish brown to dark green while the copper nanoparticle changes from reddish brown to black colour.

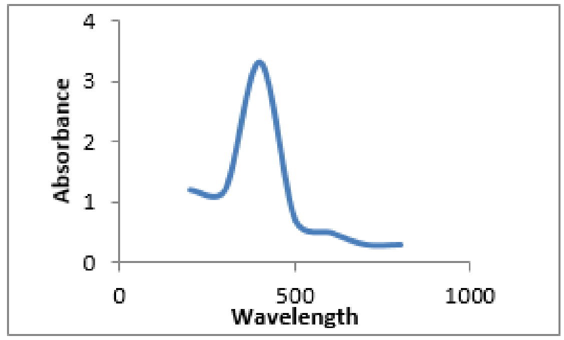

Figure 1: UV-Visible spectrum FeNPs (Eucalyptus)

The iron nanoparticles showed the highest absorbance at peak 400 mm which is due to the surface plasma vibration and excitation of the bio-reduction and capping agent present in the leaf extract. This correspond to what obtained by Suriya *et al*. (2018) which showed the highest absorbance peak at 350, 315 and 400 mm. Various report have been established that the absorption peaks of iron nanoparticles (FeNPs) should be around 280-420 mm.

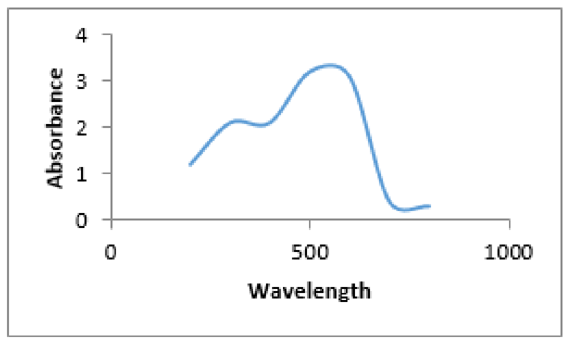

Fig. 2: UV-Visible Spectrum CuNPs (Eucalyptus)

The copper nanoparticles (CuNPS) showed the highest absorbance at a peak of 500mm in figure above which is due to the surface measure vibration and excitation of bio reduction and capping or stabilizing agent present in the leaf extract this corresponds with the literature of kiranmai et al (2017) which showed the highest absorbance peaks at 300mmand 650mm and suggest that this variation depends on the reducing agent and the type of metal salt used as a precursor.

Antimicrobial activity test results
The antibacterial activity test for copper nanoparticles (CuNPS) showed potent antibacterial activity against two bacterial strains: Gram Positive *Staphylococcus aureus* and *Klebsella preuminia* and Gram negative *Escherichia-coli* and *seudomonas aureus*. Aqueous extract of Eucalyptus containing CuNPS showed activity in all copper concentration tested against all bacterial.

The followings are the result obtained from antimicrobial activity test of green synthesized iron and copper nanoparticles (FeNPS and CuNPS) on the selected bacteria.

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Table 1: Iron nanoparticles eucalyptus antimicrobial activity

S/N	Organism	Amoxicillin	100 mg/l	200 mg/l	400 mg/l	800 mg/l
1.	Staphylococcus Aureus	19.5	7	12	14	17
2.	KlebsellaPreuminia	20	7	13	12	19
3.	Pseudomonas Aureginose	20	8	12.5	14	18
4.	Escherichia-coli	18	9	14	16	18

From the Table 1 above, all the bacteria shows an increase in bacterial inhibition growth with an increase in concentration of synthesized iron nanoparticles and they shows highest inhibition activity at 800mg. And the result is in agreement with that of Zaccheus et al, (2018).

Table 2: Copper nanoparticles eucalyptus antimicrobial activity

S/N	Bacteria	Amoxicillin 500mg/l	100 mg/l	200 mg/l	400 mg/l	800 mg/l
1.	Staphylococcus Aureus	20	8mm	11	12.7	14.2
2.	KlebsellaPreuminia	18	8mm	10	13	16.4
3.	Pseudomonas Aureginose	23	10mm	11	12.8	18.2
4.	Escherichia-coli	26	11mm	12	14	20

From the Table 2 above, it revealed that all the bacterial shows an increase bacterial growth inhibition zone with increase in concentration of the nanoparticles synthesized. And the result is similar with that Zaccheus et al, (2018).

Table 3: Copper NPS Eucalyptus FTIR

TRANSMITTANCE	PEAKS	FUNCTIONAL GROUP
89.169	3131.0	O – H Stretching
60.753	1062.3	C= O Stretching
74.817	797.7	C = C Bending
78.952	857.3	C = C Bending

The FTIR spectra of Cu-NPS (eucalyptus) displayed in Table 2 shows several peaks at 3131.0 cm^{-1}, 1062.3 cm^{-1}, 797.7 cm^{-1} and 857.3 cm^{-1}. The peak at 3131.0cm^{-1} represent O – H stretching due to the flavonoid. The absorption bond at 1062.3cm^{-1}, and 857.3cm^{-1} represent C = N and C = C stretching due to aromatic ring in the secondary metabolites present and the peak at 797.7 cm^{-1} due to C–OH corner bond symmetry stretching in the aromatic ring. The results obtained are in agreement with the literature reported by Preeti (2017) which shows that the FTIR bonds obtained indicate the presence of flavonoids, polyphenols, Triterpenes and amide present in the P. nururi leaves extract.

A shift in the absorption bands in Fourier-transform infrared spectroscopy (FT-IR) confirmed the bioactive molecules of leaves extracts have acted as expected. This is evidently noticed by comparative studies of Figures 2-4 shown below. A similar finding was earlier reported by Lalsangpuii *et al*. (2022).

Table 4: Iron NPS Eucalyptus FTIR

TRANSMITTANCE	PEAKS	FUNCTIONAL GROUP
80.315	3104.9	O – H Stretching
86.550	1617.7	C = N Stretching
75.791	864.7	C – H Bending
55.743	1066.0	S = O Stretching

The FTIR spectra of FENPS (Eucalyptus) displayed in table, it shows several peaks at 3104.9cm^{-1}, 1617.7cm^{-1}, 864.7cm^{-1} and 1066.0cm^{-1}. the peak at 3104.9cm^{-1} represent O – H stretching due to water present in the leaf extract, the absorption peak at 1617.7cm^{-1} shows C = N stretching due to the triterpenes and the other peak at 864.7cm^{-1} C – H bending due to aromatic ring in the secondary metabolites. The result obtained are in agreement with the literature reported by flora et al., (2018) which show that, the FTIR bonds obtained are characteristics of flavonoids, Triterpenes, furanoid, Sugar coumarins, tannis, Phenols and acid present in the aqueous extract of propolis.

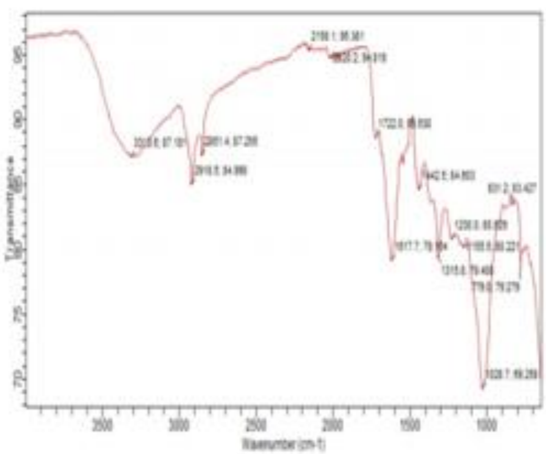

Figure 3: Eucalyptus Leaves Extract

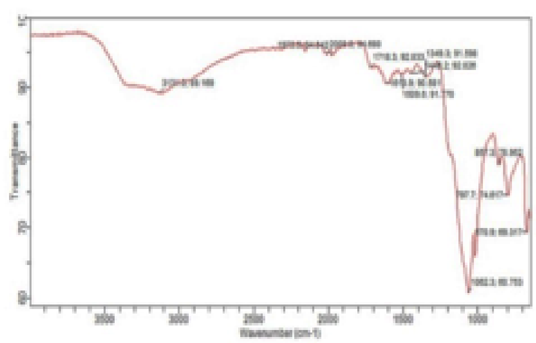

Fig. 4: Copper Nanoparticles Eucalyptus (CuNPS)

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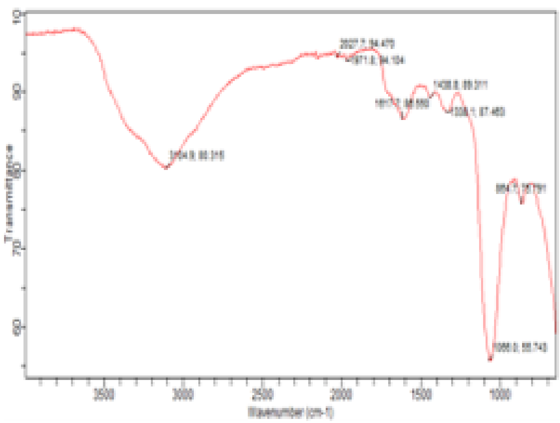

Figure 5: Iron Nanoparticle Eucalyptus (FeNPS) E

Scanning Electron Microscopy (SEM)
The figure showed the results obtained from the SEM analysis of the green synthesized iron and copper nanoparticles which described their respective morphology.

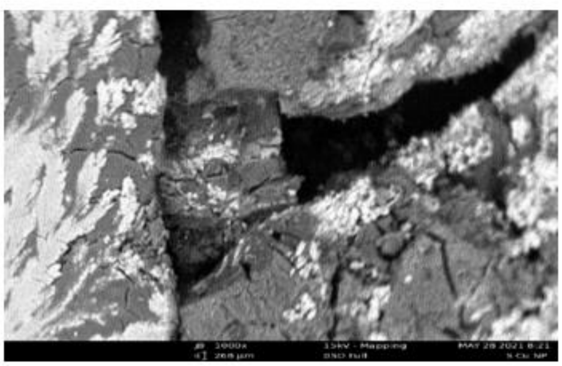

Figure 6: Iron nanoparticles Eucalyptus (Fe NPs)

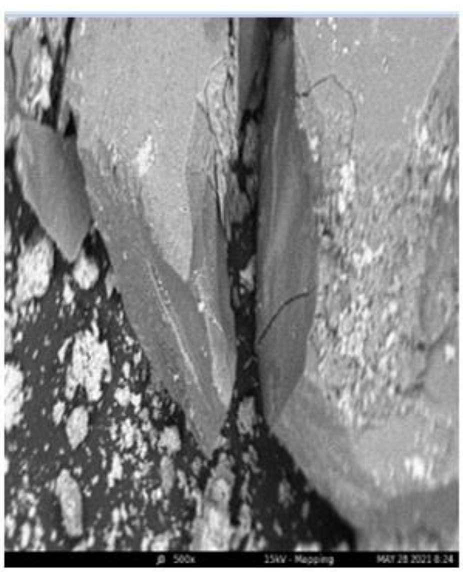

Fig. 7: Copper Nanoparticles Eucalyptus (CuNPs)

The SEM result obtained for iron nanoparticles coupled with EDX shows that iron nanoparticles have spherical granular structure which is aggregate into a larger particles with no well-defined morphology. This aggregate depends on the existence of the phyto- chemical constituents present in the leave extract and the metal precursor in question. It also shows the percentage of iron and chloride present in nanoparticles while other trace elements are due to the biological materials presents in the leaf extract. The result obtained is in agreement with literature reported by (Preeti, 2017) which show spherical and un-differential shapes for silver nanoparticles synthesized using P. nururi leaf extracts.

The SEM coupled with EDX results for copper nanoparticles shows that copper nanoparticles have mono dispersed morphology with the percentage of copper and sulphate ion present while other traces elements are due to bio reduction materials present in the leaf extract. The result obtained is in agreement with the literature reported by (kiranmai et al., 2017) which shows mono dispersed morphology for the green synthesized nanoparticles and its synergistic with antibiotics.

Conclusion
It can be concluded that copper nanoparticles were green synthesized from the leave extract of eucalyptus and characterized using UV visible spectrophotometry, FTIR, SEM coupled with EDX, XRD analysis. It shows good antibacterial when tested against gram negative and positive bacterial. Escherichia-coli and pseudomonas aureus, staphylococcus aureus and klebsella Pneumonia Escherichia-coli.

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