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DEVELOPMENT OF COPPER AND NICKEL NANOPARTICLES FROM THE BARK EXTRACT OF *BRIDELIAFERRUGINEA* PLANT FOR USE AS ANTIMICROBIAL AGENT

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ABSTRACT

The study is centered on deriving, Copper and Nickel nanoparticles via reduction using *Brideliaferruginea* bark extract. The nanoparticles were characterized using Ultra-Violet and Fourier Transformation Infrared spectroscopic techniques and were tested for antimicrobial activitiesusing eight bacteria (*E. coli, Pseudomonas aeruginosa, Shigella sp., Staphylococcus aureus, Streptococcus pnemoniae, Salmonella typhi, Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC 35218)) and seven fungi (*Aspergilusflavus, Candindaalbicans, Blastomycessp, Fusariumsp, Tricophytonsp, Aspergilusniger, Microsporium sp*.) The results of the study show that Brideliaferruginea bark extract acts as a good reducing agent for the synthesis of nanoparticles.

KEYWORDS: Nano particles, *Brideliafurruginea*, bark extract, antimicrobial etc.

INTRODUCTION

Recent trending scientific research reveals that nanoparticle has multifunctional properties and very interesting applications in various fields such as medicine, nutrition and energy [1]. The biogenic syntheses of mono-dispersed nanoparticles with specific sizes and shapes have been a challenge in biomaterial science. Also, it has created remarkable advantages in the pharmacological industry to cure various bacterial and viral diseases [2].Plant crude extract contain novel secondary metabolites such as phenolic acid, flavonoids, alkaloids and terpenoids in which these compounds are mainly responsible for the reduction ofionic species into bulk metallic nanoparticles formation [3]. These primary and secondary metabolites are constantly involved in the redox reaction to synthesizeeco-friendly nanosized particles.

Several methods are used for synthesis of nanoparticles (NPs) such as physical, chemical, enzymatic and biological. Physical methods includes plasma arcing, ball milling, thermalevaporation, spray pyrolysis, ultra-thin films, pulsed laser desorption, lithographic techniques, sputter deposition, layer by layer growth, molecular beam epistaxis and diffusion flame synthesis of nanoparticles [4].

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Plant extract mediated synthesis is an increasing focus of attention (Ali *et al.,* 2011; Processes for making nanoparticles using plant extracts are readily scalable and may be less expensive [5] compared with the relatively expensive methods based on microbial processes [6] and whole plants [7]; Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles. [8]. The source of the plant extract is known to influence the characteristics of the nanoparticles [8]. This is because different extracts contain different concentrations and combinations of organic reducing agent [9].

Nanoparticles are already used in numerous applications [10] including in-vitro diagnostics, agriculture, pharmacology, crop biotechnology, and in anti-diabetics, anti-fungicidal, anti-plasmodia, anti-inflammatory, anti-cancer, anti-viral, anti-oxidant activities but their use in medicine is mostly on an experimental basis. This research is concerned with the synthesis of metallic nanoparticles using plant extracts. In view of itssimplicity, the use of live plants or whole plant extract and plant tissue for reducing metal salts to nanoparticles has attracted considerable attention within the last 30-years. [11]. Compared with the use of whole plant extracts and plant tissue, the use of plant extractsfor making nanoparticles is simpler.

MATERIALS AND METHODS

MATERIALS

- **APPARATUS AND EQUIPMENT**

The apparatus used for this study were 125 micron mesh, weighing balance, analytical weighing balance, 100ml measuring cylinder, 250ml conical flask, Bunsen burner, Dessicator, 1000ml standard flask. The equipment used include: Centrifuge, Water bath, Soxhlet apparatus, Orbital shaker.

- **CHEMICAL MATERIALS**

Distilled water, absolute methanol, anhydrous CuSO4 and NiSO4.6H2O.

METHODS

- **PREPARATION OF POWDERED PLANT BARK**

The fresh *Brideliaferruginea* plant bark was collected and sundried for five days, the bark was separated from other solid impurities associated with it before being grinded to powder. The powdered substance was then sieved with a 125 micron sized sieve to get a finer powder.

- **BARK EXTRACT PREPARATION**

In other to extract chemical constituent from the bark of the plant, two extraction techniques were used. These techniques are Organic bark extract preparation and Aqueous bark extraction technique.

- **ORGANIC BARK EXTRACTION TECHNIQUE**

Powdered plant bark was weighed (20g) and 200ml of methanol was measured, all packed in the Soxhletapparatus and Soxhlet extraction was carried out. The extraction process was allowed to span for 72 hours for proper extraction. The extract was allowed to cool and then filtered.

- **AQUEOUS BARK EXTRACT PREPARATION**

Powdered plant bark was weighed (20g) into an already dried 250ml conical flask and 200ml of distilled water was added, The solution was shaken with the orbital shaker at 80rpm for 15 minutes until the solution thoroughly mixed. The solution was heated for 15minutes and allowed to cool for 2 hours before been filtered. The filtrate is stored in a refrigerator at 4^0C. This

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- **PREPARATION OF STOCK SOLUTIONS**

0.1M copper sulphate (CuSO4) solution was prepared by weighing 15.96 g of anhydrous copperinto a 1L standard flask and dissolved with distilled water, then made up to mark with distilled water andstored in a container. On the other hand, the stock of 0.1M NiSO4.6H2O solution was prepared by weighing 26.5 g of hydrated nickel sulphate salt into another 1L standard flask and dissolved with distilled water and made up to mark with distilled water and stored in another container.

GENERATION OF NANOPARTICLE

- **AQUEOUS BARK EXTRACTION TECHNIQUE FOR COPPER NANOPARTICLE**

From the previously prepared copper sulphate solution in 2.3, 20ml of solution was measured into a 250ml conical flask and 30ml of the aqueous plant extract was also measured into the conical flask and was shaken using an orbital shaker for three hours for thorough mixing. The solution was placed in the water bath at 60^0C for two hours, On withdrawing from the water bath, the solutions becomes cloudy and was allowed to cool in the dessicator four hours; After cooling, the solution was noticed to have precipitated, The solution was centrifuged to compact the nanoparticles for easy collection.

- **ORGANIC BARK EXTRACTION TECHNIQUE FOR COPPER NANOPARTICLES**

From the prepared 0.1M CuSO4, 20ml of solution was measured into a 250ml conical flask and 30ml of the organic plant extract was also measured into the conical flask and was shaken using an orbital shaker for three hours for thorough mixing.The solution was placed in the water bath at 60^0C for two hours, On withdrawing from the water bath, the solutions becomes cloudy and was allowed to cool in the dessicator four hours; After cooling, the solution was noticed to have precipitated, The solution was centrifuged to compact the nanoparticles for easy collection.

- **AQUEOUS BARK EXTRACTION TECHNIQUE FOR NICKEL NANOPARTICLE**

From the prepared 0.1M NiSO4.6H20 solution was measured into a 250ml conical flask and 30ml of the aqueous plant extract was also measured into the conical flask. The solution was placed in the water bath at 60^0C for two hours, On withdrawing from the water bath, the solutions becomes cloudy but not as cloudy as that of copper and was allowed to cool in the dessicator for four hours. After cooling, the solution was noticed to have precipitated, the solution was centrifuged to compact the nanoparticles for easy collection.

- **ORGANIC BARK EXTRACTION TECHNIQUE FOR NICKEL NANOPARTICLE**

From the prepared 0.1M NiSO4.6H2O solution was measured into a 250ml conical flask and 30ml of the organic plant extract was also measured into the conical flask and was shaken for thorough mixing.The solution was placed in the water bath at 60^0C for two hours, On withdrawing from the water bath, the solutions becomes cloudy but not as cloudy as that of copper and was allowed to cool in the dessicator for four hours. After cooling, the solution was noticed to have precipitated. The solution was centrifuged to compact the nanoparticles for easy collection.

The four (4) solutions were placed in a water bath for one hour at 60^0C and precipitates were observed at the bottom of the conical flask. The solutions were allowed to stand for four hours in a dessicator before they are been subjected to centrifugation for thirty minutes at 7000rpm in order to coagulate the precipitate. The solutions were decanted after centrifugation and the precipitates were then washed and dried for fifteen minutes at 105^0 C. The solid

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samples were then kept in sample bottles for characterization using different spectroscopic methods and for further antimicrobial activity test.

ANTIMICROBIAL ACTIVITIES OF NANOPARTICLES

The antibacterial potential of the synthesized copper and nickel nanoparticles from *Brideliaferruginea*against some test microorganisms was done using the agar well diffusion method. An 18-24 h old culture of each test isolate was inoculated into 5ml normal saline in atest tube and standardized. A sterile swab stick was used to apply the suspension to the surface of already prepared Nutrient Agar (NA) plates. A sterile 8mm cork borer was used in boring holes on the agar and a micropipette was used in dispensing 100 µL of the nanoparticles and 100 µL of the antibiotic into the respective labeled holes, Ciprofloxacin solution was used ascontrol. The antimicrobial activities were then determined by measuring the diameter of thezones of inhibition in milimetre.The MIC of the nanoparticles on the test isolates was determined using two-fold dilutionmethod.Sterile 8 mm cork borer was used to bore five holes onto prepared Nutrient Agar plates seededwith the nanoparticles and an antibiotic as standard. Different concentrations of the nanoparticles and the antibiotics (100%, 80%, 60%, 40% and 20%) were dispensed into each well and labeled. The preparation was left to diffuse before incubating at 37ºC for 24 h. The lowest concentration of the agent that prevented the growth of the bacteria was taken as the minimum inhibitory concentration (MIC). The zones of inhibition were observed and recorded.

RESULTS AND DISCUSSION

From figure 4.1, the UV spectra of the synthesized copper nanoparticles in aqueousmedium shows a peak around the region of 560cm^{-1} -600cm^{-1} at an absorbance range of 0.25abswhich suggests the presence of copper and a peak around 265-280nm which suggests the presence a phenolic group which is attributed to phenolic compound in the extract used in preparing the nanoparticles at an absorbance range of 0.4 abs. Similar result was presented by Prashant Praveen in 2014 [14]. Jamal Alizadeh also reported same result. Figure 4.2 shows the UV spectra of the synthesized copper nanoparticles in organic mediumwhich shows a peak around the region of 560cm^{-1} -600cm^{-1} which might likely be as a result of the presence of copper. [15-16] Fig 4 and 4.5 shows a broad but not a sharp peak around 365cm^{-1}, this is due to the presence of nickel.

Furthermore the From Figure 4.5 and 4.7, three peaks were observed, a peak was observed within the range of 3500-3200cm^{-1} which is suspected to represent the presence of an amine group present in alkaloids in the plant, a polyphenolic group present in flavonoids and tannins and an O-H group which could be present in steroid in the plant or the methanol used in preparing the extract. Another peak was noticed around the region of 1000cm-1 which is likely to account for the presence of C-O which may be present in the plant. Similar results were recorded by Akinsete *et al*., (2011) [13] and another research on a similar subject by[12] further validate the results.

Various bacteria from different sources were subjected to antimicrobial test using nanoparticles generated as substitute for antibiotics as shown in Table 1. In *Eschericia coli*, (ATCC35218), *Pseudomonas aeruginosa*, (ATCC27853) clear zones wereseen in the region of Copper and Nickel with methanolic extract which suggests the above listed bacteria are more prone to be killed by copper and nanoparticles in the organic medium. Clear zones were observed in the Copper with

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methanolic extract, Copper and Nickel with aqueous extract regions when *Shigellasp* was treated with the nanoparticles, which indicates *Shigellasp* is more sensitive to the antimicrobial strength of nickel and copper nanoparticles especially in their aqueous medium. A high sense of affinity for copper was noticed as it is sensitive to copper in the organic medium too. Clear zone was observed in the Nickel with aqueous extract regions when *Staphylococcus aereus* was subjected to the nanoparticles, It is still unclear if it has affinity for nickel nanoparticle or the organic medium as it does not replicate its strength neither in the aqueous medium of nickel nanoparticle nor in the organic medium of copper nanoparticle that it was also subjected to. *Streptococcus pnemoniae* surprisingly was overpowered by all of the four extracts as clear zone was seen in all regions, although the dimension of the clear zones in the aqueous medium was larger than that of the organic medium. *Salmonella sp* was noticed to have been overpowered by the extracts in the organic region, a clear region was also noticed in the aqueous region of nickel nanoparticle. A high affinity for nickel is noticed here too. It can be seen that the extracts in both the aqueous and the organic phases has contributed in their role as a substitute for antibiotics in the treatment bacteria. However, copper and nickel nanoparticle in their organic phase had effect on seven of the eight bacteria while copper and nickel nanoparticle in their aqueous state can boast of just two and three respectively.

In addition to this test result, various fungi from different sources were subjected to antimicrobial test using nanoparticles generated as substitute for antibiotics as shown in Table 2. In *Aspergilousflavus*, zone of inhibition were observed in the Copper with methanolic extractand Nickel with aqueous extract region when treated with the nanoparticles. *Candindaalbicans* was found to be powerless to nickel and copper in their organic and aqueous phases respectively as no zone of inhibition was observed in the Nickel with aqueous extract and Copper with methanolic extract region. Clear zones of inhibition were observed in the Nickel with methanolic extract and Nickel with aqueous extract region when *Blastomycessp* was treated with the nanoparticles, which suggests high sensitivity to nickel nanoparticle both in the organic and aqueous phase. *Fursariumspp* could only overcome the threat from nickel nanoparticle in its aqueous phase as it shows clear zone of inhibition in other regions. *Tricophytonspp* just like *Aspergiloussp* shows clear zone of inhibition in the Nickel with aqueous extract and Copper with aqueous extract region when treated with the nanoparticles. *Aspergilousniger* just like *Candindaalbican* showed clear zone of inhibition in the Nickel with methanolicextract and Copper with aqueous extract region. *Microsporum* in this context seems to be a stubborn fungus, as it could only be overcome by nickel nanoparticle in their organic phase.

Table 1.Antimicrobial activity of copper and nickel nanoparticle on bacteria

S/N	Bacteria	Extract			
		CM (mm)	NM(mm)	CW(mm)	NW(mm)
1	*E. Coli*	19.20±0.4	16.00±0.11	0.0	0.0
2	*Pseudomonas aeruginosa*	14.00±0.0	18.30±0.0	0.0	0.0
3	*Shigella sp.*	31.78±1.0	0.0	32.51±0.02	28.23±0.0
4	*Staphylococcus aureus*	0.0	16.05±0.67	0.0	0.0
5	*Streptococcus pnemoniae*	18.3±0.7	18.77±0.22	24.0±0.20	28.1±0.0
6	*Salmonella typhi*	28.90±0.13	31.0±0.0	0.0	24.20±0.04
7	*Pseudomonas aeruginosa* (ATCC 27853)	27.3±0.08	17.70±0.05	0.0	0.0
8	*E. coli* (ATCC 35218)	15.78±0.15	20.00±0.01	0.0	0.0

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Table 2.Antimicrobial activity of copper and nickel nanoparticle on fungi

S/N	Fungi		Extract		
	CM	CM	NM	CW	NW
1	*Aspergilusflavus*	24.10 ± 0.0	0.0	0.0	28.67±0.12
2	*Canadindaalbicans*	0.0	30.±0.0	27±0.17	0.0
3	*Blastomyces*	0.0	32.1±0.0	0.0	22 ± 1.0
4	*Fasurium*	26.22±0.0	24 ±0.0	14.7 ±0.03	0.0
5	*Tricophyton*	26.0 ± 0.0	0.0	0.0	17.7 ± 0.06
6	*Aspergilusniger*	0.0	18.3±0.0	21 ±0.0	0.0
7	*Microsporium sp.*	0.0	19±0.0	0.0	0.0

Key: NM= Nickel nanoparticle with methanolic extract, CM=Copper nanoparticle with methanolic extract, NW= Nickel nanoparticle with aqueous extract, CW= Copper nanoparticle with aqueous extract.

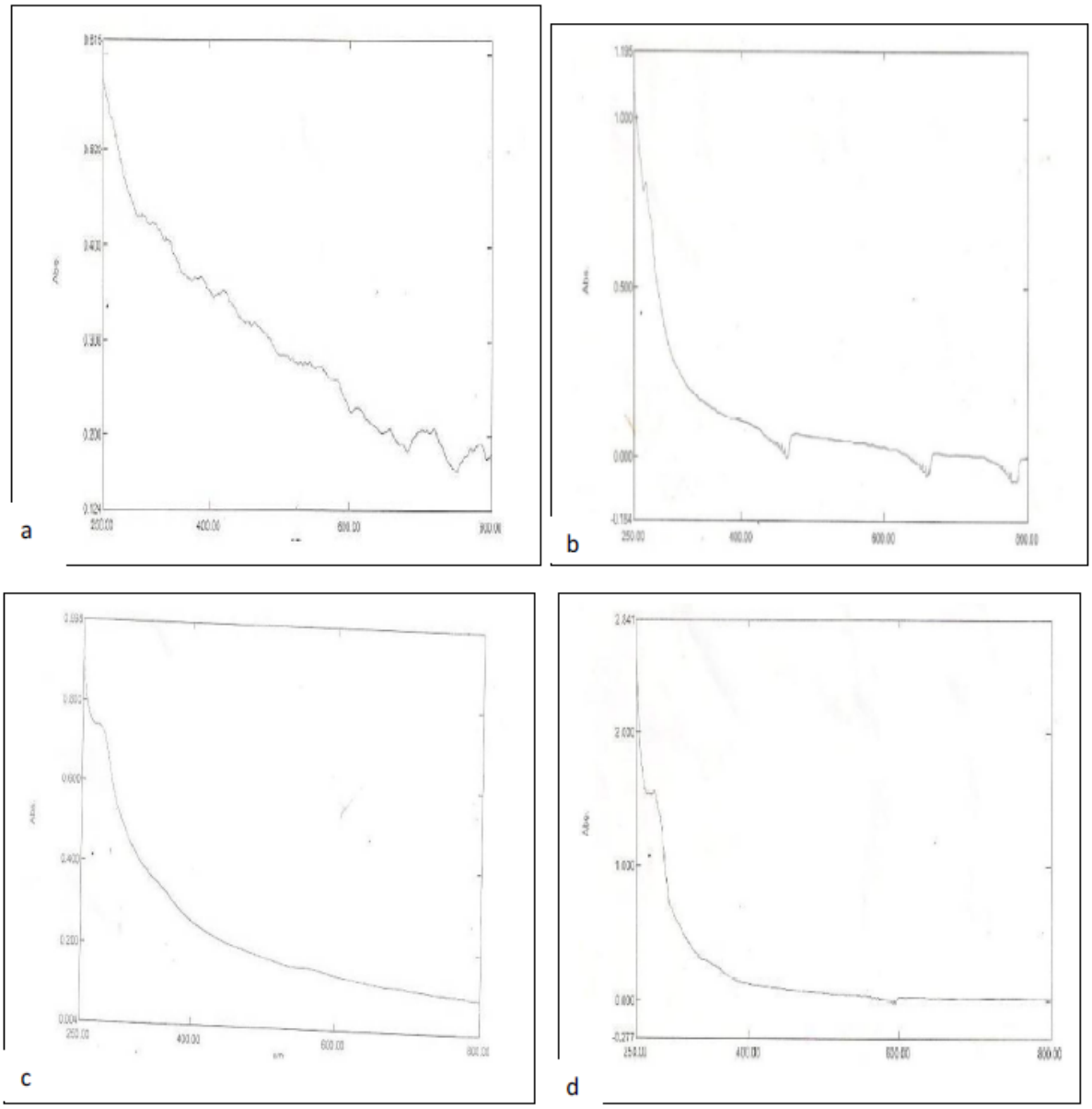

Figure 1.(a) UV Spectroscopy result for copper nanoparticle in aqeous medium; (b) UV Spectroscopy result for copper nanoparticle in organic medium; (c) UV Spectroscopy result for nickel nanoparticle in aqueous medium; (d) UV Spectroscopy result for nickel nanoparticle in organic medium

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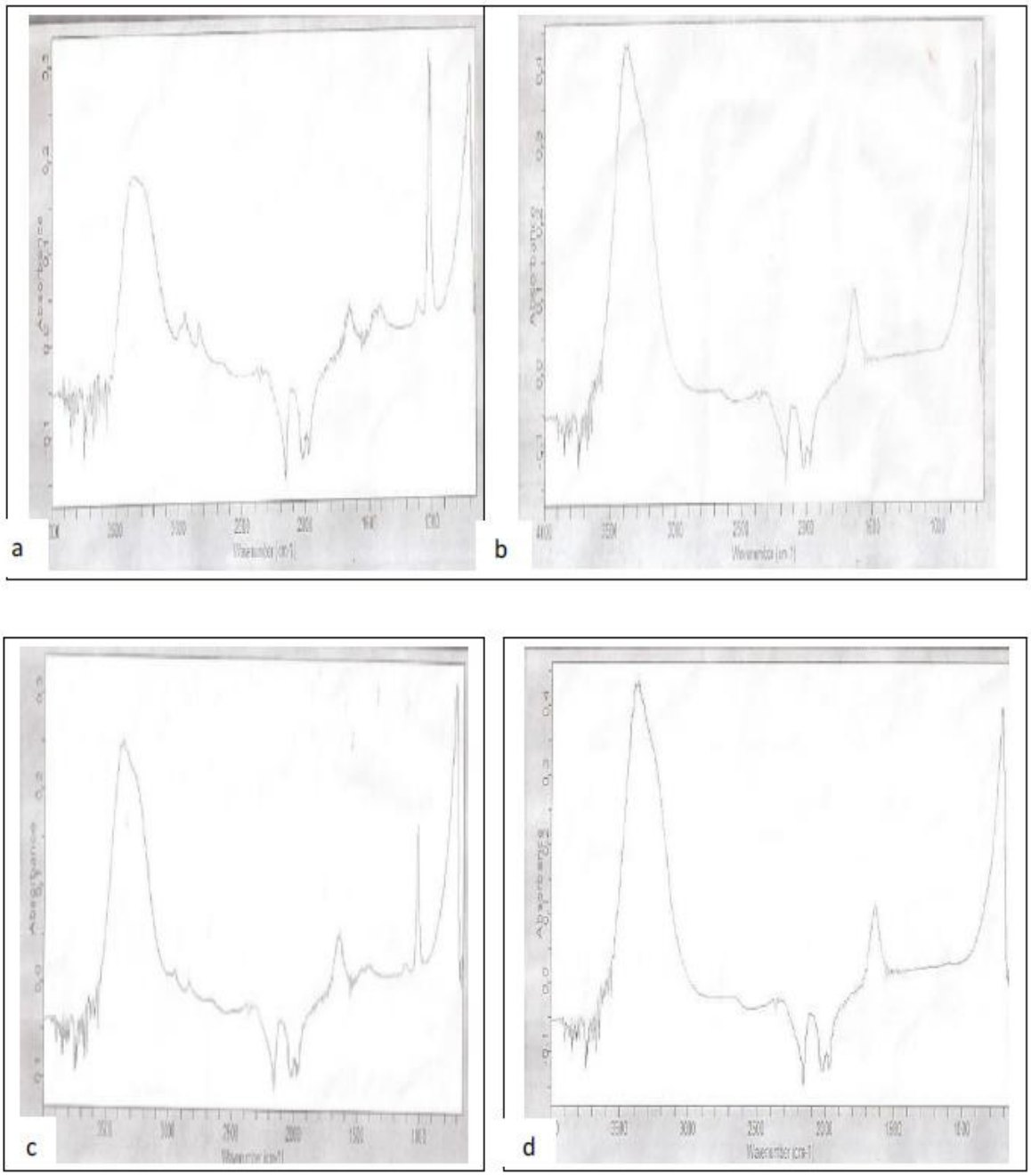

Figure 2.(a) FT-IR Spectroscopy result for nickel nanoparticle in organic medium; (b) FT-IR Spectroscopy result for nickel nanoparticle in aqueous medium; (c) FT-IR Spectroscopy result for copper nanoparticle in organic medium; (d) FT-IR Spectroscopy result for copper nanoparticle in aqueous medium

CONCLUSION

This study shows that *Brideliaferruginea* plant bark is a good reducing agent which reduces copper (ii) in CuSO4 and Nickel (ii) in NiSO4.6H2O to copper and nickel nanoparticles respectively owing to the alkaloids, flavonoids and other secondary metabolites present in the plant. The nickel and copper nanoparticles synthesized using *Brideliaferruginae* plant bark extract using biological methods which is environment friendly and commercially economical possesses high antimicrobial activity against some bacteria (*Shigellasp , Streptococuspnemoniae and Salmonelatyphi*) and some fungi (*Fusariumsp*, Candindaalbicans), Though not as effective as the conventional antibiotics.

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