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SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL ACTIVITIES OF COBALT NANOPARTICLE USING *KHAYA SENEGALENSIS* PLANT

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ABSTRACT

This research aimed at synthesis, characterization and pharmacological activities of cobalt nanoparticle using *khaya senegalensis* plant. Cobalt nanoparticles (CoNPs) were synthesized using stem bark extract of *khaya senegalensis*, via bio-reduction method. The phytochemical screening of the plant was carried out using standard methods to detect the presence of secondary metabolites. XRD was used for sizing, UV was used to find the information about the plasmonic resonance of the nanoparticles, morphology was determined using SEM, functional groups were determined by FTIR while the antimicrobial activities were determined using agar-well diffusion method. The phytochemical screening of the plant showed the presence of phenols, glycosides, tannins and flavonoids. The size of the nanoparticles synthesized using *Khaya senegalensis* was found to be 48.1 nm. The CoNPs were highly absorbed at the ultraviolet region of 394 nm. Morphology of the particles were found to be spherical at different magnifications and the biomolecule responsible for the reduction was found to be amine group whose wavelength (1315.60 cm) disappeared on the nanoparticles. The antimicrobial activities of plants and the CoNPs were carried out against *Escherichia coli*, *Salmonella typhi*, *Shigella*, *Candida tropicalis*, *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* and *Staphylococcus aureus* which showed a significant zone of inhibitions.

Keywords: Alkaloid, Augmentin, *Escherichia coli*, *Khaya senegalensis*, Nanoparticles

INTRODUCTION

Nanoparticles are materials with primary dimensions between 1 and 100 nm. The size and surface effects of metal nanoparticles have rendered it exceptionally important since its performance depends critically on their size, shape, composition and fine structure, either as alloy or core–shell [1]. Over the last decade there have been intense scientific research in the study of metal nanoparticles because of its unique properties ranging from catalytic,

electronic, optical, and structural, among others. Because of these properties, metal nanoparticles have found application in biomedical [2], catalysis [3]. electronics and optics [4]. Commercial applications of nanoparticles are increasing day by day due to great interest in nanostructure materials in every day's life.

Biological method of nanoparticles synthesis does not meet the industrial demand, chemical and physical methods method of nanoparticles synthesis are not environmentally benign and it affect animals and as well as human health and also not economically safe. Researchers used Green Nanotechnology, which is an innovative approach that leads to develop new discoveries in Green Chemistry. Green chemistry is a sustainable process which includes fundamental principles, that leads to the synthesis and stabilization of metal nanoparticles [5].

Only a tiny fraction of the existing plant species has been scientifically researched for bioactivities since 1805, when the first pharmacologically-active compound morphine was isolated from opium. Natural products and traditional medicines have already made contributions for modern medicine [6]. Some plant parts are used directly by traditional medicine practitioners. Some are extracted before use or modified and combined before administration. In most cases, where they are extracted before application, crude extracts are used. A challenge to contend with is dosage and the other is cytotoxicity issue in traditional medicine. Traditional herbs as used to cure diseases are important especially to local communities since they are easily accessible, easy to prepare and affordable to the people.

Various diseases including cancer and Alzheimer's disease had been reported to be treated with active compounds from plants [7, 8]. Researches on phytochemical analyses of plant extracts in most cases correlate the chemical constituents of the plants with their pharmacological activity [9]. Many plants in the tropical and subtropical regions have been screened for their wound healing activities. Consequently, plants having antimicrobial activity against multidrug-resistant (MDR) pathogens can be considered as a breakthrough in pharmacological industries [10].

Khaya senegalensis is a specie of trees in the *Meliaceae* family that is native to Africa. The names include Homraya, Homra, Murraya, Mahogoni in Arabic; African mahogany and dry zone Mahogany in English; Cailcedrat, Acajou d'Afrique,acajou du Sénégal in French; Madachi in Hausa; Dalehi-Kahi in Fulani; Ono in Igbo; Ogonwo in Yoruba; Kaya in Indonesian; Its trade name is Khaya wood and African mahogany.

Khaya senegalensis (Desr.) A. Juss. (African mahogany or dry-zone mahogany) has long been an important multipurpose tree in its natural range in Africa. The *Khaya senegalensis* A. Juss (Meliaceae) tree is a popular medicinal plant among the Nupes and Yorubas in Nigeria. The aqueous stem bark extract is traditionally used by these tribes in the treatment of malaria, jaundice, edema and headache. The Hausa and Fulani tribes in Northern Nigeria also use *Khaya senegalensis* to treat several human and animal ailments [11, 12]. Therefore, this research work aimed at synthesis and characterization of cobalt nanoparticle using *Khaya senegalensis* plant so as to determine the possible antimicrobial properties of the nanoparticles on the stem bark extract of *Khaya senegalensis* plant.

MATERIALS AND METHODS

Sample Collection and Preparation

The stem bark of *Khaya senegalensis* was collected in Yola South Local Government Area of Adamawa State, Nigeria. The sample was washed thoroughly with distilled water to remove dirt; cut into pieces and was allowed to dry at room temperature on clean polythene plastic bags for two weeks. The dried stem bark was ground mechanically using pestle and mortar. The sample was sieved, parked in polyethylene bags, labeled and stored for extraction, phytochemical screening, synthesis of nanoparticles and testing for antimicrobial activity.

Preparation of Stem Bark Extract of the Plants for Synthesis

Exactly 10 g of the stem bark was placed in a 250 mL flask and boiled with distilled water for 20 minutes. The mixture was allowed to cool under room temperature. The solution was filtered with Whatmann filter paper No. 1 to obtain the extract of *Khaya senegalensis* stem barks which was later used as reducing agent for the bio reduction of cobalt chloride in the synthesis of the CoNPs.

Preparation of Cobalt

Exactly 0.118 g of cobalt (II) chloride was weighed and dissolved in 100 mL distilled water in a volumetric flask to obtain 0.005 M of cobalt (II) chloride. About 50 mL of the cobalt (II) chloride solution was placed in a beaker, 20 mL of the extract was added drop wise for 1 hour with continuous stirring, colour change was observed. The product was centrifuged at 8000 rpm for 45 minutes, then filtered and rinsed with distilled water thoroughly before it was allowed to dry at room temperature [13, 14].

Spectroscopic Characterization

The following spectroscopic techniques were used in the characterization of both the extract and the nanoparticles:

UV-Visible Spectrophotometry

This refers to an absorption spectroscopy in the ultraviolet visible spectral region. This means it uses light in the visible and adjacent near UV and near infrared ranges. The absorption or reflectance in visible range directly affects the perceived colour of the chemicals involved. The UV-visible spectrophotometer (model: Dzv-8200-visible Spectrophotometer, China) analyses of the formed nanoparticles were carried out at scanning interval of 5 nm from 350 nm to 450 nm. For the extract, distilled water was used as blank while for the nanoparticles, ethanol was used as blank [14].

FT-IR Spectroscopy

FT-IR spectrometer (model CARRY 630 NBY Agilent Technologies USA) was used in this study to determine the functional groups responsible for the formation of the nanoparticles.

Qualitative Phytochemical Screening

The preliminary qualitative phytochemical screening of the stem bark extracts of *Khaya senegalensis* was carried out for saponins, flavonoids, alkaloids, phenols, tannins, glycosides and steroids [15].

Test for Tannins

About 5 mL of the solution of the extract was shaken with two drops of ferric chloride. A blue-green precipitate was formed, which showed that tannin was present.

Test for Flavonoids

About 5 mL of the solution of the extract was mixed with two drops of ammonia which gave yellow-brown colour signifying the presence of flavonoids.

Test for Saponins

The solution of the extract (5 mL) was shaken with about 5 ml of distilled water and then heated to boil. The formation of frothing, indicated the presence of saponins.

Test for Glycosides

The solution of the extract was dissolved in some glacial acetic acid containing one drop of $FeCl_3$. The solution was underplayed with concentrated H_2SO_4. No formation of a brown ring at the interphase between the acetic acid layer and H_2SO_4 layer, which indicated the absence of glycosides.

Test for Alkaloids

The solution of the extract was warmed with 1% HCl for two minutes. The mixture was filtered and few drops of Dragendorff's reagents were added. A reddish-brown colour and turbidity with the reagent indicated the presence of alkaloids.

Test for Phenols

The solution of the extract was mixed with two drops of aqueous ferric chloride. There was a formation of blue-black colouration, which indicated presence of phenols.

Microbial Analysis

The microbial analysis of the plant and CoNPs were carried out on four bacteria (*Escherichia Coli, Staphylococcus aureus, Shigella, and Salmonella typhi*) and four fungi (*Candida albica, Candida tropicalis, Aspergillus niger and Aspergillus flavus*) according to the method described by Amarnath et al. [13].

Preparation of plant extract for phytochemical screening

A sample (200 g) of powdered stem bark of *Khaya senegalensis* plant material was soaked in methanol (200 ml) for 24 h. At the end of the extraction, each extract was filtered using Whatman filter paper. The filtrate was concentrated in vacuum at 30 ^oC and stored at 4 ^oC until further use.

Antibacterial activity`

The antibacterial activity of *Khaya senegalensis* was tested against *Escherichia Coli, Staphylococcus aureus, Shigella, and Salmonella typhi* using agar well diffusion. The stem bark extract was dissolved using dimethyl sulfoxide (DMSO) and stored at 4 ^0C until use. Standard antibiotic (Augmentin) was used as control. A Well of 6 mm in diameter was created in the nutrient agar using a sterile cork borer. A freshly prepared bacterial culture was inoculated onto the surface of the agar plates using a sterile swab. About 80 µl of different

concentrations (500, 250, 125 ug/ml) of leaf extracts was added into the wells and allowed to diffuse at room temperature for 2 h. The plates were incubated at 37 °C for 18 h. The antibacterial activity of the extracts was determined by measuring the diameter of the inhibitory zones on the surface of the agar around the disc in mm. The experiment was carried out in triplicate and the mean values of the diameter of zones of inhibition were calculated

Antifungal activity

The antifungal activity of the stem bark extracts of *Khaya senegalensis* was studied prepared in DMSO against a standard (ketonazole) using agar well diffusion method. The strains of selected fungi species were maintained in culture medium of potato dextrose agar (PDA). A well of 6 mm in diameter was created in the PDA using a sterile cork borer. A freshly prepared spore solution was inoculated onto the surface of the agar plates using a sterile swab. About 80 µl of different concentrations (500, 250, 125 ug/ml) of the extracts were added into the wells and allowed to diffuse at room temperature for 2 h. The plates were incubated at 30 °C for 72 h. The antifungal activity of the leaf extracts was determined by measuring the diameter of the inhibitory zones on the surface of the agar around the disc in mm. The experiment was carried out in triplicate and the mean values of the diameter of zones of inhibition were calculated.

RESULTS AND DISCUSSIONS

Phytochemical Screening of the Plants

Table 1 shows the results of the phytochemical analysis of *Khaya senegalensis* crude extract. The phytochemical screening of *Khaya senegalensis* stem bark methanolic extract and aqueous extract revealed that favonoids, glycosides, phenols and tannins were present while terpenoids, saponins and alkaloids were absent. The absence of alkaloids on both the aqueous extract and methanolic extract correlates with the results obtained by Makut et al [12] and also a research carried out by Kubamarawa et al [16]. Phenols and tannins were found to be present also. Thus, the results obtained for glycoside and terpenoids do not correlate with the ones carried out by Makut et al [12]. Also, saponins, was found to be present in this research but were found to be absent in the research carried out by Kubamarawa et al [16]. and glycosides was present but found absent in Kubamarawa et al [16] research work. The variations in these results could be due to geographical location.

Table 1. Phytochemical screening of *Khaya senegalensis*

Phytochemical Tests	*Khaya senegalensis*	
	Methanol extract	Aqueous extract
Alkaloids	-	-
Flavonoids	+	+
Tannins	+	+
Saponins	-	-
Glycosides	+	+
Terpenoids	-	-
Phenols	+	+

Key: + = present, - = Absent

Formation of Cobalt Nanoparticles

The stem bark extracts of *Khaya senegalensis* were used for the reduction of cobalt (II) chloride solution to form cobalt nanoparticles. The reduction was monitored by a change in colour of the solution. Plate 1 indicates: A is cobalt chloride solution, B is *Khaya senegalensis* stem bark extract and C is CoNPs

The dark brown colour formation indicated the formation and presence of CoNPs in the solution as shown in Plate 1. The intensity of the colour increases with an increase in contact time. The colour is due to surface plasmon resonance of CoNPs which is attributed to the collective oscillation of conduction band electrons in response to the electric field of the electromagnetic radiation [14].

Plate 1: A is cobalt chloride solution, B is *Khaya senegalensis* stem bark extract and C is CoNPs

UV –Visible Spectroscopy

As shown in Figure 1, the sample displays an optical absorption band peak at about 396 nm, which is typical of absorption for metallic cobalt clusters due to Surface Plasmon Resonance (SPR). The observation indicated that the reduction of the Co^{+} ions took place extracellular as the peak value is lower than 430 – 500 nm. Nevertheless, the obtained SPR value is within the range of SPR values for cobalt nanoparticles.

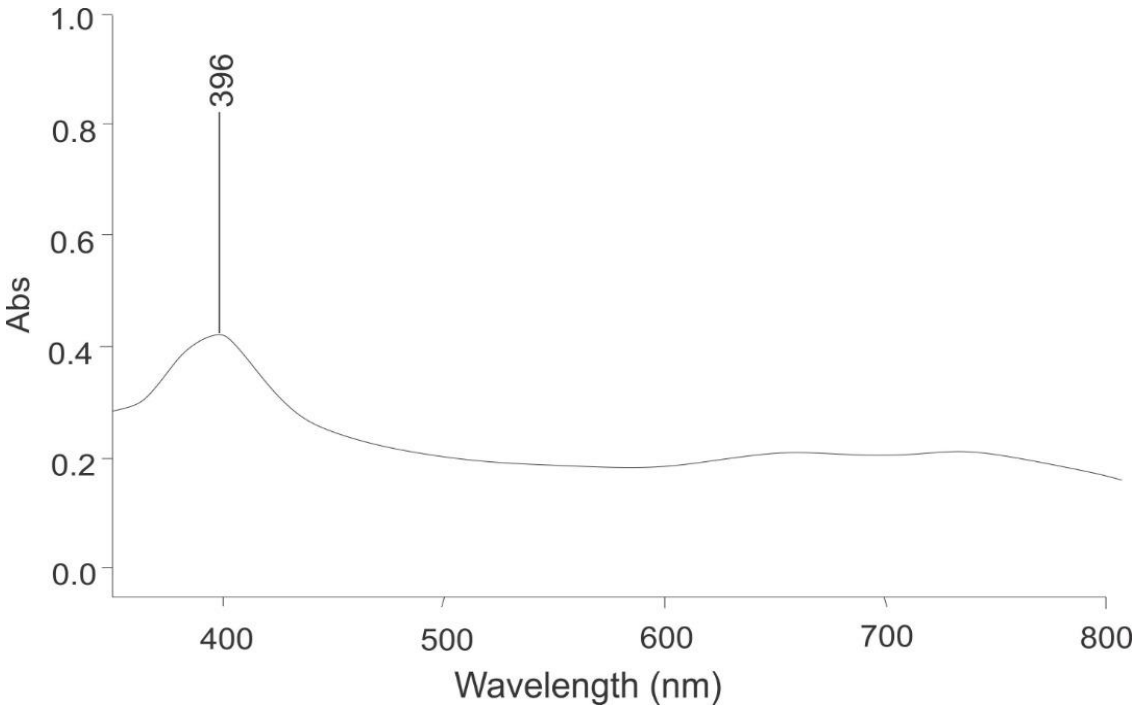

Figure 1. UV- Visible spectroscopic result of CoNPssynthesized using *Khaya senegalensis* stem bark extract.

FTIR Spectroscopy Analysis

***Khaya senegalensis* and Nanoparticle FTIR**

Figure 2 and 3 show the spectra of *Khaya senegalensis* steam bark extract and CoNPs synthesized using the plant. The IR analysis showed bands at 3261.40 cm^{-1} and 3250.20 cm^{-1}. These bands are within the range of O-H hydrogen bond stretching of alcohol for the plant and the nanoparticle. The bands at 2933.40 cm^{-1} and 2926.0 cm which appeared in both the plant and the nanoparticle spectra correspond to the acid C-H stretching. Other bands include 2102.2 cm^{-1}, 1606.50 cm^{-1} and 1602.80 cm^{-1} which can be ascribed to carbon to carbon triple bond alkyne stretching and N-H bending of amine respectively. However, the total disappearance of 1315.8 cm^{-1} bands on the nanoparticles indicated that it was responsible for the bio reduction of cobalt ion to cobalt nanoparticles.

Therefore, it can be deduced that amine group are active reducing agents in the synthesis of cobalt nanoparticles using plant extract [14, 17] though, other groups also have the potentials of reducing cobalt ions to nanoparticles.

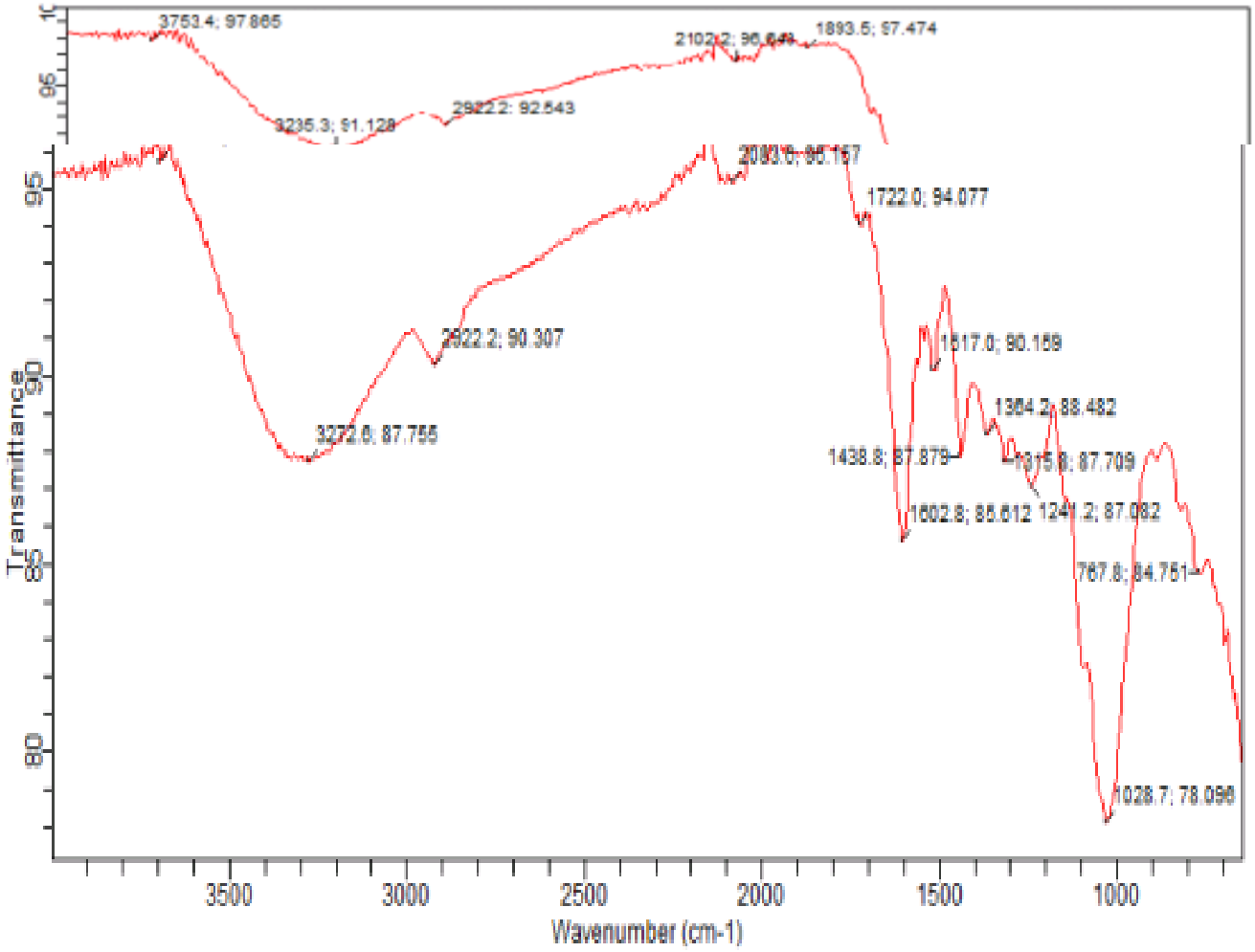

Figure 2: FTIR analysis of *Khaya senegalensis* stem bark extract.

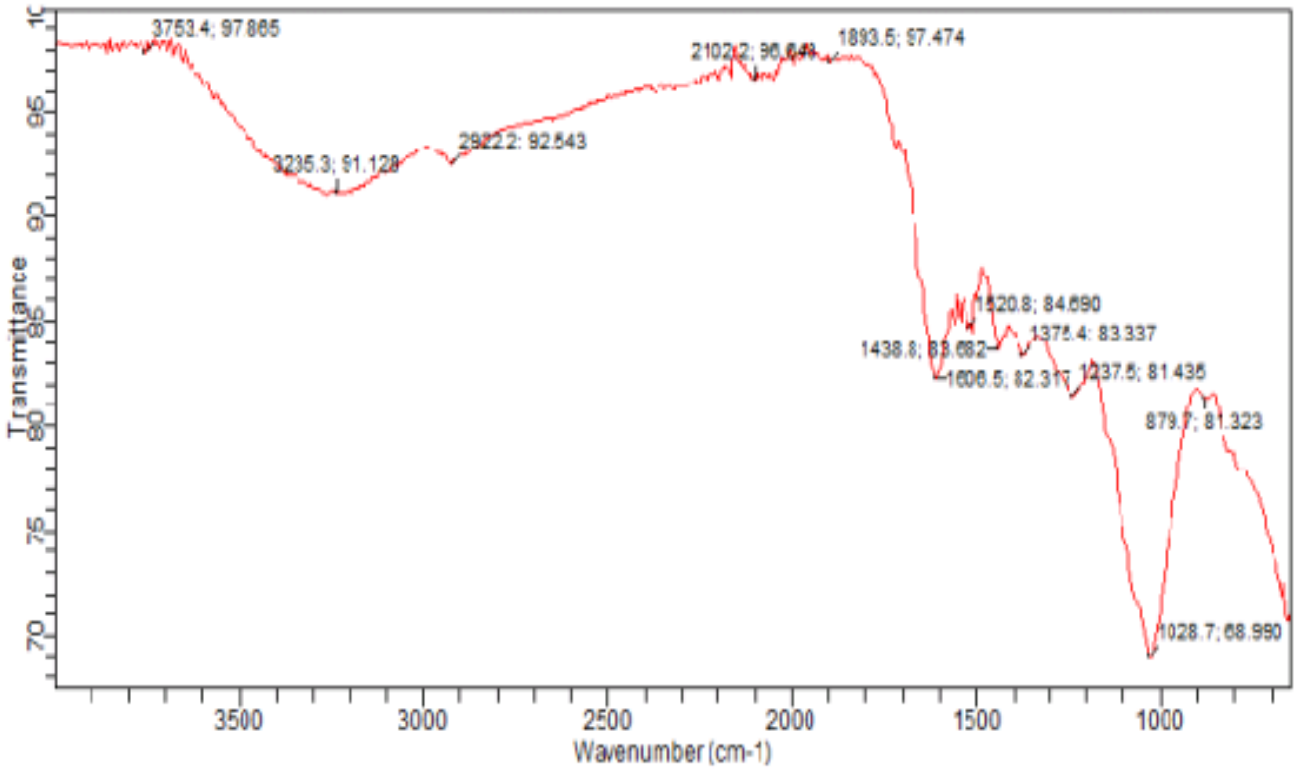

Figure 3. FTIR analysis of CoNPs synthesized using *Khaya senegalensis* stembark extract

X-Ray Diffraction (XRD) Analysis of CoNPs of *Khaya senegalensis*

Figure 4 shows XRD results of CoNPs synthesized using *Khaya senegalensis* stem bark extract. The XRD of the synthesized CoNPs using *Khaya senegalensis* shows broad peaks at values of 34.2, 38.0, 44.3 and 64.5 which are typical for the cobalt structure. Notable line broadening of the diffraction peaks is an indication that the synthesized materials are in nanometre range. Using X-Ray diffraction results, the average particle size of the nanoparticles was found to be 48.1 nm. The average particle sizes of synthesized CoNPs were calculated using Deybe-Scherrer Formula.

$D\ (nm) = K\lambda / \beta Cos\theta$

Where D =mean diameter of nanoparticles

β = the full width at half maximum value of XRD diffraction lines

λ = the wavelength of X-ray radiation source

θ = the half diffraction angle –Bragg angle

K = the Scherrer constant with value from 0.94

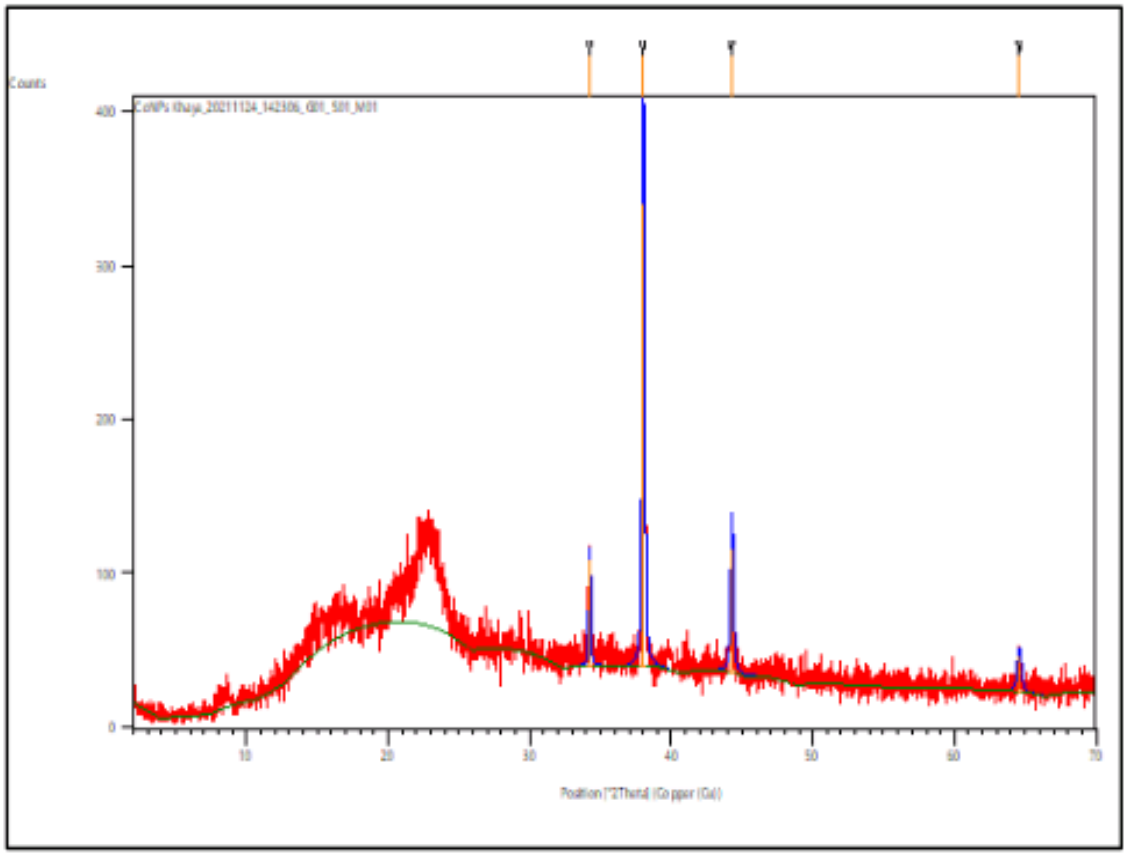

Figure 4. XRD results of CoNPs synthesized using *Khaya senegalensis* Stem bark extract

Scanning Electron Microscopy (SEM) Analysis

The morphology of the prepared nanoparticles was examined using scanning electron microscopy. Figure 5 shows the surface morphology of the cobalt nanoparticles. SEM image showed individual cobalt particles as well as a number of aggregates (200 µm,100 µm, 80 µm and 50 µm). The SEM image showed most of the nanoparticles are spherical in shape.

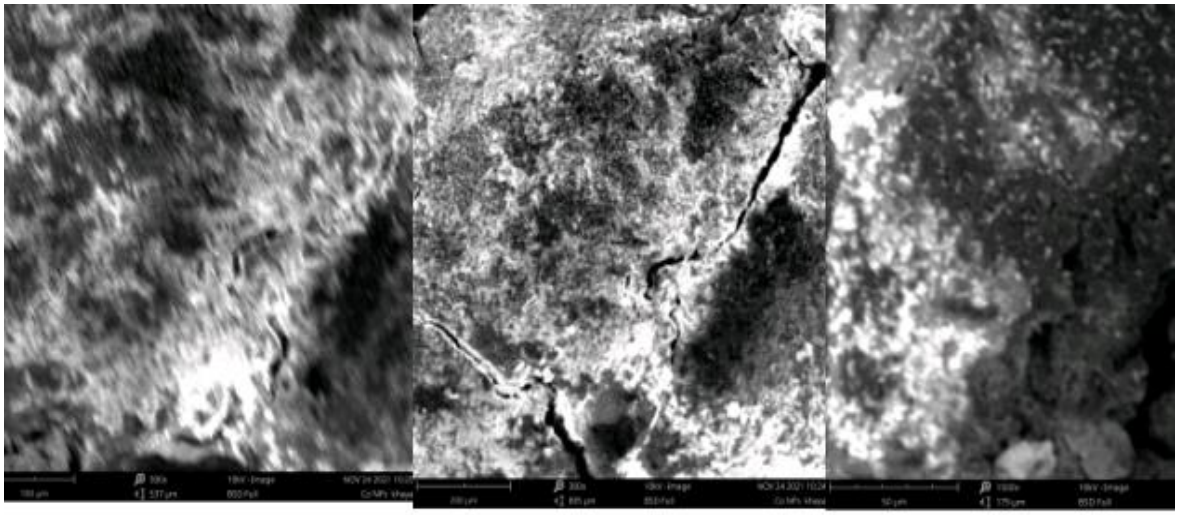

Figure 5. The SEM results of nanoparticles synthesized using *Khaya senegalensis*.

Antimicrobial Analysis

The extracts of *Khaya senegalensis* were analyzed for antimicrobial activity against four fungi and four bacteria: *Escherichia coli, Salmonella typhi, Shigella, Candida tropicalis, Candida albicans, Aspergillus niger, Aspergillus flavus and Staphylococcus aureus*. The results revealed that these plants showed antibacterial activity with varying magnitudes. The measured zones of inhibition diameter (in mm) around the discs were summarized for different concentrations of the plant extracts and nanoparticles as shown in Tables 2 and 3. The diameter of the zone of inhibition gives information about the magnitude of the susceptibility of microorganisms. The zone of inhibition above 7 mm in diameter was taken as positive result. Generally, most of the tested organisms were sensitive to the extract.

Antimicrobial Activities of the Plants

Khaya senegalensis stembark extract showed to be rich in bioactive components that can be used as a potential source of drug.

Table 2 showed that *Khaya senegalensis* stembark extract was effective against *Staphylococcus aureus*. The results were above the recommended sensitive value i.e 7 mm. As the concentration of the extract increases, the Zone of Inhibition (ZOI) also increases. This result was the same with that of *Aspergillus flavus, Aspergillus niger, Candida tropicalis* and *Candida albican*. In all these cases, the ZOI is directly proportional to the concentration of the stem bark extract of *Khaya senegalensis*. For *Salmonella typhi*, the ZOI remained constant as the concentration of the stem bark extract increases from 250 ug/mL to 500ug/mL

but there was an increase in concentration from 125 ug/mL to 250 ug/mL. *Escherichia coli (E-Coli)* showed higher ZOI at 250 ug/mL. When the concentration was increased the ZOI reduced. This suggested that a specific concentration is required for *E-coli* to be sensitive to the plant extract.

Antimicrobial Activities of Cobalt Nanoparticles

The antimicrobial activities of CoNPs synthesized using *Khaya senegalensis* showed that the nanoparticles has more antimicrobial properties than the plant extract. This could be due to significant properties of nanoparticles against bacteria and fungi. The ZOI increases as the concentration increases. At the lowest concentration (125 ug/mL), *Salmonella* showed to be non-sensitive to the nanoparticles and *Staphylococcus aureus* gave a ZOI equivalent to the acceptable sensitive value 7 mm. At 500 ug/mL *E-coli* was more sensitive to the nanoparticles than the controlled drug. Comparing the results from the nanoparticles and the controlled drug, the nanoparticles gave a result that is most similar to that of the controlled drug. These suggested that the nanoparticles have high therapeutic value at higher concentrations. Therefore it can be used as a good antimicrobial agent.

Table 2: Antimicrobial properties of *Khaya senegalensis* Stembark Extract

Organisms	**500 µg/ml**	**250 µg/ml**	**125 µg/ml**	**Augmentin 30 mg/µg/ml**	**Ketoconazole 50 mg/µg/ml**
Staph. aureus	12.33±0.67	13.33±0.88	11.33±0.88	29.00±2.08	-
Salmonella typhi	11.33±0.88	11.33±0.88	9.33±0.88	26.67±2.19	-
E.Coli	11.33±1.20	12.00±1.00	10.67±1.20	25.33±1.86	-
Shigella	17.67±1.45	12.33±1.20	14.33±0.88	20.00±2.52	-
Candida albicans	13.33±0.88	11.67±1.20	10.33±0.88	-	28.00±2.00
Candida tropicalis	12.67±0.88	12.00±1.53	9.33±0.88	-	23.00±1.15
Aspergillus niger	11.33±0.88	11.00±0.58	7.33±0.33	-	28.67±1.20
Aspergillus flavus	14.00±1.73	12.67±2.40	10.00±1.53	-	24.33±1.45

Table 3 Antimicrobial properties CoNPs of *Khaya senegalensis* Stembark Extract

Organisms	**500 µg/ml**	**250 µg/ml**	**125 µg/ml**	**Augmentin 30 mg/µg/ml**	**Ketoconazole 50 mg/µg/ml**
Staph. aureus	16.00±0.58	12.00±0.58	7.00±0.58	30.33±3.38	-
Salmonella typhi	14.33±0.67	11.00±1.15	6.33±0.33	29.67±3.18	-
E.Coli	19.67±0.88	15.00±1.15	10.00±0.58	17.00±2.86	-
Shigella	20.00±0.58	16.67±1.20	12.00±1.53	23.00±2.31	-
Candida albicans	21.33±0.88	17.33±0.88	12.67±0.88	-	24.67±1.86
Candida tropicalis	22.00±0.58	15.33±0.88	10.67±0.88	-	27.00±3.06
Aspergillus niger	23.00±0.58	17.33±0.88	13.67±0.88	-	23.67±1.76
Aspergillus flavus	23.67±0.67	19.33±1.20	15.33±0.88	-	25.67±1.45

CONCLUSIONS

CoNPs were synthesized using stem bark extracts of *Khaya senegalensis.* Synthesis was confirmed by colour changes from light brown to dark brown. The SPR value of 396 nm obtained from UV-visible spectroscopy revealed that cobalt nanoparticle was prepared as this value is within the range of other CoNPs synthesized by other researchers. Fourier transform infrared analysis revealed that aliphatic primary amine group might be responsible for the reduction of $CoCl_2$ to CoNPs. The antimicrobial activities of plants and the CoNPs against *Escherichia coli*, *Salmonella typhi*, *Shigella*, *Candida tropicalis*, *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* and *Staphylococcus aureus* show significant zones of inhibition.

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