Research Journal of Chemical Sciences ISSN 2231-606X
Vol. **8(1),** 11-17, January **(2018)** *Res. J. Chem. Sci.*

Biofabrication of cobalt Nanoparticles using leaf extract of *Chromolaena odorata* and their potential antibacterial application

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Available online at: www.isca.in, www.isca.me
Received 12th November 2017, revised 5th January 2018, accepted 15th January 2018

Abstract

Cobalt nanoparticles are gradually gaining wider applications due to their catalytic, magnetic, optical, antibacterial and biomedical properties, leading to more research on them as well as the desire to synthesize them by adopting an eco-friendly method. Here, cobalt nanoparticles were synthesized using leaf extract of Chromolaena odorata and were characterized using Ultraviolet-visible Spectroscopy (UV-vis), Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD) methods. The formation of cobalt nanoparticles was first confirmed based on a change in colour of the reaction mixture at room temperature from light brown to dark brown within 15 min. Maximum absorption peak shown at 308.00 nm in the UV-visible spectrum was due to surface Plasmon absorption of cobalt nanoparticles. The FT-IR spectrum of C. odorata leaf extract showed prominent peaks at 3280.1 (O-H stretch), 2920.0 (C-H stretch), 1625.1 (C=C stretch), 1379.1 (C-H stretch), 1222.6 (C-O-C stretch) and 1021.3 cm^{-1} (C-O-C stretch). However, the spectrum of the cobalt nanoparticles showed the absence of absorptions at 3280.1, 2920.0, 1379.1 and 1222.6 cm^{-1}, meaning that these missing functional groups were involved in the bio-reduction of cobalt ions to cobalt nanoparticles. The morphology of the nanoparticles from SEM analysis indicated irregular, cubic and hexagonal shapes of various sizes that were agglomerated. XRD analysis showed the particles to be crystalline and the average crystallite size was found to be in the range of 20-49 nm. The cobalt nanoparticles inhibited the growth of Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus and Streptococcus pyogene. The cobalt nanoparticles biofabricated from the leaf extract of C. odorata could be used in the treatment of diseases and infections caused by these pathogens.

Keywords: Cobalt nanoparticles, *Chromolaena odorata*, antibacterial application, leaf extract.

Introduction

Increasing awareness towards green chemistry and other biological processes has led to a desire to develop an eco-friendly approach for the synthesis of nanoparticles. Synthesis of metal or metal oxide nanoparticles using environmentally friendly methods without the use of harsh, toxic and expensive chemicals is the main principle of green chemistry[1]. This approach focuses on utilization of biocompatible reducing agents for synthesis of nanoparticles. The use of leaf extract, bacteria, fungi and enzymes for the synthesis of cobalt nanoparticles offer numerous benefits of eco-friendliness and compatibility for targeted applications as toxic substances are not involved. Cobalt nanoparticles possess catalytic, magnetic, optical, antibacterial and biomedical properties[2,3].

Several methods have been employed in the synthesis of cobalt nanoparticles. Some of these methods include thermal decomposition[4], ultrasonication method[2], electrochemical methods[5], DC magnetron sputtering[6], ultrasonic spray pyrolysis[3], chemical reduction method[7], and biological approaches involving microorganisms, plant extracts and agricultural wastes/biomass[8]. Some of these methods suffer some drawbacks such as unsafe reaction condition, use of expensive chemicals and instruments and longer reaction time. Chemical synthesis methods, for instance, lead to the presence of some toxic chemicals absorbed on the surface that may have an adverse effect in the medical application[9]. However, the use of plant materials in cobalt nanoparticles synthesis offer environmental friendliness and eliminate problems posed by pollution. It is also cost effective.

Chromolaena odorata is a tropical and subtropical species of flowering shrub that is native to North America and has been introduced to South America, tropical Asia, West Africa and parts of Australia. *C. odorata* is a rapidly growing perennial herb. It is a multi-stemmed shrub to 2.5 m (100 inches) tall in open areas. It has soft stems but the base of the shrub is woody. *C. odorata* has been reported to be used in traditional medicine as anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory, diuretic tonic, antipyretic and heart tonic[10,11]. Other uses of *C. odorata* in Tropical Africa include the treatment of malaria, dysentery, toothache, diabetes, skin diseases, fever and wound dressing[12,13].

The use of plant materials in the synthesis of cobalt nanoparticles has been reported by other researchers. A few of these plant materials include the leaves of *Conocarpus*

erectus[14], leaves of *Raphanus sativus var. longipinnatus*[3] and dried powdered fruits of piper longum[15]. Herein, is reported the biofabrication of cobalt nanoparticles using leaf extract of *Chromolaena odorata* and their antibacterial application.

Materials and methods

Collection of plant materials: Fresh leaves of *C. odorata* were collected from within the campus of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaf sample was identified and authenticated at the Taxonomy Section of Forestry Department of the aforementioned university. 30 g of the leaf sample was weighed out and used in the preparation of the aqueous leaf extract.

Preparation of aqueous plant extract: The collected leaves were thoroughly washed under running tap water and rinsed severally with distilled water followed by sun-drying to remove residual moisture. The dried materials were cut into fine pieces and dispersed in 200 ml of sterile distilled water in a 500 ml glass beaker and boiled at 80^0C for 15 min and were allowed to cool. After that, the solution was filtered through Whatman No. 1 filter paper (Springfield Mill. Maidstone. Kent, England) and the filtrate was used immediately for the synthesis of cobalt nanoparticles.

Synthesis of Cobalt Nanoparticles: For the synthesis of cobalt nanoparticles, 10 ml of the aqueous leaf extract was added to 90 ml of 1×10^{-3} M aqueous $Co(NO_3)_2.6H_2O$ solution in a 250 ml Erlenmeyer flask. Within 15 min. change in colour was observed from light brown to dark brown indicating the formation of cobalt nanoparticles. The cobalt nanoparticles solution obtained was purified by repeated centrifugation at 10,000 rpm for 15 min. followed by re-dispersion of the pellet in deionized water. Then the cobalt nanoparticles were dried in an oven at 80^0C and then allowed to cool. The particles were stored in an airtight container.

UV-visible Spectroscopy Analysis: The bioreduction process of cobalt ions in aqueous solution was measured by the sampling of 1 ml aliquot compared with 1 ml of distilled water used as blank and subsequently measuring the UV-visible spectrum of the solution. UV-visible spectrum was monitored on Cary Series UV-vis spectrophotometer Agilent Technology, operated within the wavelength range of 200 to 800 nm.

FT-IR spectroscopy measurement: This was carried out on *C. odorata* leaf extract and on the cobalt nanoparticles. FT-IR measurement of the samples 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 4000-650 cm^{-1}.

Scanning electron microscopy analysis: Morphology of the nanoparticles was studied using SEM analysis (MODEL - PHENOM ProX Scanning Element Microscope manufactured by PhenomWorld Eindhoven, the Netherlands).

X-ray diffraction 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-Kα radiation in a θ -2 θ configuration with a wavelength (λ) of 1.541A. The sample was made smoother and was imparted on a slide which was then charged into the machine after adjusting the machine parameters and was operated via a monitor.

Antibacterial susceptibility assay: Agar diffusion method was employed for the antibacterial susceptibility assay. The test organisms for the study were *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus pyogene*. The pure clinical isolates were obtained from the Pathology Laboratory of National Root Crops Research Institute, Umudike. All the clinical isolates were checked for purity and were maintained on nutrient broth at 4^0C in the refrigerator until required for use. Nutrient agar was poured into sterile Petri dishes and was allowed to solidify. 1 ml of the test culture was dropped on the solidified agar and the organism was spread all over the surface of the agar using a spreader. Wells of approximately 5 mm in diameter were made on the surface of the agar medium using a sterile cork borer. The plates were turned upside down and the wells labelled with a marker. Each well was filled with 0.2 ml of the cobalt nanoparticles solution. The plates were incubated aerobically at 37^0C for 24 hours. The sensitivity of the organisms to the nanoparticles was recorded. The minimum inhibitory concentration (MIC) was also determined by comparing the different concentrations of the nanoparticles having different zones and selecting the lowest concentration.

Results and discussion

UV-visible spectroscopy: The formation of cobalt nanoparticles was first confirmed based on a change in colour of the reaction mixture at room temperature from light brown to dark brown within 15 min. This was followed by UV-vis spectroscopy which is frequently used to characterise synthesized metal nanoparticles. Figure-1 shows the UV–vis absorption spectrum of the synthesized cobalt nanoparticles. The maximum absorption peak was shown at 308.00 nm due to surface Plasmon absorption of cobalt nanoparticles. The surface Plasmon absorption in the metal nanoparticles is due to the collective oscillation of the free conduction band electrons which is excited by the incident electromagnetic radiation. The change in colour of the reaction mixture was also due to this surface Plasmon resonance phenomenon which provides a convenient indication of the formation of cobalt nanoparticles.

FT-IR spectroscopy: FT-IR spectroscopy was applied to investigate the interactions between the aqueous leaf extract of *C. odorata* and the aqueous solution of the cobalt salt. The FT-

IR spectra of *C. odorata* leaf extract and that of the cobalt nanoparticles biofabricated from it are shown in Figures-2 and 3 respectively. The FT-IR spectrum of the leaf extract showed prominent absorption peaks at 3280.1, 2920.0, 1625.1, 1379.1, 1222.6 and 1021.3 cm^{-1} corresponding to O-H stretch, C-H stretch, C=C stretch, C-H stretch, and C-O-C stretch. A look at the spectrum of the cobalt nanoparticles shows that absorptions at 3280.1, 2920.0, 1379.1 and 1222.6 cm^{-1} are all missing. It, therefore, follows that these missing functional groups were involved in the bio-reduction of cobalt ions to cobalt nanoparticles. More evidence of chemical reaction is drawn from the fact that new prominent peaks appeared on the spectrum of the cobalt nanoparticles. These peaks are at 883.4 and 793.9 cm^{-1} suggesting the presence of C-H out-of-plane bending of aromatics. So, the nanoparticles are associated with other molecules. Similar observations on the association of nanoparticles with other molecules have been reported by other researchers[16]. The absorption peak at 1021.3 cm^{-1} appears broad in the spectrum of the extract but narrow and small in the nanoparticles spectrum. All these confirm that water-soluble phytochemicals present in the leaf extract of *C. odorata* have the ability to perform dual functions of reduction and stabilization of the cobalt nanoparticles.

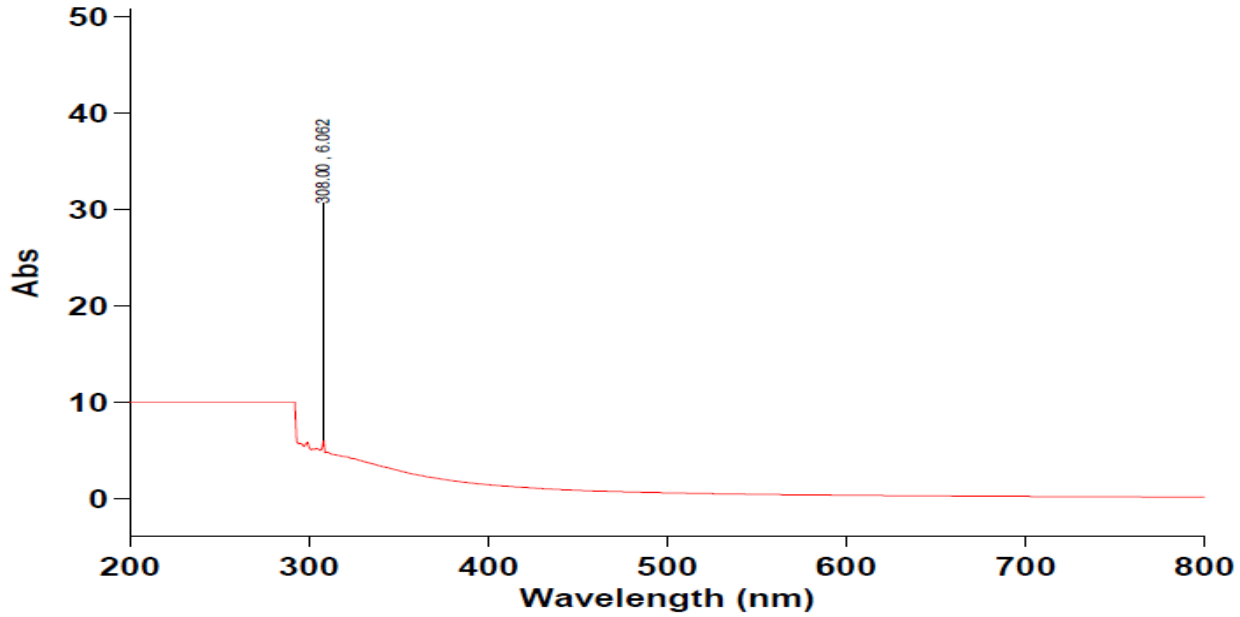

Figure-1: UV-vis absorption spectrum of cobalt nanoparticles biofabricated using *C. odorata* leaf extract.

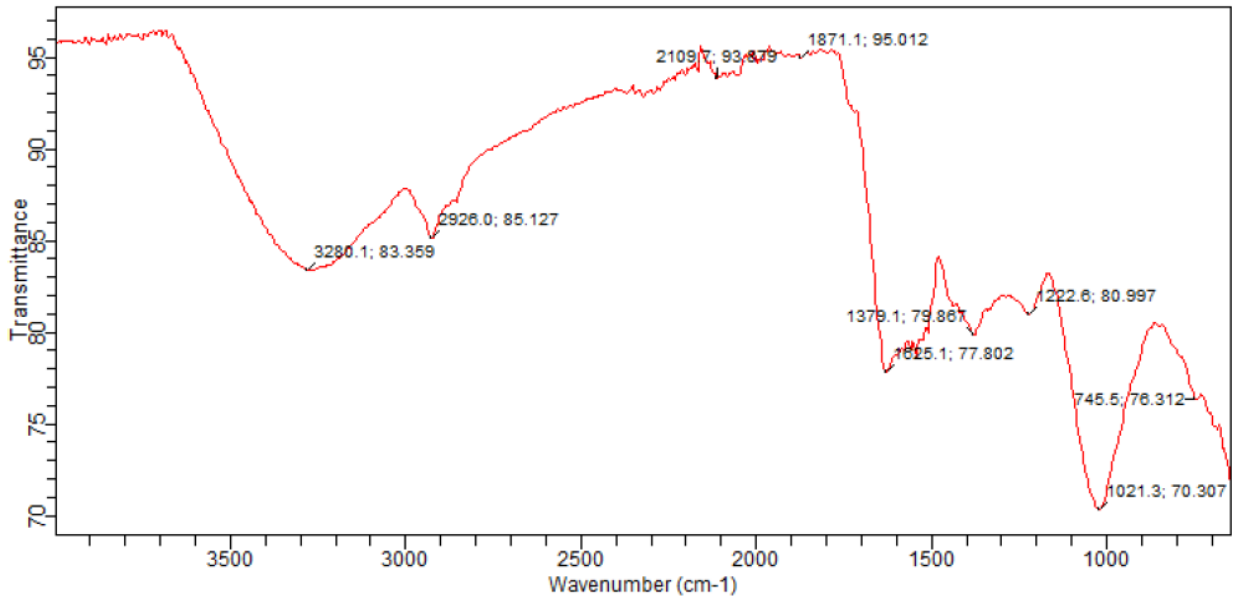

Figure-2: FT-IR spectrum of *C. odorata* leaf extract.

SEM Analysis: The SEM images of cobalt nanoparticles are shown in Figure-4. The morphology of the nanoparticles indicates irregular, cubic and hexagonal shapes of various sizes that are agglomerated. Further observations with higher magnifications reveal that these images possess smooth surfaces. At much higher magnification the images are seen as large particles which can be attributed to aggregation or clustering of smaller particles.

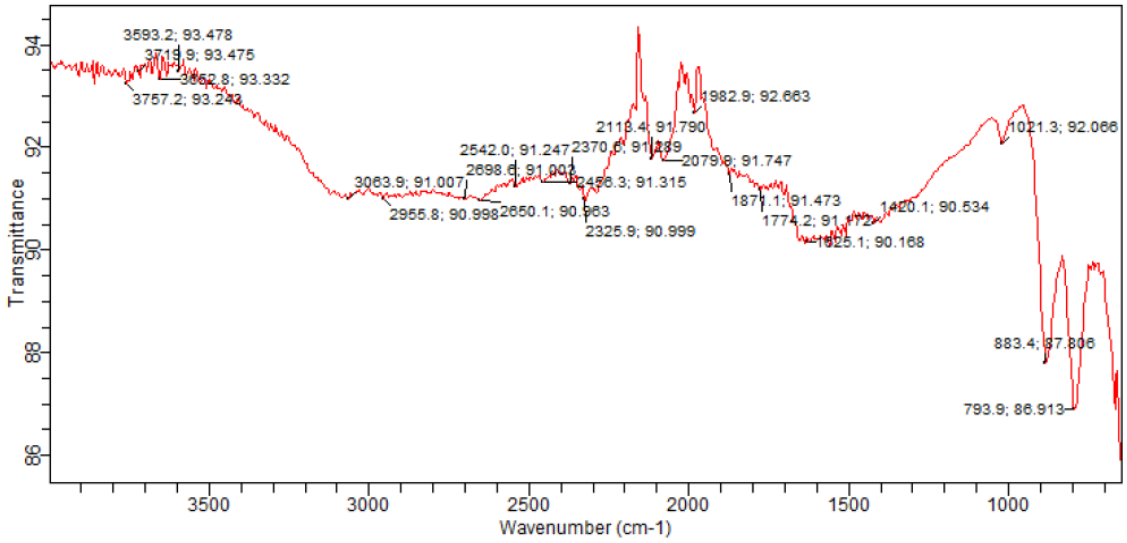

Figure-3: FT-IR spectrum of cobalt nanoparticles biofabricated using *C. odorata* leaf extract.

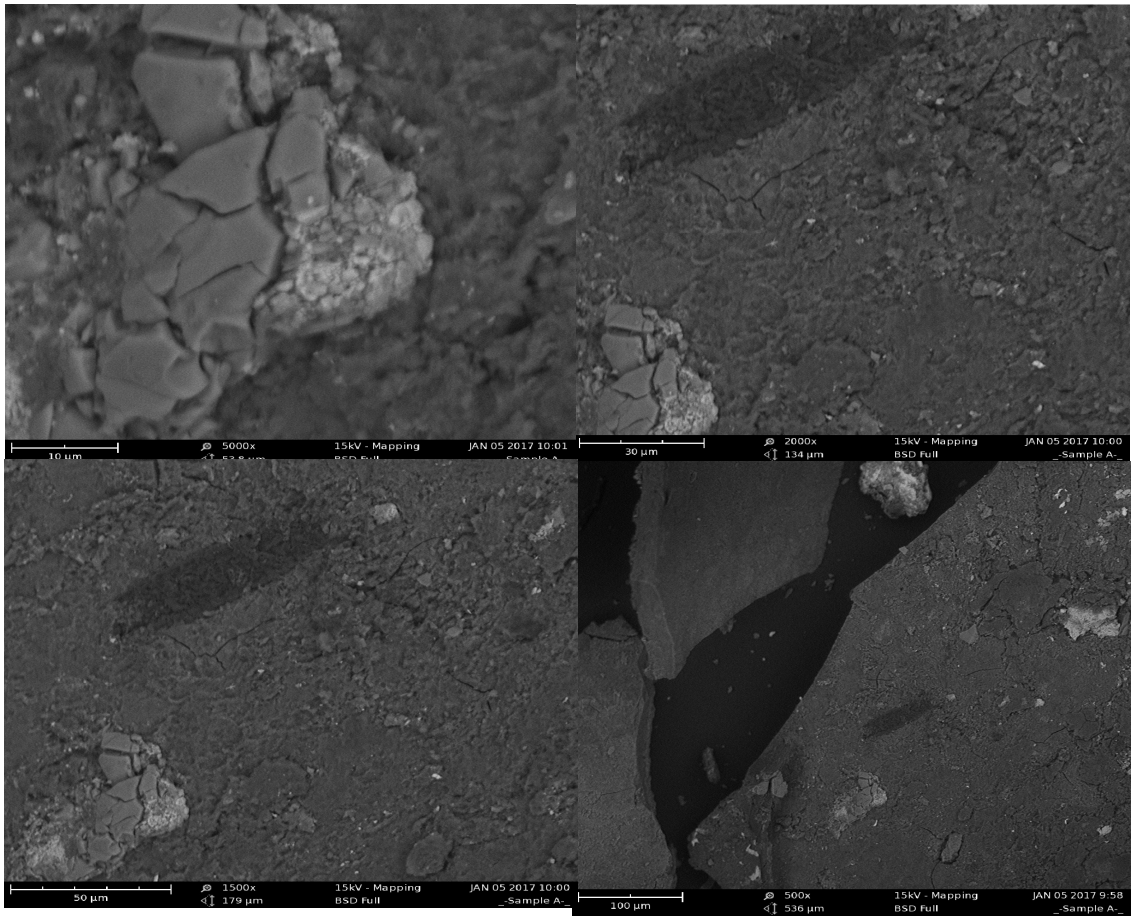

Figure-4: SEM images of cobalt nanoparticles biofabricated using leaf extract of *C. odorata* at different levels of magnification.

XRD Analysis: Figure-5 shows the XRD pattern of cobalt nanoparticles biofabricated from the leaf extract of *C. odorata*. A number of Bragg reflections with 2θ values of 9.75^0, 16.85^0, 26.34^0, 31.17^0, 36.48^0, 40.09^0, 49.27^0, 53.33^0 and 73.50^0 were observed within the angle range of $5.50\text{-}74.96^0$. The XRD pattern indicates that the cobalt nanoparticles formed are crystalline in nature with a mixed phase structure (cubic, hexagonal and irregular) of cobalt nanoparticles. The average crystallite size of the cobalt nanoparticles was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Debye-Scherrer equation shown below.

$$D = \frac{k\lambda}{\beta \cos\theta}$$

Where: D is the particle size (in nm), *k* is a constant equal to 0.9, λ is the wavelength of X-ray radiation (1.541*A*), β is the full-width at half maximum (FWHM) of the peak (in radians) and 2θ is the Bragg angle (in degrees). The average crystallite size was found to be in the range of 20-49 nm.

Antibacterial susceptibility assay: The zone of inhibition of cobalt nanoparticles biofabricated from the leaf extract of *C. odorata* against four pathogens is shown in Table-1. Two each of Gram-negative and Gram-positive bacteria organisms were used for the study. These are human pathogens capable of causing diseases ranging from skin infections, pneumonia, meningitis, bacteraemia, sepsis, toxic shock syndrome, urinary tract infections, vomiting, diarrhoea, anaemia, kidney infections, osteomyelitis, endocarditis, septicaemia, lung infection to wound infections[17]. The surfaces of the cobalt nanoparticles might have interacted directly with the bacterial outer membrane, causing the membrane to rupture thereby killing the organism. So, the antibacterial activity exhibited by the cobalt nanoparticles here is attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes. Flagyl was used as a positive control in the experiment. The minimum inhibitory concentration (MIC) of the cobalt nanoparticles is shown in Table-2. From the MIC, the degree of inhibition is summarized as *S. aureus* = *S. pyogene* > *E. coli* = *K. pneumonia*.

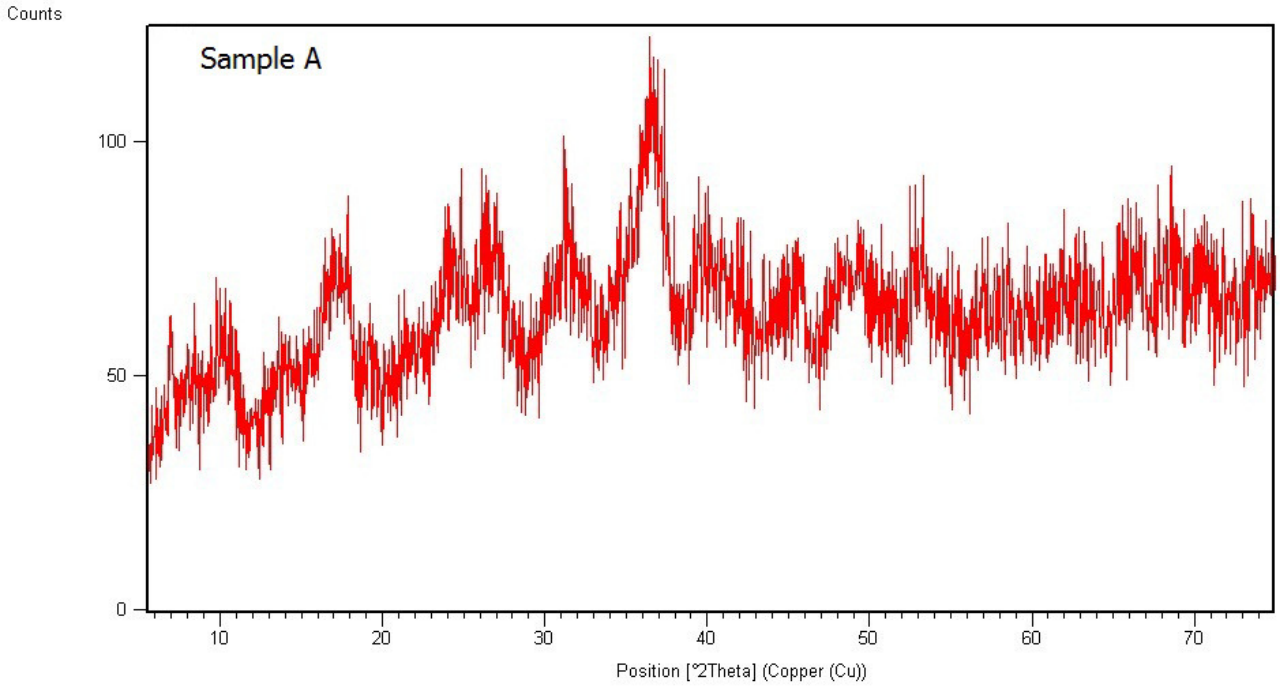

Figure-5: XRD pattern of cobalt nanoparticles biofabricated from the leaf extract of *C. odorata.*

Table-1: Zone of inhibition (mm) of cobalt nanoparticles against pathogens.

Concentration of cobalt nanoparticles (mg/ml)	*E. coli* (mm)	*K. pneumonia* (mm)	*S. aureus* (mm)	*S. pyogene* (mm)	Positive control (mm)
25.00	-	-	2.73	1.73	18.20
50.00	3.90	4.00	4.30	3.68	25.00
100.00	5.60	5.25	6.85	5.00	34.80

Table-2: Minimum inhibitory concentration of cobalt nanoparticles against pathogens.

Concentration of cobalt nanoparticles (mg/ml)	*E. coli*	*K. pneumonia*	*S. aureus*	*S. pyogene*	Positive control (Flagyl)
3.5	-	-	-	-	+
6.25	-	-	-	-	+
12.5	-	-	+	+	+
25	+	+	+	+	+

Conclusion

Cobalt nanoparticles were synthesized using leaf extract of *C. odorata* which is a green method of nanoparticles synthesis that does not introduce harmful substances into the environment and ensures cost effectiveness. The particle size was calculated to be in the range of 20–49 nm. These cobalt nanoparticles inhibited the growth of *E. coli*, *K. pneumonia*, *S. aureus* and *S. pyogene*. Therefore, it is pertinent to conclude that the cobalt nanoparticles could be used in the treatment of diseases and infections caused by these organisms.

Acknowledgement

The authors are grateful to Mr I. K. Ndukwe of Plant Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, for identifying and authenticating the plant sample.

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