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Synthesis of Cobalt Nanoparticles Biologically by *Conocarpus erectus* L. Aqueous Leaves Extract

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Abstract

Biologically, the nanoparticles of cobalt synthesized by aqueous leaves extract of *Conocarpus erectus* L. The cobalt nanoparticles appear rapidly with change in color when the leaf extract mixed with $CoCl_2.6H_2O$ (0.1mM). The cobalt characterized by UV Visible Absorption Spectrometer (UV-S), X-Ray Diffraction nanoparticles were(XRD), Fourier Transform infrared (FTIR), and Scanning Electron Microscopes (SEM).By using UV-S, the wave length was around 350-400 nm, while the size of cobalt nanoparticles was around 4.9 nm and the size particles of leave extract alone was 17.1 nm,characterized by XRD. The SEM technique represent more spherical aggregation in cobalt nanoparticles then in leaves extract alone. The technique of FTIR showed the presence of active sites for alcoholic, phenolic, amines and other compounds.
Keywords:*Conocarpus* sp., Cobaltnanoparticles, UV-S, XRD, FTIR,SEM, aqueous extract.

Introduction

Nanoscience is definedasthestudy of phenomena and the manipulation of materialsat theatomic,molecular and macromolecular scales, wherethe propertiesdifferfrom those at a larger scale (Mannino&Scampicchio, 2007).Nanoparticles have many properties : small size (1-100nm),large surface to volume ratio,chemically alterable physical properties,change in the chemical and physical properties with respect to size and shape,structural sturdiness in spite of atomic granularity,enhanced or delayed particles aggregation depending on the type of the surface modification,enhanced photoemission, high electrical and heat conductivity and improved surface catalyticactivity (Taylor & Francis, 2007).The aim of manufacturing modern materials that are scaled at nano level has led to grow the field of Nanotechnology rapidly to be applied in different aspects in terms of technology and science(Albrecht *et al*., 2006).Cobalt based NPs areadopted inbiomedicineandbiotechnology fields,such as carriers for targeteddrug delivery(Lu*etal*., 2007;Sun *etal*., 2008).Cobalt nanoparticles (CoNPs) were one of the

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importantdegradation products.(Milosev&Remskar, 2008).CoNPs are explored for a wide range of applications including catalysts(Yao *etal*., 2013), sensors(Mattila*etal*., 2014), magnetic resonance imaging (MRI) probes(Medvedeva*et al*., 2017), and antibacterial agents(Varaprasad*et al*., 2017).Several methods have been employed in the synthesis of cobaltnanoparticles. Some of these methods include thermal decomposition(Sowka*etal*., 2006), ultra-sonication method(Hoseyni*et al*., 2017), electrochemical methods(Ledo*etal*., 2006), DC magnetron sputtering(Chung and Liu, 2004), ultrasonic spraypyrolysis(Koyyati*etal*., 2016), chemical reduction methodand biological approaches involving microorganisms, plant extracts andagricultural wastes/biomass(Ullah*et al*., 2014).The use of plant materials in the synthesis of cobalt nanoparticles has been reported by other researchers (Kadhim&Abd, 2018; Alrubaie&Kadhim, 2019). The green syntheses of cobalt using leaves of *Conocarpuserectus* achieved by Ahmed*et al*.(2016). *Conocarpus erectus*is utilized against iron-deficiency, catarrh, conjunctivitis, gonorrhea, diabetes, prickly heat, fever, migraine, dying, tumors, orchitic, diarrhea, syphilis and swelling,(Ayoub, 2010; Abdel-Hameed*et al*., 2012and Shohayeb*et al*., 2013).The leaves are eaten and their decoction is inebriated for fever. The bark and the product of this species are utilized as inoculate in the treatment of the wounds, diabetes,hemorrhoids and Diarrhea (Raza *et al.*, 2016).A portion of the proven natural properties of *C. erectus* are hepaticprotective (Abdel-Hameed *et al*., 2013),antioxidant (Abdel-Hameed *et al*., 2014), anticancer (Abdel-Hameed et al;2012)and also antimicrobial (Shohayeb*et al*., 2013). This study aimed to syntheses the cobalt nanoparticles by *C. erectus*leaves which is as a save green material and to know the difference between plant extract alone and cobaltnanoparticles extract.

Materials and Methods

Preparation of aqueous leaf extract of *Conocarpus*

Fresh leaves of ***Conocarpus erectus*** were collected at March 2020 from the house gardens in Hilla ,Iraq . The specimen of the plant was identified in Plant Herbarium / Department of Biology /College of Science/ University of Babylon. The leaves of ***Conocarpus erectus***were washed , dried then ground into a fine powder by suitable grinder.

*Conocarpus*leaves extract was prepared by adding 10 gm of dry *Conocarpus* leaves powder in 100 ml sterile distilled water(w/v) and heated using magnetic stirrer at 60˚C and 700 rpm for 30 minutes , then filtered using Whatman filter paper no.1. The filtrate was collected and stored at 4˚C for further use.

Synthesis of cobalt nanoparticles and nano leaves extract

Cobalt chloride solutionof0.1mMwas prepared. Drop wise 25 ml of leavesextract has been added and colorchanges were observedafter 30 min.By adding 1M NaOH

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solution, the pH ischecked and adjusted to 12.Dispersing in sterile purified water and 700rpm centrifugation for 30minutes three times. The particles with black colorwere subsequently washed by ethanol for removing the impurities fromthe final products. After drying by vacuum oven at 60 °C forsix hour in order to obtain a black crystal. The leaves extract alone prepared in the same way of CoNPs.

Characterization of CoNPs

UV-Visible spectroscopic analysis

UV-Visible spectrum analysis necessary to go for reveals thespecific type of nanoparticle absorbing a specific wavelength of light.This property can distinguish cobalt nanoparticle from others and can also state whether it is cobalt or not present in the solution. UV-Visible spectroscopy works on the principle of light absorption depending on the concentration of particles in the solution. The UV–Vis spectroscopy measurements from 200 to 550 nm.TheCoNPs dispersedin deionized water give the highest peak between 350 -400 nm(Jayaseelan*et al*. 2012). The UV-Visible spectrum for plant extract was the same.

FTIR analyses for CoNPs and leaves extract

In FTIR, The vibration of chemical bonds can be measuredbecausechemical bonds can absorb infrared energy at specific frequencies orwavelength. The basic structure of the compound can be determined byspectral location of their IR absorption. It can also state about othermolecules being associated on the surface of nanoparticle and thuspredicts possible interaction of nanoparticles with other molecules.TheFTIR range of the dried sample was documented in the range 4000-400cm^{-1} (Sadhasivam*et al.*, 2010).

XRD analysesfor CoNPs and leaves extract

The XRD measurement was carried out for the identification of the crystal of cobalt nanoparticles .The biosynthesized CoNPswere dried powdered in order to analyze XRD pattern .The phase formation and purity of metallic nanoparticles were checked through XRD patterns which were recorded using powder X-ray diffract meter. XRD analysis was performed using at a step size of 0.02˚, scanning rate of 2˚ in 2θ/min and a 2θ range from 20 to 80 A˚ , a voltage of 40 kV and a current of 30 mA with Cu (Sadhasivam*et al*., 2010).In this technique the angles were between 26.2, 41.8 and 65.4 A˚ for*Conocorpus* particles of leaf extract without cobaltchloride. The angles were 25.1, 32.2 and 55.9A˚for the cobalt nanoparticles.Depending on the Scherrer equation: $D=k\lambda/\beta \cos\theta$, where D is the particle size, k is the Scherrer's constant (0.9 to 1.0 for spherical particle), β the width at half maxima of peaks in XRD, θ the corresponding angle for peaks and λ is the x-ray wavelength, the size of particles is determined for the average of these angles.

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Scanning Electron Microscope (SEM) Analysis

The samples of CoNPs were investigated for morphology using scanning electron microscope SEM.

Field Emission Scanning Electron Microscopy (FESEM) Analysis

The samples of CoNPs and leaves extract alone were investigated for morphology using field emission scanning electron microscope FESEM (Nath*et al*.,2018).Energy dispersive X-ray spectroscopy (EDX) was determined the quantitative and qualitative analysis may involve the formation of CoNPs and leaves extract alone.

Results and Discussion

Preparation of cobalt Nanoparticles and*Conocarpous* leave extract

The reaction between *Conocarpous* extract and salt ofcobalt chloride dehydrate solutionas shown in thefigure1.C, and the sizemaintenance of the *Conocarpous*leavesextract as shown in thefigure1.A utilized as a stabilizer and reduceragent to synthesize nanoparticles from nano-formed precursorparticles by placing them together to make each one in contact with other.The formation of a solution of dark color has been referred to occurring where the adding of sodium hydroxide (NaOH) as an accelerator to increase the decrement rate and the process of nucleation when placed in high pH=12 alkaline environment(Nishimura *et al*., 2011; Balavandy*et al*., 2015; Alrubaie&Kadhim, 2019).For the synthesis of cobaltnanoparticles, within 30 minchange in color wasobserved from light brown to dark brown indicating theformation of cobalt nanoparticles asshowed infigure 1.C. The cobalt nanoparticles after dried itin oven at 80^{0}C and then allowed to cool, the particles werebe as black crystals.

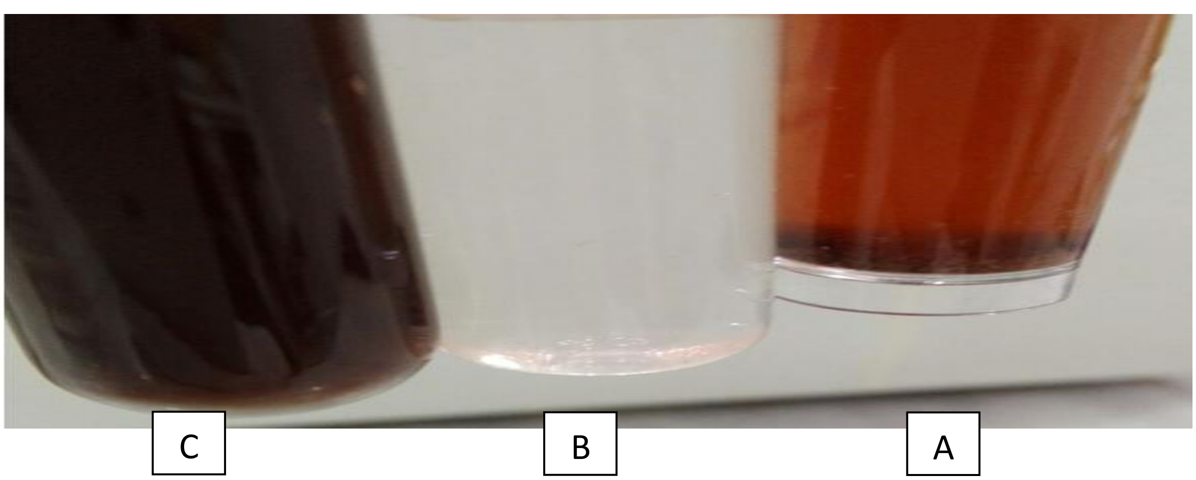

Figure 1: Synthesis stages of CoNPs by time and change the color. A: *C. erectus* leaves extract, B: $CoCl_2$, C:mixed of *C. erectus* leaves extract and $CoCl_2$ (CoNPs).

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UV-visible Spectroscopy Analysis

The formation of cobalt nanoparticles was first confirmed based on a change in color of the reaction mixture at room temperature from lightbrown to dark brown within 30 min. This was followed by UV-visspectroscopy which is frequently used to characterize synthesized metalnanoparticles.Figure2shows the UV–visabsorption spectrum of the synthesized cobalt nanoparticles.The maximum absorption peak was shown at 400nm due tosurface Plasmon absorption of cobalt nanoparticles. The surfacePlasmon absorption in the metal nanoparticles is due to thecollective oscillation of the free conduction band electronswhich is excited by the incident electromagnetic radiation. Thechange in color of the reaction mixture was also due to thissurface Plasmon resonance phenomenon which provides aconvenient indication of the formation of cobalt nanoparticles.

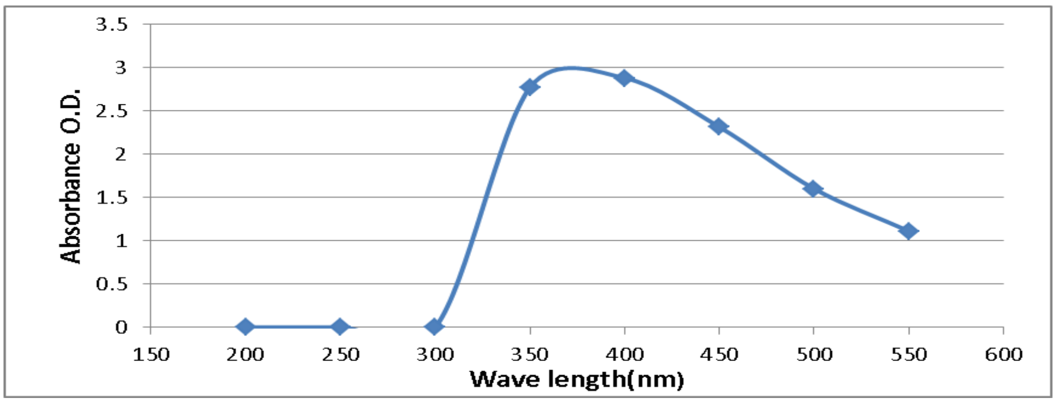

Figure (2):Absorption spectrum (nm) of CoNPs synthesized by *C. erectus* leaves extract

Fourier transform infrared (FT-IR)

FT-IR spectroscopy was applied toinvestigate the interactions between the aqueous leaf extract of*C. erectus*and the aqueous solution of the cobalt salt. The FT-IR spectra of*C. erectus*leaf extract and that of the cobaltnanoparticles bio fabricated from it are shown in figures3-A and 3-Brespectively. The FT-IR spectrum of the leaf extract (figure 3-A) showedprominent bands at 3402.43, 3248.18, 2283.72, 2113.98, 2029.11, 1620.21, 1373.32, 1188.15, 1111.00, 1072.42, 609.51, 493.78 cm^{-1}which in general corresponding to O-Hstretch, C=Ostretch, C-Ostretch,C-H stretch, and C-N. A look atthe spectrum of the cobalt nanoparticles (figure 3-B) shows that absorptionsbands at 3417.86, 3263.56, 2283.72, 2252.86, 2214.28, 2113.98, 2021.40, 1620.21, 1396.46, 1195.87, 1072.42, 624.94, 478.35 cm^{-1}which in general corresponding to O-H, C=O,C=O, C-O, C-H, and C–N where the stretching in all are missing.It, therefore, follows that these missing functional groups were involved in the bio-reduction of cobalt ions to cobalt nanoparticles. Chemical reaction is drawn from the fact that new prominent bands appeared on the spectrum of the cobalt nanoparticles. These bands aresuggesting the

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presence of C-H out-of-planebending of aromatics.So, the nanoparticles are associated withother molecules. Similar observations on the association ofnanoparticles with other molecules have been reported by otherresearchers (Caroling*et al*.,2015).The bands can be allocating to the secondary amine waging at 867.97 cm^{-1}. C – N stretching amine could be allocated to the bands at 759.95, 684.73, 609.51, 493.78,464.84cm^{-1}. The shift of bands to significantly lesser frequency give indications for the depositions of these compounds in CoNPs synthesis. For instance1620 reduced to1396 to 1195 to 1072 to 624 cm^{-1}. In addition, a substantial band acted 478 cm^{-1}, owned by Co-O vibrationalstretching, which more detection on CoNPs formation (Awwad*et al*., 2014). The existence of phenolic compounds and proteins was assured by the functional vibrations bands group as demonstrated in the FTIR spectrum. All these confirm that water-solublephytochemicals present in the leaf extract of *C.erectus* havethe ability to perform dual functions of reduction andstabilization of the cobalt nanoparticles.

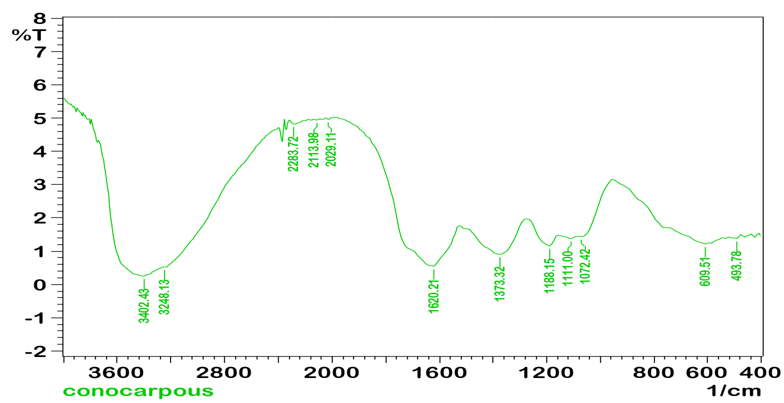

Figure 3-A: FT-IR spectrum of *C.erectus* leaf extract.

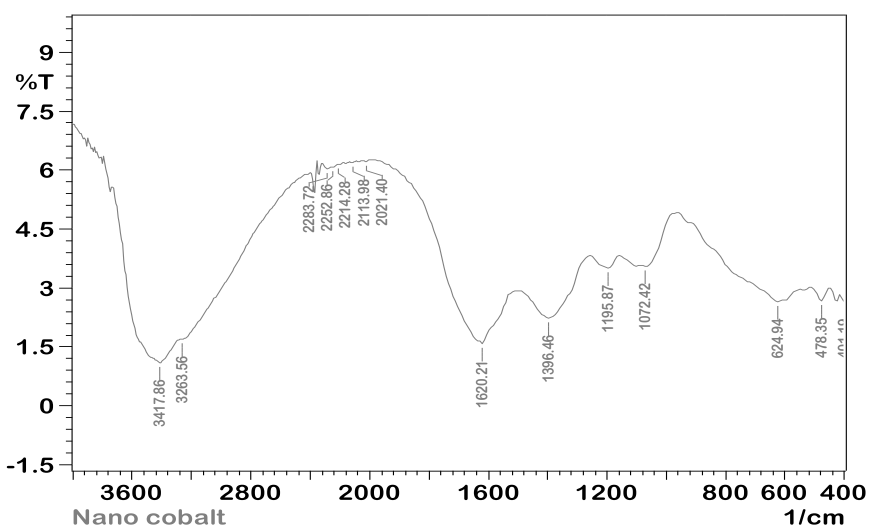

Figure 3-B: FT-IR spectrum of cobalt nanoparticles synthesized by *C. erectus* leaves extract.

X-ray diffraction (XRD)

The crystallography of CoNPs examined by X-ray diffraction,X-RD Spectra offers an insight into the crystallinity of nanoparticles figure 4.Brepresenting XRD spectra of CoNPs synthesized with extract of *C. erectus* leaves.

In this technique the angles were between 26.2,41.8 and 65.4 A°. Depending on the Scherrer's equation, the size of *C. erectus* particles of leaf extract alone, is around 17.1nm for the average of these angles 26.8A° as shown in the figure4-A. The size average of CoNPswas 4.9 nmdepending the angles were between 25.1,32.2 and 55.9A°(figure 4-B).

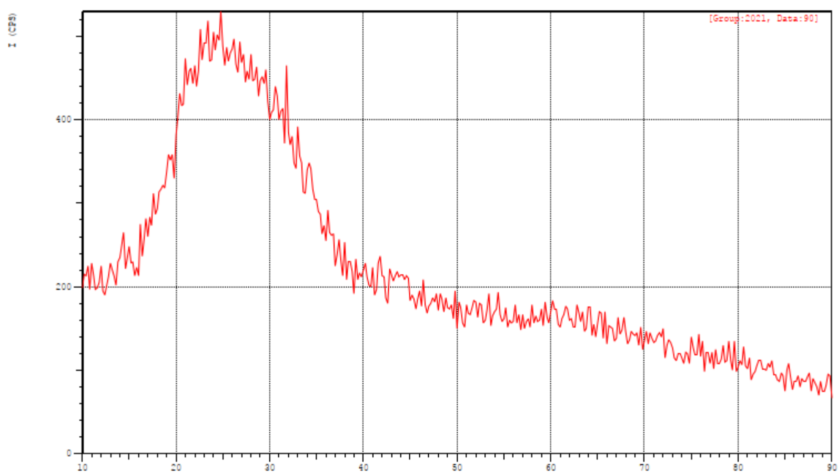

Figure 4-A: X-ray diffraction (XRD) analysis of*C. erectus* extract of leave extract.

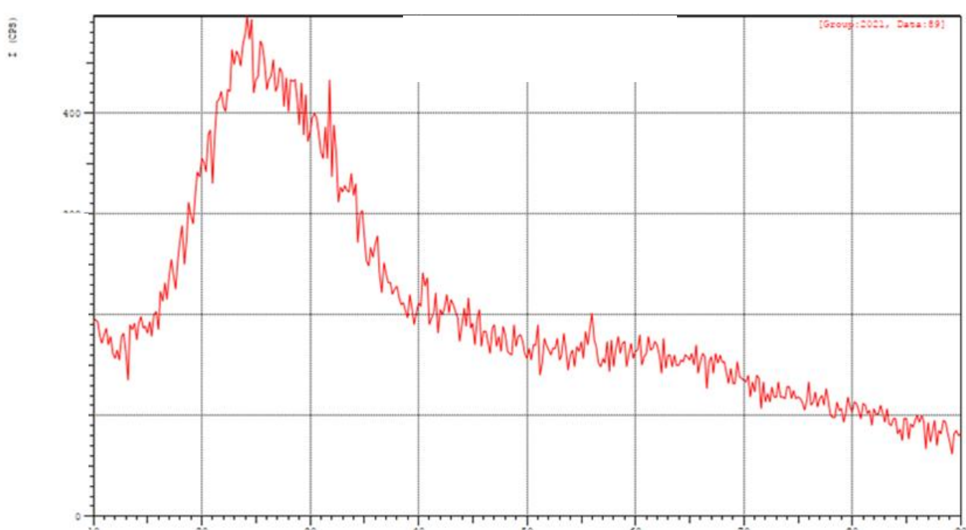

Figure 4-B: X-ray diffraction (XRD) analysis for CoNPs synthesized by *C. erectus* leaves extract.

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The anglesdegrees may not much differentbetween the plant extract alone (figure 4-A) and CoNPs (figure 4-B) butthe width at half maxima of peaks is bigger. This belong to Co element present and it make the nanoparticles smaller (4.9nm). Depending on the XRD analysis,Hafeez*etal*. (2020) refer to synthesize the cobalt oxide with 40-50 nm by *Populus ciliate*, while Diallo et al. (2015)refer to synthesize the CoNPs with a size 3.57 nm by using*Aspalathuslinearis*. This differences in size belong to differ in reducing agents (plant extracts).

SEM Analysis

The SEM images of cobalt nanoparticles are shown in figure5.The morphology of the nanoparticles indicates spherical shapes of various sizes that are agglomerated.Further observations with highersurfaces. At much highermagnification the images are seen aslarge particles which can be attributed to aggregation orclustering of smaller particles. Figure 6show themorphologyof the leave extract of *C. erectus* alone, its be larger than in figure 5 and more agglomerated.

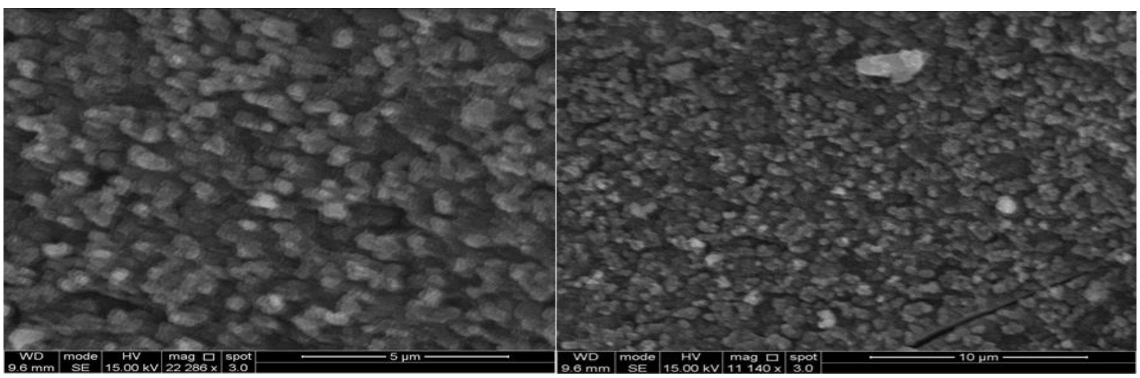

Figure5: SEM of CoNPs synthesized by *C. erectus* leaves extract in different magnifications powers.

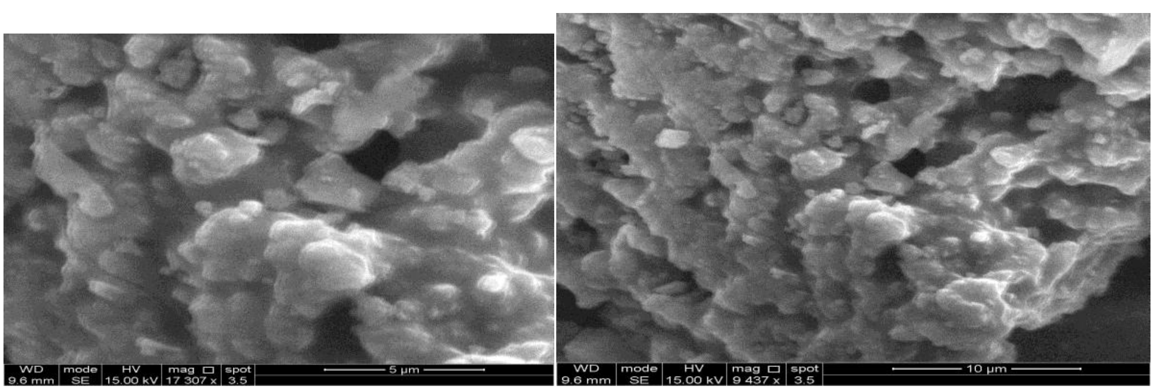

Figure6: SEM of *C. erectus* leaves extract alonein different magnifications powers.

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Nazeruddin*et al*., (2014) refer that agglomerations in the particles depend upon the nature ofthe extract, and the compounds present in the extract because biomolecule cap and stabilize the individual particle. Reactivityand attraction of the functional groups results in the formation oflarger size particles. These particles have coatings of the differentbiological compounds which have surface hydroxyl groups. Dueto intermolecular hydrogen bonding among these agents, the particles appear to be agglomerated (Bibi *et al*., 2017).

Energy dispersive X-ray spectroscopy (EDX)

The structural characterization of CoNPs was implementedutilizing an analysis of dispersive energy X-ray spectroscopy (EDX).Figure7 shows the element quantitative and qualitative analysis mayinvolve the formation of CoNPs. The obtainable composition from the analysis ofEDX were many peaks which identified asoxygen (42.09%), carbon (53.02%) , magnesium (3.28%), fluorine (0.27%), calcium (1.29%), aluminum (0.05%), and cobalt (0.01%).The C and O showed the absorption peaks ofhigher counts.The trace amounts existence of cobalt demonstrated thatplant phytochemical groups are involved in reducing and capping ofsynthesized CoNPs (Bala*et al.*,2015).The structural characterization of*C. erectus* leaves extract was implementedutilizing ananalysis of dispersive energy X-ray spectroscopy (EDX). Figure8 shows the element quantitative and qualitative analysis that existed in the aqueous leaf extract of *C. erectus*.The obtainable composition from the analysis ofEDX were many peaks which identified ascarbon (53.75%),oxygen (37.78%),Tin(total inorganicnitrogen) (0.07%), calcium (3.64%), iron(0.12%), magnesium (3.97%),sodium(0.36%).The difference between CoNPs and the aqueous leaf extract of *C. erectus*was the presence of cobalt in the cobalt nanoparticles and its trace in the aqueous leaf extract of *C. erectus*.This result may indicate that the cobalt is as trace element. The presence of cobalt chloride with the extract of *C.erectus* caused change the percentages of the rest element when compare between the figures 7 and 8. The presence high percent of oxygen may refer to form CoONPs (Bibi*et al*., 2017).

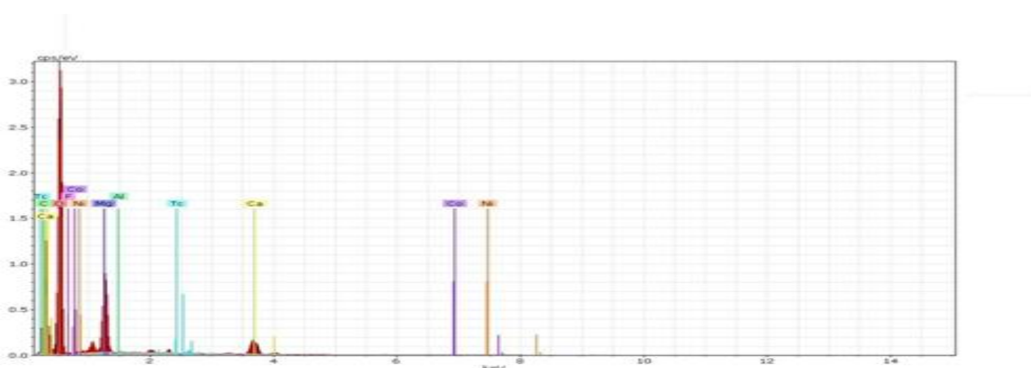

Figure7: EDX Spectrum of CoNPs synthesized by *C. eructus* leaves extract.

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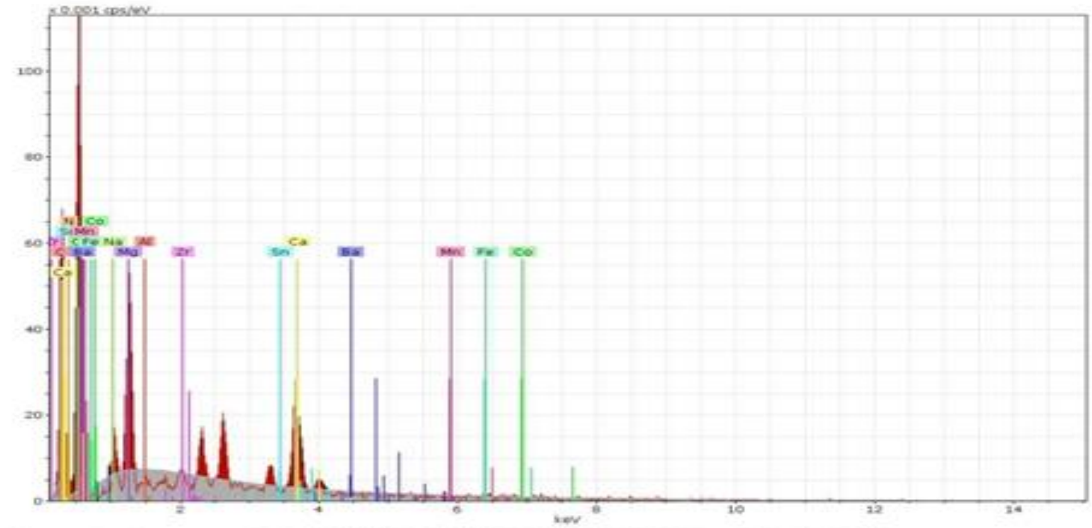

Energy

Figure8:EDX Spectrum of*C. erectus*leaves extractalone.

Conclusions

1- The synthesis of cobalt nanoparticles using leaf extract of *C. erectus* L. is a simple, efficient and represent a rapid method with very small size. 2-It is possible to synthesize an organic nanomaterials from plants parts in a simple and safe manner.

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