Cobalt Nanoparticle Synthesis, Identification, and Microbial Evaluation Using the Drumstick Leaf Extract through Sustainable Route

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Abstract: We synthesize the cobalt nanoparticles using drumstick leaf extract and cobalt chloride. We use drumstick leaf extract as both a reducing and capping agent. We synthesized the cobalt nanoparticles using an extract from drumstick leaves. We subsequently subjected these nanoparticles to analysis using advanced techniques like SEM, FTIR, UV-Vis spectroscopy, and EDX, yielding a substantial amount of valuable data. When administered in the form of nanoparticles, drumstick leaf extract has significant inhibitory effects on both gram-positive and gram-negative bacteria, such as E. coli and staphylococcus bacteria. Research is essential to understand the absorption and metabolism of vitamin B12 by cobalt. Artificial nanoparticles have a crucial role in the field of medicine, as they can serve as drug carriers or high-quality precursors.

(Received August 08, 2024; Accepted September 05, 2024)
Key Words: NPs, FTIR, UV-Vis, SEM, EDX
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1. Introduction

The word "nano-particle" was first introduced in the 1980s. Nanotechnology is an innovative technology with numerous applications. Nanoparticles are particles that have a size ranging from one to one hundred nanometers. Nanotechnology is the scientific discipline that focuses on the manipulation of matter at the atomic and molecular levels. Wet nanotechnology and Dry nanotechnology are the two classifications of nanotechnology that are distinguished by the use of living and nonliving substances. Nanoparticles can be classified into two primary types based on their chemical production: inorganic nanoparticles, which include gold (Au), silver (Ag), titanium (Ti), and zinc (Zn), and organic nanoparticles, which consist of carbon and fullerene. The nanoparticles are classified into three categories: ultrafine, fine, and coarse, based on their diameter, which ranges from 1 to 10,000 nm. In the field of medical and pharmaceutical chemistry, the use of produced nanoparticles is of utmost importance due to their potential as a valuable instrument. Nanoparticles are classified into six categories based on their physical and chemical characteristics: Lipid-based nanoparticles, Fullerenes, Metal nanoparticles, Ceramic nanoparticles, Semiconductor nanoparticles, and Carbon-based nanoparticles. Nanoparticles can exhibit many architectures, including zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), or complex configurations, depending on their dimensions. Due to extensive research on nanoparticles in the past decade, only a limited number of synthesis methods are available for nanoparticle synthesis. The methods used in this context are the Top-down methodology, Dry grinding, Wet grinding, Bottom-up method, Chemical vapour deposition (CVD) method, Physical vapour deposition (PVD) method, Liquid phase method, Chemical reduction of metal ions, Sedimentation methods, and Sol-gel process. All techniques have certain qualities such as control over particle form, size, crystal structure, enhancement of nanoparticle purity, control over aggregation, consistency of physical attributes, and greater repeatability.

The main techniques employed for the characterization of NPs include X-ray diffraction (XRD) [3], scanning electron microscopy (SEM), transmission electron microscopy (TEM), infrared (IR), polarised optical microscopy (POM), energy dispersive X-ray (EDX) [4], brunauer-emmett-teller (BET), zieta size analyzer, and energy dispersive X-ray spectroscopy (EDX). Nanoparticles possess a range of useful physicochemical qualities, including electrical, optical, magnetic, mechanical, and thermal capabilities. Additional compelling applications of NPs encompass their utilisation in the medical field, specifically for tasks such as fluorescently labelling biological entities, transporting genes, and detecting biological pathogens. These applications are valuable for identifying proteins, investigating DNA structure, developing drugs, and facilitating fluorescent biological labelling. The applications of this technology include tissue and cell engineering [12], hyperthermia for tumour destruction [13], separation and purification of biological cells and molecules [14], enhancement of MRI [15], and cellular imaging [16]. Nanoparticles (NPs) are utilised as chemical catalysts in several fields such as micro-wiring, paints, food and agriculture, electronics, energy harvesting, mechanical industries, batteries, and environmental protection. Nanoparticles have significant limitations, including challenges in their handling, toxicological impacts on plants, and unfavourable health consequences in people due to delayed exposure at various concentration levels [17]. Cobalt, a naturally occurring metal, has an atomic number of 27 and belongs to the fourth Periodic Table. Only the isotope 59Co exhibits stability within it. Cobaltite, Glaucodot, Erythrite, and Skutterudite are the primary forms of cobalt ores. Cobalt is a metallic element that has a silvery tone and is characterised by its brittleness and shiny appearance. Cobalt compounds have been utilised to create blue dyes in glass, ceramics, and coatings [18]. With a melting point of 2723 °F or 1495 °C, it exhibits a high degree of resistance to melting. The compounds mentioned are cobalt (II) carbonate (CoCO3) and cobalt (NO3)2-6 hydroxide. Cobalt-containing chemicals are utilised in the production of turbine blades for jet engines, exhaust valves, machine parts with enhanced surface hardness, gun barrels, ceramics, glass decolorizers, motors, magnetic recording media, and generators. Samarium cobalt magnets, which are permanent magnets, are produced using this material. Cobalt is mostly utilised in alloys, such as Vitallium, which exhibit high resistance to wear. These metallic alloys are essential for the fabrication of prosthetic knees and hips, as well as dental and orthopaedic implants. Cobalt nanoparticles possess unique thermochemical, physical, and optical properties. They hold significant significance in the fields of optoelectronics and optical physics. The spectral dependence of the scattering and absorption efficiency factors of cobalt nanoparticles differ greatly from those of gold or silver nanoparticles due to their broad bands or continuous spectrum. Their melting point is 1495°C and their boiling point is 2870°C. The shape of cobalt nanoparticles is spherical. Co-NPs exhibit a powdery appearance with shades of grey and black. Cobalt nanoparticles are employed in various applications such as biomedical stents, dyes, high-speed optical devices, materials for absorbing microwave and electromagnetic waves, magnetic inks, drug delivery, coating, and cobalt batteries. These substances have several applications such as light filtration, usage in Ferro fluids, production of super alloys, development of medical sensors, and manufacturing of antistatic plastic bags. Green Chemistry refers to the use of a certain set of principles aimed at reducing or eliminating the use of dangerous substances in the creation, manufacturing, and utilisation of chemical products. Sustainable chemistry, sometimes known as "green chemistry," has the ability to prevent pollution by targeting the molecular level. The significance of green chemistry lies in its ability to minimise energy use, reduce waste generation, protect the environment, maintain affordability, and incur minimal manufacturing expenses. Consequently, it is also ecologically sustainable. Due to their ability to be easily grown in large amounts, microbes are increasingly being used to produce nanoparticles [19]. Furthermore, the manufacturing of metal nanoparticles does not involve the use of toxic, abrasive, or expensive substances. The process is environmentally friendly [20]. In addition, a cost-effective approach is the production of metal nanoparticles by the use of plant extracts. Producing metal nanoparticles using plant extract is both practical and cost-effective. Plant extracts offer a secure and environmentally friendly method for synthesising metal nanoparticles. The process is sufficiently straightforward to be employed in industrial settings for the production of uniformly dispersed metal nanoparticles. This research utilises the leaves of the drumstick tree, which are commonly known as the Ben oil tree, benzoil tree, and Horseradish tree. The Drumstick tree, scientifically known as Moringa oleifera, is a member of the Family Moringaceae. It is highly sought after for its fast growth and ability to withstand drought conditions. The tree is extensively cultivated due to the culinary and medicinal value of its leaves, which are commonly used as vegetables and herbs [21]. It aids in the process of purifying water and has a tendency to grow in soil that is saturated with water. The number 23 is enclosed in square brackets. Drumstick leaves serve as a source of iron that is rich in content [24]. Leaves extract contains polyphenols [25] which possess numerous potential characteristics [26] in scientific studies.

Materials Used:

Drumstick (Moringa Oleifera) leaves gathered from the outside of the University of Engineering and Technology, Lahore, and Cobalt Chloride (CoCl2) anhydrous, with a concentration of at least 98.0% (KT), are the materials utilised in all the research projects.Drumstick (Moringa Oleifera) leaves gathered from the outside of the University of Engineering and Technology, Lahore, and Cobalt Chloride (CoCl2) anhydrous, with a concentration of at least 98.0% (KT), are the materials utilised in all the research projects.

Synthesis of Co NPs:

2.1 Preparation of Leaf extract The leaves extract was prepared by dipping 10 g of freshly washed leaves into 100 ml of double distilled water at temperatures ranging from 50 to 60 oC. After 2 hours, the solution's colour changed from colourless to a yellowish brown, indicating that the leaves were extracted. Removing the flask from the water bath was done once the extract was prepared. In order to filter the mixture, Whatman uses filter paper No. 1. The remaining extract was thereafter refrigerated and kept at 4 oC until needed.

2.2 Preparation of Cobalt Chloride (CoCl2) solution

To make a 1000 ml solution of 0.001M cobalt chloride, you'll need to dissolve 0.1298 grammes of cobalt chloride in 1000 ml of distilled water. Similar to a physical chemist, the solubility of cobalt chloride in water is quite high. This allows it to easily dissolve in water, resulting in a homogeneous solution where cobalt chloride is completely soluble. The solution was stored in a 1000 ml measuring flask, covered with aluminium foil. The solution was stored at room temperature for future research purposes.

2.3 Sample Preparation

A beaker was used to hold 20 ml of leaf extract, while a burette was filled with a 1mM solution of cobalt chloride for drop-wise addition. The beaker was placed on a hot plate with constant stirring for 48 hours. The cobalt chloride solution was carefully added to the leaves extract while stirring constantly. Various samples with different volume ratios were prepared. After the reduction of cobalt, the solution underwent a noticeable colour change from yellow to pink, suggesting the successful formation of cobalt nanoparticles. The mixture was heated and stirred on a hot plate until a paste was formed. After being dried in an oven at 60C, a black-colored Co nano powder was formed.

2. Characterization of synthesized Nanoparticles

The solution of NPs was transformed into a dry powder. Then dry powder is further fixed on a sample holder. This holder is coated with a conductive metal such as gold, using a sputter coater. With a focused fine electron beam, the whole sample is finally analyzed by scanning. The sample for UV-Vis was collected when the particles were suspended in a nanosolution. The UV analysis was performed using a double-beam spectrophotometer. A cuvet with a path length of 1cm was used. Two solutions are being analysed using UV analysis at the same time. One of the solutions used for reference was distilled water, while the other was a sample solution. Cobalt nano-powder directly runs for IR analysis. While CoCl2 was in the form of a solution, a KBr pallet was prepared and then 1 or 2 drops of cobalt nano-solution were added. Bacterial activity is mostly confirmed by using a well or spreading method by using agar media. The equipment used in this process was sterilized. After sterilization, Agar was spread on Patri dish and then samples on it. After some time, the sample prepares wells that represent the activity of the given sample.

3. Results and Discussion

4.1 UV Spectra

In the case of green synthesized cobalt nanoparticles, we get two peaks at 275 and 350 nm in UV spectroscopy. These are the characteristic peaks for cobalt nanoparticles. If the main prominent peak is obtained at 378 nm then it indicates the formation of Co nanoparticles (low dimensional beta form). UV-visible spectrum for green synthesized cobalt nanoparticles is given below.

In the absorption, and spectroscopic studies cobalt nanoparticles exhibit a broader line near to 380nm that confirms the formation of CoNPs. The absorption peaks at 270-380nm indicate the formation of cobalt nanoparticles. Above mentioned spectra indicate a fine peak at 378nm that confirms the formation of cobalt nanoparticles. The absorption peaks at 275-378 nm regions because of excitation of the surface plasmon vibrations in cobalt NPs indicated the formation of cobalt nanoparticles.

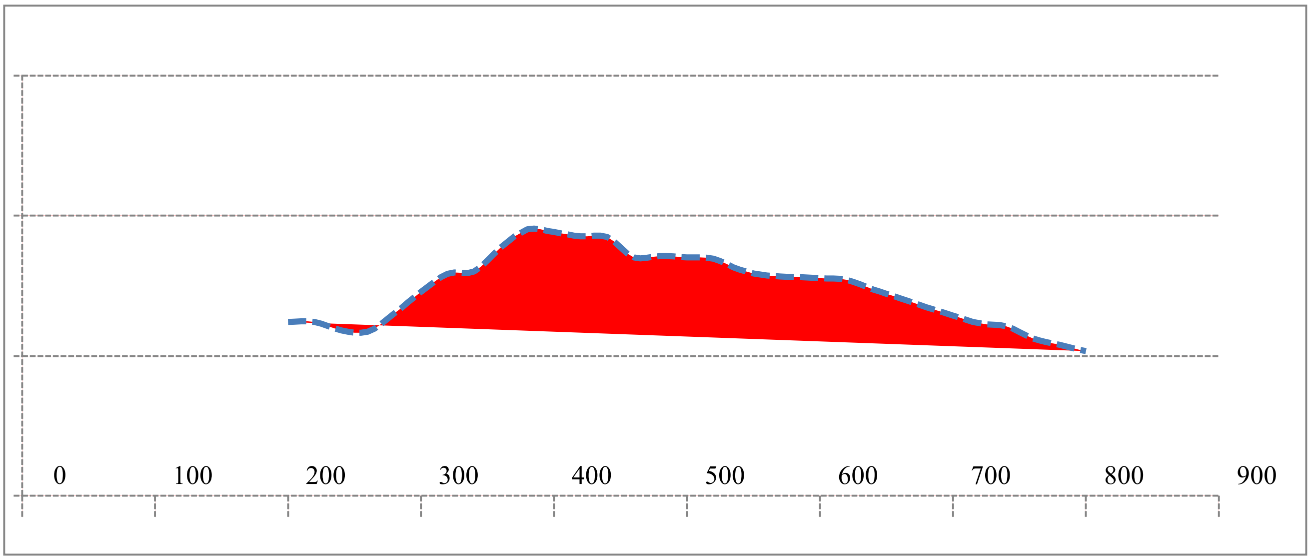

Fig: 1 UV-Visible Graph of CoNP's with Drumstick.

4.2 FTIR Analysis

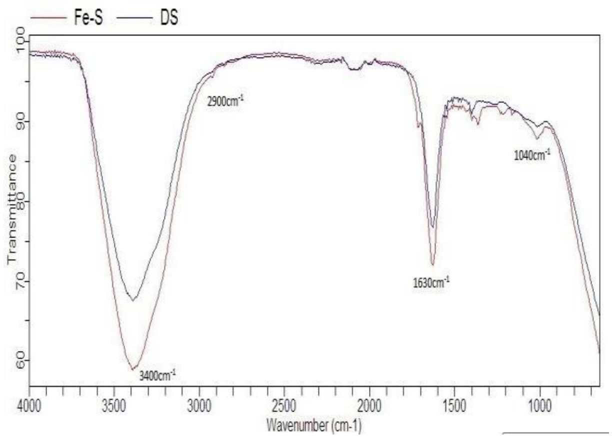

Fig: 2 FTIR Scan of CoNP's with Drumstick Tree

The provided IR spectra for the extract and Nano solution are displayed above. Various peaks are obtained that indicate the presence of functional groups in a sample. The 1040 cm^{-1} peak is indicative of primary alcohols. The peak at 1630cm-1 indicates the bending of C=C bonds. The 2900 cm^{-1} peak corresponds to the presence of Alkyl C-H bonds, while the 3400 cm^{-1} peak indicates the strength of Alcohol/Phenol O-H bonds. All these groups can be found in the cobalt nanoparticle solution. These groups were originally found in the extract solution, which functions as a capping, reducing, and stabilizing agent in the formation of nanoparticles. These are responsible for converting cobalt into cobalt nanoparticles.

4.3 Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscopic images of green synthesize cobalt nanoparticles by using Drumstick tree leaf extract is given below.

SEM images are obtained at different resolutions; different images were obtained so that we can study them properly. The minimum size calculated from these SEM images is 27.3 nm. While different-sized nanoparticles are obtained the smallest particles have this size. For the calculation of size image software is used.

4.4 Energy Dispersive X-Ray (Spectroscopy) (EDX)

Image for Energy Dispersive X-ray for Co NPs is given in Fif. 4 below.

4.5 Antibacterial Activity

Antibiotics inhibit the cell division of microorganisms. Unfortunately, the dosage of antibiotics must be increased and the treatment can be prolonged due to the low MIC of antibiotics. Our synthesized cobalt nanoparticles are also subjected to check their bacterial activity and we get superfine results. The activity of Ecoli and staphylococcus is checked and good results indicate that these cobalt nanoparticles have greater bacterial activity than the extract used to prepare these particles.

The following table 1, shows the activity of Ecoli and staphylococcus in CoNP's synthesized by using Drumstick leaf extract while the reference is extracted itself.

Table 1: Activity of Ecoli and Staphylococcus

Sample	**Staphylococcus (+)**	**E coli (-)**
	Gram-Positive Bacteria	Gram-Negative Bacteria
Reference/Extract	10 mm	7 mm
Sample/Drumstick Co NPS	15 mm	8 mm

These results clearly show that the bacterial activity of CoNPs is greater than simple extract. The bacterial activity of cobalt nanoparticles is greater than the reference i.e. simple extract. These synthesized nanoparticles can also be used in medical sciences because of nanoscale they can easily penetrate human body organs like lungs and skin. So, they can be good drug carriers shortly.

4. Discussion

The thermochemical, physical, and optical properties of cobalt nanoparticles are truly exceptional. Microbiologists find Cobalt NPs to be highly intriguing in the field of optical

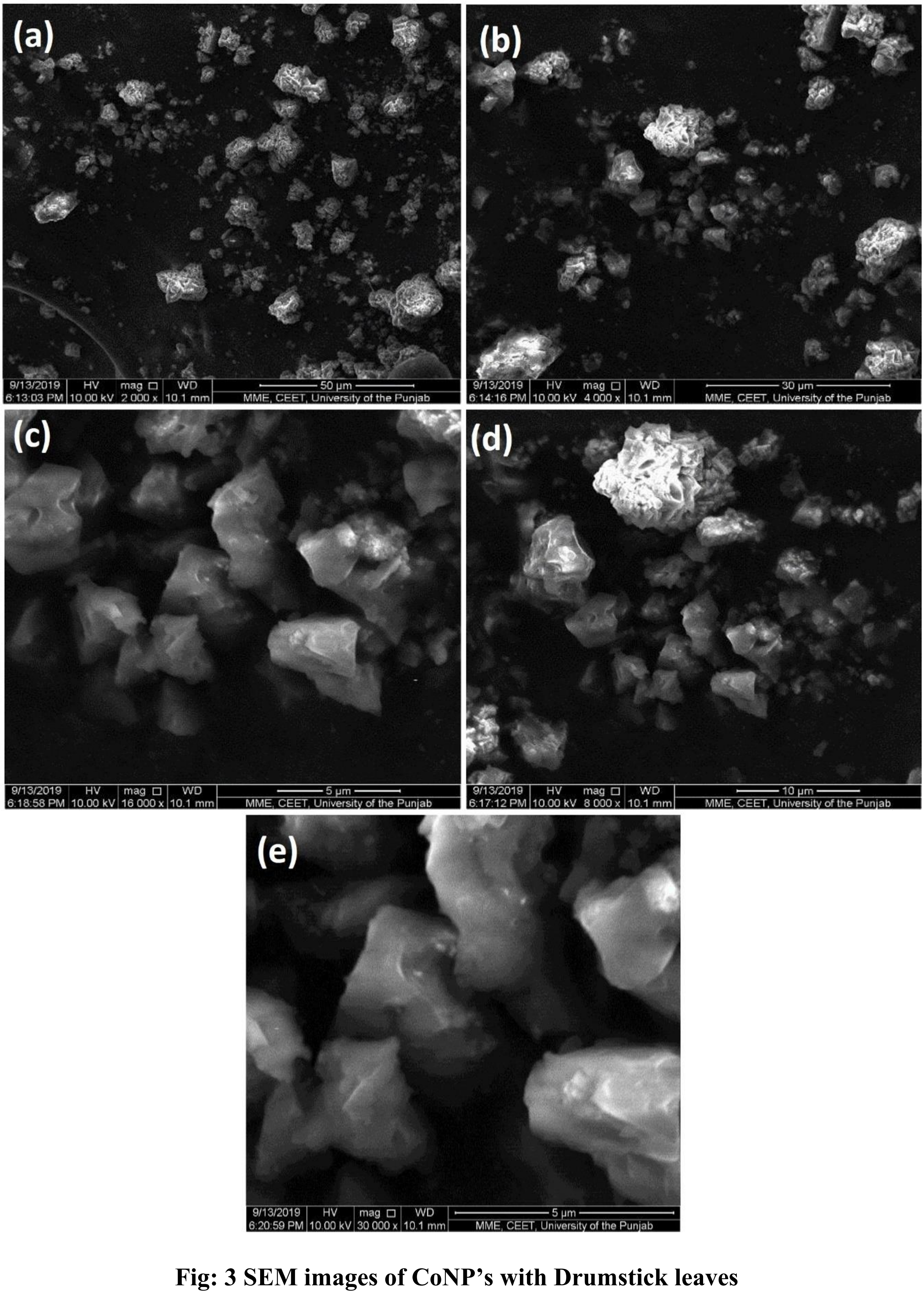

Fig: 3 SEM images of CoNP's with Drumstick leaves

physics and optoelectronics. Cobalt nanoparticles are synthesized using a green synthesis method, which is known for its environmentally friendly nature. Drumstick tree leaves extract is utilized for the synthesis of Co NPs. Plant extracts can be utilized in nanoparticle synthesis as reducing and capping agents. Producing metal nanoparticles using plant extract is a cost-effective and highly valuable method. With this method, we are able to generate a substantial quantity of NPs at an incredibly affordable price. Various techniques are employed to characterize the synthesized Co NPs.

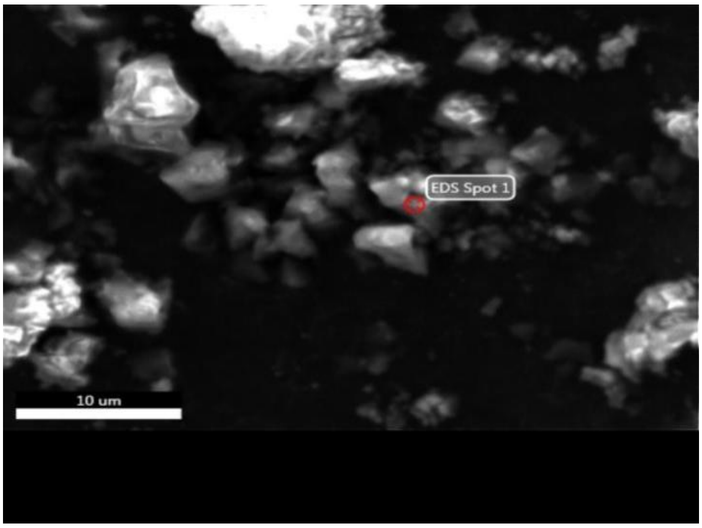

Fig: 4 Energy Dispersive X-Ray (EDX) of CoNPs

5. Conclusion

Drumstick tree leaves were utilized to synthesize cobalt nanoparticles. The nanoparticles were further analyzed using various techniques including UV-vis spectroscopy, FTIR spectroscopy, and SEM. An identifiable UV peak was observed for the Co nanoparticles. The UV absorption peaks at 378 indicate the successful synthesis of Co nanoparticles. Just like a chemist, the UV absorption observed at 378 nm provides confirmation of the synthesis of Co NPs. Phytochemicals have a significant impact as a capping and reducing agent in nano synthesis. The size of the co-synthesized nanoparticles was determined using SEM results. The SEM images revealed a range of Co nanoparticle sizes, varying from 168 nm to 295 nm. Nanoparticles synthesized using cobalt exhibited remarkable antibacterial properties. Therefore, the utilization of Co nanoparticles synthesized through drumstick mediation shows promise in the development of antibiotic drugs.

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