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Biologically Driven Synthesis of Cobalt Oxide Nanoparticles Using *Kalanchoe Pinnata* Aqueous Leaves Extract for Sustainable Biological Applications

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Abstract

The most pressing public health concern is infectious diseases, which calls for the creation of more potent infection control measures. Cobalt is a type of ultra-trace element that is necessary for many organisms' metabolism. Because cobalt nanoparticles (Co NPs) are good at targeting particular tissues and pathogenic microbes, many researchers are interested in the green production of Co NPs and their uses. This study's objectives are to create and describe CoNPs from *Kalanchoe Pinnata* leaves and verified by seeing the solution's color shift from yellow to brown, which was verified by spectrophotometric detection of optical density. The synthesized cobalt oxide nanoparticles were examined using UV-visible spectroscopy (UV) (range: 200-800 nm). The amorphous nature was identified using an X-Ray Diffraction (XRD) pattern (XRD) (size: 5 nm) whereas synthetic NPs' spherical or elliptical shape was confirmed by scanning electron microscopy analysis (SEM) (size range: 20-30 nm). The FTIR results indicate that hydroxyl group, C=O group and C-O group may function as the reducing agent which involve in synthesis. Therefore, the aim of this study was to compare and assess the antibacterial and fungicidal properties of CoNPs. The main hypothesis states that the biological activity of CoNPs is determined by the properties of stabilizing agent molecules adsorbed on their surfaces that have comparable size distributions, morphologies, and ion release profiles.

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A variety of bacterial cell types, including *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa, and P. maltophilia*, were employed as models. The most pronounced fungicidal effects were noted. The clear zone of clearance at 100 µg/mL indicates that CoO nanoparticles killed the colonies. It was found that a specific stabilizing agent can be used to modify the selectivity of CoNPs toxicity towards specific pathogens. E. coli was found to be more vulnerable to CoNP exposure than *Bacillus subtilis, Pseudomonas aeruginosa, and P. maltophilia*, regardless of CoNP size and surface.

Keywords: nanoparticles; *Kalanchoe Pinnata;* antibacterial; fungicidal properties; *Cobalt Oxide.*

1.Introduction

One of the areas of modern science that is being investigated the most is Nano chemistry, which is attracting the attention of an increasing number of scientists from a variety of fields, including chemistry, physics, biology, catalysis, and medicine. The discovery of the distinguishing features of nanoparticles, or NPs—objects with a size of less than 100 nm in at least one dimension—has enabled miniaturization in many scientific fields [1].

In the past, several techniques, including pyrolysis and abrasion, were used to create nanoparticles. Nevertheless, these methods have a number of disadvantages, such as expensive expenses, sluggish rates of synthesis, and high energy needs. For the manufacture of these materials, chemical techniques including chemical reduction and the sol gel process are typically employed. However, these techniques produce dangerous byproducts and require the use of toxic chemicals. As a result, scientists are always working to create less hazardous, environmentally friendly, economical, and clean techniques to synthesize nanoparticles. For this reason, the production of metal oxide nanoparticles using different plant extracts has recently attracted the interest of pertinent scientific communities. A further reason for the growing interest in metal oxide nanoparticle biogenesis is its ease of use in experiments and the ease with which different morphologies and sizes of nanoparticles can be produced [2].However, the green synthesis (biosynthesis) of metal nanoparticles using plants, herbs, or even microbes has garnered a lot of interest, especially for medical purposes. Green synthesis has been documented for chemical applications such catalysis and gas sensing as well as antioxidant, antibacterial, and anticancer properties. Because the method is straightforward, scalable, and non-toxic, there has been a recent surge in research on metal nanoparticles using plant extract. Certain chemical compounds possess the capacity to arrange the nanoparticles, resulting in the creation of nanostructures that resemble those created using traditional techniques [3].

The increasing need for different kinds of nanoparticles means that environmentally friendly and economically viable synthesis techniques must be developed. Because of their distinct chemical and physical characteristics and possible technological uses in a variety of disciplines, green synthesized nanoparticles have attracted a graet deal of attention from the diagnosis of diseases to catalysis. Cobalt nanoparticles, or Co NPs, exhibit a wide range of characteristics, including electrical, magnetic, and catalytic capabilities, because of their large surface area. Cobalt nanoparticles (Co NPs) have a large surface area and a variety of characteristics, such as electrical, magnetic, and catalytic. Since cobalt nanoparticles (CoNPs) have strong permanent magnetic characteristics, good catalytic activity, biological activity, and cytotoxic action, they may be effective nanoparticles. Cobalt oxide and organic metal can be created from cobalt-based nanoparticles, substances or biopolymers. Large magnetic anisotropies, superparamagnetic, and quantum size effects are also demonstrated by them below a crucial size of 20 nm. For Co-NPs to be utilized in subsequent applications in the domains of biomedicine, separation technology, catalysis, and information storage systems, they must be distinct, homogeneous in composition, uniform in size and shape, and possess a uniform crystal structure. Various liquid-phase reduction techniques, also known as in-solution techniques, offer relatively straightforward methods for achieving these characteristics. More economical, simpler to implement, and requiring no specialized equipment. Numerous technologies are accessible, including template-based methods, solvothermal and hydrothermal breakdown, microfluidic synthesis, pyrolysis, and modified polyol processes. The use of plants, plant extracts, and microorganisms in a biological (or "green") manner has recently been suggested as an alternative to risky (chemical) approaches for the synthesis of metal nanoparticles. Phenolic compounds, including tannins, flavonoids, and phenolic acids, are major contributions to the reducing or antioxidant activity of medicinal plants, fruits, or vegetables because they are believed to be involved in redox activities. Due to their redox capabilities, phenolic compounds serve as singlet oxygen quenchers, hydrogen donors, reducing agents, and metal chelators. Plant extracts contain phenolic compounds in various forms. Plant extracts contain a wide variety of phenolic chemicals. These compounds are highly reactive and take part in redox reactions. Total phenolic compounds found in plant extract may be responsible for the reduction of metal ions and the production of the corresponding metal nanoparticles [4].

In Tropical Africa, Australia, Bangladesh, India, Hawaii, and other tropical nations, kalanchoe pinnata (family Crassulaceae) is growing as a weed [4]. Fascinating plant, kalanchoe pinnata, reaches heights of three to five feet. Often referred to as the “air plant”, it features tall, hollow stems, dark green leaves with crimson trimming that is scalloped, and bell-shaped, pendulous flowers. Numerous illnesses are treated with this herbaceous plant in traditional medicine like rheumatoid arthritis inflammation, kidney stones, stomach ulcers, and respiratory conditions. The chemical components found in the plant belong to many classes, such as phenolics, glycosides, steroids, alkaloids, diterpenoidal lactones, and so on. The extraordinary pharmacological qualities include anti-allergic, anti-lipidaemic, antioxidant, anti-neoplastic, and anti-diabetic activity, among many others [5].

Figure 1: fresh leaves of kalanchoe pinnata

It tastes acidic, astringent, and has a sweet aftertaste after digestion. It has several Latin synonyms, including Bryophyllum pinnatum Linn and Bryophyllum Calycinum Salisb respectively. Other common folk names for this plant include miracle plant, air plant, panfuti (Hindi), life plant, love plant, air plant (Mexico), good luck or resurrection plant, Zakham-e-Hayat, Canterbury bells, Cathedral bells, and the well-known Katakataka because of its great flavor. The Crassulaceae family includes the medicinal plant Kalanchoe pinnata (Bryophyllum pinnatum), often known as patherchat or patharkuchi, which has long been used in ayurvedic medicine. The treatment involves the use of several plant parts, including the stems, leaves, roots, and flowers. Other qualities of the plant leaves include anti-viral, anti-bacterial, anti-inflammatory, anti-cancer, anti-diabetic, antilithic, antiproliferative, antimicrobial, antileishmanial, anthelmentic, insecticidal, anti-allergic, analgesic, antinociceptive, and wound healing. Steroids, polyphenols, and triterpenoids are among the complex chemicals found in Kalanchoe pinnata leaves [6]. It is well-known in ethnomedicine for having tocolytic and anthroposophical effects on expectant mothers. Additionally, it helps the placenta drop during childbirth. Researchers have successfully established the therapeutic potential of both the entire plant and its individual sections after thoroughly examining the plant's many elements. Only a small number of scientific validations have ever succeeded in identifying and isolating the bioactive components from different solvent extracts of the plant. The pharmacognostic qualities of the plant should be well understood before conducting additional research and clinical studies to commercialize the plant's potential pharmaceutical uses [7]. The purpose of this study was to create novice cobalt nanoparticles and evaluate their antibacterial properties using a plant extract.

2.Experimental

A popular spectroscopy method used to assess the dimensions, form, and production of noble metal-based NPs is UV-Vis spectroscopy. CoNPs exhibit broad and sharp bands in the 350–600 nm range, according to the obtained results. These bands may be connected to the surface plasmon resonance (SPR) effect of the nanostructures and are commonly seen throughout the green synthesis process. The appearance of additional broad bands in the CoNPs' UV-Visible spectroscopy analyses was also seen, and this could be related to the extracts' secondary metabolites being present on the particles' surfaces. The basis of FTIR spectroscopy is the way that metals, proteins, carbohydrates, and nucleic acids absorb infrared light. With this method, every sample displays a unique spectrum fingerprint that can be identified and distinguished from other molecules. The peaks displayed a little shift within the wave number band (4500–500 cm-1) under examination, suggesting that the extracts might have potential as caping agents, according to our previous research [8]. The structure of the nanoparticles is ascertained by SEM examination. CoO-NPs have irregular and spherical shapes, and their diameter ranges from 20 to 30 nm, as seen in the SEM image. Several irregularities and spherical cobalt chloride nanoparticles are visible in our study's SEM analysis. Many researchers also report seeing the same visuals with CoO-NPs . According to characterization data, there isn't a prominent peak in the XRD diffraction pattern, indicating that the nanoparticles aren't well crystalline because of lack of calcinations and lack of equipments at our university that are necessary to perform XRD examination the particle size was calculated in the region of 5nm using the Scherrer formula. The antibacterial activity is significantly impacted by the size of the particles and their aggregates in colloidal solutions, the media's chemical makeup, and the duration of storage for the suspension. Because of their special properties, CoO NPs can be used as an effective precursor against both gram-positive and gram-negative bacteria in a variety of ways. They can also be applied in various industries, such as biosensing, cosmetics, agriculture, food and beverage, and sterilization procedures.

3.Materials and methods

3.1 Collection of samples

Throughout the winter, in February, fresh *kalanchoe pinnata* leaves were gathered from the All seasonal nursery in the Hayatabad phase 3 Peshawar, Pakistan. From lab of Qurtuba university, cobaltous nitrate Co(NO3)2 was acquired.

3.2 Preparation of kalanchoe pinnata leaf extract

After the collection of samples, in order to get rid of the dust, these blooms were given multiple water washes. Ethanol was immersed in the leaves after they had been cleaned with double-distilled water for a duration of 1 to 2 minutes. To eliminate any remaining moisture, it was then dried in the shade at room temperature (20 °C). An incubation period of 15 minutes was conducted on a hot plate at 100°C after adding 55g of finely chopped leaves to 550mL of boiled distilled water to create a leaves extract and kept it for 24 hours. Utilizing Whatmann No 1 filter paper, the extract was filtered once it had cooled, and the filtrate was kept for later use at 4°C.

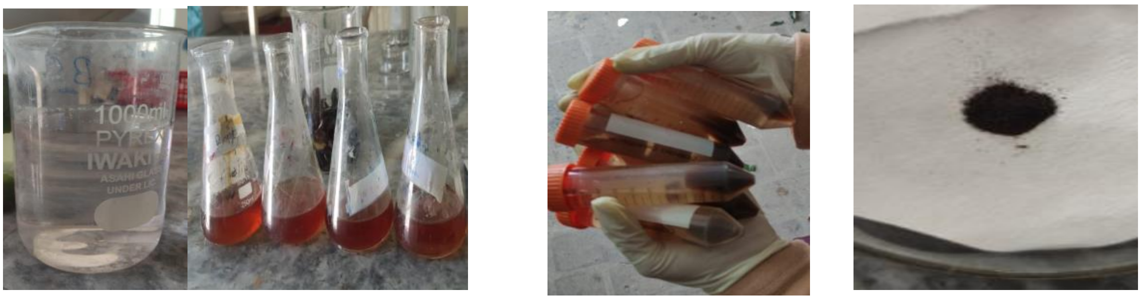
a **b** **c** **d**

Figure 2: Depicts the chopped leaves and the extract filtered solution a) dried leaves b) extract

c) After 24 hrs d) filtration

3.3 Synthesis of cobalt oxide nanoparticles

The green synthesis method was used to create CoO NPs and kalanchoe pinnata aqueous extract was employed as a capping and reducing agent. As a result, 15 mL of kalanchoe pinnata leaves extract was combined with 85 ml solution of Cobalt nitrate in 4 conical flasks equally. Place all conical flasks in a water bath for two hours. When the color of solution changed from yellowish brown to dark brown, the liquid has been evenly distributed throughout the pelcan tubes, then they should be centrifuged for half an hour. When the time is up, the particles will sink to the bottom of the bottle. Then these particles were gathered in petri dishes and they were cleaned three times using distilled water, ethanol and acetic acid. To obtain the dried precipitate, we left them in an incubator for a whole day to dry. Following that the dried particles were grinded. Ultimately, a dark brown powder was produced, and the particles were kept in an eppendorf tube for additional characterization and further biological applications.



a **b** **c** **d**

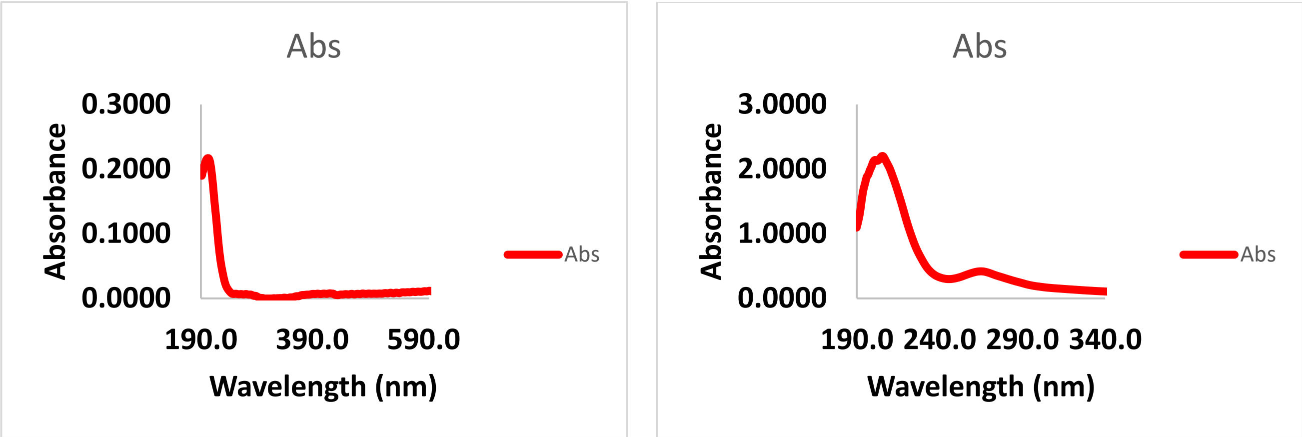
Figure 3: filtration of the extract after 24 hrs, 1molar solution of CoO and **(c)** represents centrifugation Of CoO **(d)** represents synthesis of CoO

4.Characterization of cobalt nanoparticles

4.1. UV Visible spectroscopy

UV-Visible spectroscopy is a useful technique for indirectly investigating the bio reduction of metal nanoparticles in an aqueous solution. It can contain information about the formation of NPs as well as their size, shape, and stability [9]. The presence of nanoparticles is typically detected using wavelengths between 200 and 800 nm [10].The extract was filtered through Whatman No. 1 filter paper and centrifuged for 10 minutes at 3000 rpm in order to get it ready for UV-VIS spectrophotometer examination. Using the same solvent, dilute the sample to 1:10 [11]. When sun-dried *Kalanchoe pinnata* leaves were added, the translucent aqueous extract turned reddish brown. The Cobalt nanoparticles' excitation of SPR is what causes the color changes. Cobalt nanoparticle SPR resulted in a peak with a center close to 204.0 nm. UV light was used to monitor the chemical process during green synthesis. The closest maximum absorption was detected at 204.0 nm, suggesting that cobalt nitrate was successfully converted to cobalt nanoparticles.

To prepare the extract for UV-VIS spectrophotometer analysis, it was filtered using Whatman No. 1 filter paper and centrifuged for 10 minutes at 3000 rpm. Using the same solvent, dilute the sample to 1:10. In result the peak was obtained at 206.0 nm.



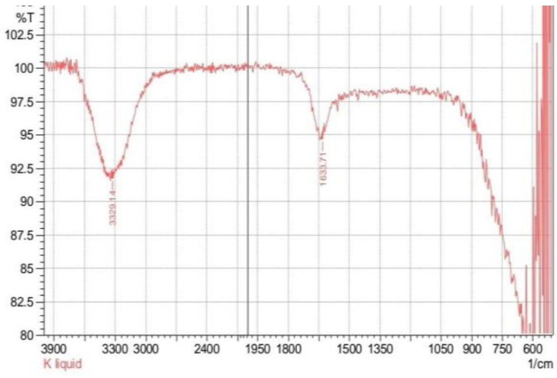
a b

Figure 4: UV Visible spectroscopy of a) CoO NPs b) Kalanchoe Pinnata leaves extract

4.2. FTIR

FTIR spectroscopy examines the chemical vibrations and infrared absorption of NPs. It can reveal details about the chemical makeup of NPs and their interactions with surrounding molecules in addition to identifying functional groups on their surface [11]. The functional groups in charge of capping and stabilizing the bio reduced CoNPs made with plant extract were found using FTIR studies. The emergence of new groups in the synthesized CoNPs was indicative of the synthesis of the particle.

Several functional groups are present in the (Fig 5) in the overlay graph of FT-IR spectra in which the hydroxyl functional group was responsible for the broad band at 3404.360 cm-1. The C=O group represents stretching peak at about 1627.92cm-1 respectively. Due to the ethers' C-O stretching, there are strong absorption bands at about 1346.313 cm−1. The peak at 673.1567cm-1 represent CoO group. In figure 1.7 the ftir spectrum of leaf extract of kalanchoe pinnata the peak of OH appears at 3329.14cm^{-1} and the peak at 1633.71 cm-1is responsible for C=O group. In figure 1.8, the FTIR spectrum of green CoO NPs, the peak of OH disappears while the new peak of C-O appears at 1346.31cm while the peak at 1543.05 cm-1 is responsible for C=O.



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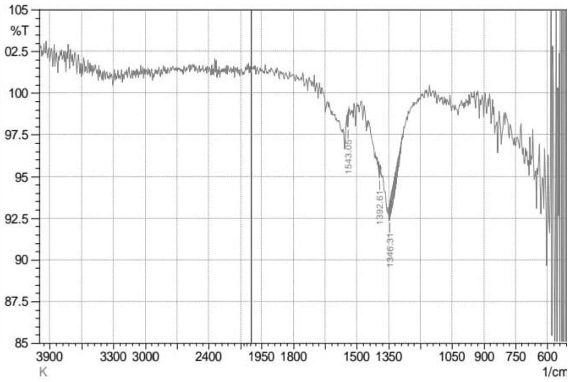

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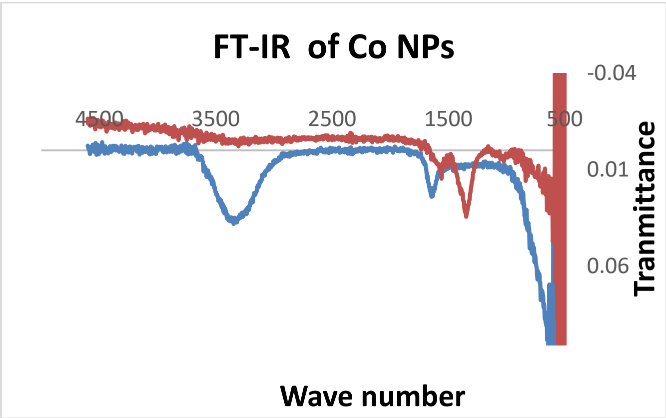


c

Figure 5: FTIR spectrum of (a) leaf extract of kalanchoe pinnata, (b) CoO NPs (c) Overlay graph of FTIR

4.3. XRD Analysis

X-ray diffraction (XRD) is a potent nondestructive method for describing crystalline materials. Along with other structural characteristics including average grain size, crystallinity, strain, and crystal defects, it offers details on phases, structures, and preferred crystal orientations (texture). A monochromatic X-ray beam dispersed at different angles from each pair of lattice planes in a sample produces constructive interference, which results in X-ray diffraction peaks. The arrangement of atoms within the lattice determines the peak intensities. The fingerprint of periodic atomic structures in a particular material is thus represented by the X-ray diffraction pattern [12].

XRD analysis was done to verify that Co3O4-NPs were synthesized. Figure shows the x-ray diffractive pattern of the biosynthesized Co3O4 nanoparticles. The (111), (002), (220), (100) and (102) planes are responsible for the Co3O4 nanoparticle diffraction peaks that appear at 2θ = 19.00°, 31.008°, 36.88°, 44.72°, 59.18°, and 65.19°. The observed Cobalt oxide nanoparticles' peaks align perfectly with the conventional JCPDS card number 042-1467's cubic structure. Cobalt oxide nanoparticles were produced as a result of the biomolecule interaction with the cobalt sources, which displayed the cobalt and oxygen nucleation sites and were attracted to one another by electron donors of plant molecules. The crystallite size of cobalt oxide nanoparticles was ascertained using the Debye-Scherrer formula [13].

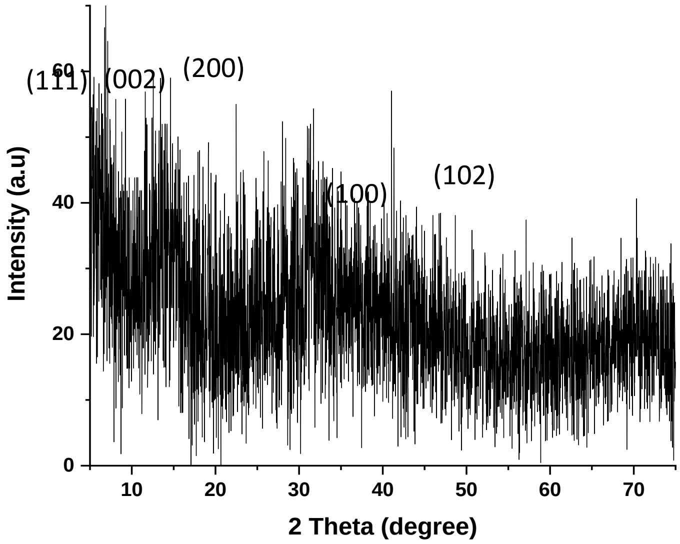
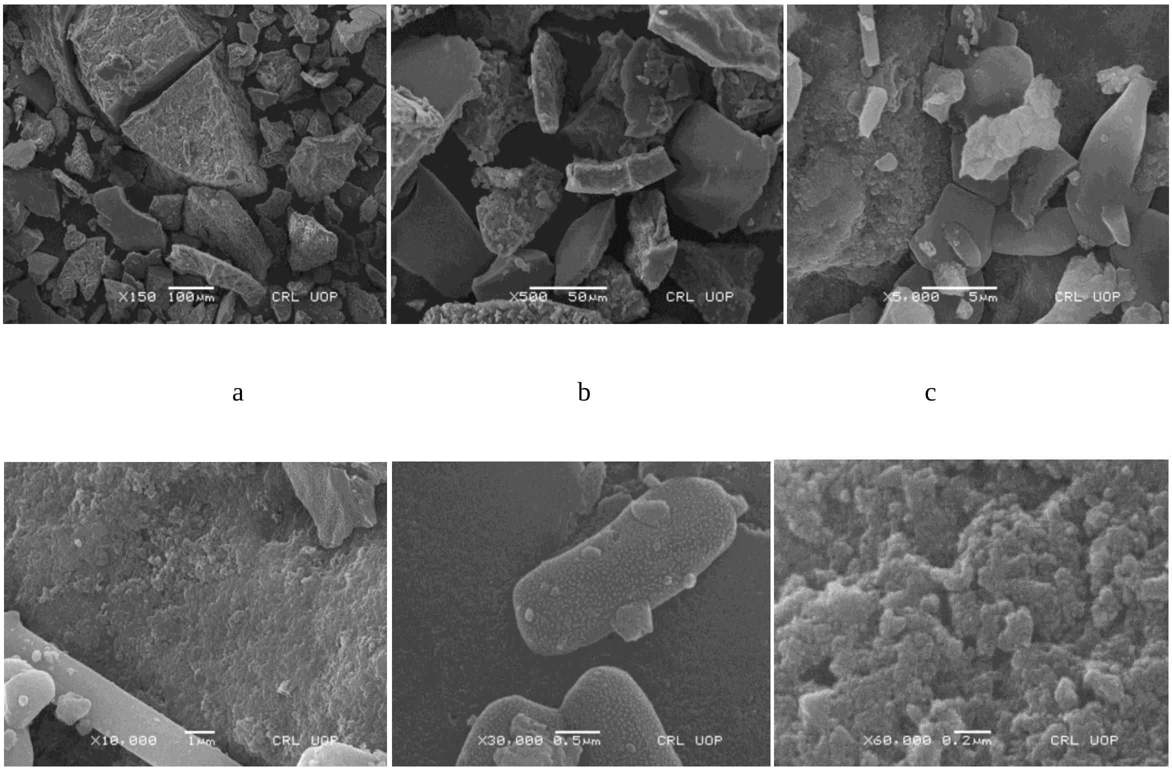


Figure 6: XRD results for KPLEx-CoO-NPs, illustrating amorphous peaks

4.4. Scanning Electron Microscopy SEM Analysis

SEM is a frequently used technology to examine the morphology, size, and structure of nanoparticles. SEM enables the characterization of NPs in terms of their size, shape, and distribution and offers details on their morphology and topography [11]. The SEM picture of biosynthesized cobalt nanoparticles is shown in Fig.1.11 where it is observed that the particles are spherical and polydisperse in nature [14].

The size range of the nanoparticles produced with green chemistry was found to be between 20-30 nm. Determining the structure of the created nanoparticles (or reaction products) is made easier with the help of the SEM examination. The SEM image showed a number of distinct and spherical Co NPs in addition to a few larger clusters. The Co NPs SEM image also showed that clustered particles were formed beyond the range of 100 nm, while spherical and somewhat irregular nanoparticles were formed within the diameter range of 20–60 nm. Several researchers have also reported similar types of images for Co NPs [11].



a b c

d e f

Figure 7: SEM images of KPLEx- CoO-NPs

5.Antibacterial activity of Co-NPs

A molecule's ability to inhibit the growth of bacteria and viruses or kill them locally without causing significant harm to neighboring tissues is known as its antibacterial and antiviral activity.

Antimicrobial compounds can therefore be categorized as bacteriostatic (having the capacity to inhibit the growth rate of germs) or bactericidal (having the capacity to kill bacteria) depending on their mode of action. Zinc oxide (ZnO), silver oxide (AgO), silver (Ag), magnesium oxide (MgO), calcium oxide (CaO), and copper oxide (CuO) are examples of metallic and metal oxide nanoparticles that have been demonstrated to exhibit antibacterial activity because of their biocompatibility, smaller particle sizes, distinctive surface morphologies, and suitability for a variety of antibacterial applications. Similar to these nanoparticles, the antibacterial activity of the CoO-NPs derived from plant extract was assessed using a range of gram-positive and gram-negative bacterial strains [15].

The antimicrobial qualities of CoO NPs have also been extensively studied (Koyyati and his colleagues 2016; Raza and his colleagues 2016; Igwe & Ekebo 2018; Shahzadi and his colleagues 2019; Kharade Suvarta and his colleagues 2020). CoO NPs are excellent antibacterial agents that can be used to treat a range of diseases caused by bacteria, including Klebsiella pneumoniae, Escherichia coli, Streptococcus pyrogens, and Staphylococcus aureus (Igwe and Ekebo, 2018). According to reports, the leaf juice of K. pinnata possesses antibiotic efficacy against Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa (Boakye-

Yiadom, 1977). Additionally, Proteus species, Klebsiella species, Shigella species, Salmonella species, Serratia marcescens, and Streptococcus pyrogenes and faecalis, as well as clinical isolates of these organisms with multiple antibiotic resistances, were all susceptible to bactericidal effects from the juice [16].

Plants bloom from November to March, and in April they bear fruit. It has a scorching potency and tastes sour, sweet, and astringent after digestion [17].

The presence of chemicals with phenolic properties suggests that the plant has antibacterial properties. According to a 2005 study by Ofokansi and his colleagues, the plant can successfully treat bacterial infections brought on by S. aureus, E. coli, B. subtilis, P. aeruginosa, K. pneumoniae, K. aerogenes, and S. typhi [17].

CoNPs were created by utilizing an aqueous extract of fresh Kalanchoe pinnata leaves and their antibacterial activity was assessed using the agar well diffusion method against a variety of pathogenic microbes, including Bacillus subtilis, Pseudomonas aeruginosa, Escherichia Coli, and P.maltophilia.These bacterias were collected from the PCSIR laboratory Peshawar. Nutrient agar can be used to cultivate a wide variety of non-fastidious microorganisms. It was first developed in response to the necessity for a standardized medium for testing dairy products, water and wastewater, and other commodities [18].

Nutrient Agar was used to cultivate the pure bacterial cultures. Using sterile cotton swabs, each strain was swabbed consistently onto the different plates. On nutritional agar plates, 8 mm diameter wells were made using sterilized micropipette tips. Using a micropipette, 100µl of the nanoparticle solution was applied to each well on each plate. The diameter of zone inhibition was measured in millimeters following a 24-hour incubation period at 37°C [19].

When applied to Escherichia, Bacillus subtilis, Pseudomonas Aeruginosa and P.maltophilia the green synthetic CoNPs exhibit outstanding antibacterial action. The outcomes show that green synthetic cannabinoids (CoNPs) made from Hibiscus cannabinus leaf extract have a potential future in medicine [4].

5.1. Result: Escherichia Coli

The majority of human diseases and tract infections are caused by harmful strains of Escherichia coli, despite the fact that the bacteria is normally common in the enteral microbiota. Furthermore E. coli resistant to a variety of fluoroquinolones and cephalosporins, which are popular antibiotics used in human medicine, have already been identified [17].

Cobalt nanoparticles have a sensitive antimicrobial impact on gram-negative Escherichia coli strains, claim Satpathy G. and Manikandan E. In addition, they show the inhibitory zone (mm) for various Co NP concentrations; for 5 µg/ml, 15 µg/ml, 25 µg/ml, and 35 µg/ml of Co NPs, the corresponding diameters are approximately 18, 20, 25, and 27 [20].

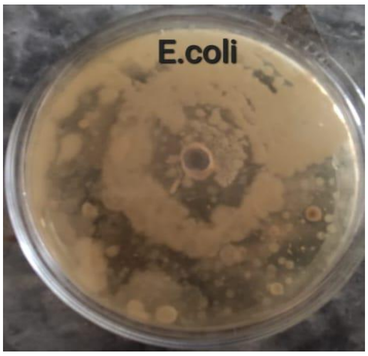


Figure 8: antibacterial effects of CoO nanoparticles against E.Coli

5.2 Bacillus subtilis

Here, cobalt oxide nanoparticles modeled after Bacillus subtilis are prepared and used as a platform for microbial hydrolase immobilization [21].

The test made using Bacillus subtilis and cross-stretched techniques at 37 °C for 24 hours. After 48 hours of follow-up civilization, the quantity of surviving spores was counted, and the bactericidal efficiency was then computed.

Figure 9: Antibacterial effect of CoO nanoparticles against B. subtilis

5.3. Pseudomonas Aeruginosa

Regarded as a significant Gram-negative opportunistic pathogen, pseudomonas aeruginosa is responsible for numerous serious acute and chronic infections that have fatality rates as high as 40% and considerable morbidity. Due to its high levels of both intrinsic and acquired resistance to several of the existing antibiotics, P. aeruginosa is an especially difficult organism to treat [11].Additionally, Co_3O_4-NPs were evaluated in relation to P.aeruginosa persister cells. According to a number of papers, it was assessed as an antibacterial and biofilm-forming inhibition agent. In hospitals and the general population, P.aeruginosa is a harmful microorganism that causes a variety of illnesses. In recent years, isolates of P. aeruginosa that are resistant to multiple drugs have emerged as a significant global public health concern [22].

Through the inoculation of an agar plate with P.Aeruginosa and subsequent application of Cobalt oxide (CoO), the cross streak method evaluates the antibacterial activity of CoO. When the CoO is incubated, its efficiency is seen by distinct zones of inhibition surrounding it. To determine the degree of antibacterial activity, these zones' sizes are useful.

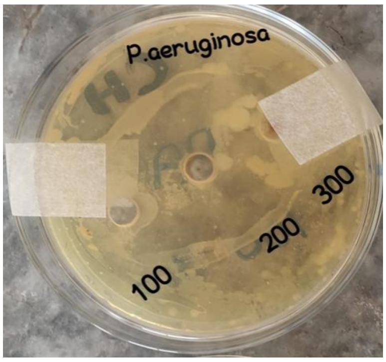


Figure 10: Antibacterial effect of CoO nanoparticles against P. aeruginosa

5.4. P.maltophilia

In 1943, a specimen of human pleural fluid led to the discovery of Stenotrophomonas maltophilia, a gram-negative bacillus that was originally known as Bacterium bookeri. (Source:) Pseudomonas maltophilia was the next classification in 1961, followed by Xanthomonas maltophilia in 1983 and Stenotrophomonas in 1999. It was then moved to the gammaproteobacteria class. One of the lesser known drug-resistant bacteria that can produce difficult illnesses is P.maltophilia. Stenotrophomonas maltophilia can generate small-colony variants in chronic infections. These variants grow slowly and can be difficult to find at times [23].

By first introducing the bacteria to an agar plate and then adding cobalt oxide (CoO), the cross-streak method evaluates the antibacterial activity of CoO against Pseudomonas maltophilia. Clear zones of inhibition surrounding the CoO after incubation show how efficient it is. These zones' dimensions aid in determining the degree of antibacterial activity.

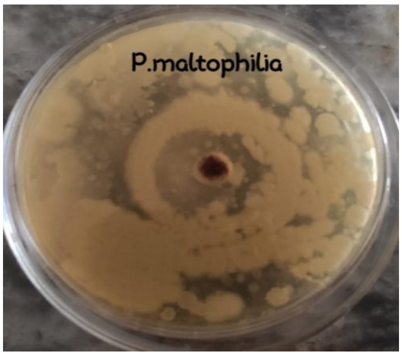


Figure 11: Antibacterial effect of CoO nanoparticles against P.maltophilia

Table 1: Zone of inhibition produced by different concentrations of CoO-NPs in antibacterial activity

Bacteria	**Inhibition zone nm m %**	***k.pinnata***
		Concentrations mg/mL
B. subtilis	**107**	**100 (mg/ml)** **1.3**
P.aeruginosa	**20**	**100 (mg/ml)** **1.2**
E.Coli	**55**	**100 (mg/mL)** **1.5 1.6**
P.maltophilia	**34**	**100 (mg/mL)** **1.5 1.4**

6.Antifungal Activity of Co NPs

Annually, fungal diseases are thought to be the cause of about 1.5 million deaths. Fungal infections are becoming more common, and even with the best care, they can have substantial fatality rates exceeding 40%.one, two This is partially because there are more individuals with HIV, more people getting hematopoietic or whole organ transplants, and more people undergoing intense chemotherapy, which increases the risk of bacterial or fungal infections. The common fungal pathogens Aspergillus fumigatus (A. fumigatus), Candida albicans (C. albicans), and Cryptococcus neoformans (C. neoformans) have acquired drug resistance, which further deteriorates the prognosis. Recently, species like Candida auris and Lomentospora prolificans that are inherently resistant to drugs have surfaced. More HIV patient deaths in poor healthcare systems are attributable to Cryptococcus spp. infections than to tuberculosis. Candida albicans is currently the fourth most prevalent iatrogenic bloodstream infection in the US [24].

6.1 Result: Candia albicans

The most common fungal pathogen in humans is Candida albicans, which can lead to invasive candidiasis, a potentially fatal condition. It is anticipated to have a diverse range of antioxidant enzymes. The conversion of thiocyanate (−SCN) to hypothiocyanite (−OSCN) and hypothiocyanous acid (NCSOH) can be catalyzed by salivary peroxidases using hydrogen peroxide (H2O2). These compounds have been demonstrated to prevent Candida albicans from surviving. The function of isothiocycanate is yet unclear to the best of our knowledge Reference [22].

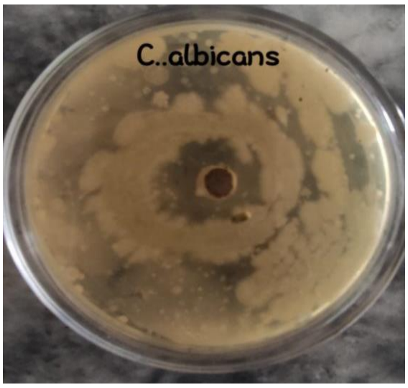


Figure 12: antifungal effects of CoO nanoparticles against C. albicans

Table 2: Zone of inhibition produced by same concentrations of CoO-NPs in antifungal activity

Bacteria	**Inhibition zone nmm%**	**k.pinnata**	
		100 (mg/mL)	
C.albicans	**62**	**1.3**	**1.5**

7.Antibiotic activity

Using Ciplox antibiotic discs conjugated with 100 µl of CoNPs and normal antibiotic discs against bacterial strains, the antibacterial activity was assessed. Irregular zones were measured in mm before incubation for 48 hours at 37 °C. When antibiotics are conjugated with CoNPs as opposed to when antibiotics or silver nanoparticles are used alone, antibacterial activity has been greatly enhanced [19].

7.1 Ciprofloxacin

Ciprofloxacin (CF) is a bactericidal drug that fights B. subtilis and Escherichia coli (E. coli). It eliminates germs by preventing DNA transcriptase, which is in charge of synthesizing both DNA and proteins, from acting. The medical industry makes widespread use of CF because of its excellent penetrability, low toxicity, and resistance Reference [25].

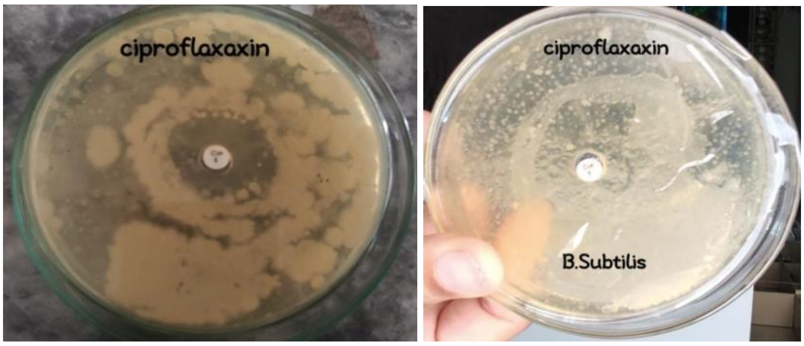


Figure 13: Antibacterial activity effect of CoO nanoparticles against ciprofloxacin

Table 1: Zone of Inhibition produced by Ciprofloxacin in CoO-NPs in antibacterial activity

Bacteria	Antimicrobial activity	Inhibition zone nmm Kalanchoe pinnata
B. subtilis	Ciprofloxacin	186+_1.5 , 1.6
E.coli	Ciprofloxacin	42+- 1.3 , 1.5

8.Discussion

Nanoparticles are particles that range in size from 0 to 100 nm. They are used in many different domains, including catalysis and medical biology. Cobalt oxide nanoparticles can be synthesized by thermal breakdown, electrochemical processes, and ultrasonic spray pyrolysis. The green method of producing nanoparticles is gaining traction since it is inexpensive, simple to use, and environmentally benign. Co_3O_4-NPs nanoparticles have cytotoxic, biomedical, and catalytic properties. Because of their high-performance permanent magnetic characteristics, they have good catalytic activity and may be effective nanoparticles. In addition, cobalt nanoparticles made using a green approach have anti-microbial properties, which makes them suitable for usage in pharmaceutical products [26].Pharmaceutical researchers have developed medications to cure a variety of ailments because to the chemical diversity of secondary metabolites present in extracts from medicinal plants. Extracts have poor solubility, low bioavailability, and limited selectivity, which makes administering them in the clinical pipeline challenging. Green nanotechnology has become a viable and sustainable answer to the contemporary issues surrounding the use of therapeutic plants in recent decades. In a previously published study, the leaves of a plant known as kalanchoe pinnata were used to synthesize Co_3O_4-Nps. In a recent work, cobalt nitrate and hexahydrate were used as a source of cobalt to create cobalt oxide nanoparticles. Different techniques were employed to characterize the produced nanoparticles [24].

9.Conclusion

Research on the environmentally friendly production of bioactive nanostructures has been quite active in recent years. This work established for the first time that CoNPs could be green-synthesized using polar and non-polar extracts from K. pinnata. According to the results, it was found that stirring and temperature throughout the experiment were favorable for obtaining CoNPs with various structural configurations [8].

This study uses plant extract from Kalanchoe pinnata to create cobalt nanoparticles in an environmentally friendly manner. The plant extract's ability to function as a stabilizing and reducing agent is due to the various chemicals included in it. The production of dark brown color and the appearance of the surface plasmon resonance band in an investigation using a UV-visible spectrophotometer were the first indicators of synthesized cobalt nanoparticles. The functional groups in charge of capping and stabilizing the bioreduced CoNPs made with plant extract were found using FTIR studies. XRD analysis was done to verify that Co3O4-NPs were synthesized. Nanoparticle size, shape, and distribution are confirmed by the SEM image. It was discovered in this study that higher concentration

s of cobalt nanoparticles were associated with enhanced anti-bacterial and anti-fungal activities. Because of their superior antibacterial and antifungal properties, the biosynthesized cobalt nanoparticles can be used in biomedical applications to treat a variety of illnesses [15].

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