Research Article

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Biosynthesis and screening of cobalt nanoparticles using citrus species for antimicrobial activity

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Abstract: The synthesis of the nanomaterial is crucial for its characteristics, as well as physiological features. Green nanoparticle production, which reduces metal ions using natural extracts rather than industrial chemical agents, has been created to lower costs, minimize pollution, and improve environmental and human health safety. An aqueous and methanolic extract of a combination of *Citrus sinensis* and *Citrus limetta* peel was utilized for the green synthesis of cobalt nanoparticles. UV–Visible, Fourier-transform Infrared, X-ray diffraction (XRD), scanning of electron microscopy (SEM) and other techniques were employed to describe the prepared cobalt nanoparticles. The crystal structure of cobalt nanoparticles was revealed by XRD study. The SEM images of the cobalt nanoparticles revealed the usual subdivision dimensions of 20–30 nm. Using the well diffusion method, the antibacterial activity of the crude extract derived from the combination of the two plants mentioned above and the biosynthesized cobalt nanoparticles was assessed. The result showed that the crude extract and cobalt nanoparticles exhibited antibacterial activity and cobalt nanoparticles exhibited much higher activity than the crude extract. Overall, these findings revealed that formulated cobalt nanoparticle treatment considerably prevented the development of different micro-organisms.

Keywords: cobalt nanoparticle, green synthesis, antibacterial activity, micro-organisms

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1 Introduction

The tradition of therapeutic plants is closely tied to common knowledge that is extensively shared without scientific validation and based primarily on empirical evidence. This approach seeks to condense dependence on the conventional healthcare system, adopt less expensive alternatives, and prevent and manage illnesses [1]. Nevertheless, because this kind of customary knowledge is typically passed down via years of use, it is still important to consider and do more research [2]. Plant material can be prepared and administered in several conducts. For instance, plant substances, which can be made from any part of the plant, are manufactured more commonly than infusions, sometimes known as teas. Foliage and fruit macerates are another example [3]. It is vital to assess each preparation method's physicochemical properties, such as the compounds to be extracted solubility and thermostability, before extracting a particular set of molecules [4].

Numerous commercially significant fruits in the *Citrus* genus are grown all over the world for their high nutritional and therapeutic value [5]. One of the largest families in the Sapindales order is the Rutaceae, which contains citrus. Citrus blossoms and leaves usually have strong fragrances, and their extracts are rich in useful flavonoids and other chemicals that perform well as fungicides, insecticides, and medications. [6–8]. Some of the most significant fruit trees that are cultivated worldwide are found in the *Citrus* genus [9]. Oranges, or *Citrus sinensis*, are some of the most popular fruits to eat worldwide. The zesty flavor of fresh oranges offers several health advantages. This miraculous fruit is good for the natural resistance, weight loss, and skin. As everyone knows, oranges are ironic in other essential phytonutrients including vitamin C. Orange peel also contains a portion of polyphenols, which guard against a numeral of illnesses. Because limonene is a certainly taking place molecule, it also has significant anti-cancerous qualities. Additionally, the orange peel's essential oil possesses anti-inflammatory qualities that support our immune system [10]. Another name for *Citrus limetta* is sweet lemon. Owing to their many medicinal benefits, its constituents are only used for a range of therapeutic purposes in both Asian and Western countries. Owing to its pharmacological characteristics, it is acknowledged as one of the superfoods in India. In the past, people have utilized sweet lemon to boost innate immunity, ward against scurvy, and treat skin and hair issues. Previous studies have shown that the presence of 15 different powerful compounds is what gives sweet lemon pulp its anti-inflammatory and antioxidant qualities. Limonene is one of these beneficial compounds present in sweet lemons. It is the primary ingredient in *C. limetta* and has pharmacokinetic and pharmacodynamic properties [11].

In terms of environmental sustainability, biotech and nanomaterials together, or bionanotechnology, appear to function as a substitute for a variety of applications [12]. Nature aids nanotechnology-finished biotic procedures which encourage the contact among the microstructure and the media in which it will be distributed, which are aided by medicinal plant resources. Bio-nanotechnology presenting itself as a safe, ecologically friendly solution that can solve major challenges. It is employed in the food industry to find preservatives with no cytotoxicity, in the environmental industry to develop possible antibacterial substances via the collaboration of the natural dispersive medium and nanostructure, and in environmental remediation and recovery approaches [13–15].

Recently, biological methods of nanoparticle synthesis have drawn more interest than physical and chemical ones [16]. The absorbance maxima of the aqueous mixed extract were located at 460 nm in the UV–Visible spectra of the cobalt nanoparticles generated in the reaction media. The particles are Pol disperses, as evidenced by the large signal widening observed between 350 and 480 nm [17,18]. Chemical processes including chemical reduction and other methods are typically used in the manufacturing of these materials. However, these procedures produce dangerous by-products in addition to requiring the usage of substances that are cytotoxic. Thus, herbal cuttings and microorganisms are utilized today to produce nanomaterials [19]. The biosynthesis of nanoparticles is advantageous over microorganisms because it eliminates the requirement for the challenging procedure of safeguarding cell cultures and can be effectively scaled up for large-scale nanoparticle manufacturing. Because of this, researchers are still working to develop safe, affordable, environmentally acceptable, and clean ways to manufacture nanoparticles [20,21]. Because of this, current studies in the relevant scientific domains have concentrated on producing metallic oxide nanomaterials using completely new plant extracts. Moreover, because of its straightforward scientific basis and the simplicity with which nanoparticles of various diameters and topologies may be generated, there is a dramatic increase in interest in the biosynthesis of metallic oxide nanoparticles [22]. It is thought that flavonoids and other phenolic compounds contribute to the reducing or antioxidant qualities of medicinal plants. When the metal ions in the plant extract are reduced by phenolic chemicals, individual metal nanoparticles are produced [23–25].

Cobalt is among the most auspicious bimetal because of its numerous applications, and scientists from an inclusive variety of fields have long been interested in learning more about it [26,27]. Cobalt nanoparticles are now believed to have potential applications in electromagnetic acceptance, including high-frequency circuitry and mobile phones [23,28]. Cobalt nanomaterials even have an inherent advantage in healthcare-related industries like magnetic resonance imaging and medicine delivery, where the highest standards of quality and purity are required to prevent any changes to their response stability or magnetism [29–31]. In addition, there has been a rise in the claim of manufacturing knowledge to switch the emergence and spread of bacteria resistant to drugs. One important public health issue that may be present is antibiotic resistance [32,33]. Many common metal nanoparticles, including iron, titanium, and silver, have been broadly considered in this field because of their potent antibacterial qualities. Developing new natural medicines and materials connected to nanotechnology, like biologically derived antimicrobial silver, gold, and cobalt nanoparticles, is an excellent solution to tackle this problem.

Citrus fruits were well known for their healing qualities and were utilized to address a range of illnesses, including gout, scurvy, digestive problems, and skin diseases [34]. They were also employed as an antidote to poison and as an antiseptic during the plague. Cobalt nanoparticles are an effective choice for finding new antimicrobials because of the benefits of creating green synthesis to create nanoparticles and their undeniable antibacterial efficacy across a variety of diseases. The purpose of this research was to evaluate the antibacterial effects of newly synthesized cobalt nanoparticles made from several citrus plants. We emphasized the usage of these plant components' potential in the development of various metallic nanoparticles in addition to their antibacterial properties.

2 Methodology

2.1 Preparation of extract

Orange and sweet lemon peels, *C. sinensis* and *Citrus limeetta*, were bought from the local Bhopal, Madhya Pradesh, India market. After repeatedly washing the orange and sweet lemon peels in water to get rid of any dust or debris, they were allowed to dry at room temperature in the shade before being ground into a powder in a grinder. Additionally, the two grinded ingredients were considered evenly (about 50 g each) and appropriately combined in a 1:1 ratio before extraction assembly was used to prepare the methanolic and aqueous extracts. After filtering the extract using filter paper, it was refrigerated for storage.

2.2 Biosynthesis of cobalt nanoparticle

Cobalt nitrate was used as a precursor to make nanoparticles. Dissolve 5 g of Co $(NO_3)_2 \cdot 6H_2O$ in 100 mL of deionized water and stir for 30 min. To synthesize cobalt nanoparticles, a glass flask containing 40 mL of cobalt salt solution and 60 mL of the previously generated extract was frenzied at 80°C for 2.5 h. The mixture took on a rich brown hue. Stir the mixture at room temperature for 30 min. Centrifuge the cobalt nanoparticles to collect them and then thoroughly wash them with ethanol and deionized water to remove any leftover impurities. In a vacuum oven, the cobalt nanoparticles are dried for an entire day at 60°C [35].

2.3 Characterization of nanoparticles

2.3.1 UV–Visible spectroscopy

Many different substances can be studied using UV–Visible spectroscopy, including biological molecules, inorganic chemicals, and organic molecules. Since it may offer evidence around the electronic structure and bonding of the sample, it is a helpful technology for food quality assurance and pharmaceutical industries along with organic and biotic studies [36].

2.3.2 Fourier-transform infrared (FTIR) spectroscopy

An FTIR spectrophotometer with 64 scans in the transmittance method and a resolution of 4 cm^{-1} was practiced to examine the synthesized cobalt nanoparticles.

2.3.3 Scanning of electron microscopy (SEM)

Approximately 10 µL aliquots of the synthesized cobalt nanoparticle samples were placed on slides coated with 1% polylysine and then they were post-treated with the chemicals already mentioned in a previous study for about 2 h. Afterwards, the plates were subjected to increasing concentrations of alcohol to simulate dehydration [37]. Following that, they were coated in gold, subjected to SEM examination, and critical point CO_2 dehydration.

2.3.4 X-ray diffraction (XRD)

XRD can be practiced to analyze a broad variety of materials, such as metals, minerals, ceramics, and pharmaceuticals. Given its ability to provide information on the sample's crystal structure, phase composition, and lattice parameters, it is a valuable tool for materials research, quality assurance, and process improvement in industrial settings. Using CuKα radiation and a diffractometer operating at room temperature, 40 kV, and 20 mA, this investigation was carried out. X-ray photons from a powder sample were collected every 2 h using a Mythen 1 K detector [38].

2.4 Screening of antimicrobial activity

2.4.1 Test microorganism

Escherichia coli (MTCC No. 1698), *Streptococcus pneumonia* (MTCC No.655), *Pseudomonas aeruginosa* (MTCC No. 7925) were produced according to their toxicological and medical importance and preserved for further study.

2.4.2 Antimicrobial assay

The process of antibacterial testing determines a substance's ability to stop or completely eliminate the growth of bacteria. It is widely used to evaluate the effectiveness of antibiotics and other antimicrobial medications in treating and preventing bacterial infections. Antibacterial testing can be done with a variety of bacterial strains. It is important to keep in mind that the results of antibacterial testing may vary depending on the type of bacterium, how the test is conducted, and the specifics of the experiment. Therefore, to ensure reliable and consistent results, many testing could be necessary.

After sterilizing the nutrient agar medium for 15 min at 121°C and 15 pounds of pressure, the media was transferred onto Petri dishes. A 5-mm-diameter cork holder was drilled into the cemented plates. The antimicrobial assay was performed on the well-equipped plates. Using the well diffusion method, the antibacterial activity of crude extract and synthesized cobalt nanoparticles of several concentrations, 250, 200, 150, and 100 µg/ml was evaluated against *E. coli*, *S. pneumonia*, and *P. aeruginosa* [39].

Using the streak plate approach, various carefully chosen bacterial strains were added to the prepared culture plates for inoculation. Using a sterile syringe, several samples were poured into the well. To check for bacterial activity, the plates were incubated for 24 h at 37°C. Testing was done on various bacterial strains using varying concentrations of the crude extract and cobalt nanoparticles made from different extracts. The zone of inhibition was calculated by measuring the circumference of the inhibition zone, which included the well diameter. Four distinct fixed orientations were used to calculate the readings and average results.

2.5 Statistical analysis

Primer was the program used to explore the data. An analysis of variance was conducted between the various concentrations, with a significance level of $P < 0.05$. It was possible to determine the data's mean and standard deviation.

3 Results

3.1 Biosynthesis of cobalt nanoparticles by *C. sinensis* and *C. limetta* peel

Once cobalt nanoparticles have developed, the color of the fluid changes. The hue of the solution was used to track the biogenesis of cobalt nanoparticles after three days. The orange suddenly turning grey indicates the reduction. The intensity of the color shift was highest on the second day, and there was no change observed on the third day, suggesting that the biosynthesis of cobalt nanoparticles had stopped. The acquired color of a solution can reveals whether the nanoparticles are spherical, circular, or rod-shaped. Because surface plasmon resonance is excited, cobalt nanoparticles exhibit an orange-brown color in aqueous solution and methanol. The resulting solution color reformed from yellowish to dark orange and orange to dark brown when combined with the aqueous solution and methanol of the cobalt ion complex respectively. The production of the cobalt nanoparticles was caused by the decrease of cobalt ions, which is shown in Figure 1.

$$\text{Extract } + Co(NO_3)_2 \rightarrow Co_3O_4 \text{ nanoparticle.}$$

3.2 UV–Visible spectroscopy

In the UV–Visible spectrum, the absorbance maxima of the cobalt nanoparticles of methanolic and aqueous extract showed absorbance maxima at 510 and 460 nm, respectively. The large enlargement of the signal between 350 and 480 nm and between 480 and 550 nm indicates that the particles are Pol dispersed.

3.3 FTIR spectroscopy

The numerous functional groups present in the biosynthesized cobalt nanoparticles of aqueous combination extract

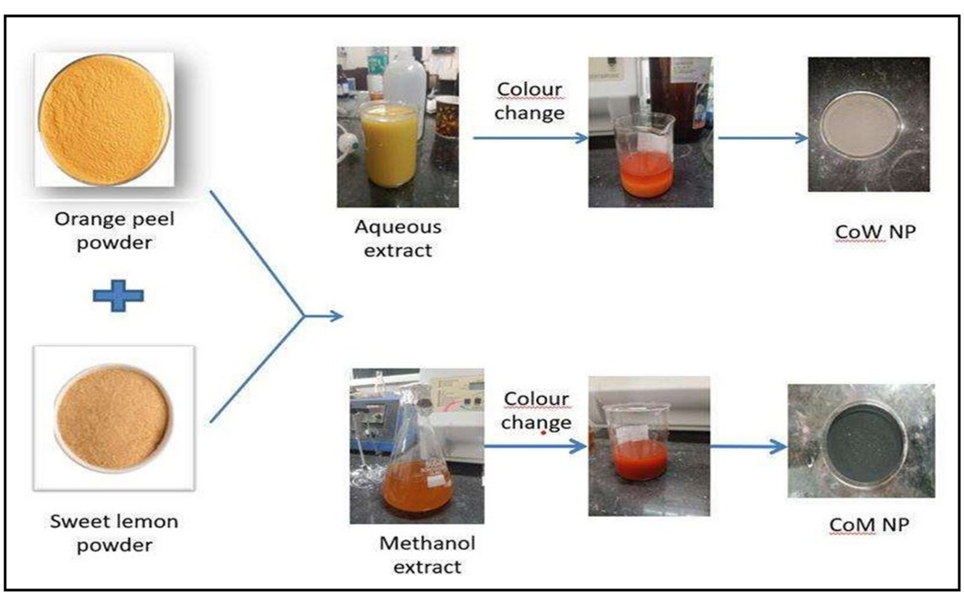


Figure 1: Biosynthesis of cobalt nanoparticles from the combined extract of peel of orange and sweet lemon in aqueous (CoW) and methanol (CoM).

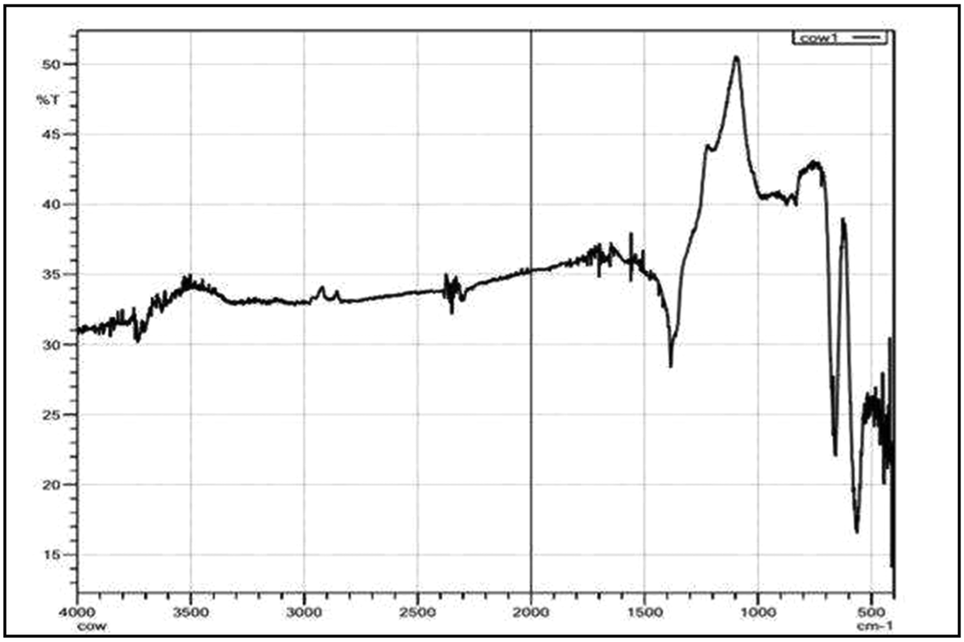


Figure 2: FTIR spectra of biosynthesized cobalt nanoparticle of aqueous combined extract of orange and sweet lemon peel.

of orange and sweet lemon peel were identified using FTIR analysis. The average peak size of hydroxyl groups in phenolic compounds was around 1,200 cm^{-1}. The carbonyl group, amide group, C–O of alcohols or phenols, and Co_3O_4 at 1,322, 800, 600, and 400 cm^{-1}, respectively, can be attributed to further noteworthy peaks. Additionally, the FTIR measurement of the biosynthesized cobalt nanoparticles of the methanolic combined extract of orange and sweet lemon peel, as shown in Figures 2 and 3, yields nearly identical peaks.

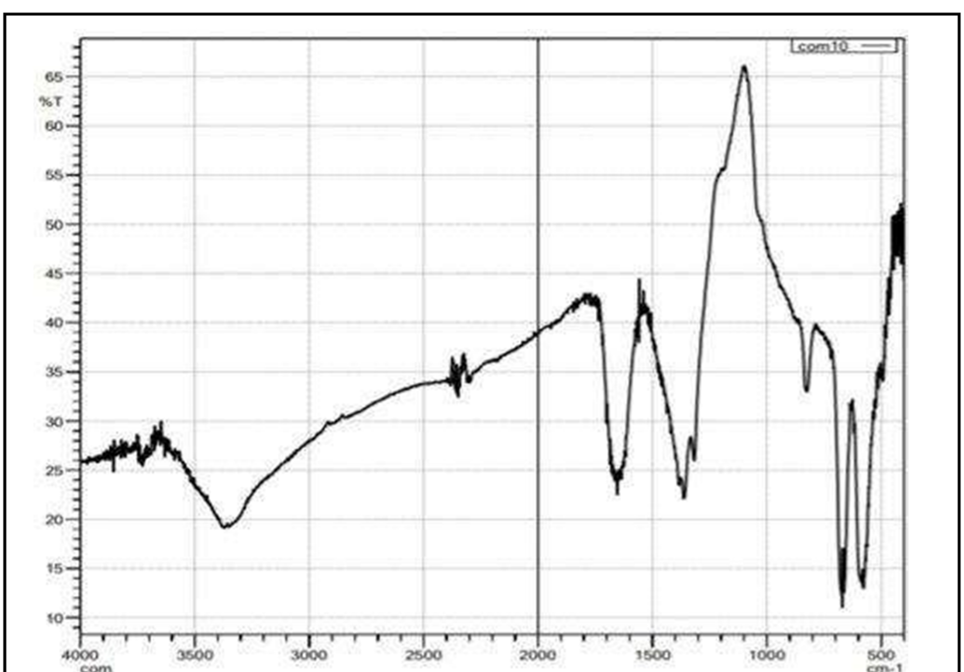


Figure 3: FTIR spectra of biosynthesized cobalt nanoparticle of methanolic combined extract of orange and sweet lemon peel.

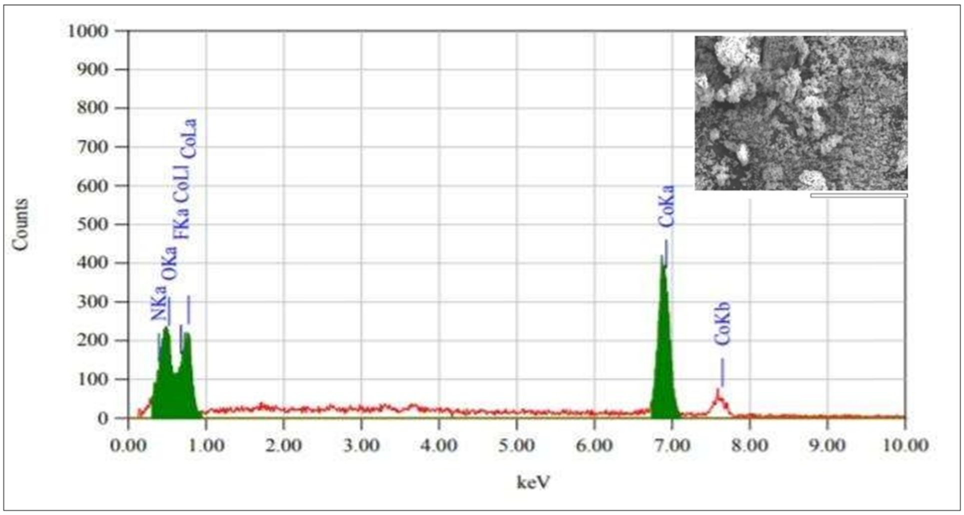


Figure 4: SEM image and EDS analysis of biosynthesized cobalt nanoparticles.

3.4 SEM

The cobalt nanoparticles' nanoparticle-like shape, which denotes well-uniform particles with narrow size allocations between 20 and 30 nm, is clearly visible in the SEM image of the particles displayed in Figure 2. Biosynthesized nanoparticles are more effective at killing bacteria because of their flat surface, which facilitates better contact with bacterial cell walls. Such behaviors of smooth-surfaced nanoparticles have already been demonstrated. The elemental composition of the cobalt nanoparticles was assessed by EDS analysis. The synthesized cobalt nanoparticles are shown by the prominent peaks. The elemental composition of the nanoparticles is 17.03 wt% carbon and 82.97 wt% cobalt. The compositional results of the EDX analysis demonstrate great compositional uniformity across the nanoparticles and are in good accord with theoretically predicted values (Figure 4).

3.5 XRD

With powder XRD, the effective biogenesis of cobalt nanoparticles was verified. It is clear from Figure 5 that the characteristic peaks observed in the XRD image supported the resulting diffraction pattern in a biosynthesized cobalt nanostructure. The angles of the various diffraction peaks are 35, 36, 2, 42, and 65 degrees, respectively. The particle size was discovered to vary between 10 and 20 nm.

3.6 Antimicrobial activity

Various amounts of synthesized cobalt nanoparticles were tested against various species of bacteria, as well as one control group. The control group does not include any extracts or nanoparticles. By measuring the diameter of the inhibition zone surrounding the well, including the well diameter, the zone of inhibition was computed. It was discovered that, when tested against various bacterial strains, the zone of inhibition of the biosynthesized cobalt nanoparticles in both extracts was significantly higher than that of the crude extract (Tables 1 and 2; Figures 6 and 7).

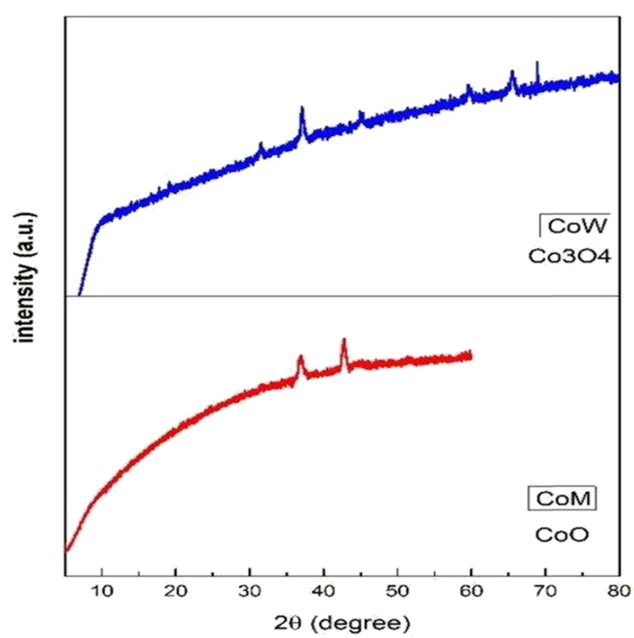


Figure 5: XRD spectra of biosynthesized cobalt nanoparticles.

Table 1: Presenting zone of inhibition (ZOI) in aqueous medium against different microorganism

Microorganisms	ZOI (mm)	ZOI of extract (mm)				ZOI of cobalt nanoparticles (mm)			
	Control	250 μg/ml	200 μg/ml	150 μg/ml	100 μg/ml	250 μg/ml	200 μg/ml	150 μg/ml	100 μg/ml
S. aureus	00.00	19.10 ± 0.65	18.89 ± 0.76	20.45 ± 1.23	17.17 ± 0.39	30.24 ± 0.23	29.84 ± 0.14	28.00 ± 0.49	27.56 ± 0.28
P. aeruginosa	00.00	23.60 ± 2.64	22.86 ± 1.09	24.10 ± 1.76	20.42 ± 1.81	33.38 ± 0.54	34.95 ± 1.28	35.61 ± 0.40	36.94 ± 0.59
E. coli	00.00	17.12 ± 0.67	9.00 ± 1.29	10.97 ± 2.34	8.17 ± 1.89	27.65 ± 0.98	26.00 ± 1.36	24.41 ± 0.76	20.82 ± 0.10

4 Discussion

This study explains the fundamental principles of "green" chemistry, as well as "green" ways for producing metal nanoparticles from bioresources. The most popular method for producing these nanoparticles is a "bottom-up" approach that employs a wide range of organic solvents, poisonous chemicals, and non-ecological reagents under high pressure and temperature. Alternative, cost-effective, and secure solutions are therefore required. "Green" synthesis mitigates the detrimental influence on the environment and human health by preventing contamination during the early stages of chemical processes [40,41]. Many innovative biological techniques have been devised, utilizing plants, algae, fungi, bacteria, viruses, and so on. Plants are an excellent source of precursors for the production of metal nanoparticles and other "green" resources since they are abundant, easy, and nontoxic. Plant suitability is governed by elements such as total protein content and antioxidant capabilities. To enhance the practical use of "green" nanoparticles, it is necessary to study their physical and chemical properties, stability, and activity. This study discusses the synthesis of magnetic nanoparticles using several techniques such as sol–gel, hyperthermal, and auto-combustion. Reductants are commonly derived from plant extracts such as *C. limetta* (Sweet lemon) and *C. sinensis* (Orange) peels. Metal nanoparticles can be created using a "green" process, allowing for more exact sizes and shapes. These revolutionary "green" technologies have the potential to drastically reduce both environmental pollution and health dangers. SEM, UV, FTIR, and XRD demonstrated the successful biogenesis of cobalt nanoparticles.

Depending on the bacteria tested and the exposure dose, cobalt nanoparticles demonstrated bacterial activity against various bacterial strains. *Staphylococcus aureus* is a significant bacterial human pathogen with a wide range of clinical symptoms. Infections are widespread in both community and hospital settings, and treatment remains difficult to manage due to the introduction of multidrug-resistant strains such as methicillin-resistant *S. aureus*. *S. aureus* can be found in the environment as well as in normal human flora, where it lives on most healthy people's skin and mucous membranes (most commonly in the nose area). *S. aureus* does not often infect healthy skin; nevertheless, if permitted to enter the bloodstream or internal tissues, these bacteria can cause a variety of potentially dangerous illnesses. Typically, transmission occurs through direct contact. However, some infections are transmitted by different means [42].

E. coli is commonly found in the enteral microbiota; nonetheless, unhealthful strains are the leading cause of disorders in humans and are also the most responsible for tract infections. Furthermore, *E. coli* has been shown to be resistant to numerous cephalosporins and fluoroquinolones, which are common antibiotics used in human medicine [43].

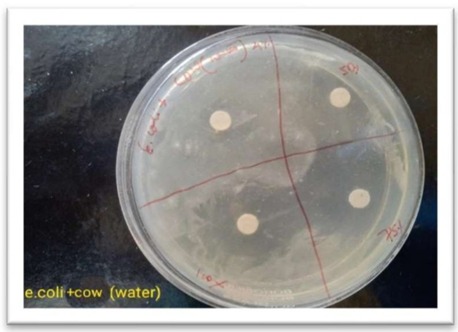
P. aeruginosa is a gram-negative, aerobic, non-spore-producing rod that can cause a range of infections in both immunocompetent and immunocompromised individuals. Its proclivity to induce infections in immunocompromised hosts, high adaptability, antibiotic resistance, and a diverse set of dynamic defenses make it an extraordinarily difficult organism to treat in modern medicine [44].

Table 2: Presenting zone of inhibition (ZOI) in methanol against different microorganisms

Microorganisms	ZOI (mm)	ZOI of extract (mm)				ZOI of cobalt nanoparticles (mm)			
	Control	250 g/ml	200 μg/ml	150 μg/ml	100 μg/ml	250 μg/ml	200 μg/ml	150 μg/ml	100 μg/ml
S. aureus	00.00	12.83 ± 0.19	9.94 ± 2.64	10.80 ± 1.90	8.69 ± 2.45	27.00 ± 1.54	23.82 ± 0.23	20.57 ± 0.78	18.63 ± 0.31
P. aeruginosa	00.00	13.34 ± 2.68	11.59 ± 1.90	10.56 ± 2.06	10.51 ± 1.72	20.00 ± 0.47	20.98 ± 0.62	18.16 ± 1.04	19.37 ± 0.52
E. coli	00.00	18.43 ± 0.58	15.18 ± 1.34	17.94 ± 0.39	13.75 ± 0.26	27.00 ± 2.36	20.57 ± 0.27	22.60 ± 0.38	20.98 ± 0.61

(a)

(b)



(c)

Figure 6: Images of antibacterial activity of discs of different concentrations of Co nanoparticles prepared from aqueous extract on (a) *S. pneumonia*, (b) *P. aeruginosa*, and (c) *E. coli.*

(a)

(b)

(c)

Figure 7: Images of antibacterial activity of discs of different concentrations of Co nanoparticles prepared from methanolic extract on (a) *S. pneumonia*, (b) *P. aeruginosa*, and (c) *E. coli.*

Chemicals like the cobalt nanoparticles that were biosynthesized in this work have the potential to take the role of antibacterial therapies, as bacterial resistance is a significant public health concern.

5 Conclusion

The current study's conclusion is to produce cobalt nanoparticles by combining two plant extracts – one from oranges and the other from wonderful lemons – in various solvent combinations. Cobalt nanoparticles are often produced using a variety of chemical synthesis procedures, including sol–gel, chemical reduction of oxides, and plasma-chemical synthesis. Despite being multistep procedures, these chemical modes are routinely used to manufacture cobalt nanoparticles; nonetheless, they are not environmentally benign and produce toxic byproducts. Plant extract is not used in the manufacture of cobalt nanoparticles; hence, no hazardous compounds are created. Overall, our findings indicated that treatment with biosynthesized cobalt nanoparticles considerably inhibited the growth of numerous bacteria. The utility of biosynthesized cobalt nanoparticles in biological applications, such as the treatment of infectious diseases, became clear.

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Conflict of interest: All authors declared no conflict of interest.

Ethical approval: The conducted research is not related to either human or animals use.

Data availability statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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