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Biosynthesis of Cobalt Oxide Nanoparticles Utilizing *Ficus religiosa* Leaf Extract and its Characterization and Biological Activity

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Abstract: ***The aim of the study, a facile green approach to synthesize cobalt oxide nanoparticles by using Ficus religiosa leaf extract. The structural properties of the as - synthesized cobalt oxide nanoparticles were characterized by UV - Vis spectrophotometry, Fourier Transform Infrared (FT - IR), X - ray diffraction (XRD), Scanning Electron Microscopy (SEM), Energy dispersive X - ray spectroscopy (EDX) and Transmission Electron Microscopy (TEM). The antibacterial activity of the synthesized cobalt oxide nanoparticles when tested against two - gram positive bacteria and one - gram negative bacteria was evaluated and good results were obtained. The antifungal activity when tested against two fungi showed a very good result.***

Keywords: green synthesis, cobalt oxide nanoparticles, antibacterial activity, antifungal activity

1. Introduction

One of the notable active areas of investigation in modern material science is in nanotechnology. In the last several years, there have been substantial developments in nanotechnology in relation to the, “green synthesis” of nanoparticles using plant parts extracts, human genes, and microorganisms. Green nanotechnology refers to the incorporation of green chemistry and green engineering principles in nanotechnology. There are many methods in which nanoparticles can be synthesized [1 - 3]. However, conventional methods (physical and chemical) for producing nanoparticles create toxic by - products, that may be hazardous to the environment.

In recent years, the synthesis of environmentally friendly nanoparticles that do not produce toxic waste has been of interest [4, 5]. This can be done through biological nature with biotechnology tools that are relatively safe and ecologically good for fabrication instead of conventional chemistry methods. Green nanotechnology refers to the synthesis of nanoparticles or nanomaterials using biological routes, notably microorganisms, plants, viruses, or their by - products, such as proteins and lipids. Nanoparticles made by green technology are much more advantageous than nanoparticles made by traditional methods because they do not require costly chemical precursors and use less energy, producing waste products that are eco - friendly, at the same time [6]. The plant extracts from seeds, stem, roots, leaves and fruits contain phytochemical compounds for the reduction of metal ions into nanoparticles [7]. All these green synthesis consequences are traditionally believed to be activated by active compounds such as polyphenols, flavonoids and other secondary metabolites [8].

Cobalt oxide nanoparticles are frequently used in industry for a range of applications, because of low - cost, environmentally - friendly, and greater surface area per unit weight compared to their bulk material counterparts. A green method for the synthesis of cobalt oxide nanoparticles is to utilize the leaf extract from the *Ficus religiosa* plant, which is a convenient method for reducing the metal in solutions with plant extracts. The use of a green route for the synthesis of cobalt oxide nanoparticles in comparison to conventional materials is really straightforward, and less toxic, and gives certain high yields. *Ficus religiosa* has been venerated for its sacred importance in Hindu mythology in India for many centuries, the leaf extract possesses a variety of activity (anti - inflammatory, anti - oxidant, anti - allergic, anthelmintic) due to the presence of compounds, such as polyphenols and flavonoids, which make suitable replacement for toxic reducing agents for cobalt oxide synthesis [9]. However, literature indicates that an alkaline medium ($pH > 11$) was more beneficial in comparison to acidic and neutral conditions for the synthesis of cobalt oxide nanoparticles due to the mono - dispersed and highly stable system for the production of cobalt reducing ions in the solution [10].

This study reports on the green synthesis of cobalt oxide nanoparticles using *Ficus religiosa* leaf extract, evaluates its antimicrobial activity, and discusses its structural and morphological characteristics.

2. Review of Literature

In the previous studies, various plant parts are used to synthesize the nanoparticles. The plants such as *Aspalathus linearis [*11], *Azadiracht indica [*12], *Calotropis giganta [*13], *Euphorbia heterophylla [*14], *Moringa oleifera [*15], *Piper nigrum [*16], *Tamarind [*17] and *Taraxacum officinal [*18] were used for nanoparticles synthesis. Phytochemicals can condense metal salts into metal nanoparticles. Among the nanomaterials, the metallic nanoparticles, which have antibacterial and antitumor properties, open new avenues to combat and prevent different types of tumour's and other infectious diseases. Its inexpensive nature, environment friendliness and its considerable role in the synthesis and production of cobalt nanoparticles are in wide use in various sectors [19].

3. Materials and methods

Materials: Chemicals were procured from Kevin Laboratories, Chennai, analytical grade cobalt nitrate ($Co(NO_3)_2.6H_2O$, 98% purity), hydrochloric acid, sodium hydroxide. Various strains of bacteria were used in testing, including *Staphylococcus aureus*, *Enterococcus*, and *Escherichia coli*, alongside fungal strains *Aspergillus niger* and *Candida albicans* obtained from the King Institute of Preventive Medicine and Research Centre, Guindy, Chennai, Tamilnadu.

Preparation of *Ficus religiosa leaf* extract: Fresh and fully grown leaves of *Ficus religiosa* were collected from Chennai, Tamilnadu. The leaves were washed with water a few times to remove soil and contaminants, and subsequently washed thoroughly in double distilled water. The washed leaves were chopped into small pieces, air - dried over 5 to 10 days in a shaded area, and uniformly dried in an electric blender. Dried leaves were ground into a fine powder, which was then stored in an air - tight container till next use. Ten grams (10 g) of the leaf powder were mixed with 250 ml of double distilled water. The leaf powder suspension was then heated on a water bath for 30 minutes at 80 - 90°C and then allowed to cool for 1 h before centrifugation in order to get rid of the fine tissues in the leaf extract. The supernatant was carefully filtered through Whatman No.1 filter paper to remove all the biomass left in the leaf decocted. The filtrate was centrifuged at 1200 rpm for 15 min; the pale brown solution was made to pH - 11 by adding 0.1 NaOH solution and then set aside for further use [20].

Synthesis of Cobalt Oxide Nanoparticles: Copper nitrate solution of 0.1M was prepared in a 250 ml Erlenmeyer flask using double distilled water, with *Ficus religiosa* leaf extract being added gradually under uniform stirring, maintaining a 5: 5 ratio for 30 minutes. Eventual colour change might be observed, and the time it took for the reaction to occur was noted. A cloud of particles was observed with a clear colour change of the solution indicating the presence of nanostructures formation. The solution was further centrifuged, and the residue obtained was dried in a hot air oven for 24 hours at 100ºC. The synthesized cobalt oxide nanoparticles were kept in the muffle furnace at 350ºC for 4 hours. Freshly synthesized cobalt oxide nanoparticles were stored in air - tight containers for further characterization [21].

Characterization: The absorption properties of the biosynthesized cobalt oxide nanoparticles and the leaf extract was examined by UV - Vis spectrometer (Elico SL210) was monitored between 200 and 650 nm. FT - IR measurements was done using Perkin - Elmer spectrophotometer to determine the different types of chemical bonds between bioactive compounds of extract and cobalt oxide nanoparticles and the samples were scanned from 400 – 4000 cm^{-1}. The nature of the particles was studied using X - ray diffractometer (D8 Bruker Advance) using CuKα radiation at a scan rate of 0.02%/min with constant time of 2 min. The morphology and particle size of the synthesized cobalt oxide nanoparticle was elucidated from the scanning electron microscopy (JEOL - 6390LA) and transmission electron microscopy (HEOL - JEM 2100) fortified along selected area electron diffractometer.

Anti - microbial activity of cobalt oxide nanoparticles: The anti - microbial activities of cobalt oxide nanoparticles from the *Ficus religiosa* leaf extract were evaluated the Agar Well Disc Diffusion method [22]. The agar well diffusion technique was used to assess the antibacterial efficacy of CoONPs against clinically relevant bacterial pathogens (*Staphylococcus aureus*, *Enterococcus*, and *Escherichia coli*). Using the agar well diffusion technique CoONPs were evaluated for their antifungal efficacy against clinically relevant fungal pathogens (*Aspergillus niger* and *Candida albicans*).

4. Results and Discussion

Characterization of Plant mediated synthesis of CoONPs
UV – Vis studies

The formation of cobalt oxide nanoparticles is supported by their strong surface plasmon resonance absorption shown in the UV - Vis spectra. The UV - Vis spectra of *Ficus religiosa* leaf extract and green synthesized CoO NPs are shown in Figure 1. The peak maximum observed at 234 nm in the extract - theoretical peak was shifted to 301 nm indicating the phytocompounds in the extract reduced the cobalt ions into cobalt oxide nanoparticles effectively. The smaller peak that is observed may be from small organic molecules in the reaction mixture. The UV data can help support characterization of the plant extract - mediated cobalt oxide nanoparticles [23]. Using the Planck's equation, the band energy gap of cobalt oxide was determined to be 4.12 eV.

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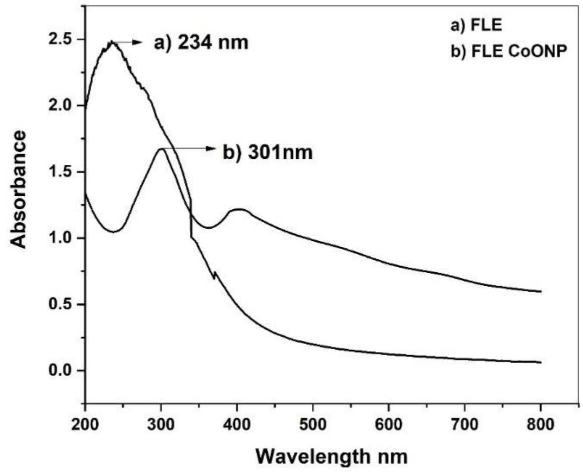

Figure 1: UV - Visible absorbance spectra of a) *Ficus religiosa* leaf extract and b) CoO NPs

FT – IR spectroscopy analysis

The FTIR spectrum of *Ficus religiosa* leaf extract show peaks at the wave length of 3329 cm^{-1}, 2215 cm^{-1} and 1620 cm^{-1} (Figure 2a). A band in the region of 3329 cm^{-1} is due to the stretching vibration of amino and hydroxyl groups (- NH and - OH). The band in the region of 2215 cm^{-1} is assigned to the stretching vibration of C - H bands due to the presence of CH_2 - CH_3 groups in the structure of leaf extract. The shouldered peak appearing at 1620 cm^{-1} is due to stretching vibrations of carbonyl compounds which indicates the presence of fatty acids present in the leaf extract. The FTIR spectra of cobalt oxide nanoparticles is also showing a number of peaks. In the Figure 2b, the broad peak at 3316 cm^{-1} with decreased transmittance because cobalt metal ions reduce the various - OH group containing group to alcohols, - C - H stretching vibration of alkyl deformation also reduced by cobalt ions. Peak at 2021 cm^{-1} denotes the C - H stretching H - bonded alkane. The peaks cantered at 1618 cm^{-1} is due to stretching and bending vibration of - OH group of the water molecules. The peak at 971 cm^{-1} is due to the stretching vibration of NO_3^-, which may be due to the adherence of cobalt nitrate salt, which was used as precursor material for the synthesis procedure. The observed peak at 636 cm^{-1} is represented with Co - O and O - Co - O stretching frequency that confirmed the configuration of cobalt oxide nanoparticles [24]. The comparative spectra of the leaf extract and cobalt oxide nanoparticles indicate that the phenolic compounds in the leaf extract interact with cobalt metal, allowing for the stabilization and dispersion of cobalt oxide nanoparticles [25].

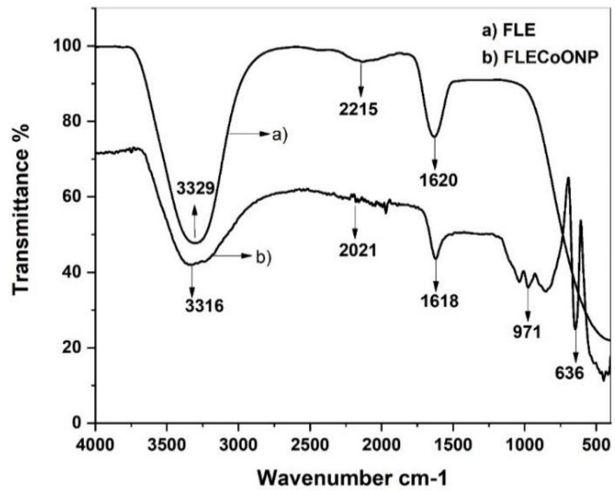

Figure 2: FT - IR spectra of a) *Ficus religiosa* leaf extract (FLE) and b) Cobalt oxide nanoparticles (FLE CoO NPs).

X – ray diffraction (XRD) analysis

The XRD pattern of biogenic cobalt oxide NPs manifests a clear monoclinic structure whose characteristic 2θ are 19.02º, 31.32º, 36.79º, 44.90º, 59.42º and 65.37º respectively; their respective planes were expected to be (111), (220), (311), (222), (511) and (440). It is apparent that all diffraction peaks can, in general, be indexed to JCPDS card no.073 - 1701 [26].

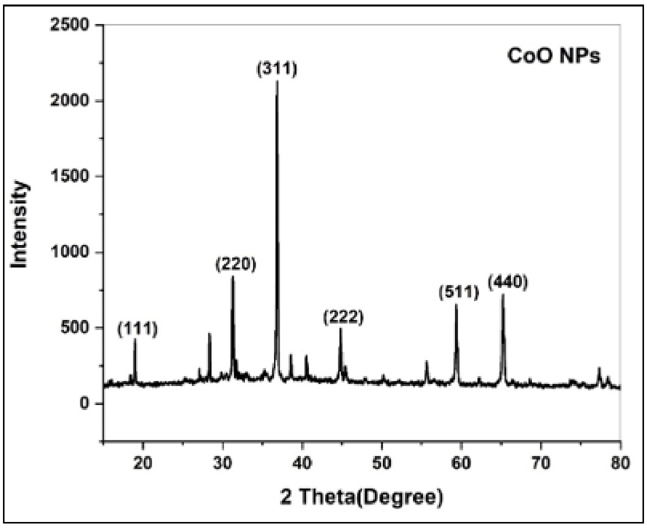

Figure 3: X - ray diffraction spectrum of Cobalt oxide nanoparticles

The typical crystallite size of the cobalt oxide nanoparticles was calculated by using the Debye - Scherer equation and the value of 'd' (the interplanar spacing between the atoms) is calculated using Bragg's equation. The calculated results were depicted in Table 1.

Table 1: Structural parameters of the biosynthesized Cobalt oxide nanoparticles.

2 Theta	Theta	d – spacing	FWHM	Crystallite Size D (nm)	D nm (Average)
19.02	9.51	4.6623	0.1293	62.3068	34.97 nm
31.32	15.66	2.8537	0.1885	43.7625	
36.79	18.40	2.4404	0.3541	23.6424	
44.90	22.45	2.0171	0.3096	27.7673	
59.42	29.71	1.5542	0.3075	29.7485	
65.37	32.69	1.4262	0.4174	22.6154	

Scanning Electron Microscopy (SEM)

The characterization of cobalt oxide nanoparticles through SEM, seen in Figure 4, was an extensively undertaken analysis to verify their morphology and size distribution. The morphology of cobalt oxide nanoparticles in terms of their external characteristics exhibited irregular, random, and cubo - hexagonal shape with various levels of openness or agglomeration (Figure 4a). The SEM morphology demonstrated the agglomeration of CoO NPs. SEM images taken at a higher magnification demonstrated that these were smooth morphology of cobalt oxide nanoparticles. The size distribution for average and particles were confirmed using Gaussian fitting of the size distribution histogram in Report 4b. Biogenic synthesized CoO NPs sizes were observed to be between 60 – 160 nm in diameter with an average size of 120 nm.

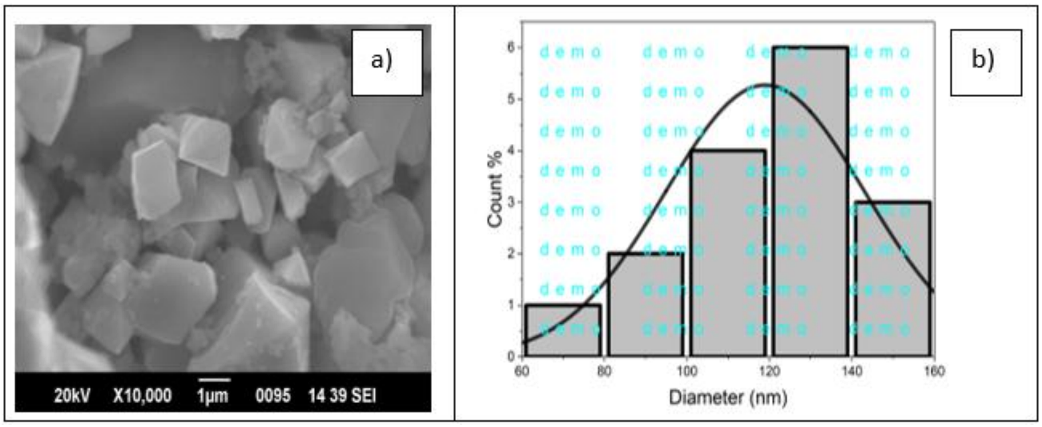

Figure 4: SEM images of green synthesized CoONPs (a) and Particle size Histogram (b).

Energy Dispersive X - ray (EDX) analysis

The spectra of elemental composition exhibited the formation of strong peaks of Co in the nanoparticles produced (Figure 5). Peaks appeared sharp in the spectrum around 2 - 3 KeV and 6.5 - 7.5 KeV. The elemental composition of biosynthesized cobalt oxide nanoparticles suggest that Co is the major constituent, with 84.52 wt. % and 59.71 At. %. The cobalt oxide nanoparticles also contained oxygen in the amount of 15.48 wt. % and 40.29 At. %. The biosynthesized cobalt oxide nanoparticles produced exhibit a high degree of purity in agreement with studies that appeared in the literature [27]. The percent weight and atomic weight percent is shown in Table 2.

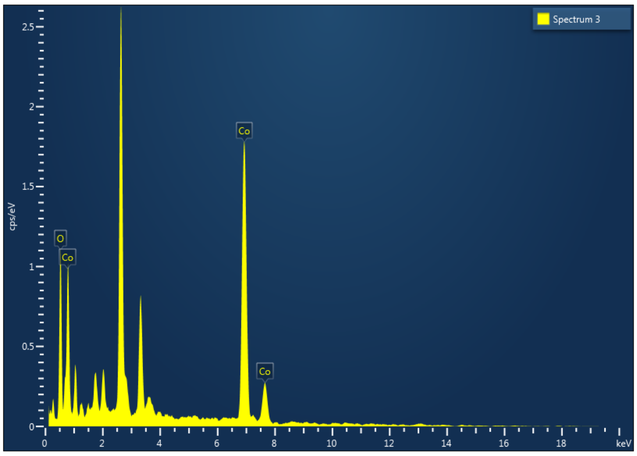

Figure 5: EDX spectrum of Calcium oxide nanoparticles

Table 2: Constituent elements and their percentage values.

Element	Line type	Weight %	Atomic %
O	K series	15.48	40.29
Co	K series	84.52	59.71

Transmission Electron Microscope (TEM) analysis

The high - resolution transmission electron microscopy study of the nanoparticles shows polydispersity in shapes and sizes, but most of the nanoparticles have a nearly spherical orbit. The porous nanomaterials have random distributions of particle sizes ranging from 160 to 300 nm, have irregular shapes, and show poorly aggregated crystalline features (Figure 6a) [28]. Figure 6b, shows a SAED pattern for the single crystals. In addition, the SAED pattern shows a spot diffraction pattern indicating that the cobalt meso crystals are arranged more or less in the same lattice direction. The SAED patterns consist of well - defined rings which could be indexed with a face - centered cubic cobalt phase. Importantly, this method did not find representations of other phases. The particle size distribution in average sizes was calculated using the Gaussian fitting of size histogram distribution shown in a form of Figure 6c. The range of particle sizes for biogenic synthesized CoO NPs was between 160 - 360 nm, with an average particle size of 220 nm.

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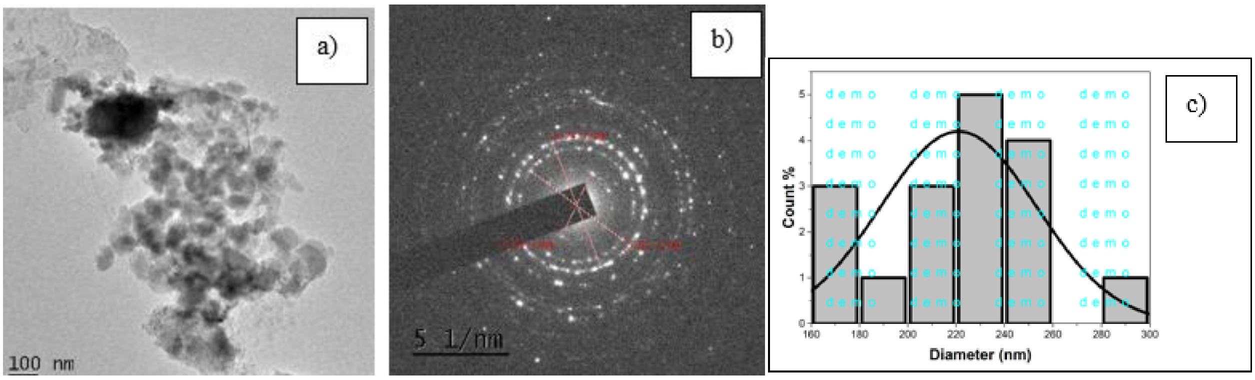

Figure 6: a) TEM image b) SAED pattern and c) Particle size histogram of CoONPs

Anti – Bacterial activity

Mueller – Hinton Agar well diffusion method

The bio synthesized cobalt oxide nanoparticles exhibited good anti - bacterial activity against bacterial strains. The zone of inhibition of cobalt oxide nanoparticles was observed 26, 30, 34 and 38 mm for *Staphylococcus aureus (*Gr +ve) bacteria. The zone of inhibition of cobalt oxide nanoparticles was noted 20, 28, 30 and 34 mm for *Enterococcus (*Gr +ve) bacteria. The zone of inhibition was observed 22, 24, 25 and 28 mm for *Escherichia coli (*Gr - ve) bacteria (Table 3). Figure 7, showed that green synthesized cobalt oxide nanoparticles have excellent anti - bacterial activity against Gram positive (*Staphylococcus aureus* and Enterococcus) bacteria than Gram negative (*Escherichia coli*). From the Figure 7, the zone of inhibition of bacterial growth was not observed at lower concentrations, ZOI is increased as the dose of cobalt oxide nanoparticles is augmented. This represents the dose dependent antibacterial activity of cobalt oxide nanoparticles against clinically significant pathogens. Further, the results are analogous with standard antibacterial drug Gentamycin (10 µg/well).

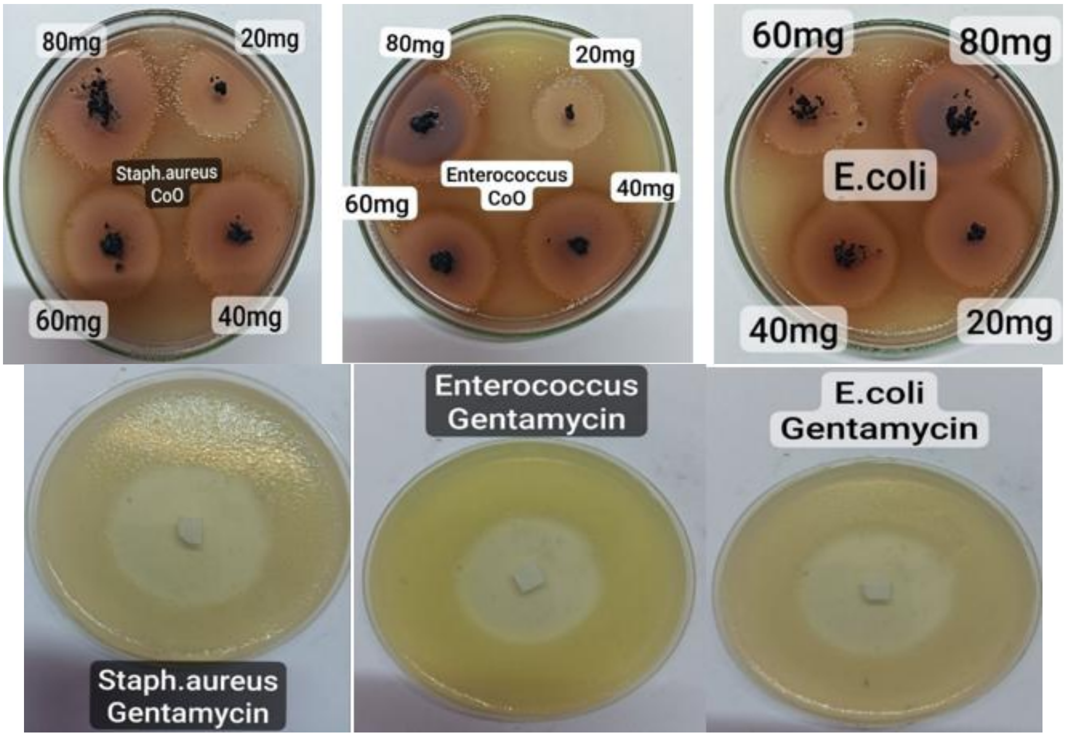

Figure 7: Well diffusion method antibacterial activity against *Staphylococcus aureus, Enterococcus*, and *Escherichia coli*, using green synthesized cobalt oxide nanoparticles and Standard is Gentamycin 10 µg/well

Table 3: Zone of Inhibition of green synthesized cobalt oxide nanoparticles

Concentration (mg)	Zone of Inhibition (mm)		
	Staphylococcus aureus	*Enterococcus*	*Escherichia coli*
20	26	20	22
40	30	28	24
60	34	30	25
80	38	34	28
Gentamycin (10 mg)	40	40	40

Anti – Fungal activity

Potato Dextrose Agar well diffusion method

The bio synthesized cobalt oxide nanoparticle demonstrated significant anti - fungal activity against the tested fungal pathogens (*Aspergillus niger* and *Candida albicans*). Among the two tested fungal pathogens, cobalt oxide nanoparticle demonstrated highest antagonism against *Aspergillus niger* with a ZOI of 32 mm 80 mg/ml concentration (as shown in Figure 8.). Based on the agar - well diffusion assay, it was confirmed that the green synthesized cobalt oxide nanoparticle is a potent anti - fungal agent with significant antagonism against human fungal pathogens (Table 4) [29].

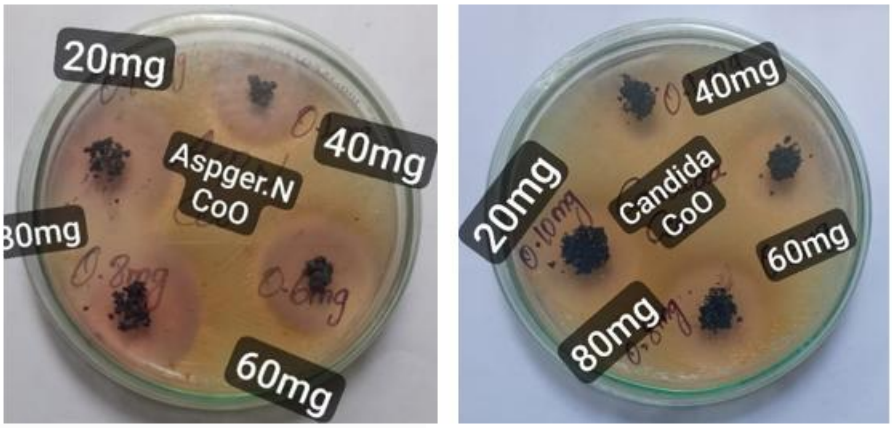

Figure 8: Well diffusion method antifungal activity against *Aspergillus niger* and *Candida albicans using* green synthesized cobalt oxide nanoparticles and Standard is Clotrimazole 10 mg/well

Table 4: Zone of Inhibition of green synthesized cobalt oxide nanoparticles

Concentration (mg)	Zone of Inhibition (mm)	
	Aspergillus niger	*Candida albicans*
20	19	11
40	23	13
60	28	15
80	32	18

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