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Synthesis of Cobalt Oxide Nanoparticles from the Leaf Extracts of *Eruca Vesicaria* its Characterization Antibacterial and Antifungal Activity

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ABSTRACT

The leaf extracts of *Eruca vesicaria* was used to synthesize the cobalt oxide nanoparticles by the co-precipitation method. The synthesized nanoparticles were characterized by UV-Visible Spectroscopy, Fourier Transform (FTIR), Scanning electron Microscopy (SEM), EDX analysis. UV -visible spectroscopy showed the strong absorption band at 200-350 nm. The synthesized cobalt nanoparticles were tested for antibacterial activity in *Bacillus subtilis and Proteus vulgaris* and the antifungal activity in *Candida Albicans and Aspergillus Fumigatus*. For the synthesized cobalt oxide nanoparticles the minimum inhibitory concentration were also tested against the two bacterial and the fungal species for the least concentration. The gram-positive bacteria *Bacillus subtilis* showed maximum zone of inhibition (16.5±0.7). In fungal studies the maximum zone inhibition showed by *Candida albicans* (12.5±0.7).

KEYWORDS: Cobalt oxide; *Eruca vesicaria*; Antibacterial; Anti-fungal, Surface functionalisation; Co-precipitation method.

1. INTRODUCTION

Green Chemistry and other biological processes are becoming more popular and this has leads to a need to develop an environmental friendly strategy for creating nanoparticles. The main goal of green chemistry is to produce metal, metal oxide or even their bimetallic nanoparticles in an environmentally friendly manner without using harmful or expensive chemicals. This approach focuses on using biocompatible reducing agents for nanoparticle synthesis as reported in numerous literatures [1]. Different physical and chemical methods are used in nanotechnology for the preparation of nanoparticles. These nanoparticles can be used in a variety of industries, agriculture, environment, nanomedicine, nanoelectronics, and biomaterials development [2]. The use of inorganic nanomaterials as antibacterial precursors, which are long lasting, resistant to high temperatures, active and have a high selectivity compared to other organic antibacterial agents has come to light with the development of modern nanotechnology processes [3] Laser – ablated methods can also be used to create nanoparticles can be employed as nano weapons against bacteria and fungi, but their effectiveness also depends on their dosage and size [4]. Due to its environmentally benign, non-hazardous, and non-toxic manufacturing processes, green synthesis of metal oxide, nano particles using plant has captured the attention of scientists and researchers (5). Cobalt oxide based nano particles are used in biotechnology and medical applications, such as carriers for targeted medication delivery (6). Since nano particles have a larger surface area to volume ratio, they aggregate and absorb tremendous energy. Different phytochemicals found in plants function as capping, stabilizing, and reducing agents, minimising the aggregation of CO_3O_4 nanoparticles and giving these nano particles a distinctive property of stabilising the synthesis processes and influencing the structural shape (7). Cobalt oxide has recently been described in composites with graphene and CO_3O_4 that aid to increase the substrate' dimensional stability (8). Despite the remarkable qualities of cobalt metal nano particle, their smaller surface area and a bigger crystalline size prevented their practical use on a broad scale in actual applications. Because of the components and composition of the phytochemicals in the extract vary, it has been observed that the features of the prepared nanoparticles rely on the kind and part of the plants that are used for synthesis (9).

Eruca Vesicaria is an edible annual plant in the family Brassicaceae used as a leaf vegetable for its fresh, tart, bitter and peppery flavour. Its other common names include garden rocket. *Eruca Vesicaria* is an annual plant with rigid stem, more or less bristly, hairs, usually ramified, often reddened in the upper part. The leaves are antiscorbutic, diuretic, stimulant and stomachic.

2. EXPERIMENTAL METHODS

2.1 MATERIALS USED

Cobalt chloride solution, *Eruca Vesicaria* leaves, deionized water and Whatmann no.1, beaker were among the components used in this work.

2.2 PREPARATION OF AQUEOUS LEAF EXTRACT OF ERUCA VESICARIA

In Sathyamangalam, Erode (Dt), Tamil Nadu in December 2022 fresh *Eruca Vesicaria* leaves are collected. *Eruca Vesicaria* leaves were cleaned, dried and then ground into a fine powder using a mortar.

Eruca Vesicaria leaves extract were made by mixing 10 gm of dry *Eruca Vesicaria* leaf powder with 125 ml of sterile distilled water, and kept in magnetic stirrer at 700rpm for 30 minutes and then filtering the mixture using whattmann filter paper no.1. For later usage, the filtrate was collected and kept at 4℃.

2.3 SYNTHESIS OF COBLAT NANOPARTICLE USING PLANT EXTRACT

Cobalt oxide nanoparticles were synthesized by 50 ml of the aqueous leaf extract taken in a beaker and add 100ml of NaOH solution dropwise and kept in a magnetic stirrer without any disturbance. After 15 mins add 0.1 M cobaltous chloride solution is added to the reaction mixture. Within 5 minutes, the colour change was observed which indicates that the formation of cobalt oxide nano particles. The solution can be centrifuged at 7000 rpm for 15 minutes. Then the cobalt nanoparticles were dried in an oven at 800℃ and then allowed to cool. The particles were stored in an air tight container.

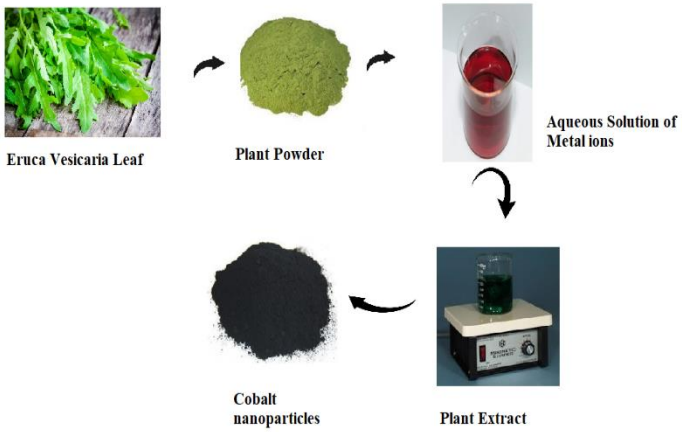

FIGURE 1: Schematic representation of cobalt oxide nanoparticle synthesis using *Eruca Vesicaria* plant extract.

3. CHARACTERIZATION OF THE COBALT OXIDE NANO PARTICLES

3.1 FT-IR

The vibration of chemical bonds can be measured in FT-IR spectrum. The FTIR range of the dried sample was documented in the range 4000-400cm^{-1}. The basic structure of the compound can be identified in FT-IR spectrum.

3.2 UV VISIBLE SPECTROSCOPY ANALYSIS

The results obtained from the UV-VIS spectrophotometer shows that the wavelength can be operated in the range of 200 to 800 nm.

3.3 SEM ANALYSIS

The results obtained from the scanning electron microscope shows that the surface morphology of the nanomaterial (Cobalt oxide nano particles) was characterized.

3.4 EDX ANALYSIS

The results obtained from the EDX Analysis shows that the estimated elemental composition of Cobalt oxide (Co_3O_4) nanoparticles.

4. RESULTS AND DISCUSSION

4.1 UV VISIBLE SPECTROSCOPY

Due to the colour change in the reaction mixture from light brown to dark brown the formation of cobalt nanoparticles was confirmed. The UV Visible spectrum of Co_3O_4 nanoparticles revealed a strong absorption band in visible region around 200-350 nm (10). From the leaves of Eruca vesicaria plant extract Co_3O_4 Nanoparticles was synthesized that shows the strong absorption bands at 200-350 nm that confirms the cobalt oxide nanoparticles was formed.

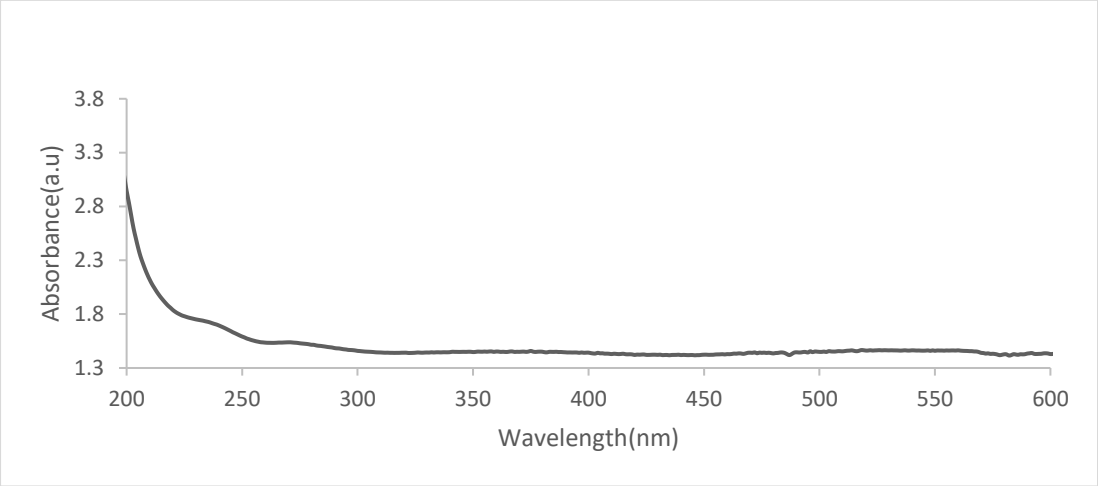

FIGURE 2: UV-Visible spectrum of Co_3O_4 Nanoparticles from the leaves extract of *Eruca Vesicaria.*

4.2 FT-IR

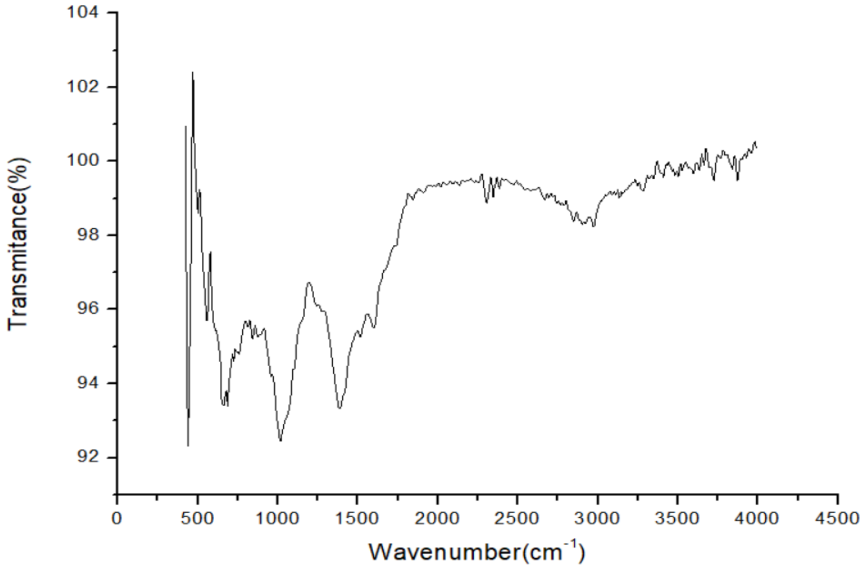

FIGURE 3: FT-IR Spectrum of Co_3O_4 Nanoparticles from the leaves extract of *Eruca Vesicaria.*

In FTIR spectrum, the leaf extract of *Eruca Vesicaria* shows peaks at 3100 cm^{-1}, 2400 cm^{-1} and 1700 cm^{-1} and 1067 cm^{-1} which shows the presence of hydroxyl group, carbonyl group, C-O of phenols and C-H groups respectively. The FTIR Spectrum of green Co_3O_4 Nanoparticles shows strong peak at the wavelength of 3832 cm^{-1} shows O-H groups (11). The peak at 1700 cm^{-1} is due to C=O Stretching of polysaccharides (12).

4.3 SEM

By using Scanning electron microscope the morphology of Co_3O_4 Nanoparticles was determined. The SEM figure represents several irregulars Co_3O_4 Nanoparticles. SEM images of Co3O4 nanoparticles at different magnification, which clearly exhibit the nanoparticles like morphology indicate well uniform particles with narrow size distribution lies in the range of 35nm.The synthesized nanoparticles surface is very smooth, which facilitates the better contact with the bacterial cell wall and hence increases bacterial killing ability of NPs.

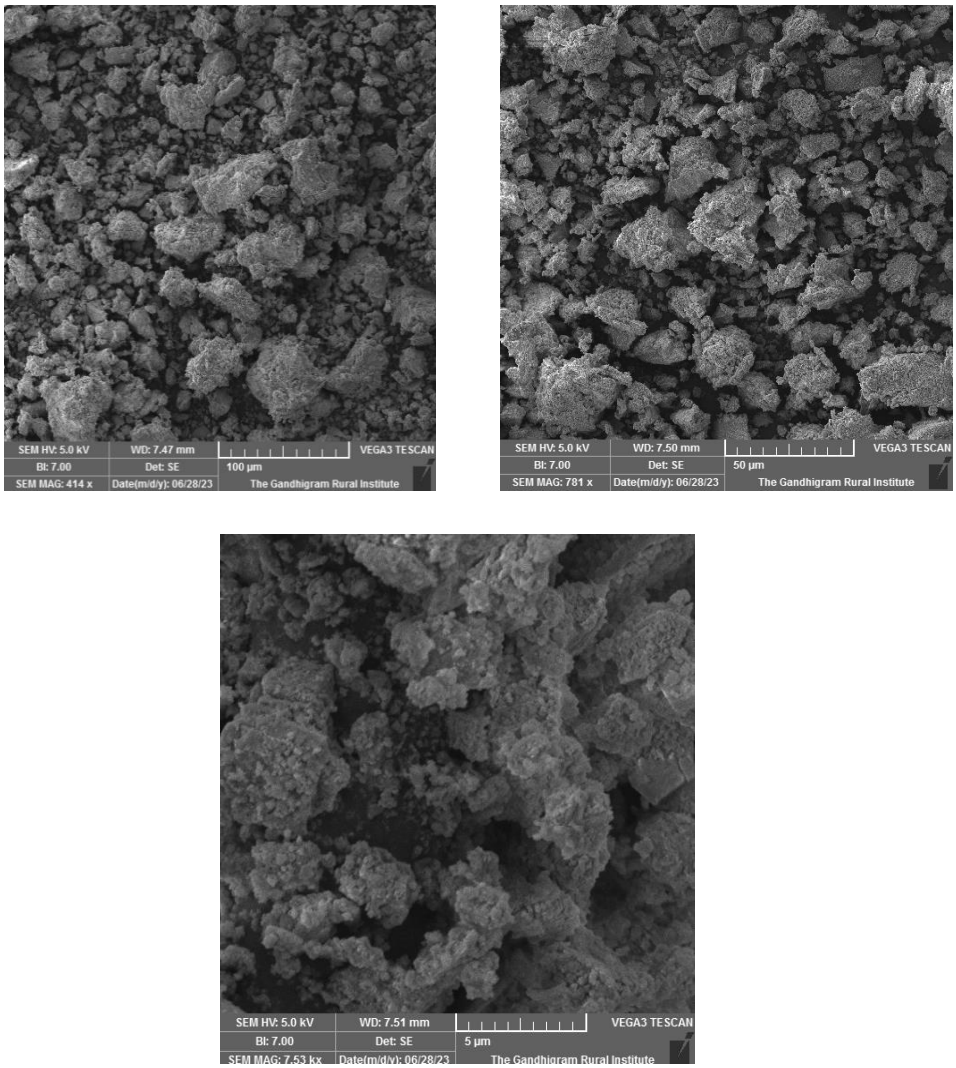

FIGURE 4: SEM image of Co_3O_4 Nanoparticles from the leaf extract of *Eruca Vesicaria*

4.4 EDX

In EDX Analysis, the elemental composition of synthesized nanoparticles from the plant extract of Eruca vesicaria was studied in Fig 5.In this figure, the peaks indicate the Co and O of the synthesized nanoparticles. The elemental composition of the nanoparticles shows 22 weight per cent cobalt and 15 weight per cent oxygen corresponding to Cobalt oxide (Co_3O_4).

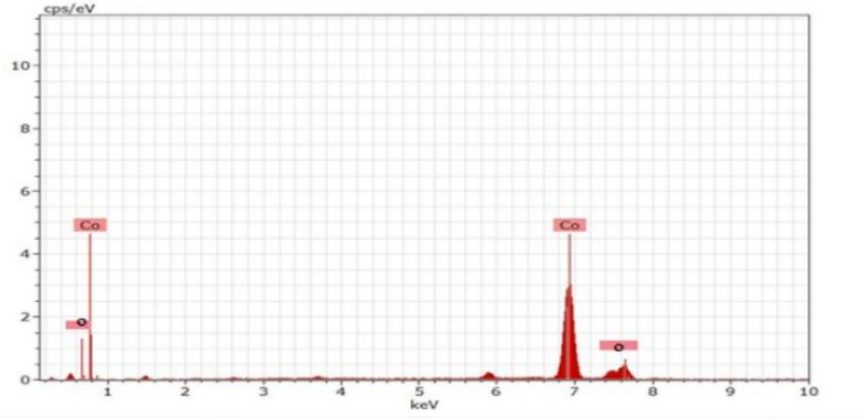

FIGURE 4: EDX image of Co_3O_4 nanoparticles synthesized from the leave extract of

Eruca Vesicaria.

Anti-bacterial activity

Cobalt oxide nanoparticles produced good antibacterial activity against *Bacillus subtilis* and *Proteus vulgaris* (12-13). The gram-positive bacteria *Bacillus subtilis* showed maximum zone of inhibition (16.5±0.7). Antimicrobial and Antibacterial activities of CO_3O_4 Nanoparticles are presented below.

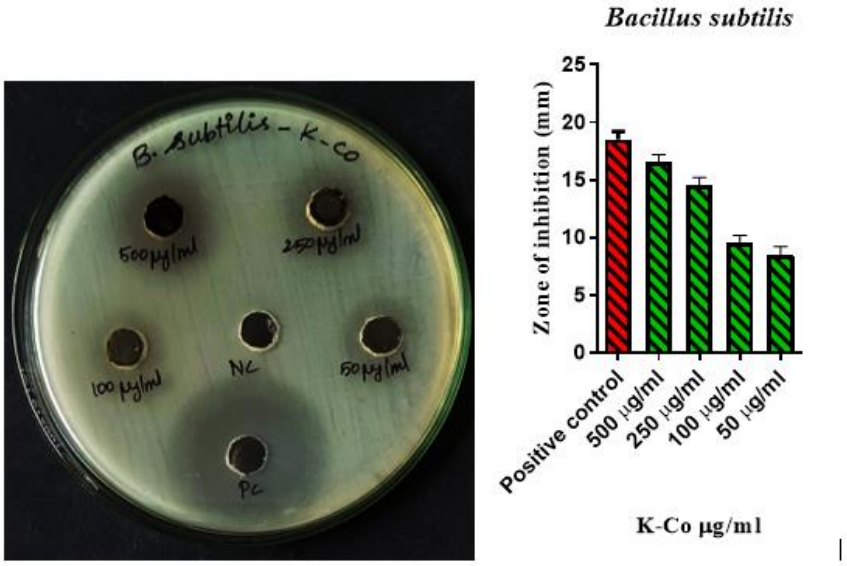

FIGURE 5:Effect of sample K-Co against *Bacillus subtilis.*

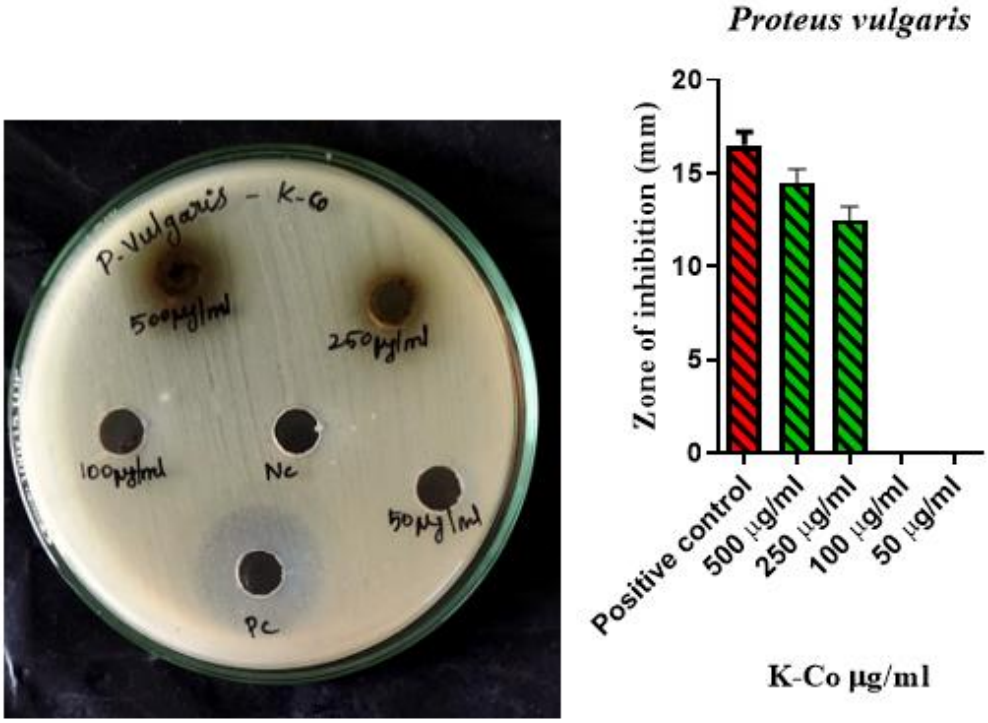

FIGURE 6: Effect of sample K-Co against *Proteus vulgaris.*

TABLE 1. SD± Means of zone of inhibition obtained by sample K-Co against *Proteus vulgaris* and *Bacillus subtilis.*

S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	*Bacillus subtilis*	K-Co	16.5±0.7	14.5±0.7	9.5±0.7	8.5±0.7	18.5±0.7
2.	*Proteus vulgaris*		14.5±0.7	12.5±0.7	0	0	16.5±0.7

Anti-fungal activity

Antifungal activities of Co_3O_4 are presented in Table 1. Cobalt Oxide nanoparticles produced good anti-fungal activity against *Aspergillus fumigatus and Candida albicans* (14-15). Among both classes of fungal *Aspergillus fumigatus and Candida albicans*, the maximum zone inhibition showed by *Candida albicans* (12.5±0.7).

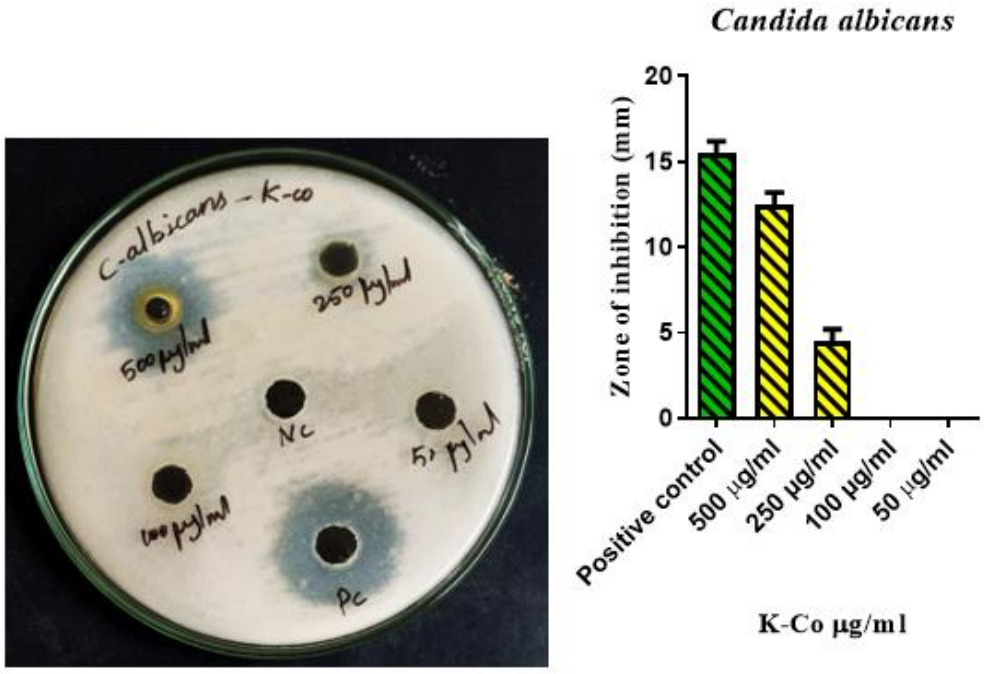

FIGURE 7: Effect of sample K-Co against *Candida albicans*

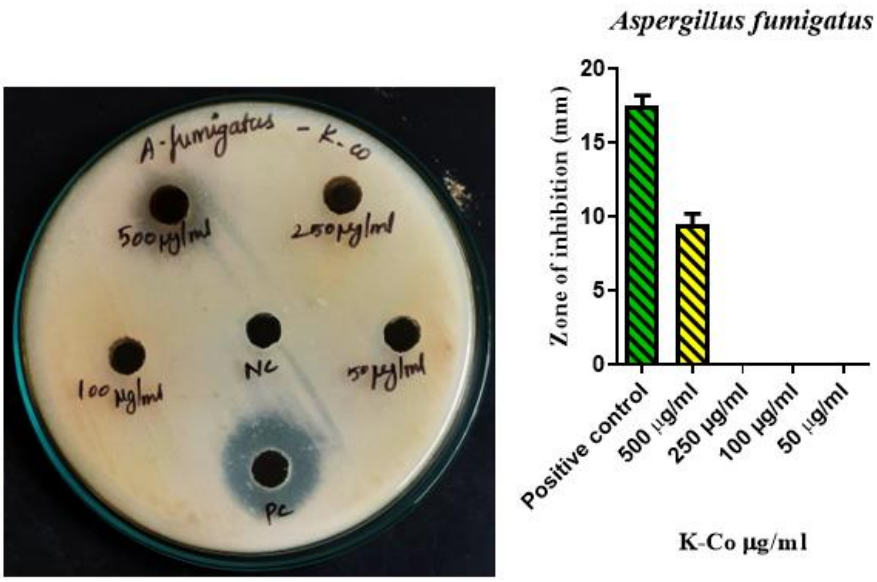

FIGURE 8: Effect of sample K-Co against *Aspergillus fumigatus.*

TABLE 2. SD± Means of zone of inhibition obtained by sample K-Co against *Aspergillus fumigatus* and *Candida albicans.*

S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	*Aspergillus fumigatus*	K-Co	9.5±0.7	0	0	0	17.5±0.7
2.	*Candida albicans*		12.5±0.7	4.5±0.7	0	0	15.5±0.7

CONCLUSION

The cobalt oxide nanoparticles was synthesized by using the leaves extracts of *Eruca Vesicaria* plant extract. The synthesized cobalt oxide nanoparticles were analysed UV- Visible spectroscopy, FT-IR, SEM techniques. These techniques revealed the successful synthesis of cobalt oxide nanoparticles. Antibacterial activities of synthesized cobalt oxide naoparticles were analysed against gram positive bacteria *Bacillus subtilis* and gram negative bacteria *Proteus vulgaris*. The Anti-fungal activity of synthesized Cobalt oxide nanoparticles was analyzed against *Aspergillus fumigatus and Candida albicans*. It was found that by increasing concentration of cobalt oxide nanoparticles, antibacterial activity was increased.

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