



# Biological Synthesis of Cobalt Oxide Nanoparticles Using *Ziziphus oenopolia* Leaf Extract

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## ABSTRACT

In this article, cobalt oxide nanoparticles were synthesized from the leaf extract of *Ziziphus oenopolia* by using cobaltous chloride hexahydrate. The prepared samples were characterized and subjected to the antimicrobial activity of some pathogens. The first confirmation of the formation of the cobalt oxide nanoparticles resulted from the reaction mixture's color change from light brown to dark brown at room temperature. Further, UV-Visible spectrum analysis showed a strong absorption band at the visible region around 269 nm, confirming the formation of stable cobalt oxide nanoparticles. From the FT-IR spectrum, the peaks at 3626, 2978, 1689, 1519, 1396 and 578  $\text{cm}^{-1}$  show the presence of OH stretching, C=O group, C=C stretching, C-C bonds and cobalt oxide nanoparticles. The X-ray diffraction showed their crystalline nature with a face-centered cubic structure; the surface morphology revealed an irregular shape. The antibacterial studies have been carried out against four bacterial strains such as *Staphylococcus aureus*, *Streptococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*; *E. coli* showed the highest zone of inhibition. The antifungal studies were exhibited against *Candida albicans*, *Candida vulgaris*, *Aspergillus flavus* and *Aspergillus niger*; *Aspergillus niger* showed the highest zone of inhibition.

**Keywords:** Plant-mediated synthesis; Cobalt oxide nanoparticles; Antimicrobial activity; *Ziziphus oenopolia* leaf.

## 1. INTRODUCTION

Numerous physical and chemical techniques are used in nanotechnology to create nanomaterials with special qualities (Muhammad *et al.* 2020). These nanoparticles can be used in a variety of industries, including agriculture, environmental protection and advancements in biomaterials, nanomedicine and nanoelectronics (Syed *et al.* 2022). The synthesis and use of metal and metal oxide nanoparticles have generated significant attention owing to their distinct characteristics stemming from their size, shape, dimension and morphology (Dhaneswar and Bhaskar, 2023). One of the most significant areas of study in material science is nanotechnology. When compared to their bulk materials, nanoparticles exhibit remarkable physical and chemical properties because of their high surface area-to-volume ratios (Majid *et al.* 2020). In the twenty-first century, nanotechnology has become more popular and is expanding by leaps and bounds, since it can modify and use the properties of assemblies at the nano-scale of different molecules. Additionally, scientists can change the chemical composition of these assemblies' chemical, physical and biological characteristics, enabling their fabrication at a regulated size range of 1-500 nm (Panneerselvam *et al.* 2011). In recent years, nanotechnology has become increasingly significant in contemporary research (Kalpana *et al.* 2016). Designing, characterizing, creating, applying and manufacturing structures and systems by manipulating size and shape at the nanoscale is known as nanotechnology (Aliyaa and

Wisam, 2019). Since the size and shape have a major impact on nanoparticles' physico-chemical properties, much research has been done to control their size and shape. Because of their strong resistance to oxidation and corrosion, cobalt nanoparticles have a wide range of potential uses in daily life. Cobalt nanoparticles have been created using a variety of physical and chemical processes, such as thermal breakdown, high-temperature solution phase, reduction and micro-emulsion hydrothermal. From that nanoparticle biosynthesis has developed into a prominent nanotechnology offshoot compared to conventional synthesis methods, which require the use of high pressure, temperature, energy and chemical additives. This method is more economical and environment-friendly (Ismat *et al.* 2017). Nobel metal nanoparticles gained attention in recent years because of their special optical, electrical, mechanical, magnetic and chemical properties that differ markedly from those of bulk substances (Joy and Johnson, 2015). Recent research has demonstrated that plants may serve as a promising source for the safe manufacture of nanomaterials; they are effectively employed in the synthesis of many greener nanomaterials. Nanoparticles exhibit distinctive characteristics in their size, shape and dispersion. Additionally, metallic nanoparticles have emerged over the years and gained a lot of popularity because of their distinctive characteristics and uses. Most metals in transitions such as platinum, gold, silver, cobalt, iron and copper, among others, have been used in plant-mediated nanoparticle synthesis (Mela *et al.* 2022). For the synthesis of cobalt oxide nanoparticles, the leaf

extract of *Ziziphus oenopolia* has been used. It is a plant in the family Rhamnaceae. Commonly known as jackel jujube, small-fruited jujube (or) wild jujube, it is a flowering plant with a broad distribution through tropical and sub-tropical Asia and Australia.



Fig. 1: Leaves of *Ziziphus oenopolia*

Family	<i>Rhamnaceae</i>
Species	<i>Z. oenopolia</i>
English	Jackel Jujube
Tamil	Suraimullu
Botanical name	<i>Ziziphus oenopolia</i>

## 2. EXPERIMENTAL METHODS

### 2.1 Materials

Cobaltous chloride hexahydrate ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ) and sodium hydroxide (NaOH) were purchased from Merck. The leaves of *Ziziphus oenopolia* were collected from Sathyamangalam, Erode District, Tamilnadu, India.

### 2.2 Preparation of Leaf Extract

The leaves were collected and washed with tap water followed by distilled water. Washed leaves were dried at room temperature for 30 days. Then the dried leaves were powdered. The powdered leaves of 10 g were taken in a beaker along with 250 ml of distilled water; the mixture was stirred for 4 h in a magnetic stirrer. Then the solution was filtered using Whatmann No. 1 filter paper and stored in a refrigerator for further use.

### 2.3 Synthesis of Cobalt Oxide Nanoparticles

40 ml of leaf extract was taken in a beaker and 25 ml of 0.1 M aqueous solution of cobaltous chloride was added to it and the mixture was placed in a magnetic stirrer for 2 h, and then 0.01 M NaOH solution was added drop-by-drop as a catalyst till the pH of the mixture reached 9, and the color change was observed, indicating the formation of cobalt oxide nanoparticles. The solution can be centrifuged at 7000 rpm. Finally, the obtained precipitate was washed with water simultaneously 3-4 times and the obtained residue was air dried for 6 h at 75 °C. Finally, ethanol can be added to remove the impurities. The residue was further crushed. Finally, a dark brown colored powder was obtained; the powder was stored in an airtight container for further characterization.

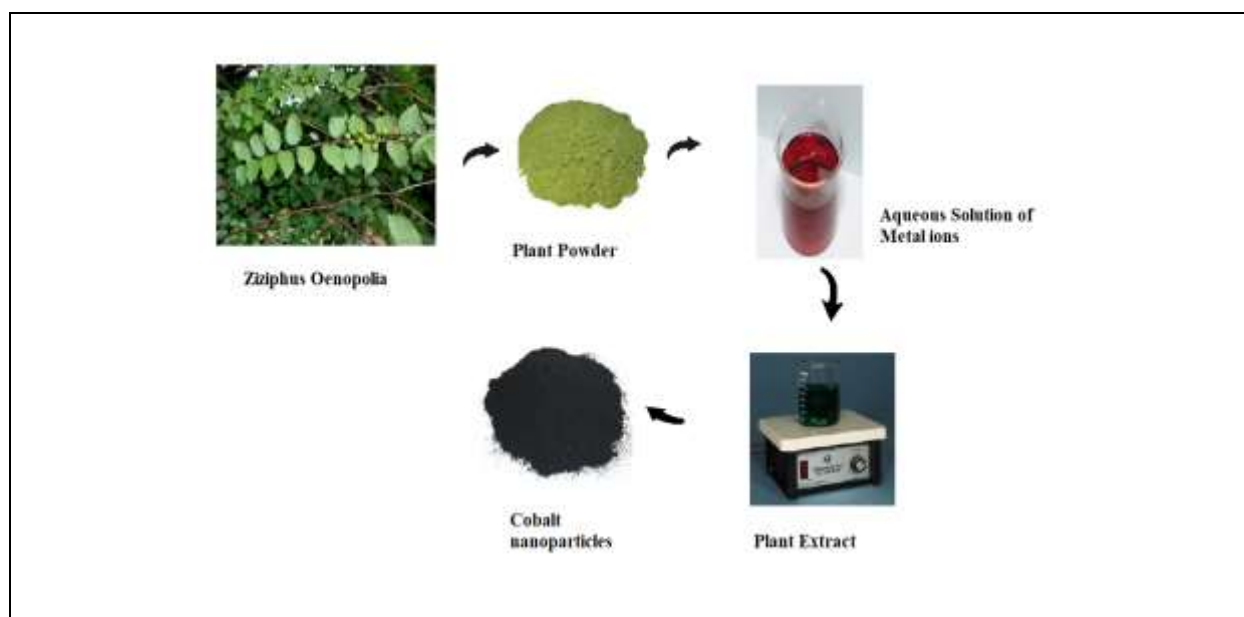


Fig. 2: Synthesis of Cobalt oxide nanoparticles

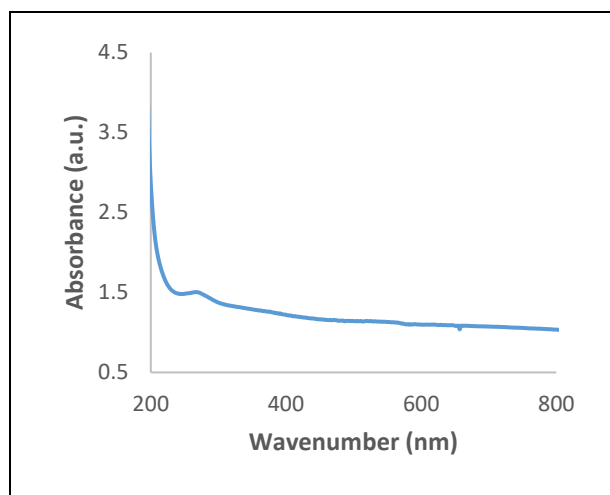
## 2.4 Characterization

The synthesized cobalt oxide nanoparticles were characterized using various techniques. The absorbance spectra of the cobalt oxide were acquired by UV-Visible spectrophotometer. Fourier Transform Infrared Spectrophotometer (FTIR) was used to find out the presence of specific functional groups. An X-ray Diffractometer was used to analyze the crystalline nature and phase identification. The morphology and elemental composition were analyzed using SEM and EDX.

## 3. RESULTS AND DISCUSSION

### 3.1 UV-Visible Spectroscopy Analysis

The first confirmation of the formation of the cobalt nanoparticles resulted from the reaction mixture's color change at room temperature. Fig. 3 shows the UV-visible spectrum of synthesized cobalt oxide nanoparticles; they showed strong absorption in the visible region of 200 – 350 nm (Nur *et al.* 2019).



**Fig. 3: UV-visible spectrum of Synthesized cobalt oxide nanoparticles**

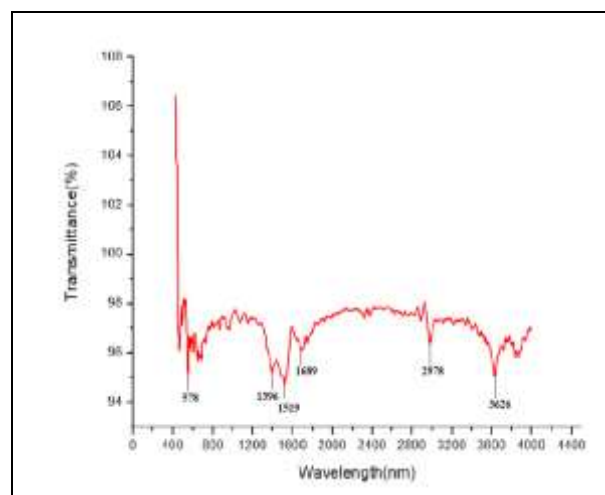
The synthesized cobalt oxide nanoparticles from the leaf extract show strong absorption bands at 269 nm, confirming the formation of stable cobalt oxide nanoparticles.

### 3.2 Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

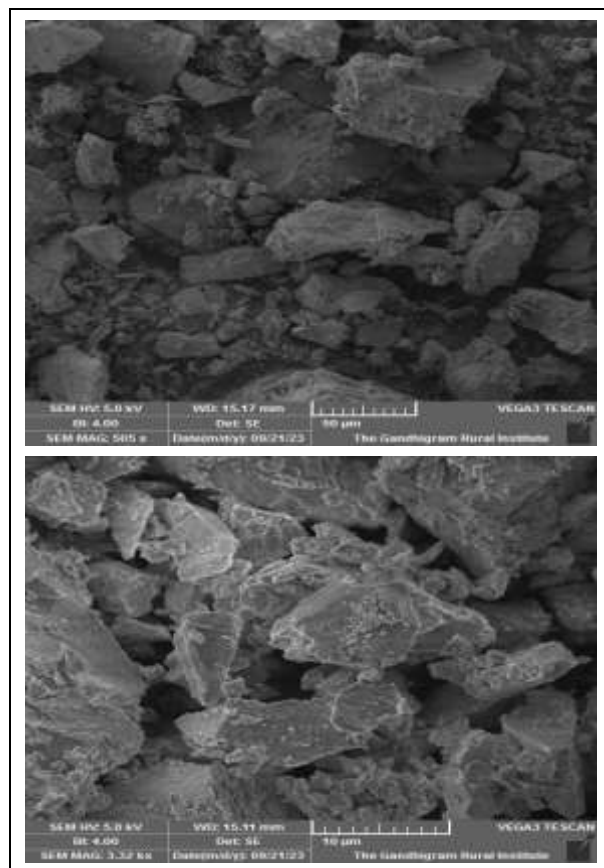
FT-IR analysis was used to find out the functional groups that are present in the synthesized cobalt oxide nanoparticles. The FT-IR spectrum is shown in Fig. 4.

The FT-IR spectra of synthesized cobalt oxide nanoparticles show strong peaks at the wavelength of 3626  $\text{cm}^{-1}$  and 2978  $\text{cm}^{-1}$  corresponding to O-H

stretching vibration of phenolic compounds (Suman *et al.* 2013). The peaks at 1689  $\text{cm}^{-1}$  show the presence of C=O group. The peak at 1519  $\text{cm}^{-1}$  shows the presence of C=C stretching (Sivaji and Palaniyandi, 2019) and the peak at 1396  $\text{cm}^{-1}$  indicates the presence of C-C bonds (Majid *et al.* 2020); the one at 578  $\text{cm}^{-1}$  confirms the presence of cobalt oxide nanoparticles.



**Fig. 4: FT-IR spectrum of Synthesized cobalt oxide nanoparticles**



**Fig. 5: SEM images of Synthesized cobalt oxide nanoparticles**

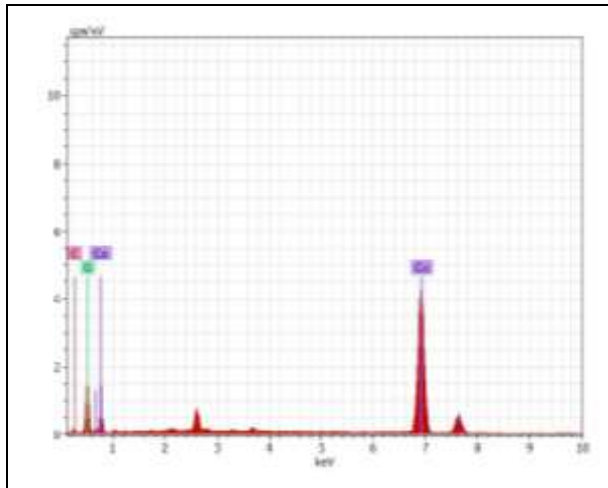


Fig. 6: EDX image of Synthesized cobalt oxide nanoparticles

### 3.3 Scanning Electron Microscopy (SEM) Analysis

The morphology of cobalt oxide nanoparticles was determined using SEM analysis. It shows irregular cobalt oxide nanoparticles. SEM images at different magnifications are shown in Fig. 5.

### 3.4 EDX Analysis

EDX analysis was done to determine the elemental composition of synthesized cobalt oxide nanoparticles. It was carried out by using internal standards at the energy from 0-10 eV. EDX spectra (Fig. 6) showed strong signals of cobalt and oxygen in prepared samples. It was confirmed that the cobalt oxide nanoparticles were pure, since no additional peaks were observed.

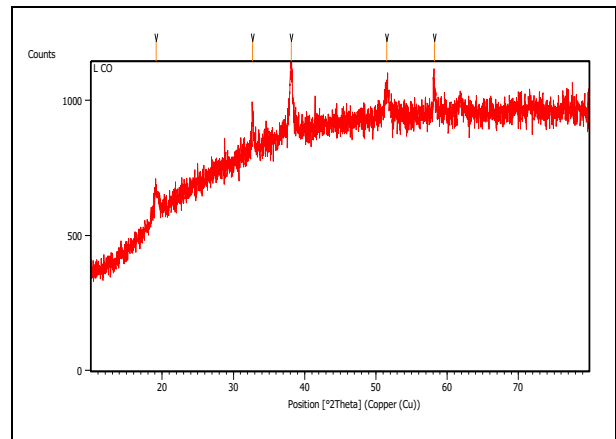


Fig. 7: XRD image of Synthesized cobalt oxide nanoparticles

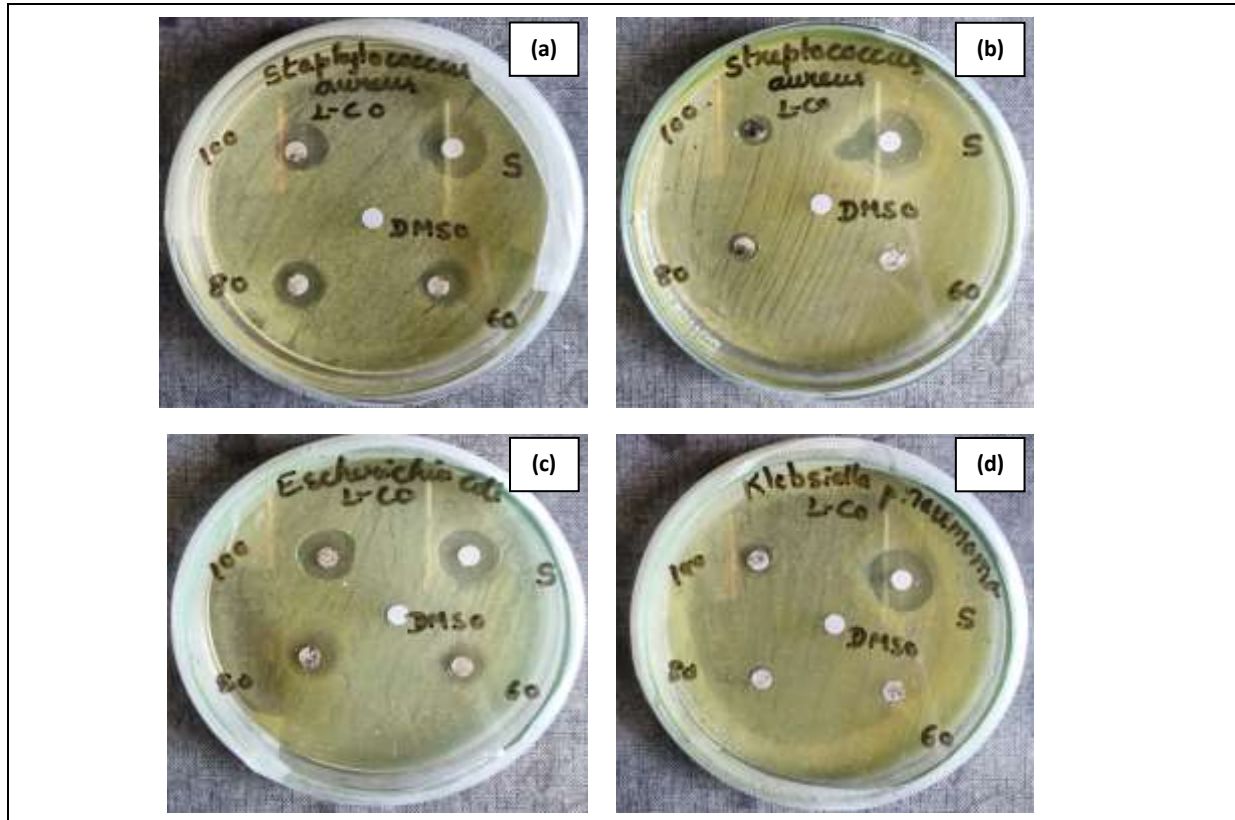


Fig. 8: Effect of samples of cobalt oxide nanoparticles synthesized from leaf extract of *Ziziphos oenopolia* against: (a) *Staphylococcus aureus* (b) *Streptococcus aureus* (c) *Escherichia coli* and (d) *Klebsiella pneumoniae*



### 3.5 XRD Analysis

XRD examination was performed to determine the crystalline structure; significant peaks at Bragg's angle or  $2\theta$  values of 19.1, 32.6, 38.1, 51.4 and 58.2 corresponded to lattice planes of (110), (211), (220) and (320) that can be correlated to the face-centered cubic phase of  $\text{Co}_3\text{O}_4$  nanoparticles. The average particle size of cobalt oxide nanoparticles was 27 nm, calculated using Debye-Scherrer formula.

## 4. ANTIMICROBIAL ACTIVITY

### 4.1 Antibacterial Activity by Disc Diffusion Method

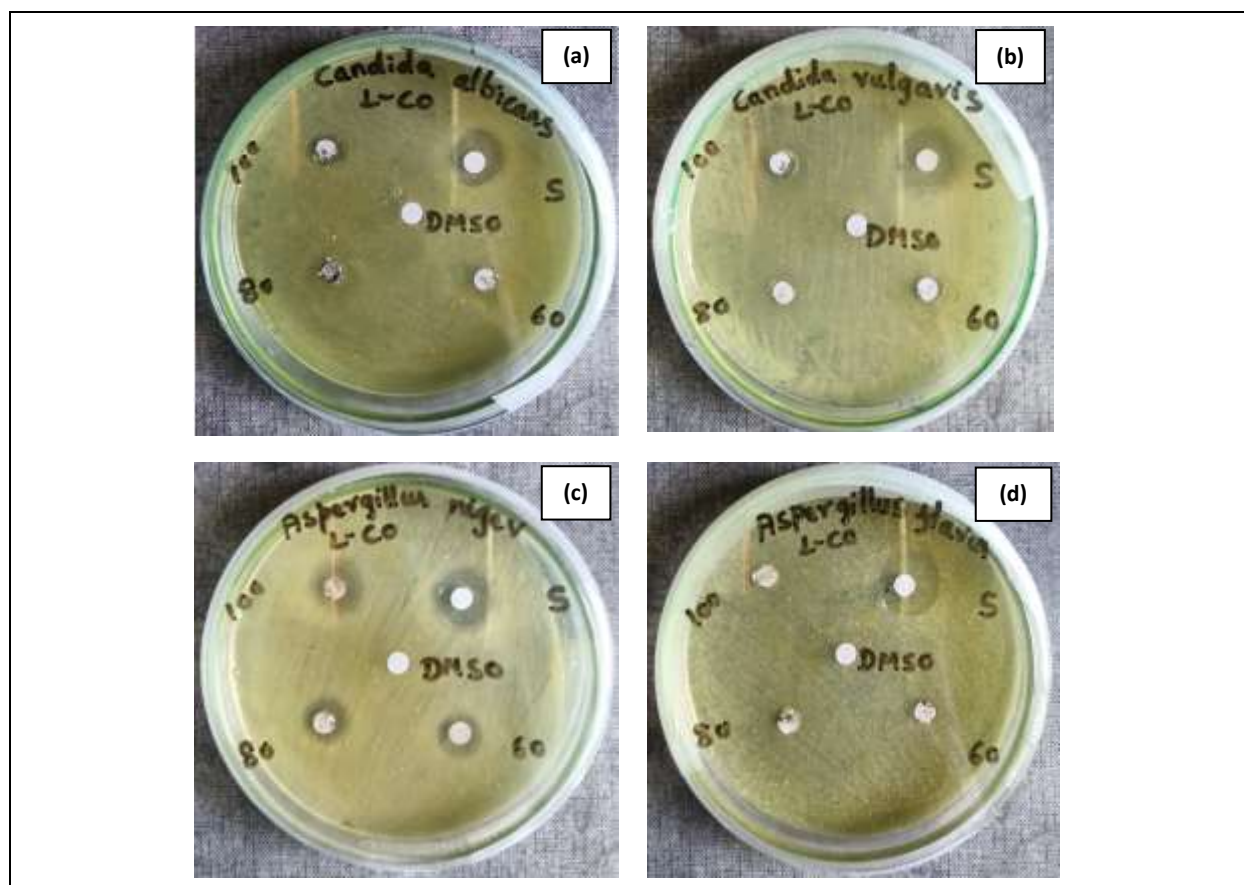
Antibacterial activity of synthesized cobalt oxide nanoparticles exhibited against two gram-positive bacterial strains (*Staphylococcus aureus*, *Streptococcus*

*aureus*), and two gram-negative bacterial strains (*Escherichia coli* and *Klebsiella pneumonia*) was studied (Fig. 8).

The antibacterial activity of cobalt oxide nanoparticles is depicted in Fig. 10. At  $100\ \mu\text{g/ml}$ , *E. coli* showed the highest zone of inhibition at 6 mm compared to other bacteria.

### 4.2 Antifungal Activity by Disc Diffusion Method

Antifungal activity of synthesized cobalt oxide nanoparticles exhibited against *Candida albicans*, *Candida vulgaris*, *Aspergillus niger* and *Aspergillus flavus* was studied (Fig. 9). Antifungal activity of cobalt oxide nanoparticles is depicted in Fig. 11. At  $100\ \mu\text{g/ml}$ , *Aspergillus niger* showed the highest zone of inhibition at 5 mm compared to other fungi.



**Fig 9:** Effect of samples of cobalt oxide nanoparticles synthesized from leaf extract of *Ziziphus oenopolia* against: (a) *Candida albicans* (b) *Candida vulgaris* (c) *Aspergillus niger* and (d) *Aspergillus flavus*

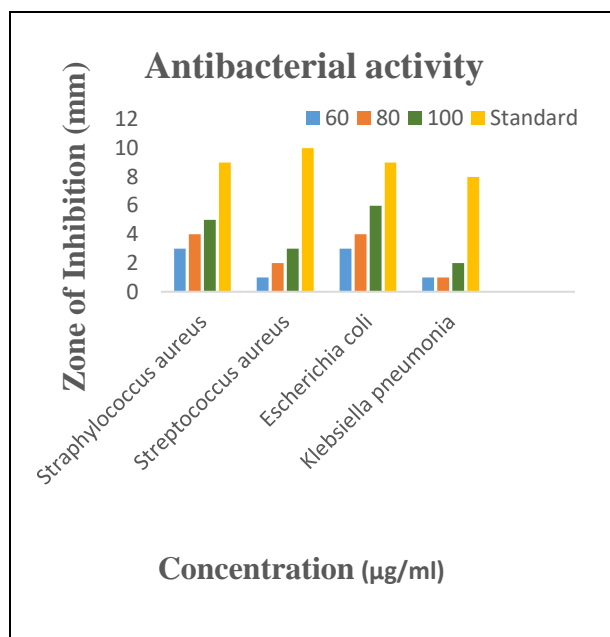


Fig. 10: Anti-bacterial activity of *Staphylococcus aureus*, *Streptococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*

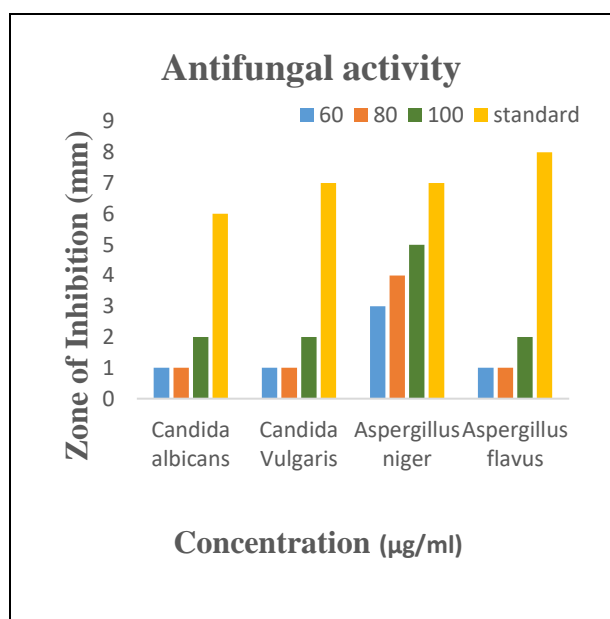


Fig. 11: Anti-fungal activity of *Candida albicans*, *Candida vulgaris*, *Aspergillus niger* and *Aspergillus flavus*

## CONCLUSION

The leaf extract of *Ziziphus oenopolia* showed its efficacy in synthesizing cobalt oxide nanoparticles at room temperature. UV-Visible spectrum of synthesized cobalt oxide nanoparticles showed strong absorption bands. FT-IR spectrum analysis confirmed the presence of phenolic groups and cobalt oxide nanoparticles. SEM image revealed that cobalt oxide nanoparticles have an irregular shape with a diameter range of 20 to 30 nm.

EDX analysis showed the major peaks of cobalt and oxygen elements in the synthesized cobalt oxide nanoparticles; furthermore, the nanoparticles showed good antibacterial activity against *E. coli* and antifungal activity against *Aspergillus niger*.

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## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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