Synthesis and characterization of copper oxide nanoparticles using *Brassica oleracea var. italic* extract for its antifungal application

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**Keywords:** antifungal, *Brassica oleracea var. italic*, copper oxide nanoparticles, green synthesis

**Abstract**

Green synthesis has emerged as a reliable, sustainable and ecofriendly protocol for synthesizing a wide range of nanomaterials and hybrid materials. In this paper, we report the synthesis of Copper oxide nanoparticles by a simple biological route using the extract of *Brassica oleracea var. italic* and copper (II) acetate as the metal precursor. The synthesized copper oxide nanoparticles were characterized using UV–visible spectroscopy, FTIR spectroscopy, FESEM, EDAX, and XRD techniques. UV–Visible analysis shows a characteristic peak around 220 nm for copper oxide nanoparticles. FTIR spectroscopy was used to characterize various capping and reducing agents present in the plant extract responsible for nanoparticle formation. The surface morphology was characterized using FESEM. The EDAX and XRD pattern suggested that prepared copper oxide nanoparticles were highly pure. The average particle size was calculated as 26 nm using the XRD technique. Further, the nanoparticles were found to exhibit the highest antifungal activity against *Aspergillus niger* and *Candida Albicans*.

1. **Introduction**

Recently Metal Oxide nanoparticles have a challenging base for research as wide varieties of applications attract researchers to explore its properties especially due to their ability to alter the physical, optical and the electronic properties of compounds. Copper oxide nanoparticles gains special interest among the oxides of transition metals because of their efficiency as nano fluids [1], sensors [2], antimicrobial applications [3], catalysis [4], super conductors [5] energy storage systems [6] and as anticancer agent [7]. So far, Copper oxide nanoparticles have been synthesized using chemical, physical, biological and hybrid methods. As these methods employ expensive, toxic chemicals which make them unsuitable for biomedical applications synthetic methods based on naturally occurring biomaterials provide an alternative means for obtaining these nanoparticles suitable for biological applications.

Green synthesis of copper oxide nanoparticles by various plant extracts like *Catha edulis* [8], *Psidium guajava leaf extract* [9], *Syzygium alternifolium* [10] *Azadirachta indica leaves* [11], *Populus ciliata* [12] etc has been reported so far. The major advantage of using plant extracts for copper oxide nanoparticles (NPs) synthesis is that they are easily available, safe, and nontoxic in most cases. Copper oxide NPs has been proven to have the highest microbial toxicity compared to other metal oxides [13, 14]. In this study copper oxide nanoparticles has been synthesized using flowers of *Brassica oleracea var. italic* for the first time with the help of greener protocols. Further its efficiency against fungi like *Aspergillus niger* and *Candida Albicans* were analyzed using disc diffusion method. The role of biological components like glucosinolates, polyphenols, flavonoids, vitamins and mineral nutrients [15] present in *Brassica oleracea var. italic* to act as reducing agents was also explored.
2. Methods

2.1. Preparation of Brassica oleracea var. italic extract
Fresh and healthy florets of Brassica oleracea var. italic (broccoli) were collected from Madurai district, Tamilnadu. It was rinsed thoroughly with tap water followed by distilled water to remove all the dust and unwanted visible particles and cut in to small pieces. About 10 g is weighed and transferred into 250 ml beakers containing 100 ml distilled water and boiled for about 20 min. The extract was filtered with normal filter paper and then through whatmann No1 filter paper to remove particulate matter and to get clear solution. It is refrigerated (4°C) in 250 ml Erlenmeyer flasks for further experiments [16].

2.2. Preparation of copper oxide nanoparticles
About 50 ml of 0.1 M Copper acetate Cu(CH₃COO)₂ H₂O was mixed with 10 ml of Brassica oleracea var. italic extract (1:5 ratio), heated (80°C) and stirred at 400 rpm for about 1 h. The solution was aged for 24 h. Nanoparticles prepared was centrifuged at 6000 rpm for 15 min and dried at 80°C [17].

2.3. Disc diffusion method for antifungal activity
The antifungal activity of synthesized copper oxide NPs was done using standard disc diffusion method. The fungal stains used in the study are Candida albicans & Aspergillus Niger. The inoculums for the disc diffusion method were prepared using a suitable broth and the medium was dried at 30°C–35°C prior to the application. The standardized inoculums were transferred in to the sterilized petri dish prepared earlier. The excess of inoculums was removed by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid. The petri dish was dried at room temperature for 24 h with lids closed [18].

The petri dish was divided into parts in which the samples were placed with the help of sterile forceps. DMSO solvent is used as control. About 10 μg ml⁻¹ of standard Flucanazole was required to obtain a uniform lawn. Different amounts of copper oxide nanoparticles were prepared through series of dilution and approximately 100 μg ml⁻¹ was being used for evaluating its antifungal activity. Then petri dishes were placed in the refrigerator at 4°C or at room temperature for one hour for diffusion and incubated at 37°C for 24 h. Finally, the zone of inhibition produced by different samples was measured using a standard scale.

2.4. Characterization
Bio synthesized copper oxide nanoparticles were characterized using UV–vis spectra, between the wave lengths 190–800 nm in a spectrometer (Beckman-Model No.DU-50, Fullerton) having a resolution of 1 nm. FTIR spectra were recorded with a Perkin Elmer Spectrum Express version 10,300. The Scanning data were obtained from the average of 47 scans in the range 4000–400 cm⁻¹ with the resolution of 4 cm⁻¹. The Crystal structure of the produced copper oxide nanoparticles was determined and confirmed by using x-ray diffraction meter (Model PW 1710 control unit Philips Anode Materials Cu, 40 kV, 30 MA, optics Automatic divergence slit) with Cu kβ radiation λ = 1.5405Å over a wide range of Bragg angles (30° ≤ 2θ ≤ 80°). The FESEM observation was carried out using the Quanta FEG-250 SEM instrument equipped with EDAX for elemental analysis.

3. Results and discussion

3.1. The UV–vis absorption spectra
The UV–vis absorption spectra of copper oxide NPS was given in figure 1. It shows a peak at 220 nm which is attributed to the formation of cuprous oxide nanoparticles which is in close agreement with the earlier reports [17].

3.2. FTIR spectrum of copper oxide NPS
The FTIR measurements for the synthesized copper oxide NPs is given in figure 2 which helps in identifying possible biomolecules that acts as reducing and capping agents. The strong peaks observed at 3441, 1633, 1046 and 1403 cm⁻¹ corresponds to OH, C=O, C–O and aliphatic C–H stretching vibrations respectively. The peaks at 620 and 686 cm⁻¹ corresponds to Cu(I)–O vibration of Copper oxide nanoparticles [19].

3.3. XRD analysis
The peak positions (figure 3) with 2θ values of 32.5°, 35.6°, 38.7°, 48.9°, 58.3°, 66.4°, 68.1°, 72.5° and 75.1° can be assigned to the planes (–111), (002), (111), (–202), (202), (–311), (113), (311) and (400) which corresponds to monoclinic phase of Copper oxide (JCPDS card no 45-0937) [20]. The average particle size D is estimated
using Debye–Scherrer equation by determining the width of (−111) peak.

\[ D = \frac{0.9 \lambda}{\beta \cos \theta} \]
where $\lambda$ is the x-ray wavelength, $\beta$ is full width half maximum and $\theta$ is Braggs angle

$$D = \frac{0.9 \times 1.5408 \times 10^{-10}}{5.1496 \times 10^{-3} \times 0.999} = 26.95 \times 10^{-9}$$

### 3.4. SEM—EDAX analysis

The surface morphology the copper oxide NPs synthesized using *Brassica oleracea var. italic* was shown in figure 4. It shows the presence of nanoparticles with more or less uniform shape but varying in sizes and also no aggregation is seen. The purity was analyzed using EDAX spectrum (figure 5) which shows highest peak for copper and oxygen indicating that the copper oxide nanoparticles synthesized is highly pure.

### 3.5. Anti-fungal activity

In general copper oxide NPs are effective against a wide range of pathogens [21]. The antifungal activity was measured by disc diffusion method using fungi stains such as *Candida albicans & Aspergillus Niger*. Flucanazole was kept as the standard and the zone of inhibition was noted. A series of dilutions for the synthesized copper oxide nanoparticles were carried out as per the procedure [22] and it was found that the minimum inhibitory
concentration of copper oxide nanoparticles is 100 $\mu$g ml$^{-1}$. The zone of inhibition for the copper oxide nanoparticles is shown in the figure 6.

From the table 1 it is inferred that the synthesized copper oxide NPs show a better antifungal activity. The size and concentration of the copper oxide nanoparticles play a vital role in exhibiting antimicrobial activity [21].

| Organisms          | Flucanazole [10 $\mu$g ml$^{-1}$] | Copper oxide nanoparticles [100 $\mu$g ml$^{-1}$] |
|--------------------|----------------------------------|-----------------------------------------------|
| Aspergillus niger  | 10                               | 9                                             |
| Candida albicans   | 9                                | 9                                             |

Figure 6. Antifungal activity of synthesized copper oxide nanoparticle and plant extract.

Table 1. Antifungal activity of copper oxide NPs.

4. Conclusion

A simple green synthesis of copper oxide Nps using Brassica oleracea var. italic extract was given in the present study. The UV visible spectra shows a peak at 220 nm indicating the formation of copper oxide Nps. It is further confirmed using FTIR which shows a band at 620 and 686 cm$^{-1}$ corresponding to Cu–O bond. The size of the nanoparticles was calculated using XRD which was found to be 26 nm. The surface morphology and shape of copper oxide nanoparticles were analyzed using FESEM. The purity of NPs was confirmed by EDAX technique. The copper oxide Nps synthesized using the green method showed excellent antifungal activity. The exact mechanism and the cytotoxic nature of the nanoparticles should be investigated further for its effective application.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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