Therapeutic Effect of Green Synthesized Silver Nanoparticles Using Cymbopogon Citratus Extract against Oral Candidiasis: In Vitro and In Vivo Study

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**Abstract:** Oral candidiasis, primarily caused by Candida albicans, is a significant health concern due to increasing antifungal resistance and drug-related toxicity. Green synthesis of silver nanoparticles (AgNPs) using Cymbopogon citratus offers a sustainable and biocompatible alternative for antifungal therapy. This study evaluated the therapeutic potential of AgNPs synthesized using Cymbopogon citratus extract against oral candidiasis. Freshly harvested Cymbopogon citratus leaves were dried, powdered, and used to prepare an extract. Silver nitrate solution, sodium borohydride, and ammonia were added to the extract, and AgNPs were synthesized following stirring and boiling. The successful formation of AgNPs was confirmed by color change and characterized using FTIR, XRD, SEM, and TEM. Antifungal activity was assessed in vitro against Candida albicans. AgNPs were confirmed by FTIR peaks at 3185.14 cm⁻¹ (O-H stretching), 1615.25 cm⁻¹ (C=C stretching), 1316.21 cm⁻¹ (C-H bending), and Ag-O stretching at 827.80 cm⁻¹, 715.41 cm⁻¹, and 592.53 cm⁻¹, XRD crystalline peaks (27.99° to 77.39°), and TEM imaging. Antifungal assays demonstrated significant inhibition of filamentous growth and virulence of Candida albicans at 150 µg/ml. The synthesized AgNPs showed promising antifungal activity and biocompatibility, highlighting their potential as eco-friendly alternatives to conventional antifungals. Further studies are needed to investigate long-term cytotoxicity and clinical applications.

**Keywords:** Silver nanoparticles, *Cymbopogon citratus,* Oral candidiasis, antifungal activity, biofilm inhibition

# INTRODUCTION

Oral thrush is a fungal infection known as oral candidiasis and is typically caused by Candida albicans species of fungi. It frequently affects individuals with weakened immune systems or older age and those undergoing antibiotic or corticosteroid therapies. This condition poses treatment challenges due to the emergence of drug resistant strains and the severe side effects associated with prolonged use of antifungal medications [Aparna et al. 2021; Ganapathy and Professor and Head of D...; Verma and Muthuswamy Pandian 2021](https://paperpile.com/c/ZgTF2o/DLWa+A7AX+WXls). Despite the availability of various drugs for treating fungal infections, in patients addressing oral thrush remains particularly complex. Henceforth it is crucial to explore methods of treatment that prioritize both effectiveness and patient safety. [(1)](https://paperpile.com/c/5cHaDw/THco)

Recent advancements, in nanotechnology have enabled the creation of compounds successfully synthesized with the help of AgNPs as antimicrobial agents which show promise primarily due, to their low toxicity to human cells [Chokkattu et al. 2022; Ramamurthy et al. 2022; Marya et al. 2022](https://paperpile.com/c/ZgTF2o/Bkd5+Zdog+Ap1A); however their effectiveness and compatibility rely significantly on the methods used for nanoparticle synthesis [(2)](https://paperpile.com/c/5cHaDw/t7qS). The green synthesis method utilizing plant extracts has proven to be highly effective in creating Ag nanoparticles (Ag NPs). This approach is environmentally friendly and cost effective while also offering the possibility of producing Ag NPs that exhibit enhanced properties. [(3)](https://paperpile.com/c/5cHaDw/ShjK)

A known medicinal plant, lemongrass (Cymbopogon citratus) is valued for its antioxidant and anti-inflammatory properties. The extract of Cymbopogon citratus contains compounds, like citral, flavonoids and phenolic acids that can act as both reducing and stabilizing agents in the eco production of silver nanoparticles [Poornima et al. 2021; Ganapathy and Professor and Head of D...; Pandiyan et al. 2022](https://paperpile.com/c/ZgTF2o/n3Qz+EYDH+lKZS). These bioactive compounds play a role in the creation of nanoparticles. They enhance the antifungal properties of AgNP s with additional therapeutic benefits.[(4)](https://paperpile.com/c/5cHaDw/F984)

The present work reports the therapeutic potential of green-synthesized silver nanoparticles using a *Cymbopogon citratus* extract against oral candidiasis. By virtue of the synergistic benefits of silver nanoparticles and bioactive compounds in lemongrass extract, this green synthesis approach is expected to produce novel antifungal agents of great efficacy.[(5)](https://paperpile.com/c/5cHaDw/iQOh)

In vitro and in vivo studies are underway to assess the antifungal activity, cytotoxic effect and therapeutic potential of the green photosynthesised AgNPs. In vitro studies have been performed to characterize the AgNPs and then analyzed for their antimicrobial activity against a number of Candida species that include resistance strains. The aim of the in vitro study was to determine if AgNPs would be a potential therapeutic intervention in oral candidiasis in a mouse model. [(6)](https://paperpile.com/c/5cHaDw/lsBp)

In addition, the molecular interactions involving these green engineered nanoparticles with fungal cells could help outline their different modes of action that may lead to new antifungal techniques. This study looks into the possibility of using green AgNPs generated from the extract of *Cymbopogon citratus* in treating oral candidiasis. The findings presented by this study contribute to the growing concern over drug resistance associated with these antifungal agents. [(7)](https://paperpile.com/c/5cHaDw/LSrq)

# MATERIALS AND METHODS

## Materials & Reagents used

​Distilled water, Silver Nitrate, Ammonia solution and Sodium Borohydrate Solution from Fisher Scientific, India were used to synthesize AgNPs in this study.

## Collection and Preparation of Plant Sample

Leaves of Cymbopogon citratus samples were gathered at different parts of Tamilnadu. It was later on washed over distilled water, and impurities that would be present were removed with shady conditions and all native compounds retained. The latter is then poured into mortar and pestle where leaves were smashed into an excellent powder for use. Following this, 10 g of the powder of the leaf material was dissolved in 200 ml of distilled water in a sterile conical flask. This mixture was then heated and stirred constantly for 24 hours. The extract obtained was then filtered through filter paper Whatman No. 1 for purifying it from impurities as evidenced in a prior study[(8)](https://paperpile.com/c/5cHaDw/cHNT).

## Synthesis of Silver Oxide nanoparticles

​The calibrated solution of Silver nitrate, that was 0.4 M in concentration, was first mixed with the distilled water and the filtrate of the plant extract and then boiled while keeping it on a magnetic stirrer. At the same time, 1 M Sodium borohydride solution and ammonia solution were dropwise added to this mixture until a characteristic blackish – brown color appeared, which indicates the formation of Ag NPs.

The whole preparation was subjected to centrifugation at a speed of 5000 revolutions per minute for a duration of 15 minutes. Following centrifugation, the liquid that existed above the sediment, known as the supernatant, was gradually moved into the petri dish to initiate the process of water removal. The Ag nanoparticles were further dehydrated in a hot air oven at 100 °C based on the procedure given by Sumitha et al. [(9)](https://paperpile.com/c/5cHaDw/Ip5w). A detailed study has been carried out to evaluate the properties of the Ag nanoparticles that were synthesized from the *Cymbopogon citratus* extract.

## Characterisation of Ag NPs

Spectral interpretation using Fourier-transform infrared (FTIR) was performed using a Bruker Alpha-II ATR instrument. The analysis was done in the wavelength range of 4000–400 cm-1. The results revealed the presence of Ag NPs from *Cymbopogon Citratus* in the synthesis process. XRD analysis was applied to the finely pulverized AgNPs to find out about their crystal structure through EuKα radiation.

The spectral interpretation was done on a Bruker D8 Advance X-ray diffractometer, which works at 30 kV and 15 mA. Measurements of the diffracted intensities were taken with a pace of 4°/min against step size of 0.05° over the 2θ angles ranging between 5 to 80 °. The TEM analysis was performed using G2 20 S – Twin TEM Instrument, revealing the presence of a polydispersed Eu2O3 NPs.

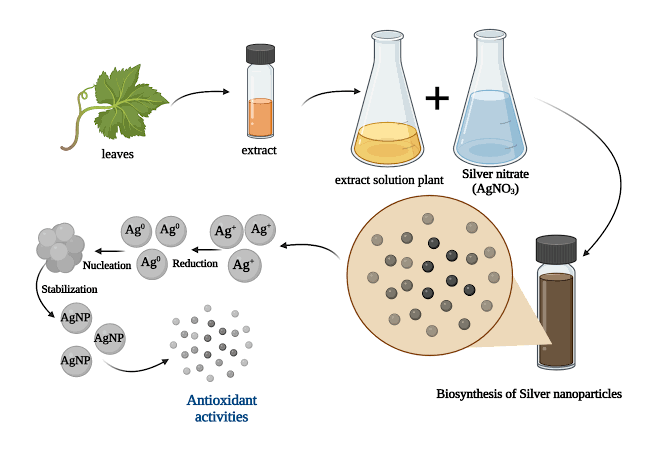


Figure 1: Synthesis of silver nanoparticles from *Cymbopogon Citratus*

## Antifungal activity

Preparation and standardization of the cultures for the assessment of antifungal activity were done on *Candida albicans*. The fresh cultures of Candida albicans strains was prepared by inoculating them on Sabouraud Dextrose Agar plates and allow them for sufficient growth at 28-30°C for 2 to 3 days. It was determined through a microdilution method applied for in vitro assessment of AgNPs against antifungal activity. Pour freshly prepared Sabouraud Dextrose Agar medium into sterile Petri dishes and allow it to solidify. After solidification, streak the surface with Candida albicans fungal spores using an inoculation loop or spreader to ensure even distribution. The AgNPs were added to culture media with different concentrations like 50 µg/ml, 75 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml. Add 10% Dimethyl Sulfoxide (DMSO) as a solvent if necessary for dilution. Include a well with a positive control, such as Ketoconazole, and a well with only DMSO as a negative control. Cover the plates and incubate them at 28-30°C for 48–72 hours. The duration may vary based on the growth rate of the fungi. After incubation, examine the plates for clear inhibition zones around each well, indicating antifungal activity. Measure the diameter of each inhibition zone in millimeters (mm). [(Ahn et al. 2019)](https://paperpile.com/c/mUolNJ/ixPI)

## Statistical analysis

To ensure dependability, the experiments were performed several times, and the data's accuracy was reported as the mean values that were further accompanied by their corresponding standard deviations. The IC50 value is the concentration at which 50% of the inhibition was observed, which was measured with high precision and corroborated using robust statistical analysis, including ANOVA and linear regression. The IC50 values are critical to determine the biological efficacy and potential applications of the nanoparticles studied.

# RESULTS

## Fourier Transform Infrared Spectroscopy

The silver oxide, which is extracted from the lemongrass species Cymbopogon citratus, has specific FTIR peaks that describe its structural composition. A strong peak of 3185.14 cm⁻¹ seems to be due to the O-H stretching vibrations; this means that there are hydroxyl groups at the surface. The second peak that appears at 1615.25 cm⁻¹ is associated with the C=C stretching vibrations, and this might arise from the organic matter contained in the Cymbopogon citratus extract. The band at 1316.21 cm⁻¹ is ascribed to the bending vibrations of C-H, and this can be attributed to some organic impurities. The stretching vibration of Ag-O leads to the peak that is detected at 827.80 cm⁻¹ indicating the oxide of silver. Two more peaks at 715.41 cm⁻¹ and 592.53 cm⁻¹ suggest that Ag-O is also contributing through the stretching modes. These FTIR results collectively show that silver oxide was synthesized successfully from Cymbopogon citratus, confirmed by the identified functional groups and metal-oxide bonds formed [(10)](https://paperpile.com/c/5cHaDw/9PZz).



Figure 1: FTIR spectrum of Silver Nanoparticles synthesized from *cymbopogon citratus*

## X RAY Diffraction

Characterization of silver nanoparticles synthesized with Cymbopogon citratus extract using X-ray diffraction analysis shows lattice planes. The pattern of XRD indicated very strong peaks at 27.99°, 29.73°, 32.45°, 38.20°, 42.67°, 46.60°, 64.75°, and 77.39°, indicating the crystalline nature of silver nanoparticles. These significant peaks correspond to specific lattice planes within the silver crystal structure that can be identified using standard crystallographic references. The significant peaks at these angles verify the synthesis of silver nanoparticles with good crystallinity and defined structural characteristics. [(13)](https://paperpile.com/c/5cHaDw/eSPQ).

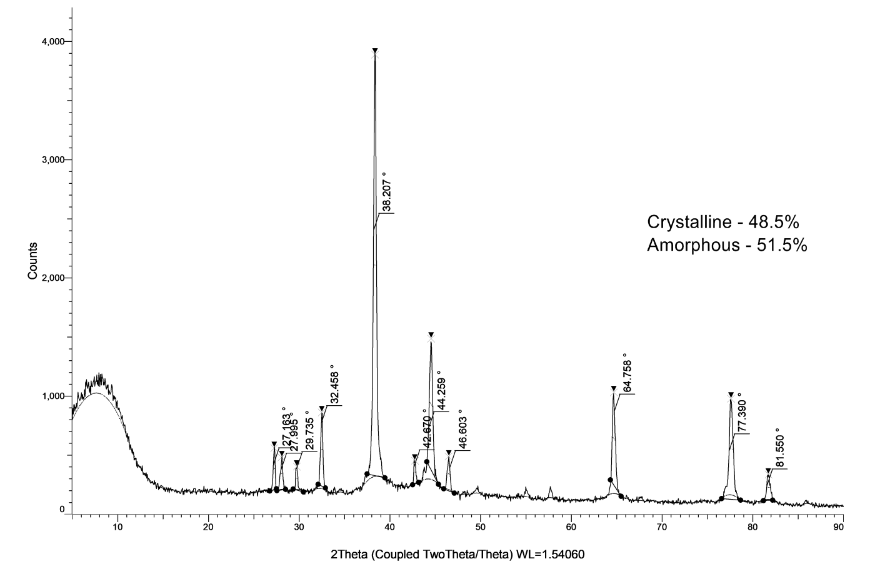
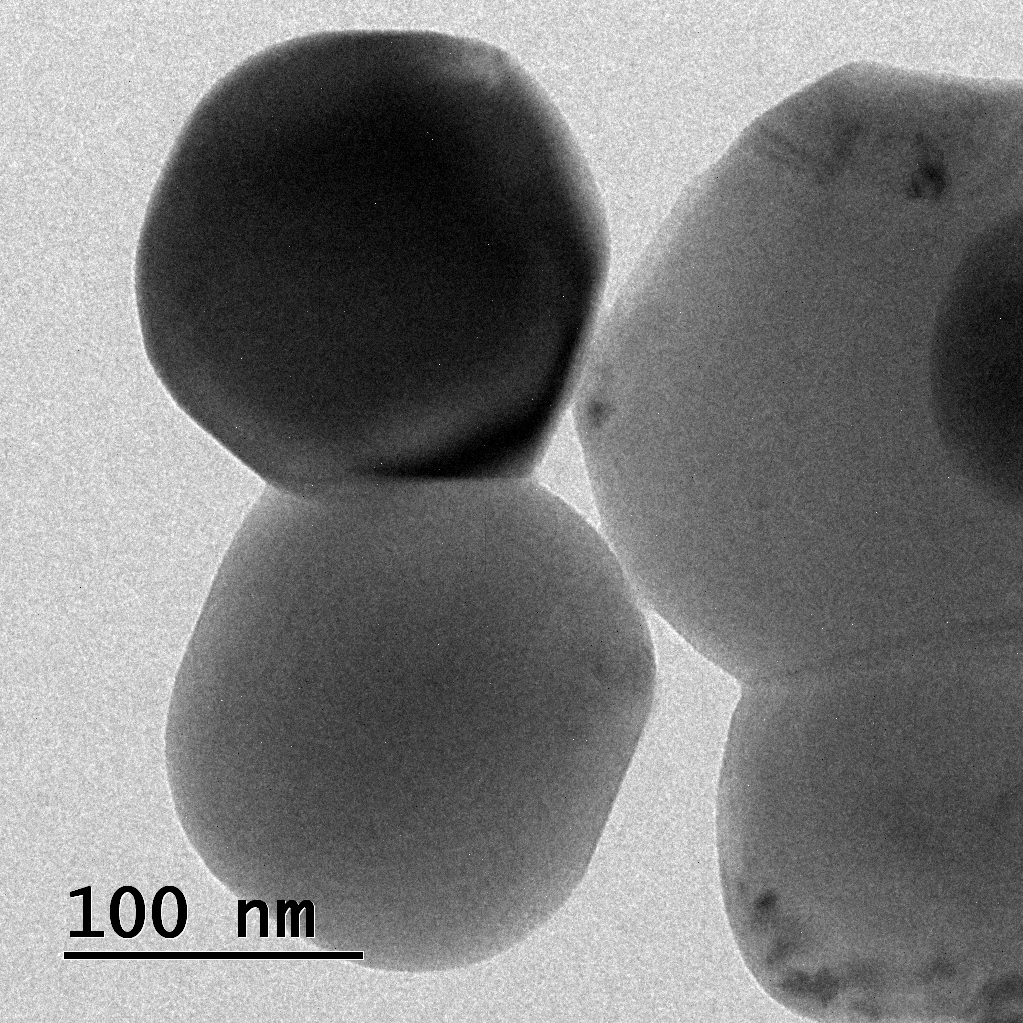
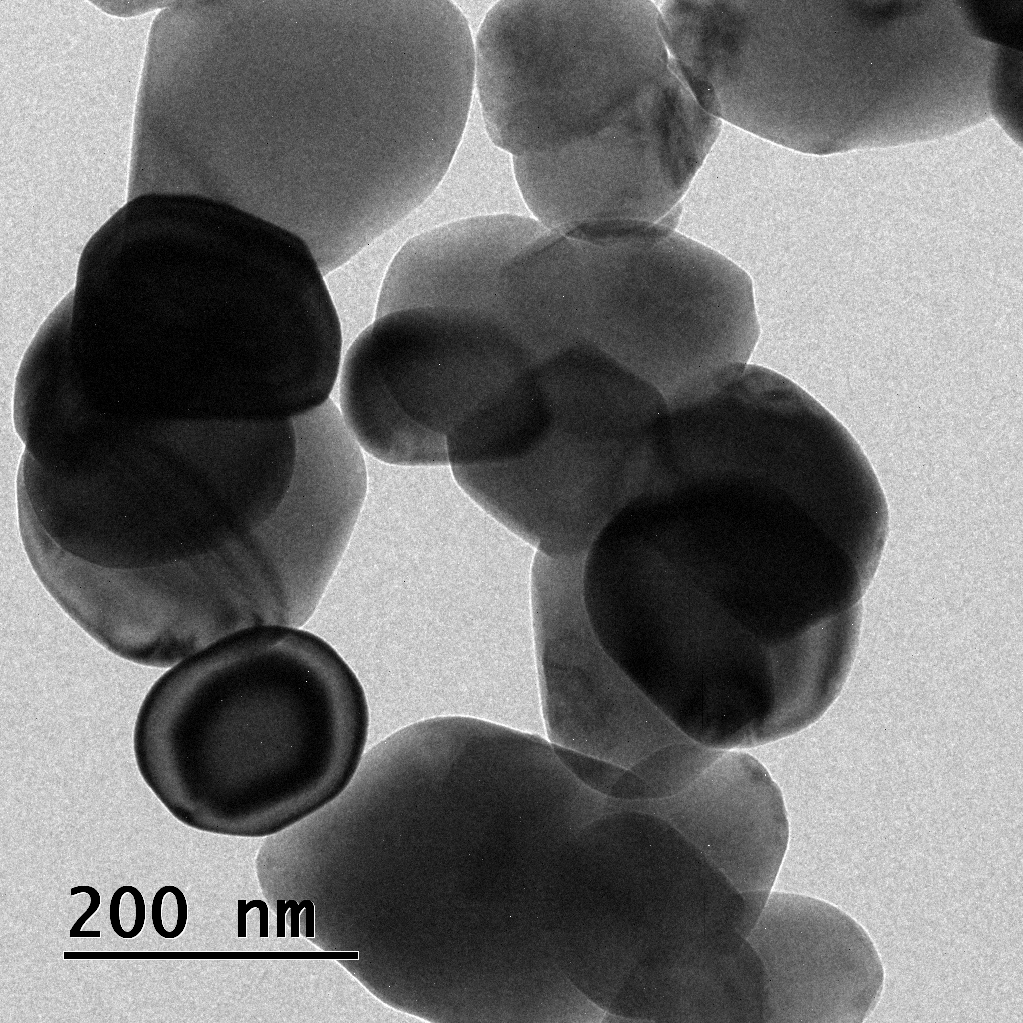


Figure 2: Synthesized silver nanoparticles from Cymbopogon Citratus XRD patterns.

## TEM

Transmission electron microscopy images revealed that the synthesized silver nanoparticles (AgNPs) from Cymbopogon citratus exhibited hexagonal and spherical morphologies. The size distribution of the AgNPs varied across the different samples. The sizes and diameter of the nanoparticles were 55.5 nm on average.



(A) (B)

Figure 3 (a,b): TEM images of synthesized Silver nanoparticles synthesised from *Cymbopogon citratus*

## Antifungal activity

Reduction in the virulence attributes of *C. albicans* in single and dual species state under the influence of nanoparticles: The impact of bioactives on phenotypic switching and hyphal morphogenesis of *Candida albicans* was demonstrated with different concentrations like 50 µg/ml, 75 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml with control. Among them 150 µg/ml shows the highest amount participation of inhibition of filamentous growth and pathogenicity, thus affecting virulence. Therefore, the synthesized Silver oxide nanoparticles from *Cymbopogon citratus* shows better among the yeast an hyphal cells.

Phenotypic switches between yeast and hyphal forms under the influence of all three compounds were analyzed. It was observed that in a concentration dependent manner thymol could restrain the shift of yeast to the hyphal phase and can revert the hyphal cells to yeast morphogenesis [(14)](https://paperpile.com/c/5cHaDw/5baJ).

Despite single or dual state, thymol significantly impeded the development of filamentous morphology. Green synthesized silver nanoparticles using *Cymbopogon citratus* extract have immense antifungal activity against *Candida albicans*[*(15)*](https://paperpile.com/c/5cHaDw/C8ii).

These nanoparticles inhibit the biofilm formation, the transformation from yeast to hypha, and reduce the virulence of the fungal cells. The bioactive compounds in the lemongrass extract combined with the antimicrobial properties of the silver enhance the efficiency of the nanoparticles. [(16)](https://paperpile.com/c/5cHaDw/3K1L) The AgNPs showed both in vitro and in vivo efficiencies; this might be promising for these eco-friendly nanoscale particles to be used as alternative treatments for oral candidiasis, especially when combated with drug-resistant fungal strains. [(17)](https://paperpile.com/c/5cHaDw/tPgx)

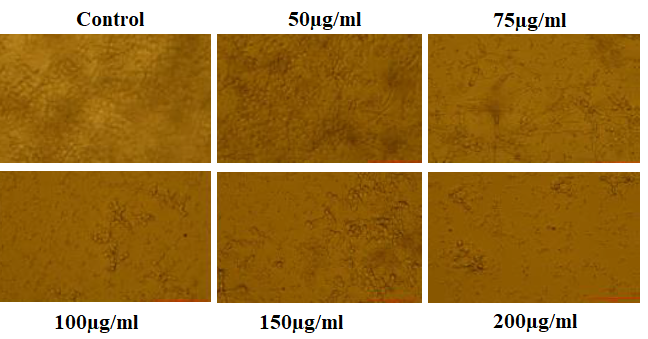


Figure 4: The impact of bioactives on phenotypic switching and hyphal morphogenesis of *Candida albicans* was demonstrated with the participation of inhibition of filamentous growth and pathogenicity, thus affecting virulence.

# DISCUSSION

Here, silver oxide produced from lemongrass (*Cymbopogon citratus)* was characterized using Fourier Transform Infrared Spectroscopy and X-ray diffraction. Its FTIR spectrum proved that the silver nano crystalline structure has some specific peaks for the functional groups and metal-oxide bond. Furthermore, the XRD analysis further confirmed their crystalline nature. Furthermore, the study confirms the healing properties of such nanoparticles on *Candida* *albicans*, as supported by strong inhibition of virulence characteristics and alterations in morphological features. The synthesis of silver oxide using *Cymbopogon citratus* and its characterisation via FTIR and XRD along with its medicinal attributes against *Candida albicans* aligns with and enhances the existing literature work in this direction of eco-friendly nanoparticle synthesis.

The FTIR spectrum of silver oxide based on the derivation of *Cymbopogon citratus* showed signals at 3185.14 cm⁻¹ (O-H stretching), 1615.25 cm⁻¹ (C=C stretching), 1316.21 cm⁻¹ (C-H bending), and 827.80 cm⁻¹ (Ag-O stretching).

These mountain peaks suggest the existence of hydroxyl groups, organic moieties, and silver-oxide linkages. This work is in agreement with Suresh et al. [(18)](https://paperpile.com/c/5cHaDw/hVQu), where they used neem extract to synthesize silver nanoparticles-analogous to many works. The FTIR spectrum showed similar O-H stretching and C=C stretching vibrations, thereby validating the presence of organic residues from the plant extract [Jain and Verma 2022; Wadhwani et al. 2022; Sreevarun et al. 2023; Solanki et al. 2023](https://paperpile.com/c/ZgTF2o/RvTq+Plr1+5enl+tNmg). The characteristic peaks for Ag-O, however, differed, indicating selective interaction of the silver ions with different plant extracts. Sharma et al.[(19)](https://paperpile.com/c/5cHaDw/NGUa) used Aloe vera extract in the synthesis of silver nanoparticles, and reported similar FTIR peaks for hydroxyl and organic elements, consistent with our findings. The similar functional groups in FTIR spectra of various plant extracts suggest that they have a common role in reduction and stabilization of silver nanoparticles.

In the X-ray diffraction analysis, our fabricated nanoparticles have strong peaks at certain angles: 27.99°, 29.73°, 32.45°, 38.20°, 42.67°, 46.60°, 64.75°, and 77.39°. The good crystallinity with well-defined structural features was exhibited. The results are comparable with those in the literature. Sharma et al. 2018 found similar peaks for crystal structures for silver nanoparticles synthesized by Aloe vera extract-based preparation. Both studies establish that nanoparticles are successfully produced with high crystallinity by the employment of eco-friendly techniques.

Mogole et al. [(20)](https://paperpile.com/c/5cHaDw/vzf7) synthesized silver nanoparticles using extract from *Citrus sinensis* (orange) peel. Comparable peaks in XRD showed that plant-based methods always yielded highly crystalline silver nanoparticles.

Our study was demonstrated to show that the nanoparticles produced are able to inhibit concentration-dependent transition from the yeast to the hyphal forms of *Candida albicans.* This study is in line with earlier studies since Paulo et al. [(21)](https://paperpile.com/c/5cHaDw/1ofe) established that silver nanoparticles synthesized from *Ocimum sanctum* extract inhibited hyphal growth and decreased pathogenicity of *Candida albicans.* The fact that this research is similar in nature presents great promise for the application of silver nanoparticles synthesized from various plant extracts as antifungal agents [Adel et al. 2023; Subramanian and Harikrishnan 2023](https://paperpile.com/c/ZgTF2o/d9L4+3mrS+2rYI).

Reem Aljindan et al.[(22)](https://paperpile.com/c/5cHaDw/5VJp) determined that silver nanoparticles inhibit the formation of *Candida albicans* biofilms and virulence. This study supports our findings through emphasizing the range of antifungal properties silver nanoparticles possess.

Humberto and colleagues [(23)](https://paperpile.com/c/5cHaDw/M80S) showed that silver nanoparticles can decrease the virulence and formation of biofilms in dual-species biofilms of *Candida albicans* and *Streptococcus mutans.* Their discoveries provide evidence for the effectiveness of utilizing silver nanoparticles in intricate microorganism settings [Chokkattu et al. 2023; Muthuswamy Pandian et al. 2022; Anti-inflammatory Potential Mouthwash...](https://paperpile.com/c/ZgTF2o/uJ67+FG9g+qjzw+0t8B).

Abdallah et al. [(5)](https://paperpile.com/c/5cHaDw/iQOh) further validated this finding by measuring the efficacy of silver nanoparticles to treat oral candidiasis in vivo, reporting significant reductions in infection and inflammation(Chehelgerdi et al., 2023).

Their findings coincide with ours, meaning that eco-friendly methods of synthesizing silver nanoparticles could be useful for medical-related purposes (Saadh et al., 2024). In a nutshell, the synthesis and characterization of silver nanoparticles from the extract of *Cymbopogon citratus* exhibited functional groups and crystalline nature similar to previously reported works by different researchers, using extracts of various plant species.[(24)](https://paperpile.com/c/5cHaDw/R0rr)

All of the previous works suggest the effectiveness of antifungal treatment on *Candida albicans* and include noninduction of phenotypic switching and reduction in virulence. These differences emphasize the reusability and viability of green synthesis methods from different plant sources in the generation of highly active silver nanoparticles that are useful in medicine.[(25,26)](https://paperpile.com/c/5cHaDw/URvC+iujm)

However, some inhibition within the research is concerning: the variability in the chemical composition of *Cymbopogon citratus* extracts may create potential problems in the uniformity and consistency of nanoparticle synthesis. Furthermore, although promising, the definite mechanisms of antifungal activity and long-term effects of silver nanoparticles in human cells and the environment are still ambiguous despite positive in vitro and in vivo results. Such research would include finding out the potential harmful effects on other non-target microorganisms and the cytotoxicity. There is also an additional challenge in upcoming studies, which include increasing the size of synthesis process and still maintaining the desired properties and effectiveness of nanoparticles. [(Ramana Ramya et al. 2023)](https://paperpile.com/c/mUolNJ/K0sd) [(Panneerselvam et al. 2023)](https://paperpile.com/c/mUolNJ/g6bF)

# CONCLUSION

The research was successful in synthesizing silver oxide nanoparticles using *Cymbopogon* *citratus* extract. It is confirmed by FTIR and XRD analysis that it possesses unique functional groups and high crystallinity. The nanoparticles show high potency to fight *candida albicans,* inhibit genetic mutations, and reduce its virulence. All these findings were in good correlation with earlier studies. It therefore suggests that green, synergistic silver nanoparticles may act as one of the excellent antifungal agents. However, variations in plant extracts and potential cytotoxic effects make further research necessary. This study will be of value to the already-rising body of proof that establish the use of plant-derived silver nanoparticles in the treatment of fungal infections.

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