

**Evaluation of Antifungal Activity of Mint on Fluconazole Resistant Candida Glabrata**

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**Abstract**

**Introduction :** Antifungal resistance shown by different species of Candida have affected management of candidiasis drastically. This has led to the need for newer safer therapeutic alternatives for their management. Phytochemical agents have been long known to possess numerous medicinal activities.

**Aim :** To evaluate the efficacy and resistance of fluconazole and to compare the antifungal effect of Mentha piperata in Candida glabrata.

**Materials and methods :** The organism is inoculated into specific medium and extract was added at serial dilutions and incubated to evaluate the zone of inhibition.

**Results :** The extract showed statistically significant and superior antifungal activity to fluconazole in fluconazole resistant Candida glabrata.

**Conclusion :** Although the extract showed superior antifungal activity in resistant Candida glabrata strains, further studies are needed to evaluate these extracts in patients to see if their efficacy is impeded by any systemic or local factors in the body.

**Keywords:** Candida glabrata, Antifungal agent, Mint, Fluconazole, antimicrobial resistance

**Introduction**

Candida glabrata was considered as a non-pathogenic commensal of the oral cavity and other mucosal surfaces in the early 1990s. Increased administration of broad-spectrum antibiotics, immunosuppressants and cancer

therapy led to suppressed immunity in the host providing a favourable environment for the growth of the organism.(1) These organisms are currently known to cause Candidiasis in immunocompromised individuals like hospitalised patients, individuals suffering from diabetes, AIDS and those undergoing cancer therapy. *Candida glabrata* is the second common cause of superficial and systemic Candidal infections especially nosocomial infections, with an innate resistance to common antifungal drugs like azoles.(2) It possesses colonising frequency even higher than *Candida albicans* and a higher colonising capacity on dental appliances like dentures. This organism has an ability to withstand high oxidative stress making it a successful human pathogen as well as it has ability to form biofilms especially the yeast form which disseminates and aids in spreading of the disease.(3)

Irrespective of innumerable studies on the pathogenesis, mode of invasion and various other virulence factors if *Candida* still only a handful of Pharmacological agents exist for their management. The usage of these agents are further limited by the susceptibility profile of the fungal species, the adverse effects as well as tolerance by the patient.(4) To overcome these disadvantages, multiple drug targets are being tried due to lack of newer classes of pharmacotherapeutic agents or targets. The minimum inhibitory concentration of *Candida glabrata* is higher than that of *albicans* and ultimately various resistant strains were also identified.(5,6)

As a product of unethical usage of azoles and other antifungal agents in both management and prevention of Candidiasis, there is a surge in the frequency of cases of Candidiasis refractory to treatment owing to emergence of resistant strains.(5) Fluconazole is the drug of choice in predisposed individuals or those at high risk for developing these infections and also in immune compromised individuals due to various reasons. Recently

there were strains showing development of mutation in ERG11 gene, which is the target site for Fluconazole there by leading antifungal resistance to azole drugs.(5)

The collective administration of both synthetic pharmacotherapeutic agents as well as natural phytochemical products can be a potential therapeutic strategy to reverse as well as overcome multi drug resistance in Candidiasis.(7) Use of herbal agents in treatment of these infections will provide us with a cheaper and simpler alternative which may even prove to be a better alternative to these chemotherapeutic agents. These agents may help in restoring their previous susceptibility of the organism to the drugs or can be used alone as therapeutic agents obtained from organic or herbal plants like coriander, mint, pomegranate etc. As these natural agents can be prepared at home and they can serve as a cheaper alternative and also has fewer side effects on comparison with the antifungal agents that are used, owing to the variety of functionally relevant secondary metabolites produced by these plants or extracts. The aim of present study was to evaluate the efficacy and resistance of fluconazole and to compare the antifungal effect of *Mentha piperata* to fluconazole in *Candida glabrata*.

### Materials and Methods

#### Method to extract herbal component

Take suitably sized *Mentha piperita* (powder or pieces) in an extractor. Add alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85 degree for 3-4 hours. Filter the extract through a filter (preferably 10µm pore size) suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. The filtrate obtained is then stored.

#### Method to assess growth of microorganisms

The organism is inoculated into specific culture medium, HiChrome Candidal Differential Agar media and then incubated for 24-48hours. The growth of the organism was indicated by the presence of yellowish creamy, smooth and raised colony detachable from the agar surface following incubation in the culture medium for 24-48 hours. The presence of the organisms was then confirmed in KOH and PAS stain.

Method to assess efficacy and resistance of Candida glabrata to fluconazole.

The resistance of Candida glabrata species to fluconazole will be assessed by incorporating fluconazole antifungal disc into the culture medium and zones of inhibition are checked for after 20-24hrs of incubation and if insufficient growth is observed then the culture will be incubated again for 48hrs. The resistant species will fail to show zone of inhibition even after 48hrs and these strains are isolated for further study.

Method to assess antifungal efficacy of Mint on Candida glabrata

The fungal strains were procured and inoculated in the specific culture medium and standard concentrations (25, 50, 75, 100, 125, 150, 175 and 200 µl) of the herbal extract was added to the culture and incubated to assess the antifungal activity of the extracts by estimating the zone of inhibition. The growth of the organism was indicated by the presence of yellowish creamy, smooth and raised colony detachable from the agar surface following incubation in the culture medium for 24-48 hours. The presence of the organisms was then confirmed in KOH and PAS stain.

### Statistical Analysis

The obtained raw data were then subjected to statistical analysis. The raw data did not follow the normal distributions and hence non-parametric statistical analysis was done for evaluating the statistical significance.

Pearson's correlation test was done as the study required comparison between the extract and fluconazole. The test enabled comparison of the efficacy of mint and fluconazole against Candida glabrata.

### Results

The study was conducted to determine the anticandidal properties of Mentha piperata on fluconazole resistant Candida glabrata by evaluating the zone of inhibition against the organisms.

Among the natural extracts, the herbal extracts failed to produce a zone of inhibition from a concentration of 25 µl till 75 µl. At the same time, showed increase in the diameter of the zone of inhibition from a concentration of 100 µl till 200 µl.

Even though fluconazole resistant strain of Candida glabrata was used in the study, mint showed an inferior anti-fungal action to the fungal strain at lower concentrations. Even the though the strains were resistant, the zone of inhibition was produced by Fluconazole at a concentration of 250mg comparable to that by the mint at a concentration of 200µl. Since analysis of the values between the positive control and the study group showed p value less than 0.001 in all these extracts, they are considered statistically significant. There is a significant difference between antifungal property of fluconazole and mint against fluconazole resistant Candida glabrata. (Figure1) (Table 1-2).

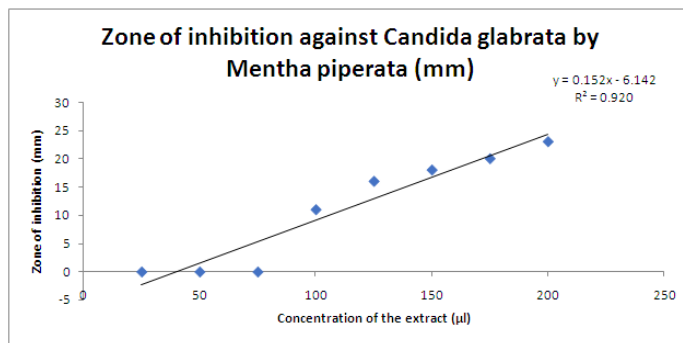


Figure 1: Zone of inhibition against *Candida glabrata* by *Mentha piperata* (mm)

Table 1: Zone of inhibition against *Candida glabrata* by *Mentha piperata* (mm)

Concentration of the extract (µl)	Zone of inhibition (mm)
25	0
50	0
75	0
100	11
125	16
150	18
175	20
200	23
Positive control (Fluconazole)- 250mg	26
Negative control (Ethanol)-200	0

Table 2: Comparison of the antifungal property of Fluconazole and *Mentha piperata* against fluconazole resistant *Candida glabrata*

Anti-fungal agents/ Extract	Zone of inhibition	Concentration	P-value	Significance
Fluconazole	26	250mg	0.000	Significant
<i>Mentha piperata</i>	23	200 µl		

**Discussion**

Antimicrobial resistance is one of the biggest therapeutic challenges in the management of various infectious diseases faced worldwide in the past decade. Injudicious use of antimicrobial agents has led to the development of various resistant microorganisms which have given rise to more devastating infections. Though a better understanding of these infections were attempted, still no exact method for its management or reversal have been found yet thereby necessitating search for novel, safer alternatives.(8,9) Phytochemical agents or herbal therapeutic agents were the main treatment modalities for any disease in the older times. With the knowledge of the deleterious effects of these agents we are again redirected

our roots to find herbal agents to treat many conditions. These herbal agents were used to cure infections, cancer, to fasten wound healing etc. These natural agents are regaining their lost therapeutic values due to adverse effects of synthetic pharmacotherapeutic modalities and emergence of resistant strains of various infectious pathogens. Various herbal plants have medicinal action in their leaves, fruits or roots. They contain numerous bioactive molecules which confer to it numerous antibacterial antifungal and anti-oxidative potential.(10,11)

We conducted an in-vitro study to assess antifungal activity of *Mentha piperata* against Fluconazole resistant *Candida glabrata* and the efficacy of the extracts were then compared with fluconazole and among each other. Only few studies have been done to assess the efficacy of antifungal agents against *Candida glabrata*. Our study is the first of its kind to assess the antifungal action of mint against Fluconazole resistant *Candida glabrata*. In the current study, mint showed significant antifungal activity in higher concentrations and was significantly superior to fluconazole.

Mint is an antimutagenic and chemopreventive plant material which is a part of the culinary and cosmetics world. It has long been proven that mint possess antiparasitic, antibacterial, analgesic, bug repellent properties. The main component present in it is menthol and pulegone. Deyab et al (2018) performed a study comparing mint and Apple cider vinegar on *Candida* associated denture stomatitis and inferred that mint has anticandidal activity. The molecules bind to the cell membrane thereby disrupting it which further decreases the production of ergosterol by the cell, providing the fungicidal activity to mint.(12) Githaiga et al (2018) performed an in vitro study to evaluate the antibacterial properties of mint and to conduct oil distillation to

evaluate the essential components of mint. They reported that mint possessed significant antibacterial properties and that tannins, alkaloids Anderson's were the major components of it.(13) In our study, mint showed superior antifungal action to fluconazole and showed a greenish zone of inhibition surrounding the colonies in comparison to the clear zone produced by Coriander sativum. The current study was in accordance with the study conducted by Erdogan et al (2016) where they investigated antifungal activity of mint, lavender and thyme against *Verticillium dahliae* Kleb and reported that mint and thyme showed similar antifungal activity in a dose dependent manner.(14) The results of the present study is similar to the study conducted by K Y Wenji et al 2018, who reported that mint leaves have potent antifungal activity against *Candida albicans* at a concentration of 80%. (15) The study provided a natural at the same time safer alternative to the existing pharmacotherapeutic agents which are known to have numerous side effects. These Phytochemical agents are available in our country and are part of our culture and hence will be cheaper and can be easily available to rich and poor alike. Also, being natural agents these may be used as a prophylactic measure in predisposed individuals without the fear of side effects. The main limitation of the study is that being an in vitro study we were unable to assess the effect of systemic factors or systemic illness on the mode of action on these agents. Since candidiasis is most commonly arising as a superadded infection, most of the primary disorders involved were either bacterial infections, systemic conditions like diabetes, HIV etc. It is not known if these herbal agents may have antagonistic or synergistic effect with any other medication administered for different purpose. One another limitation of the present study was the use of crude herbal extracts. Also since it was an in vitro study the effect of other conditions in the oral cavity

or body that may act as confounding factor in the effectiveness of the drug could not be evaluated.

### Conclusion

The current study was conducted to evaluate the antifungal activity of *Mentha piperata* and against *Candida glabrata*. The herbal extract in the study showed potent antifungal activity against *Candida glabrata* and the values were found to be statistically significant. These agents can thus be a safer and cheaper alternative to the existing therapeutic systems without any of the harmful side effects. These drugs were also superior to fluconazole. Mint extracts may be used alone or can be used as a multi drug combination with fluconazole which will reduce the dosage needed for managing the condition. Further studies are needed to evaluate these extracts in patients to see if their efficacy is impeded by any systemic or local factors in the body. Also, drug interactions with medications given for other purposes should be evaluated as this infection is more prevalent in patients who are suffering from Diabetes, Immunosuppression, Acquired Immunodeficiency Syndrome etc which was a major limitation faced as the current study was an in vitro study.

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