Evaluation of antifungal activity of mint, pomegranate and coriander on fluconazole-resistant *Candida glabrata*

Lekshmy Jayan, N Priyadharsini, R Ramya, K Rajkumar

Department of Oral and Maxillofacial Pathology and Microbiology, SRM Dental College, Chennai, Tamil Nadu, India

**Abstract**

**Introduction**: Antifungal resistance shown by different species of *Candida* has affected the 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**: The aim of this study was to evaluate the efficacy and resistance of fluconazole and to compare the antifungal effects of *Coriandrum sativum*, *Mentha piperita* and *Punica granatum* in *Candida glabrata*.

**Materials and Methods**: The organism was inoculated into a specific medium, and extracts are added at serial dilutions and incubated to evaluate the zone of inhibition.

**Results**: All the three extracts showed statistically significant and superior antifungal activity to fluconazole in fluconazole-resistant *C. glabrata*.

**Conclusion**: Although the extracts showed superior antifungal activity in resistant *C. 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**: Antifungal resistance, *Candida glabrata*, candidiasis, coriander, fluconazole, herbal extracts, mint, phytochemical therapy

**INTRODUCTION**

Self-treatment causes more harm than good; the thirst to self-diagnose and relying on Internet sources for health problems has led to an unethical use of various pharmacotherapeutic agents. One of the reasons for an upsurge in the development of antimicrobial resistant organisms in the past decades is the increased use of antimicrobials and steroidal agents resulting in immunosuppression which knocks down the harmony of the microflora enabling these organisms to thrive. The first name that comes to mind when we talk about an opportunistic infection is candidiasis caused by fungal organisms of the genus *Candida*. In the recent decade, these organisms present with the dilemma of antifungal resistance to majority of the antifungal drugs used against them. Although these organisms can be present in normal individuals without causing disease, in susceptible individuals, it can lead to localized or in rare cases disseminated candidiasis.[1]

*Candida glabrata* was considered as a nonpathogenic 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 favorable environment for the growth of the organism.[2] These organisms are currently known to cause candidiasis in immunocompromised individuals like hospitalized patients, individuals suffering from diabetes, AIDS and those undergoing cancer therapy. C. 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.[3] It possesses colonizing even higher than Candida albicans and a higher colonizing 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 the ability to form biofilms, especially the yeast form which disseminates and aids in the spread of the disease.[4]

The comparable appearance of the fungal cellular structure in candidal species to that of human cells by virtue of their eukaryotic nature adversely affects the treatment of candidal infections. Even with innumerable research on the pathogenesis, the mode of invasion and virulence factors of Candida, only handful of chemotherapeutic agents are effective in its management. The usage of these agents is further limited by the susceptibility profile of the fungal species, the adverse effects as well as tolerance by the patient.[5] To overcome these disadvantages, multiple drug targets are being tried due to the lack of newer classes of pharmacotherapeutic agents or targets. The minimum inhibitory concentration of C. glabrata is higher than that of albicans, and ultimately, various resistant strains were also identified.[6,7]

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 the emergence of resistant strains.[6] Fluconazole is the drug of choice in predisposed individuals or those at high risk for developing these infections and also in immunocompromised individuals due to various reasons. Recently, there were strains showing the development of mutation in ERG11 gene, which is the target site for fluconazole, thereby leading antifungal resistance toazole drugs.[6]

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 multidrug resistance in candidiasis.[8] It is always better to find the answer to our questions in the wisdom provided by our ancestors; the usage of herbal extracts and their usage in the 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 such as coriander, mint and pomegranate. The natural agents have an added effect of being cheaper and that they can be prepared at home without the need of any fancy equipment. Unlike, the synthetic pharmacotherapeutic agents these natural agents have fewer side effects. All these advantages can be attributed to the presence of the secondary metabolites produced by these extracts. The aim of the present study was to evaluate the efficacy and resistance of fluconazole and to compare the antifungal effects of Coriandrum sativum, Mentha piperita and Punica granatum in C. glabrata.

MATERIALS AND METHODS

Method to extract herbal component
Take suitably sized M. piperita, C. sativum and P. granatum (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°C and 85°C for 3–4 h. Filter the extract through a filter (preferably 10 μm pore size) suitably 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 a specific culture medium, HiCrone candidal differential agar media, and then incubated for 24–48 h. 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 h. The presence of the organisms was then confirmed in KOH and periodic acid–Schiff (PAS) stain.

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

518
Method to assess antifungal efficacy of mint, pomegranate and coriander 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 extracts were 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 h. 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, nonparametric statistical analysis was done for evaluating the statistical significance. Pearson's correlation test was done, as the study required a comparison between different groups. The test enabled a comparison of the efficacy of different groups of extracts among themselves and with fluconazole against C. glabrata.

RESULTS

The study was conducted to determine the antifungal properties of C. sativum, M. piperita and P. granatum extracts on fluconazole-resistant C. glabrata by evaluating the zone of inhibition as well as the minimum inhibitory concentration for these extracts 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, it showed an increase in the diameter of the zone of inhibition from a concentration of 100 µl till 200 µl. C. sativum had the largest zone of inhibition and the lowest minimal inhibitory concentration among the three extracts studied.

Table 1: Zone of inhibition against Candida glabrata by Punica granatum (mm)

| Concentration of the extract (µl) | Punica granatum | Coriandrum sativum | Mentha piperita |
|----------------------------------|-----------------|--------------------|-----------------|
| 25                               | 0               | 0                  | 0               |
| 50                               | 0               | 0                  | 0               |
| 75                               | 0               | 0                  | 0               |
| 100                              | 05              | 15                 | 11              |
| 125                              | 14              | 16                 | 16              |
| 150                              | 16              | 17                 | 18              |
| 175                              | 19              | 23                 | 20              |
| 200                              | 21              | 26                 | 23              |

Positive control (fluconazole) - 250 mg
Negative control (ethanol) - 200 mg

Table 2: Comparison of the antifungal property of fluconazole and Punica granatum, Coriandrum sativum and Mentha piperita against fluconazole-resistant Candida glabrata

| Antifungal agents/extract | Zone of inhibition (mm) | Concentration | P value | Significance |
|--------------------------|-------------------------|---------------|---------|--------------|
| Fluconazole              | 26                      | 250 µl        | 0.000   | Significant  |
| Punica granatum          | 21                      | 200 µl        | 0.000   | Significant  |
| Coriandrum sativum       | 26                      | 250 µl        | 0.000   | Significant  |
| Mentha piperita          | 23                      | 200 µl        | 0.000   | Significant  |

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. Although a better understanding of these infections was attempted, still no exact method for its management or reversal has been found yet, thereby necessitating search for novel, safer alternatives. 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 have given rise to more devastating infections.
were used to cure infections, cancer, to fasten wound healing, etc. These natural agents are regaining their lost therapeutic values due to the 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 its numerous antibacterial antifungal and antioxidative potential.[11,12]

We conducted an in vitro study to assess the antifungal activity of three herbal extracts, i.e., *C. sativum*, *M. piperita* and *P. granatum* against fluconazole-resistant *C. glabrata*, and the efficacy of the extracts was then compared with fluconazole and among each other. Only a few studies have been done to assess the efficacy of antifungal agents against *C. glabrata*. Our study is the first of its kind to assess the antifungal action of coriander, mint and pomegranate against fluconazole-resistant *C. glabrata*. In the current study, all the three extracts showed comparable antifungal property without any statistically significant difference in their action. However, all the extracts were significantly superior to fluconazole.

*C. sativum* or coriander is one of the commonly used culinary spices which contain bioactive chemical compounds such as linalool, cedrene, cymene and pinene. These phytochemical compounds confer to them various medicinal activities such as anticarcinogenic, antibacterial, antifungal, neuroprotective action and antidiabetic. One of the bioactive molecules in coriander and linalool has been found to play a remarkable role in regulating these biological effects.[11,13] Msaada et al. (2014) reported that coriander has potent antioxidant properties as a product of high concentrations of flavonoids, tannins and polyphenols.[14] Chaudhary et al. in a study for evaluating antibacterial properties of coriander concluded that it is a potent antibacterial agent and it shows significant inhibition of *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Lactobacillus*, *Salmonella*, and the extract had rich flavonoids, alkaloids, glycosides and amino acids but lacked reducing sugars.[15] Liu et al. (2016) showed that mint possesses antibacterial activity against Gram-positive *Staphylococcus aureus* and *Bacillus cereus* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* and antifungal activity against *C. albicans* but showed mild cytotoxicity against human embryonic kidney cell lines.[16] In the current study, the antifungal activity of *C. sativum* was superior to that of

| Natural extract used | Zone of inhibition (mm) | Concentration (µl) | Correlation value | P    | Significance    |
|----------------------|-------------------------|-------------------|------------------|------|-----------------|
| *Punica granatum*    | 21                      | 200               | 0.960            | 0.867| Insignificant   |
| *Coriandrum sativum* | 26                      | 200               | 0.956            | 0.866| Insignificant   |

Table 3: Comparison of antifungal property of *Punica granatum* and *Coriandrum sativum* against fluconazole-resistant *Candida glabrata*

Graph 1: Zone of inhibition against *Candida glabrata* by *Punica granatum* (mm)

Graph 2: Zone of inhibition against *Candida glabrata* by *Coriandrum sativum* (mm)
fluconazole in fluconazole-resistant species of *C. glabrata*. In comparison with the other extracts, visually coriander showed a clear zone of inhibition around the colonies, suggesting complete lysis of the organisms which were absent in the other two extracts.

The seed and peel or skin of pomegranate are usually thrown away, but recently, they have been found to possess biochemical molecules of medicinal value. It is also shown to exert estrogen-like actions also.[17] Punicalagin and ellagic acid are the most significant components of these particular plant extracts which are believed to be responsible for these medicinal properties.[18] Patel et al., in a study where they evaluated whether pomegranate possesses antibacterial properties on Gram-positive and Gram-negative bacteria as well as antifungal activity on *C. albicans*, inferred that pomegranate inhibits both the Gram-negative and Gram-positive organisms and *Candida*. The bioactive metabolites present in Pomegranate has two modes of action by which it exerts its antifungal properties. The first is by binding to the cell membrane thereby destroying the bacterial cell membrane. Secondly, these molecules inhibit the membrane integrated enzyme molecules resulting in triggering of apoptosis.[19] Rongai et al. (2019) compared different genotypes of pomegranate to evaluate the antifungal properties by assessing inhibition of mycelial growth and reported that wild genotypes CREA-FRU6, CREA-FRU11 and CREAM-FRU76 have potent antifungal activity.[20] Rizwan et al. performed a study evaluating the anticandidal activity of pomegranate peels and Darehald root bark and inferred that both the extracts showed superior antifungal activity in comparison with fluconazole and voriconazole.[21] In the current study, although pomegranate lacked potent antifungal action against *C. glabrata* at lower concentrations at higher concentrations, it showed statistically significant antifungal action superior to fluconazole. Mbatha (2018) also reported similar results from his study on pomegranate skin and concluded that in addition to fungicidal action, pomegranate also inhibits germ tube formation at subtherapeutic levels in *C. albicans.[22]* The results of the present study contradicted the results obtained by Nicole et al. (2014) who reported that pomegranate extract lacked antifungal property against *Candida* species. However, in our study, pomegranate showed comparable antifungal effects to coriander and mint at higher concentrations.

Mint is an antimutagenic and chemopreventive plant material which is a part of the culinary and cosmetic world. It has long been proven that mint possesses antiparasitic, antibacterial, analgesic, bug repellent properties. The main component present in it is menthol and pulegone. Deyab et al. 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.[23] Githaiga et al. 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 etc., were the major components of it.[24] 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 *C. sativum*. The current study was in accordance with the study conducted by Oktay Erdogan et al. where they investigated the 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.[25] The results of the present study are similar to the study conducted by Wenji et al. 2019, who reported that mint leaves have potent antifungal activity against *C. albicans* at a concentration of 80%. 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. Furthermore, being natural agent, 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 or systemic conditions such as diabetes and HIV. It is not known if these herbal agents may have an antagonistic or synergistic effect with any

![Graph 3: Zone of inhibition against *Candida glabrata* by *Mentha piperita* (mm)](image-url)
other medication administered for different purposes. One another limitation of the present study was the use of crude herbal extracts. Furthermore, since it was an in vitro study, the effect of other conditions in the oral cavity or body that may act as a confounding factor in the effectiveness of the drug could not be evaluated.

The need of the hour is the discovery of newer drugs in treating infections, especially antifungal targets in the treatment of candidal infections, as they are the ones with the most resistance to all the antifungal drugs used in their management.[14,15]

CONCLUSION

The current study was conducted to evaluate the antifungal activity of C. sativum, M. piperita and P. granatum against C. glabrata. All the herbal extracts used in the study have shown potent antifungal activity against C. 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. These extracts may be used alone or can be used as a multidrug 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 factor in the body. Furthermore, 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 and acquired immunodeficiency syndrome, which was a major limitation faced as the current study was an in vitro study.

Financial support and sponsorship Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Fritz H, Kayser, K. A. Biens, J. Eckert. Medical microbiology. 1st edition. Medical publications. 2005.
2. Subhan M, Faryal R, Macreadie I. Stain resistance in Candida glabrata. Biotechnol Lett 2018;40:1389-94.
3. Li L, Redding S, Dongari-Bagtzoglou A. Candida glabrata: An emerging oral opportunistic pathogen. J Dent Res 2007;86:204-15.
4. Rodrigues CF, Henriques M. Oral mucositis caused by Candida glabrata biofilms: Failure of the concomitant use of fluconazole and ascobic acid. Ther Adv Infect Dis 2017;4:10-7.
5. Thaczu D. Drug resistance. Aidsmap 2018;5:1112-22.