Antifungal effects of tulsi, garlic, cinnamon and lemongrass in powder and oil form on *Candida albicans*: An *in vitro* study

Mitul Prajapati¹, Monali Shah¹, Amena Ranginwala¹, Prakhar Agrawal¹, Dhruval Acharya², Shreya Thakkar²

¹Department of Oral and Maxillofacial Pathology, Ahmedabad Dental College and Hospital, ²Oral Pathologist, Private Practitioner, Ahmedabad, Gujarat, India

**Abstract**

**Introduction:** The use of plants for treating diseases is as old as the human species. Medicinal plants have been a major source of therapeutic agents for alleviation and cure of diseases. **Objectives:** The objective of the study was to evaluate and compare the antifungal activity of garlic, cinnamon, lemongrass and tulsi in powder and oil form at different concentrations on *Candida albicans*.

**Materials and Methods:** Powder and oil of garlic, cinnamon, lemongrass and Tulsi dissolved in inert solvent dimethyl formamide to obtain different concentration. Stock solution of different concentration was inoculated on Petri plates containing *C. albicans* and incubated at 30°C for 48 h. The inhibition zones were measured in millimeters using Vernier caliper. The collected data were analyzed using statistical test like mean value and one-way analysis of variance.

**Results:** Maximum zone of inhibition for the *C. albicans* was 42 mm at concentrations of 50% for the oil of lemongrass; followed by cinnamon 40 mm, garlic 24 mm and tulsi 20 mm. The *P* value obtained 0.050, 0.040, 0.036 and 0.031 were found to be statically significant for *C. albicans* at 20%, 30%, 40% and 50% concentrations of the various oil preparations, respectively. The *P* value obtained 0.043, 0.033, 0.032 and 0.027 were found to be statically significant for *C. albicans* at 20%, 30%, 40% and 50% concentrations of various plant powder, respectively.

**Conclusions:** Lemongrass and cinnamon oil shows best antifungal effect against *C. albicans* as compared to garlic and tulsi. Compared to powder preparations, the oil preparations are better to inhibit the growth and higher the concentrations, greater the zone of inhibition seen in all the plant extracts and in oil.

**Keywords:** *Candida albicans*, medicinal plants, plant preparations

**INTRODUCTION**

*Candida albicans* represent the most permeative fungal pathogen colonizing humans. *C. albicans* exists in two forms – pseudohyphae and yeast forms – a trait known as dimorphism. The yeast form is believed to be innocuous, but the hyphae form is usually associated with invasion into the
host tissue. This transition from a benign yeast type to highly invasive hyphae type depends on changes in the host defences. Candidiasis is a most commonly observed opportunistic infection in oral cavity often referred to as thrush.11 The
antimicrobial properties of various plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives. A few of them are cinnamon (Cinnamomum zeylanicum), garlic (Allium sativum), tulsi (Ocimum tenuiflorum) and Lemongrass (Cymbopogon).  

Garlic (A. sativum) is species in the onion genus, Allium. It has been used as both food and medicine in many cultures for thousands of years. Garlic has antiseptic, antimicrobial and antifungal properties. It is a good source of Vitamin B6, Vitamin C, manganese and phosphorus. The sulfur-containing compound alliiin, ajoene, diallyl polysulfide’s and Maillard reaction products are attributed to the therapeutic effects of garlic. Cinnamon (Cinnamomum verum) is a spice obtained from the inner bark of several trees from the genus Cinnamomum. It is principally employed in cookery as a condiment and flavoring material. It has a antioxidants, anti-inflammatory, antiviral, antibacterial, antifungal and anti-allergic properties. A tulsi (Ocimum sanctum) plant of Indian Basil occupies an important place in the Hindu religion. The name tulsi denotes “the incomparable one.” Tulsi is used in many conditions such as fever, common cold, sore throat, and respiratory disorders. It has a germicidal, bactericidal, antiedematous, antimicrobial and anti-inflammatory properties. Lemongrass (Cymbopogon) is plants in grass family. Lemongrass has hypnotic, anticonvulsant, antioxidant, antifungal, antibacterial, analgesic, antiemetic, antitussive and antiseptic effects. It helps in digestion, improves oral health and controls bad breadth.  

Hence, this study attempts to summarize the **in vitro** study of the antifungal properties of various plants such as cinnamon (C. zeylanicum), garlic (A. sativum), tulsi (O. tenuiflorum) and Lemongrass (Cymbopogon) on C. albicans.

### MATERIALS AND METHODS

The present study was design to evaluate and compare the antifungal activity of garlic, cinnamon, lemongrass and tulsi in powder and oil form at different concentrations on C. albicans.

**Sample collection**

Fresh garlic bulbs, cinnamon bark sticks and leaves of lemongrass and tulsi were collected from local market in Ahmedabad and 100% pure form of essential oils of same plants were purchased from Devinez company. Thirty-two Sabouraud dextrose agar media for C. albicans were taken.

**Preparation of plant extracts (powder form)**

Garlic bulbs, cinnamon bark sticks and leaves of lemongrass and tulsi were collected and cleaned twice using distilled water and ground to fine powder using a mechanical grinder. Twenty gram of each plant powder dissolved in 100 ml of distilled water in sterile mortar and pestle and then filtered using Whatman No. 1 filter paper and collected in a 250 ml glass flask. Flasks were then plugged with cotton and kept in refrigerator at 4°C for 24 h and then filtered and kept in a hot air oven for 5–7 days at 30°C ± 2°C to completely evaporate the solvent and to get a black shining crystal powder form. One gram of each extract was diluted with 10 ml of an inert solvent dimethyl formamide (DMFO) to obtain 10% concentration. This concentration was further diluted to obtain 20%, 30%, 40% and 50% concentrations and stored in sterile test tube.

**Preparation of plant oils**

Essential oils of tulsi, garlic, cinnamon and lemongrass were purchased from the local market in pure 100% form. One milliliter oil of all plant was dissolved in 10 ml of DMFO to obtain 10% concentration. This concentration...
was further diluted to obtain 20%, 30%, 40% and 50% concentrations and stored in sterile test tube. The stock solution of positive control – voriconazole was also prepared by dissolving 100 mg in 10 ml of sterile distilled water to get the 10 mg/ml. Stock solutions of each plant extracts and oils were labeled.

**Preparation of culture media**

Freeze dried form of the microorganism *C. albicans* was obtained from Microbial Type Culture Collection, Chandigarh. The ampules containing freeze dried forms of microorganism were opened and content mixed with distilled water (0.4 ml). The obtained mixture was stirred using a sterile stirrer and kept standing for half an hour. Each prepared mixture then aseptically inoculated and evenly spread using sterile “L” rod or “Swab” on the surface of sterile culture media plate to obtain the primary Culture plates. For *C. albicans*, the culture media plate sabouraud dextrose agar (90 mm) was incubated for 30°C for 48 h and used for secondary culture procedure. Blank discs were used to prepare respective control for the test of each plant extract and oils.

### Table 2: Statistical analysis of various plants powder at 10%, 20%, 30%, 40% and 50% on *Candida albicans*

| Concentration (%) | Mean±SD                  |
|-------------------|--------------------------|
| 10                | 13.5000±5.44671          |
| 20                | 15.2500±5.73730          |
| 30                | 19.5000±6.60808          |
| 40                | 22.5000±7.72442          |
| 50                | 26.0000±8.28654          |
| Total             | 19.3500±7.69330          |

SD: Standard deviation

### Table 3: One-way ANOVA analysis for the mean comparison of various plant powder at 10%, 20%, 30%, 40% and 50% on *Candida albicans*

| Mean square | F   | Significant |
|-------------|-----|-------------|
| Between groups | 105.200 | 2.242 | 0.113 |
| Within groups   | 46.917 |  |          |

### Table 4: Statistical analysis of various plant oils at 10%, 20%, 30%, 40% and 50% concentration on *Candida albicans*

| Concentration (%) | Mean±SD                  |
|-------------------|--------------------------|
| 10                | 15.0000±6.83130          |
| 20                | 18.7500±8.05709          |
| 30                | 23.0000±8.90693          |
| 40                | 27.0000±10.03328         |
| 50                | 31.5000±11.12055         |
| Total             | 23.0500±10.07067         |

SD: Standard deviation

### Table 5: One-way ANOVA analysis for the mean comparison of various plant oils at 10%, 20%, 30%, 40% and 50% on *Candida albicans*

| Mean square | F   | Significant |
|-------------|-----|-------------|
| Between groups | 170.300 | 2.051 | 0.139 |
| Within groups   | 83.050 |  |          |
impregnated. Required concentration of the various plant extracts and oils such as 10%, 20%, 30%, 40%, and 50% applied immediately on the surface of inoculated plates. A comparison antibiotic (positive control) test was made using commercial disk of voriconazole for \textit{C. albicans}. Negative control of inert solvent DMFO was introduced into same plate. The fungal strains were incubated at 30°C for 48 h. The plates were duplicated for each concentration. Finally, the inhibition zones (diameter of translucent zones, from the center of each disk) were measured in millimeters using Vernier caliper [Figures 1-8].

Table 6: Statistical comparison (one-way ANOVA) of various plant powder at different concentration on \textit{Candida albicans}

| Concentration (%) | Plant extracts | Inhibition zone (mm) | SD  | P    |
|-------------------|----------------|----------------------|-----|------|
| 10                | Cinnamon       | 13                   | 0.042 | 0.054 |
|                   | Garlic         | 12                   | 0.045 |      |
|                   | Lemongrass     | 21                   | 0.026 |      |
|                   | Tulsi          | 8                    | 0.068 |      |
| 20                | Cinnamon       | 16                   | 0.072 | 0.043 |
|                   | Garlic         | 12                   | 0.096 |      |
|                   | Lemongrass     | 23                   | 0.050 |      |
|                   | Tulsi          | 10                   | 0.115 |      |
| 30                | Cinnamon       | 24                   | 0.083 | 0.033 |
|                   | Garlic         | 16                   | 0.124 |      |
|                   | Lemongrass     | 26                   | 0.076 |      |
|                   | Tulsi          | 12                   | 0.165 |      |
| 40                | Cinnamon       | 28                   | 0.110 | 0.032 |
|                   | Garlic         | 18                   | 0.172 |      |
|                   | Lemongrass     | 30                   | 0.103 |      |
|                   | Tulsi          | 14                   | 0.221 |      |
| 50                | Cinnamon       | 32                   | 0.129 | 0.027 |
|                   | Garlic         | 21                   | 0.197 |      |
|                   | Lemongrass     | 34                   | 0.122 |      |
|                   | Tulsi          | 17                   | 0.244 |      |

SD: Standard deviation

Table 7: Statistical comparison (one-way ANOVA) of various plant oil preparation at different concentration on \textit{Candida albicans}

| Concentration (%) | Plant extracts | Inhibition zone (mm) | SD  | P    |
|-------------------|----------------|----------------------|-----|------|
| 10                | Cinnamon       | 16                   | 0.043 | 0.064 |
|                   | Garlic         | 12                   | 0.057 |      |
|                   | Lemongrass     | 24                   | 0.028 |      |
|                   | Tulsi          | 8                    | 0.085 |      |
| 20                | Cinnamon       | 24                   | 0.067 | 0.050 |
|                   | Garlic         | 14                   | 0.115 |      |
|                   | Lemongrass     | 27                   | 0.060 |      |
|                   | Tulsi          | 10                   | 0.161 |      |
| 30                | Cinnamon       | 30                   | 0.089 | 0.040 |
|                   | Garlic         | 18                   | 0.148 |      |
|                   | Lemongrass     | 31                   | 0.086 |      |
|                   | Tulsi          | 13                   | 0.206 |      |
| 40                | Cinnamon       | 35                   | 0.115 | 0.036 |
|                   | Garlic         | 21                   | 0.391 |      |
|                   | Lemongrass     | 36                   | 0.111 |      |
|                   | Tulsi          | 16                   | 0.251 |      |
| 50                | Cinnamon       | 40                   | 0.139 | 0.031 |
|                   | Garlic         | 24                   | 0.232 |      |
|                   | Lemongrass     | 42                   | 0.132 |      |
|                   | Tulsi          | 20                   | 0.278 |      |

SD: Standard deviation

**Statistical analysis**

The collected data were analyzed using statistical tests such as mean value and one-way analysis of variance.

**RESULTS**

The present study was carried out to evaluate the antifungal activity of plants such as cinnamon (\textit{C. Zeylanicium}), garlic (\textit{A. sativum}), tulsi (\textit{O. tenuiflorum}) and lemongrass (\textit{Cymbopogon}) in powder and oil form at various concentrations (10%, 20%, 30%, 40% and 50%) against \textit{C. albicans}. In the present study, positive control for \textit{C. albicans} – Voriconazole showed the zone of inhibition of 36 mm for all the concentrations. The negative control showed no zone of inhibition at all. The present study showed that the maximum zone of inhibition for the \textit{C. albicans} was 42 mm at concentrations of 50% for the oil of lemongrass, followed by cinnamon 40 mm, garlic 24 mm and tulsi 20 mm [Table 1]. The collected data were analyzed using statistical test such as for powder preparations mean value [Table 2], One-way ANOVA [Table 3] and for oil preparations mean value [Table 4], One-way ANOVA [Table 5]. The P value obtained 0.043, 0.033, 0.032 and 0.027 were found to be statically significant for \textit{C. albicans} at 20%, 30%, 40% and 50% concentrations of various plant extracts (powder), respectively. The P value obtained 0.054 was found to be statistically nonsignificant for the \textit{C. albicans} at 10% concentration of the various plant extracts [Table 6]. The P value obtained 0.050, 0.040, 0.036 and 0.031 were found to be statistically significant for \textit{C. albicans} at 20%, 30%, 40% and 50% concentrations of the various oil preparations, respectively. The P value obtained 0.064 was found to be statistically nonsignificant for the \textit{C. albicans} at 10% concentration of the oil preparations [Table 7]. Compared to powder preparations of various plants, the oil preparations are better to inhibit the growth at different concentrations and also the higher the concentrations, greater the zone of inhibition seen in all the plant extracts and in oil.

**DISCUSSION**

Candidiasis is a common opportunistic fungal infection of oral cavity, which is most commonly caused by the fungus \textit{C. albicans}. It is most commonly seen in patients with an impaired immune system. All commercially available antifungal drugs with prolonged use may have negative effect on human health, and hence an alternative therapy with minimal side effects is desirable. Hence, the search for the alternative product continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals. Ayurveda is the traditional nature...
healing system of India and is quickly gaining popularity. The present study was undertaken to assess the role of various plant extracts and essential oils such as tulsi (O. sanctum), cinnamon (C. verum), garlic (allium sativum), and Lemongrass (Cymbopogon) against C. albicans. Our study showed that lemongrass oil and powder both have an antifungal activity on C. albicans. The inhibitory zone increases with the increase in concentration of lemongrass oil and powder. These findings are in accordance with Pokpong Amornvit et al.,[6] who demonstrated antifungal activity at the concentration of 0.20% or at higher level of lemongrass oil by well-diffusion method. These findings are also similar to Tyagi and Malik,[7] Abe et al.,[8] Basera et al.[9] (2019), and Madeira et al.[10] (2019), and Madeira et al.[10] also found inhibition activity of tulsi powder on C. albicans is increased with increase in the concentration of lemongrass powder. Our study showed that cinnamon oil and powder both have an antifungal activity on C. albicans. Fani and Kohanteb[11] found inhibition zone of 8 mm, 13 mm, 27 mm and 54 mm at concentration of cinnamon oil at 12.5%, 25%, 50% and 100%, respectively, on C. albicans which is similar to our findings. Mahmood[12] found antifungal activity of cinnamon powder with higher concentration which is similar to our study. Allicin, an active compound in garlic, has antifungal activity. It downregulates the putative virulence gene, SIR2 in C. albicans.[13] Our study showed that garlic oil and powder both have an antifungal activity on C. albicans. These findings are in accordance with, Lemar[14] (2005) who found antifungal activity of Garlic oil at 50% concentration which shows 25 mm zone of inhibition and antifungal activity of garlic powder at 50% concentration which shows 20 mm zone of inhibition. Shuford et al.[15] and Shuford[16] (2005) found similar zone of inhibition activity on C. albicans with using higher concentration of garlic powder. Our study showed that tulsi oil and powder both have an antifungal activity on C. albicans. Devkatte et al.[17] found inhibition zone of 12 mm at 25% concentration of tulsi oil on C. albicans which is similar to our findings. Arora et al.[18] found inhibitory activity of tulsi powder on C. albicans at higher concentration. Pathak[18] found inhibitory zone increase with the increase in concentration of tulsi powder on C. albicans which is similar to our study. Subramaniam et al.[19] found inhibitory zone of 9.10 mm, 11.13 mm and 13.45 mm at 30%, 60% and 90% concentration of tulsi powder. The addition of antimicrobial and antifungal agents to dentifrices, mouthwashes and varnishes increases the effect of mechanical oral hygiene procedure. When this antimicrobial and antifungal agent derived from plant, the undesirable effects of synthetic drugs can be overcome. Moreover, these phytochemicals produce other biological activities such as induction of immunity, which indirectly reduces the risk of oral diseases. Recently, some plants have also been shown to potentiate the activity of antimicrobial and antifungal agents against resistant strains, introducing the concept of resistance modification.

CONCLUSIONS

The present study showed that, as compared to powder preparations, oil preparations are better to inhibit the growth. Higher the concentrations greater the zone of inhibition is seen in all the plant powder and in oil. Lemongrass and Cinnamon oil shows best antifungal effect against C. albicans as compared to garlic and tulsi. Our study suggests that the inclusion of natural products with antimicrobial and antifungal properties in routine diet may helpful in combating and preventing various infectious diseases. However, further studies with multidisciplinary approach on a larger scale and with clinical trials will aid in giving clear evidence to confirm the antimicrobial and antifungal action and general safety.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Bommanavar SB, Gugwad S, Malik N. Phenotypic switch: The enigmatic white-gray-opaque transition system of Candida albicans. J Oral Maxillofac Pathol 2017;21:82-6.
2. Bayan I., Kouliivand PH, Gorji A. Garlic: A review of potential therapeutic effects. Avicenna J Phytomed 2014;4:1-14.
3. Rao PV, Gan SH. Cinnamon: A multifaceted medicinal plant. Evid Based Complement Alternat Med 2014;2014:642942.
4. Cohen MM. Tulsi-Ocimum sanctum: A herb for all reasons. J Ayurveda Integr Med 2014;5:251-9.
5. Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann AS. Scientific basis for the therapeutic use of Cymbopogon citratus, stapt (Lemon grass). J Adv Pharm Technol Res 2011;2:3-8.
6. Amornvit P, Choontharungkaj S, Srithavaj T. Lemongrass-incorporated tissue conditioner against Candida albicans culture. J Clin Diagn Res 2018;12:ZC50-2.
7. Tyagi AK, Malik A. Liquid and vapour-phase antifungal activities of selected essential oils against Candida albicans: Microscopic observations and chemical characterization of Cymbopogon citratus. BMC Complement Altern Med 2010;10:65.
8. Abe S, Sato Y, Inoue S, Ishibashi H, Maruyama N, Takizawa T, et al. Anti-Candida albicans activity of essential oils including
Lemongrass (*Cymbopogon citratus*) oil and its component, citral. Nihon Ishinkin Gakkai Zasshi 2003;44:285-91.
9. Basera P, Lavania M, Agnihotri A, Lal B. Analytical investigation of *Cymbopogon citratus* and exploiting the potential of developed silver nanoparticle against the dominating species of pathogenic bacteria. Front Microbiol 2019;10:282.
10. Madeira PL, Carvalho LT, Paschoal MA, de Sousa EM, Moffa EB, da Silva MA, et al. *In vitro* effects of lemongrass extract on *Candida albicans* biofilms, human cells viability, and denture surface. Front Cell Infect Microbiol 2016;6:71.
11. Fani MM, Kohanteb J. Inhibitory activity of *Cinnamomum zeylanicum* and *Eucalyptus globulus* Oils on *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida* species isolated from patients with oral infections. J Dent 2011;11:14-22.
12. Mahmood SN. Antifungal activity of *Cinnamomum zeylanicum* and *Eucalyptus microtheca* crude extracts against food spoilage fungi. Euphrates J Agric Sci 2012;4:26-39.
13. Said MM, Watson C, Grando D. Garlic alters the expression of putative virulence factor genes SIR2 and ECE1 in vulvovaginal *C. albicans* isolates. Sci Rep 2020;10:3615.
14. Lemar KM, Passa O, Aon MA, Cortassa S, Müller CT, Plummer S, et al. Allyl alcohol and garlic (*Allium sativum*) extract produce oxidative stress in *Candida albicans*. Microbiology (Reading) 2005;151:3257-65.
15. Shuford JA, Steckelberg JM, Patel R. Effects of fresh garlic extract on *Candida albicans* biofilms. Antimicrob Agents Chemother 2005;49:473.
16. Devkatte AN, Zore GB, Karuppayil SM. Potential of plant oils as inhibitors of *Candida albicans* growth. FEMS Yeast Res 2005;5:667-73.
17. Arora T, Kang RS, Mann JS, Khurana NS, Aggarwal R, Walia G. Antimicrobial activity of herbal extracts against recalcitrant endodontic pathogens: An original *in vitro* study. Saint Int Dent J 2015;1:28-32.
18. Pathak AK. Anti-candida activity of aqueous extracts of some herbs. Indian J Fund Appl Life Sci 2012;2:1-6.
19. Subramaniam G, Tewari BB, Comathinayagam R. Studies of antimicrobial properties of different leaf extracts of Tulsi against human pathogens. Am Int J Contemp Res 2014;4:149-157.