Research Article

Antifungal activity of ginger oil against clinical isolates of *Candida* species

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ABSTRACT

Introduction: The use of essential oils from herbs and spices is a novel antimicrobial treatment to reduce the initial microorganism loads. In herbs and spices, there are many antimicrobial compounds exhibiting a wide range of activities against bacteria, yeasts, and fungi. Essential oils from plants have been suggested as natural preservatives. Thus, the aim of the present study is to assess the antifungal activity of ginger oil against clinical isolates of *Candida* species. **Materials and Methods:** A total of 20 non-repetitive clinical isolates of *Candida* species were collected from different samples of immunocompromised individuals attending a medical hospital, in Chennai. They were characterized by carbohydrate fermentation and assimilation tests and confirmed. Isolates were preserved in semisolid Sabouraud chloramphenicol semisolid stock and stored at 4°C until further use. *Candida* species were further characterized using HiCrome candida differential agar (HiMedia, Mumbai). **Results:** Clinical isolates of *Candida* sp. were inhibited from 0.125% to 1% of ginger oil. The minimal inhibitory concentration of ginger oil was appeared to be 0.25% for *Candida* spp. **Conclusion:** The ginger oil is found to have antifungal activity against *Candida* spp. Since any toxic or irritant property of ginger oil has to be under consideration, ginger oil can be used as alternative and complementary agent for controlling fungal infections.

KEY WORDS: Antifungal, *Candida* species, Chloramphenicol, Cytoprotective, Ginger oil, Medicinal plants, Sabouraud agar

INTRODUCTION

Medicinal plants are used as pharmaceuticals, nutraceuticals, cosmetics, and also food supplements.[1] Plant-derived products have been used for medicinal purposes for centuries. In traditional Indian medicine or Ayurveda, *Zingiber officinale* and many other herbs have been used as medicine. One of the most common infectious diseases of humans is urinary tract infections, caused by *Candida* species, which are responsible for more than 80% of cases worldwide.[2] About 6–20% of the human populations are experiencing asymptomatic bacteriuria (ABU), depending on age and gender, and nearly 50% of women suffer from cystitis in their lifetime. Many ABU *Candida* isolates are phylogenetically associated with virulent uropathogenic *Candida* strains, and some may have evolved from pathogenic strains by virulence attenuation.[3] Epidemiological studies also revealed a rise in local fungal infections of chronic wounds (diabetic foot, burn, bedsore, and cancer ulceration), with the involvement of *Candida albicans* biofilm. In the past two decades, the increased prevalence of fungal infections has been reported. Fungi cause both superficial and internal mycoses. Systemic fungal infections constitute a major community health problem in many parts of the world, both in developed and developing countries. Along with other potential infections, *Candida* affecting oral environment becomes common, leading to oral thrush and denture stomatitis. *Candida* species are capable of causing a variety of deep-seated and superficial infections. *Candida* sp. is considered as one of the most hospital-acquired infections. Due to the increase in multidrug-resistant strains of *Candida*, it becomes necessary to search for new sources of antifungal agents and compounds that inhibit these resistance mechanisms. This has led to a search for therapeutic alternatives,

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

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Received on: 19-06-2018; Revised on: 22-07-2018; Accepted on: 04-09-2018

particularly among medicinal plants and compounds isolated from them used for their empirical antifungal properties. The *in vitro* antimicrobial evaluation of lozenges containing extract of garlic and ginger was carried out. Results indicated that there was inhibition of growth by Nystatin tablet, but garlic and ginger combination only inhibited the growth of laboratory strains of *C. albicans*. The use of essential oils from herbs and spices is a novel antimicrobial treatment to reduce the initial microorganism loads. In herbs and spices, there are many antimicrobial compounds exhibiting a wide range of activity.[4] Hence, there is a great demand for novel antifungals belonging to a wide range of structural classes, selectively acting on new targets with fewer side effects. One approach might be the testing of plants traditionally used for their antifungal activities as potential sources for drug development. Medicinal plants are not only important to the millions of people for whom traditional medicine is the only opportunity for health care and to those who use plants for various purposes in their daily lives but also as a source of new pharmaceuticals.[5]

Ginger is an important spice in Thailand. In 2001, Thailand grew more than 30,000 million tons of ginger. It is widely used as an ingredient in the food, pharmaceutical, cosmetic, and other industries. Garlic and ginger have been known to possess immunological and cytoprotective effects in the biological host. Ginger contains a unique flavor derived from both non-volatile and volatile oils. The pungent compounds are gingerol and shogaol, while zingiberene is a predominant component of oils. Some volatile compounds having antimicrobial properties present in ginger are α-pinene, borneol, camphene, and linalool.[6] The medicinal properties have been mainly used for treating the symptoms of vomiting, diarrhea, light-headedness, blurred vision, dyspepsia, tremors, decrease in body temperature, and high blood pressure. Furthermore, 6-gingerol and 6-shogaol can reduce the viability of gastric cancer cells.[7,8] Ginger extracts have been reported to inhibit the growth of *Listeria monocytogenes*, *Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus*, *Bacillus subtilis, Fusarium moniliforme,* and *Mycobacterium* sp.[9] The garlic and ginger lozenge demonstrated pronounced antifungal activity against the laboratory isolate of *C. albicans* but not against the clinical isolates of the same organism while nystatin gave inhibition zone diameter values of 21–41 mm. *Escherichia coli* and *Staphylococcus aureus* strains were resistant. Ginger oils showed very good inhibition of *Salinicoccus roseus*, *Halococcus turkmenicus,* and *Halococcus morrhuae* isolated from salt-cured fish.[10] Thus, the present study indented to determine the antibacterial activity of ginger oil against clinical isolates of *Candida* sp.

MATERIALS AND METHODS

Candida Isolates

A total of 20 non-repetitive clinical isolates of *Candida* species were collected from different samples of immunocompromised individuals attending a medical college, in Chennai. They were characterized by carbohydrate fermentation and assimilation tests and confirmed. Isolates were preserved in semisolid Sabouraud's chloramphenicol semisolid stock and stored at 4°C until further use.

Characterization of *Candida* Species

Candida species were further characterized using HiCrome agar (HiMedia, Mumbai).

Preparation of Hichrome Agar

CHROM agar *Candida* (HiMedia, Mumbai) was prepared following manufacturer's instructions. About 21.02 g of HiChrome *Candida* differentiation agar base (modified) was suspended in 500 ml of distilled water. It was heated to boiling gently to dissolve the medium completely. Then, it was allowed to cool to 50°C, and rehydrated (one vial) contents of Hichrome *Candida* selective supplement were added under aseptic precautions. It was mixed well and poured into Petri dishes. Isolates were identified on Hichrome agar based on the characteristic color of the colony by subculturing from Sabouraud's chloramphenicol agar plates, and the *Candida* Hichrome plates were incubated at 37°C for 24–48 h.[8] Based on color produced by the isolates, speciation has been made.

Detection of Antifungal Activity of Ginger Oil against Clinical Isolates of *Candida* spp.

Antifungal activity of ginger oil was tested against *Candida* isolates by minimum inhibitory concentration method. Mueller Hinton broth was supplemented with 0.002% (V/V) tween 80 (HiMedia, Mumbai) to enhance the dispersion of the essential oil. Agar dilution method was performed to attain the different concentrations of essential oils such as 0.03%, 0.06%, 0.125%, 0.25%, 0.5%, 1%, and 2% in Mueller Hinton Agar.

Viability controls were performed on the fungal strains in the liquid medium under the same assay conditions. The plates were sealed and incubated at 35°C ± 2°C for 24–48 h. The minimal inhibitory concentrations (MICs) of the products used in the biological assays were defined as the lowest concentrations capable of visually inhibiting the fungal growth in the wells, as compared to the growth under control conditions.

Media containing various concentrations of essential oils were poured over the sterile Petri dishes and allowed to dry. Media without essential oil were served as control plate. Spot inoculation of 0.5 McFarland

standard turbidity adjusted isolates was made on the plates and incubated at 37°C for overnight. The lowest concentration of the essential oils that completely inhibited the growth of isolates was considered as MIC.[11]

RESULTS

Characterization of *Candida* Species by Hichrome *Candida* Agar

A total of 20 clinical isolates of *Candida* sp. were seeded on to Hichrome *Candida* agar, and the results were tabulated based on pigment production [Table 1, Figures 1 and 2].

Sample Wise Distribution of *Candida* Species

Of the 20 clinical isolates of *Candida* sp., 6/20 (30%) were from oral thrush, 5/20 (25%) from urine, 3/20 (15%) from sputum, 3/20 (15%) from vaginal swab, 2/20 (10%) from ear swab, and one (5%) from wound swab [Figure 3].

We have observed that clinical isolates of *Candida* sp. were inhibited from 0.125% to 1% of ginger oil.

The MIC of ginger oil was appeared to be 0.25% for *Candida* spp [Table 2].

DISCUSSION

Plant oils used as cooking and flavoring agents are increasingly claimed to have broad-spectrum antimicrobial activity. Selected oils have been suggested to have potent antimicrobial activity against skin infections, insect bites, chicken pox, colds, flu, measles, sinus congestion, asthma, bronchitis, pneumonia, tuberculosis, and cholera, probably due to their phenolic, alcoholic, and terpenoid constituents.

The various therapeutic effects of ginger have been reported which includes antiemetic activity, antiulcer, antiplatelet, antipyretic, anti-inflammatory, and antioxidant activity.[11] The antifungal activity of ginger has been attributed to gingerol and shagelol derived from the ethanolic extracts of ginger.[12]

The study results are comparable with other studies, suggesting that different antifungal agents are present in the ginger extract. In the ginger rhizome, there are several components which have antibacterial and antifungal effects.[13] The study done by Supreetha.S on antifungal therapy has shown that ethanol itself has antifungal activity against *Candida albicans*. However, there was an increased inhibition zone when an ethanolic extract of ginger was used when compared to ethanol alone and this was statistically significant.[14]

According to the study by Anupama *et al.*, these oils required more than 1.0% to exert fungicidal effect.

Table 1: Isolated Candida species

Candida species	Color
C. albicans	Green
C. tropicalis	Blue
C. krusei	Pink dry colonies
C. kefyr	Pale
C. parapsilosis	Pale

C. albicans: Candida albicans, C. tropicalis: Candida tropicalis, C. krusei: Candida krusei, C. kefyr: Candida kefyr, C. parapsilosis: Candida parapsilosis

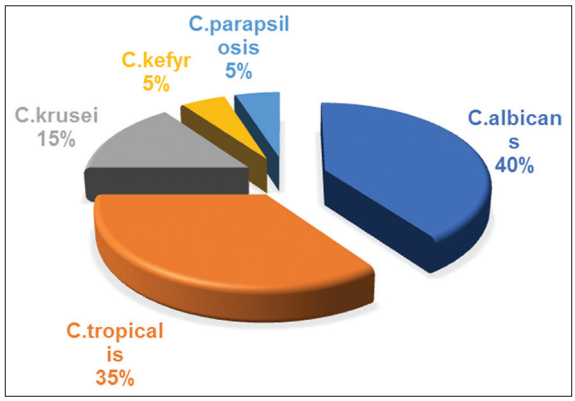

Figure 1: Pie chart showing the distribution of *Candida* species

Figure 2: Representative picture showing *Candida* species on Hichrome *Candida* agar

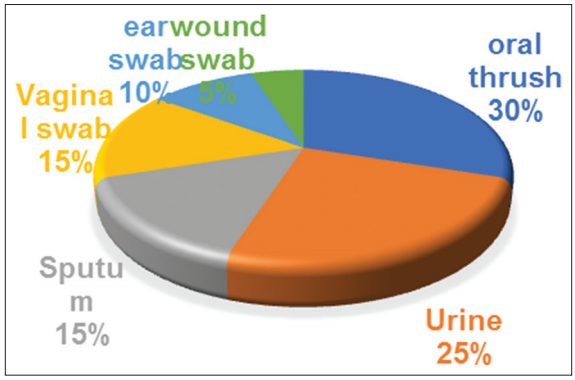

Figure 3: Result of antifungal activity of ginger oil against clinical isolates of *Candida* species

Table 2: The MIC of ginger oil against isolated *Candida* species

Dilutions of ginger oil	0.03%	0.06%	0.125%	0.25%	0.5%	1%	2%
Number of organisms	0	0	3 (15)	2 (10)	9 (45)	6 (30)	0

MIC: Minimal inhibitory concentration

Minimum fungicidal concentration of these oils ranged from 1.0% to 3% except in the case of jasmine, ginger, and juniper oil, which were not fungicidal up to the 3% concentration.[15]

The higher content of geranial and other oxygenated compounds makes fresh ginger oil more potent than dry ginger oil. The content of hydrocarbon compounds is more in dry ginger oil compared to fresh ginger oil.[16] Earlier studies have reported that monoterpene compounds are more active than sesquiterpene compounds. Dry ginger oil had higher content of sesquiterpene hydrocarbons. Especially when this is compared to the results obtained for the standard nystatin tablet where the *C. albicans* strains showed susceptibility. It is worthy to note that, in most cases of infection, a combination of antimicrobial activity and one or more other biological effects, such as immunomodulation, could be responsible for the overall effect of a natural product.[17]

A study conducted by Prakasam *et al*. from Chennai in 2014 demonstrated that Acinetobacter strains were inhibited from 0.06 to 0.25%, 0.25 to 1%, and 0.125 to 1% for clove, peppermint, and eucalyptus oils, respectively. In clove oil, 14/50 (28%) isolates were inhibited at 0.06%, 25/50 (50%) at 0.125%, and 11/50 (22%) at 0.25%. In peppermint oil, 34/50 (68%) isolates were inhibited at 0.25%, and 12/50 (24%) and 4/50 (8%) were at 0.5% and 1% concentrations of peppermint oil, respectively. In eucalyptus oils, 10/50 (20%) isolates were inhibited at 0.125%, 18/50 (36%) at 0.25%, 16/50 (32%) at 0.5%, and 6/50 (12%) at 1%, respectively. Thus, the MIC of clove oil was found to be 0.06%, 0.25% for peppermint oil, and 0.125% for eucalyptus oil.[18,19] In contrast, in our study, we used ginger oil against *Candida* sp. isolates. 15% of isolates were inhibited at 0.125%, 10% were at 0.25%, 45% were at 0.5%, and 30% were at 1% of essential oil.

Thus, the MIC of ginger oil against *Candida* sp. was found to be 0.125%. The resistance of clinical strains of *C. albicans* isolated from prosthesis users to the antifungal standard nystatin, which is used for the treatment of *Candida*-associated denture stomatitis, demonstrates the importance of investigating the efficacy and mode of action of novel products on this clinically relevant pathogen. Such research will facilitate the discovery of new antifungal agents.[20]

In some essential oils such as eucalyptus and clove oils, there has been much research and documented some toxic and irritant properties. In spite of this, most of these oils are available as whole oils or as part of pharmaceutical and cosmetic products. However, the studies on toxic and irritant properties of essential oils are imperative, especially when considering any new products for human administration.[21]

The addition of citral to the growth medium at a subinhibitory concentration of 4 μg/mL (MIC/8) did not alter the resistance of the clinical strains or the standard to nystatin, as assessed by proliferation. Furthermore, there are no studies in the literature investigating the ability of citral to modulate nystatin activity. The ability of unconventional compounds to increase the antimicrobial activity or reverse the resistance of microorganisms to drugs classifies the compounds as modifiers of antifungal activity.[22]

It is, therefore, likely that a combination of these biological effects of garlic and ginger and the demonstrated antimicrobial effect may explain its usefulness in the management of oropharyngeal infections, especially those of fungal origin in folklore medicine.

CONCLUSION

The ginger oil is found to have antifungal activity against *Candida* spp. However, the studies on toxic and irritant properties of essential oils are imperative, especially when considering any new products for human administration. This can be used as alternative and complementary agent for controlling the infections. Nowadays, the indiscriminate use of commercial antimicrobial drugs has caused multiple drug resistance in human pathogenic microorganisms. The resistance strains of *C. albicans* have become a cause of major health concerns and require novel antifungal agents to tackle this problem.

REFERENCES

1. Sharma S, Vijayvergia R, Singh T. Evaluation of antimicrobial efficacy of some medicinal plants. J Chem Pharm Res 2010; 2:121-4.
2. Ronald A. The etiology of urinary tract infection: Traditional and emerging pathogens. Dis Mon 2003;39:71-82.
3. Foxman B. Epidemiology of urinary tract infections: Incidence, morbidity, and economic costs. Am J Med 2002;113:5-13.
4. Mabbett AN, Ulett GC, Watts RE, Tree JJ, Totsika M, Cheryl-lynn YO, *et al.* Virulence properties of asymptomatic bacteriuria *Escherichia coli*. Int J Med Microbiol 2009;299:53-63.
5. Roller S, editor. Natural Antimicrobials for the Minimal Processing of Foods. 1st ed. San Diego: Elsevier; 2003. p. 176-200.
6. Ravindran PN, Babu KN, editors. Ginger: The Genus Zingiber.

1st ed. Boca Raton: CRC Press; 2016. p. 966.
7. Kumar MD, Gopinath P. Antibacterial activity of ginger oil against clinical isolates of *Escherichia coli*. Int J Sci Dev Res 2018; 3:14-7.
8. Ishiguro K, Ando T, Maeda O, Ohmiya N, Niwa Y, Kadomatsu K, *et al.* Ginger ingredients reduce viability of gastric cancer cells via distinct mechanisms. Biochem Biophys Res Commun 2007;362:218-23.
9. Yamada Y. Identification of antimicrobial gingerols from ginger (*Zinglber officinale roscoe*). J Antibact Agent 1992;20:309-11.
10. Prasad MM, Seenayya G. Effect of spices on the growth of red halophilic cocci isolated from salt cured fish and solar salt. Food Res Int 2000;3:793-8.
11. Prakasam G, Bhashini M, Lakshmipriya N, Ramesh S. *In-vitro* antibacterial activity of some essential oils against clinical isolates of *Acinetobacter baumannii*. Indian J Med Microbiol 2014; 32:90-1.
12. Jain J, Tikare S, Mahuli A. Antifungal activity of ginger extract on *Candida albicans*: An *in-vitro* study. J Dent Sci Res 2011; 2:18-21.
13. Atai Z, Atapour M, Mohseni M. Inhibitory effect of ginger extract on *Candida albicans*. Am J Appl Sci 2009;6:1067-9.
14. Wayne PA. National Committee for Clinical Laboratory Standards. Vol. 12. Performance Standards for Antimicrobial Disc Susceptibility Testing; 2002. p. 1-53.
15. Anil S, Shukla YN, Sushil K. Recent development in plant derived antimicrobial constituents-a review. J Med Aromat Plant Sci 2000;22:349-405.
16. Sinha GK, Gulati BC. Antibacterial and antifungal study of some essential oils and some of their constituents. Indian Perfum 1990;34:126-9.
17. Shuford JA, Piper KE, Steckelberg JM, Patel R. *In vitro* biofilm characterization and activity of antifungal agents alone and in combination against sessile and planktonic clinical *Candida albicans* isolates. Diagn Microbiol Infect Dis 2007;57:277-81.
18. Bonjar S. Evaluation of antibacterial properties of some medicinal plants used in Iran. J Ethnopharmacol 2004;94:301-5.
19. Hammer KA, Carson CF, Riley TV. *In-vitro* activity of essential oils, in particular *Melaleuca alternifolia* (tea tree) oil and tea tree oil products, against *Candida* spp. J Antimicrob Chemother 1998; 42:591-5.
20. Lawless J. The Illustrated Encyclopedia of Essential Oils: The Complete Illustrated Guide to the use of Oils in Aromatherapy and Herbalism. Shaftesbury, Dorset, UK: Element; 1995.
21. Gupta S, Ravishankar S. A comparison of the antimicrobial activity of garlic, ginger, carrot, and turmeric pastes against *Escherichia coli* O157:H7 in laboratory buffer and ground beef. Foodborne Pathog Dis 2005;2:330-40.
22. Coutinho HD, Costa JG, Lima EO, Falcão-Silva VS, Siqueira-Júnior JP. Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L. and chlorpromazine. Chemotherapy 2008;54:328-30.

Source of support: Nil; Conflict of interest: None Declared