

In Vitro evaluation of antimycotic activity of oregano essential oil on candida species from isolation of patient with chronic periodontitis

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ABSTRACT

Background: Candida albicans is an opportunistic fungi mainly causing mucosal and opportunistic infections in immunocompromised people. Origanum vulgare is an herbal plant that has been used as a food flavouring agent in many countries. It has strong antiseptic and antimicrobial activity due to the presence of carvacrol and thymol, which directly inhibit germination and hyphal formation in candida. Periodontitis is one of the first diseases to be related to mixed-species biofilms in humans. C. albicans produces virulence factors that may play a role in the incidence of periodontal disease, such as penetrating ability, colonisation and growth of candida species in the oral mucosa and periodontal pocket.

AIM: Is to evaluate the antimycotic activity of oregano essential oil on candida species isolated from patient with chronic periodontitis
Materials And Methods: The study was conducted at Saveetha dental college. Ethical clearance for the study was obtained from the Institutional Ethical Committee. Swabs were obtained from the subgingival plaque tooth surface of patients who had clinical symptoms of chronic periodontitis. For the isolation of Candida from the clinical samples, the obtained samples were plated onto candida chromagar. The isolated species of candida is then investigated for antimycotic activity. The antimycotic activity of oregano essential oil on Candida species isolates was examined using an agar well diffusion assay.

Result And Discussion : Antimycotic activity of the Oregano essential oil against clinical isolates of Candida albicans are shown in the table. The findings reveal that oregano essential oils showed a zone of inhibition of 21 mm against Candida albicans at concentration of 100µL compared to the standard ketoconazole which shows a zone of inhibition of 25 mm. As the concentration of the extract increases the zone of inhibition increases.

Conclusion: Based on the results presented, it is possible to conclude that the essential oil extracted from Oregano may represent a good alternative for the treatment of candidiasis due to its appreciable antifungal action against Candida spp in vitro.

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INTRODUCTION

Candida albicans is a member of our natural microflora, or the bacteria and fungi that dwell in and on our bodies(1). It's present in the gastrointestinal tract, the mouth, and the vaginal area. Candida albicans is the most common organism in humans. Albicans is the Latin word for "white," and it is the name of the species. When cultivated on a plate, yeast appears white. It can also cause white spots in the case of some illnesses, such as oral thrush(2). Candida albicans is an opportunistic fungi of humans. C. albicans has the ability to colonise practically every human tissue and organ, causing mucosal and systemic infections in immunocompromised people(3).

KEYWORDS:

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Origanum vulgare is a member of the Verbenaceae family and is used as a flavouring agent in food and alcoholic beverages. It also contains antibacterial, antiviral, antifungal, and insecticidal qualities(4). Oregano essential oil has an antioxidant with antibacterial effects against germs and fungus. The antimicrobial or antifungal properties of essential oils may be due to the properties of terpenes/terpenoids, which are capable of disrupting the cell membrane, causing cell death, or inhibiting the sporulation and germination(5) . As a response, Essential oil, like other phytochemicals, may inhibit microbial growth and biofilm formation(6).

Periodontitis is one of the first diseases to be related to mixed-species biofilms in humans(7). It is a bacterial microorganism-induced chronic inflammatory illness that entails a severe chronic inflammation that destroys the tooth-supporting structure and can lead to tooth loss(8). It can also result in other health issues. The accumulation of germs in the form of dental plaque is always the cause of periodontitis. More than 700 distinct species of bacteria thrive in a healthy mouth, the majority of which are absolutely harmless and live in harmony with their "host." A variety of microorganisms, including bacteria, fungus, and possibly viruses, make up the dental "plaque biofilm". Although *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, collectively known as the "red complex," have been found as the most periopathogenic bacteria linked to various kinds of periodontitis around the world, Several investigations have found that in chronic periodontitis patients, yeasts, notably *Candida albicans*, colonise the subgingival space at a higher rate than in periodontally healthy people(9). *C. albicans* produces virulence factors that may have a role in the occurrence of periodontal disease, such as penetrating ability(10).

Polymorphonuclear cells are inhibited by the epithelium and triggering lysis of monocytes .The fungus may function directly, in collaboration with subgingival bacterial pathogens, or indirectly, by promoting the generation of pro-inflammatory cytokines, which accelerate periodontal attachment loss and, as a result, periodontal disease. Smoking, diabetes, HIV/AIDS, family medical history, and certain drugs are the other factors that enhance the risk of this disease(11). Therefore *C. albicans* has been found in periodontal pockets in 7.1 percent to 19.6 percent of chronic periodontitis patients. This is mostly accomplished by the virulence factors found in these bacteria, which help them colonise and multiply in the oral mucosa and periodontal pockets(12).

Thus the aim of the study is to evaluate the antimycotic activity of oregano essential oil on candida species from isolation of patients with chronic periodontitis .

MATERIALS AND METHODS

Collection and speciation of oral isolates of Candida

The study was conducted at Saveetha dental college .Ethical clearance for the study was obtained from the Institutional Ethical Committee. Swabs were obtained from the subgingival plaque tooth surface of patients who had clinical symptoms of chronic periodontitis. For the isolation of *Candida* from the clinical samples, the obtained samples were plated onto candida chromagar.

The isolated species of candida is then investigated for antimycotic activity. The antimycotic activity of oregano essential oil on *Candida* species isolates was examined using an agar well diffusion assay.

Agar well diffusion assay

Agar well diffusion method is used to evaluate the antimycotic activity of essential oils. The microorganisms is is cultured as a lawn on SDA agar ,and the agar plate surface is inoculated by evenly distributing a volume of the microbial inoculum throughout the agar surface .Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20-100 uL) of the oregano essential oil at different concentrations were introduced into the well. Then, agar plates were incubated at 37 °c for 24 hr. After the incubation period, plates were removed and zones of inhibition were recorded.

RESULT

In our present study Antimycotic activity of the Oregano essential oil against clinical isolates of *Candida albicans* are shown in the table 1. The Antimycotic activity of Oregano essential oil was established by comparing it to a known Antimycotic drug such as standard ketoconazole. An agar well diffusion assay was used to determine the antifungal susceptibility.

The zone of inhibition against *Candida albicans* at various oregano essential oil concentrations is shown in the table below. With an increase in concentration, the zone of inhibition significantly decreases. The results demonstrate that at concentrations of 25 µL, 50 µL, and 100 µL, oregano essential oils inhibited *Candida albicans* with zones of inhibition of 11 mm, 17 mm, and 21 mm, respectively. However, the zones of inhibition decreased with increasing concentration.

Table 1: Zone of inhibition against *Candida albicans* at different concentrations of oregano essential oil is tabulated .

Concentration of oregano essential oil	<i>Candida albicans</i>
25 µL	11 mm
50 µL	17 mm
100 µL	21 mm
Standard ketoconazole	25 mm

DISCUSSION

Microorganisms have evolved resistance to numerous antibiotics, leading to a huge clinical challenge in the treatment of infectious disorders. Due to the widespread usage of commercial antimicrobial medications for treating infectious diseases, the organisms' resistance grew(13). The researchers were compelled by this circumstance to look for novel antimicrobial compounds from a variety of sources, including medicinal plants. The basis for identifying which essential oils and plant oils may be beneficial for particular medical issues comes from the traditional usage of plants as alternative medicines(14).

In our present study antimycotic properties of the novel medicinal plant Oregano essential oil against oral isolates of *Candida* from chronic periodontitis patients were assessed. *O. vulgare* has been widely used as an antimicrobial agent against human and animal infections with *Candida*. *O. vulgare* has been investigated for its antifungal properties against *Candida* spp. isolated from systemic diseases(15). On reviewing the existing literature, it was discovered that no studies had been done using isolates of *Candida* from patients with chronic periodontitis. Other studies have shown that the essential oils of thyme (*T. vulgaris*), which is high in thymol, and oregano (*O. vulgare*), which is high in carvacrol, suppress the growth of *Candida albicans*, whereas the effects of other thyme chemotypes that are lower in phenolic components are poor Giordani et al(16). In another study, carvacrol-containing oregano essential oil was found to inhibit *C. albicans* development both in vitro and in vivo(17).

In our study the antimycotic activity of the *O. vulgare* essential oil was confirmed by comparing it with the known antifungal agent like standard ketoconazole. Antimycotic susceptibility testing was carried out using agar well diffusion method. Antimycotic activity showed a zone of inhibition as 21 mm for *O. vulgare* essential as compared to 25 mm for standard ketoconazole against *Candida albicans*. As the concentration of the extract increases the zone of inhibition increases.

This finding proved the experimental oregano essential oil has antimycotic properties and showed that it has activity that is comparable to standard ketoconazole. In previous research Vinaya et al. (2018) found that Oregano essential oil shows a zone of inhibition of 30mm against the *Candida* spp isolated from *Candida* associated denture stomatitis patients(18). According to Rosato et al. and Souza et al. evaluations, *O. vulgare* essential oil appeared to be successful at inhibiting every *Candida* species in their study(19)(20).

The oregano essential oil's additional antifungal action is directly correlated with its phenolic alcohol content, supporting the generally held notion that their antimicrobial effectiveness is influenced by their chemical components. The essential oils, especially those rich in phenolics, can infiltrate the phospholipid layer of the bacterial cell wall and modify the permeability and function of the cell membrane proteins by binding to and blocking their normal actions. Therefore, it is crucial that the plant essential oils and their constituents be

further researched with regard to their potential as antimicrobial agents given the current urgent need for new antimicrobials(21-30)

CONCLUSION

Based on the results presented, it is possible to conclude that the essential oil extracted from Oregano may represent a good alternative for the treatment of candidiasis due to its appreciable antifungal action against *Candida* spp in vitro. In this preliminary study, *O. vulgare* was discovered to have strong anti-candidal capabilities against the oral isolates of *Candida* spp. However, the authors recommend more extensive investigation into this unique plant essential oils before it may be considered for therapeutic purposes in the oral cavity.

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Authorship contribution

KCK compiled the manuscript RVG conducted the study LT designed the study

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