ORIGINAL RESEARCH

The Antifungal Effects of Two Herbal Essences in Comparison with Nystatin on the *Candida* Strains Isolated from the Edentulous Patients

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Abstract

Aim: The aim of this study is to evaluate the anticandidal effects of essential oils derived from fennel (*Foeniculum vulgare*) and cumin (*Cuminum cyminum*) on *Candida* strains isolated from edentulous patients.

Materials and methods: *Candida* samples were isolated from 30 edentulous patients and the strains were identified using the CHROMagar method. Using the macro-broth dilution method and punched-hole tests, the effectiveness of fennel and cumin essential oils (prepared through distillation by water) was clarified. Nystatin was used as a positive control.

Results: Nystatin (44 µg/mL) had the strongest antifungal effect, followed by cumin [minimum inhibitory concentration (MIC) = 662 µg/mL; minimum fungicidal concentration (MFC) = 630 µg/mL] and fennel (MIC = 1,074 µg/mL; MFC = 1,227 µg/mL). The average diameter of the fungal growth inhibition zone was 23 mm for Nystatin, 14 mm for cumin essential oil, and 5 mm for fennel essential oil.

Conclusion: The anti-*Candida* effects of fennel and cumin show promise as alternatives to conventional drugs for the treatment of *Candida* infections.

Keywords: Antifungal effect, *Candida*, Cumin, Fennel, Nystatin.

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Introduction

Historically, the prevalence of fungal infections has been lower than that of viral and bacterial infections; however, in recent decades, fungal infections have become more common.[1]

Opportunistic fungal pathogens of the genus *Candida* coexist in the gastrointestinal tract, mucus, skin of humans, and animals.[2] This fungus is also found in the environment. In cases where host resistance is primarily or secondarily impaired due to predisposing disease factors, the *Candida* fungus is capable of causing disease in any region of the body. Oral candidiasis, which is caused by *Candida* species, is a common opportunistic infection observed in denture wearers. The small number of antifungal drugs, their toxicity, and the development of drug-resistance in some *Candida* species are major issues in the treatment of these conditions.[3,4]

The use of broad-spectrum antibiotics, immunosuppressive drugs, and radiotherapy has led to an increased incidence of candidiasis. Other risk factors are increasing age, diabetes, salivation disorders, denture use, smoking, Sjögren's syndrome, cancers, and immunodeficiency syndromes, including AIDS.[2,5]

Nystatin, which is a tetra-n-macrolide first isolated from *Streptomyces noursei* in 1950,[6] is a dry yellow powder that is sparingly soluble in water (4 mg/mL). This drug inhibits fungal growth and shows strong fungicidal activity at high concentrations and acidic pH. Nystatin shows excellent inhibitory activity against *Candida* yeasts, especially *Candida albicans*[7,8] but has disadvantages including unpalatable taste and poor systemic uptake. It can cause kidney problems, rash, nausea, diarrhea, vomiting, and epigastric pain. Furthermore, sweeteners added to overcome the bitter taste of Nystatin mouthwash can increase the risk of dental decay.[9–11]

There is a long history of the use of plant-derived natural products for the treatment of various human diseases. The antimicrobial effects of plants are generally mediated by compounds such as phenol, saponin, and flavonoids, which function by acting on the plasma membrane or structural enzymes of microorganisms.[12,13]

Foeniculum vulgare, or fennel, is a medicinal plant of the *Apiaceae* or *Umbelliferae* family. The plant has almost cylindrical fruit (0.5–1 cm in length) that is light green to yellow in color. This fruit consists of two parts joined by five longitudinal lines and a black spot in the middle. The most important medicinal part of *Foeniculum vulgare* is its seed, which is separated after the fruit is harvested.[14] *Foeniculum vulgare* has anti-inflammatory, analgesic, antispasmodic, diuretic, and expectorant properties. The main ingredients of this plant include trans-anethole, alpha-pinene, limonene, and various flavonoids. These compounds have antioxidant properties.[15,16]

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Conflict of interest: None

Cuminum cyminum, or cumin, is a member of the parsley family. This small, herbaceous plant (15–50 cm high) has long, slender, white roots, and straight and branched shoots with binary divisions. The fruit contains tannin (7% oil and 13% resin), aleurone, and essential oils (2.5–4%).[17] This semi-wild plant grows in a vast region of the Mediterranean, Saudi Arabia, and Iran. Its important compounds are sabinene, flavonoids, polysaccharides, coumarin, cuminaldehyde, pinene, and terpinene. *Cuminum cyminum* is used in traditional medicine to treat gastrointestinal diseases, as a carminative, to facilitate digestion, and in pulmonary diseases, for the treatment of coughs.[13,18,19]

All chemical medicines can cause unwanted side effects. Antifungal drugs such as Nystatin are cytotoxic and the sensitivity of some *Candida* species is decreasing.[20,21] To avoid these disadvantages, new antifungal agents are required to deal with strains that are resistant to commonly used antibacterial agents and herbal products may act as substitutes for synthetic drugs. Considering the contradictory results of studies on the medicinal properties of plants, the present study was designed to evaluate the antifungal effects of essential oils derived from fennel and cumin in comparison with those of Nystatin.

Materials and Methods

Sampling

Patient Selection

Ninety completely edentulous patients wearing complete dentures for at least 2 years were randomly selected from two dental centers. Information relating to individual demographics (age, sex, drug use history, smoking habits, and stomatitis symptoms) in addition to factors relating to denture wear (such as how long patients had used dentures, cleaning methods used, daily use frequency, and vertical dimensions) were collected from patients in response to questionnaire forms provided by the dentists involved in the study. All participants had dentures for both the mandible and the maxilla.

Inclusion criteria were as follows: (1) men and women aged over 35 years; (2) wearing a complete set of denture prostheses; (3) individuals wearing the prostheses for more than 2 years; (4) people volunteering to participate in this study. Exclusion criteria were as follows: (1) aged less than 35 years; (2) use of dentures for less than 2 years; (3) use of antifungal drugs; (4) patients with a history of diabetes or any impairment of the immune system; (5) people who declined to participate in this study.

Sample Collection

A sterile swab was rubbed along the palate of the patients. The swab was then rubbed on CHROMagar *Candida* media (Biolife Italiana, Milano, Italy) and incubated at 35 °C for 48 hours. *Candida* strains were then identified according to the color of the cultured fungus. The colonies were then subcultured in the Sabouraud dextrose agar (SDA; Merck, Germany) and incubated at 35 °C for 48 hours. A total of 30 *Candida* strains isolated from 90 edentulous patients were tested.

Preparation of Essential Oils from Plants

Essential oils were extracted from plants by distillation with water (hydrodistillation) using the Clevenger apparatus. Dried herbs (500 g) mixed with 700 mL of water was poured into the volumetric flask for extraction. The refrigerant cold water flow was established, and the volumetric flask was placed into the electric heater. After 2 hours of distillation, the heater was turned off and the Burt output valve was opened. The plant sap was removed and, then, its essential oil was collected in a separate container, which was labeled with details of the essential oil, including the weight:volume ratio, the plant name, the plant collection site, the name of the experimenter, and the date of the extraction of the essential oils. The glass lid was sealed with paraffin wax and stored in the refrigerator at 4 °C until it was used. The purity of obtained essential oil was assumed to be 100% and was diluted to 8% in distilled water.

Punched-hole Test

The antifungal activity was evaluated using the punched-hole test. First, a well with a diameter of approximately 6 mm was created on plates containing SDA (a depth of 4 mm). A suspension was prepared from *Candida* strains (cultivated for 24–48 hours) in phosphate-buffered saline or distilled water to a turbidity equivalent to 0.5 McFarland. Using sterile cotton swabs, the plate surface was uniformly inoculated with the *Candida* suspension, and 40 µL of each essential oil and Nystatin were added to the holes. After incubation at 30–35 °C for 24–48 hours, the diameter of the fungal growth inhibition zones was measured.

Antifungal-susceptibility Testing of Yeast using the Macro Broth Dilution Method

Preparation of the Inoculum

The inoculum (a candidate isolated from the patients) was cultured in the SDA medium at 37 °C for 48 hours. When a diameter of at least 1 mm was reached, the *Candida* colonies were used to inoculate 2 mL of sterile distilled water. The number of yeast cells was counted using a hemocytometer until the final concentration of suspension reached 1 × 106 cfu/mL (equivalent to 0.5 McFarland).

Preparation of Sabouraud Dextrose Culture Medium Containing Solvent Dimethyl Sulfoxide (DMSO)

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) tests showed that solvent DMSO had no effect on *Candida* growth at concentrations lower than 5%; therefore, this was used as a solvent in the culture medium.

Preparation of the Working Solution and Serial Dilution of Essential Oils

Two-fold serial dilutions of essential oil of each plant (0.015–8% v/v) were prepared in SDA medium containing 2% DMSO. Eleven test tubes of 16 × 100 mm sterile were prepared and the provided medium was added to tube nos 2–11. In tube no. 1, 1,840 µL of medium with 160 µL of essential oil were spilled. One milliliter of tube no. 1 was added after mixing and uniforming to tube no. 2. Then 1 m: of tube no. 2 went to tube no. 3 and then continued to tube no. 10. After mixing, 1 mL is removed from tube no. 10 and discarded.

Subsequently, 50 µL of the inoculum was added. Controls containing inoculum, solvent, and essential oil or solvent-culture control and culture medium without inoculum and essential oil were also set up. All samples were incubated at 30–35 °C for 8 hours.

MIC and MFC Tests

The MIC is the lowest drug concentration in which no visible fungal growth is seen after incubation.

The MFC is the lowest concentration of drug in which no significant fungal growth is visible after incubation.

To determine the MFC, 20 µL of each of the MIC tubes in which the yeast did not grow was cultured on SDA plates at 30–35 °C for 48 hours or until growth was observed.

Data Analysis

Data were presented as the mean ± standard deviation of three separate experiments. Differences relative to Nystatin were analyzed by *t* tests using SPSS software. A *p* value of <0.05 was considered to indicate statistical significance.

Results

The diameters of the fungal growth inhibition zones are presented in Table 1. Based on the MIC and MFC results, Nystatin had the strongest antifungal effect at its lowest concentration (0.44 µg/mL), followed by *Cuminum cyminum* (MIC = 0.630 µg/mL and MFC = 0.662 µg/mL) and *Foeniculum vulgare* (MIC = 1.227 µg/mL and MFC = 1.074 µg/mL) (Fig. 1).

The antifungal effect of Nystatin was significantly higher than that of *Cuminum cyminum* ($p < 0.001$) and *Foeniculum vulgare* ($p < 0.001$). Furthermore, there was a significant difference between the antifungal effects of essential oils of *Cuminum cyminum* and *Foeniculum vulgare* ($p < 0.001$).

Discussion

In this study, we assessed the *in vitro* antifungal effects of essential oils derived from fennel and cumin using Nystatin as a reference. We concluded that the essential oils of these plants have effective anti-*candida* properties comparing with that of Nystatin.

The results of the present study show that *Cuminum cyminum* essential oil is effective in the inhibition of fungi, which is in accordance with the results reported by Naeini et al.[20] in 2014 on the antifungal activity of *Cuminum cyminum* and *Salvadora persica* extracts against pathogenic *Candida* strains. Our results are also consistent with those reported by Khosravi et al. in 2011 on inhibition of aflatoxin production and growth of *Aspergillus parasiticus* by essential oils extracted from *Cuminum cyminum*, *Ziziphora clinopodioides*, and *Nigella sativa*.[21] In 2011, Ridawati et al. examined the antifungal activity of *Cuminum cyminum* essential oil on four standard species of *Candida* fungi (*C. parapsilosis*, *C. orthopsilosis*, *C. metapsilosis*, and *C. etchellsii*). MIC and MFC tests revealed the strong antifungal activity of *Cuminum cyminum*, although the effects were significantly weaker than those of Nystatin.[22]

In this study, we also demonstrated that *Foeniculum vulgare* inhibits the growth of *Candida* fungi, which is in accordance with the results reported by Naeini et al.[23] This group studied the antifungal effects of a herbal extract mixture (*Nigella sativa*, *Foeniculum vulgare,* and *Camellia sinensis*) against *Candida* species isolated from denture wearers. Their results indicated that *Nigella sativa* and *Foeniculum vulgare* have remarkable antifungal activities against these *Candida* species.[23] Our results are also consistent with those reported by Gulfraz et al. on the composition and antimicrobial properties of essential oil of *Foeniculum vulgare*. Fennel oil inhibited the growth of some *Bacillus* bacterial species, *Escherichia coli*, *Klebsiella pneumonia*, *Micrococcus lutus*, *Pseudomonas pupida*, and *Pseudomonos syringae*, with the lowest MIC values for *C. albicans* (0.4% v/v).[24]

Pai et al.[16] studied the antifungal efficacy of *Punica granatum*, *Acacia nilotica*, *Cuminum cyminum,* and *Foeniculum vulgare* on *C. albicans*. They reported a slight antifungal effect based on the small diameter of the inhibition zone (4 mm) in the presence of *Cuminum cyminum* ether extract.[16] In the present study, the diameter of the inhibition zone was 14 mm due to the higher concentrations of the active ingredient of the essential oil compared with that in the ether extract. Thus, much better results were observed using the extract obtained in this study, and its use in pharmaceutical applications should be considered and verified in future studies.

Shah et al. showed that ethane *Foeniculum vulgare* extract administered orally at different doses and at different times had no toxic effects and no external, hematological, or spermatogenetic morphological changes were observed in mice. This study suggested that *Foeniculum vulgare* can be used as an antifungal drug without harmful side effects.[25]

According to the result of this study, *Cuminum* has more marked antifungal effects than fennel. These findings are in accordance with those of Kamble et al., who investigated the antifungal activities of essential oils derived from 20 spices including fennel and cumin for against *Aspergillus niger*, *C. albicans*, *C. blankii*, *C. cylindracea*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *Saccharomyces cerevisiae*. Essential oils from cumin, thyme, cassia, and allspice showed a greater antifungal activity than the other oils investigated.[26]

Since all these results are based on *in vitro* studies of essential oils, *in vivo* studies and clinical trials are required for further verification. Furthermore, long-term randomized controlled trials are needed to evaluate the possible disadvantages of herb-derived medicines. Further investigation of the effects of these essential oils on different species of *Candida* and their efficacy in comparison with other antifungal medications is also warranted.

Table 1: Diameter of the fungal growth inhibition zone in punched-hole tests (mm)

Foeniculum vulgare	*Cuminum cyminum*	*Nystatin*
5	14	23

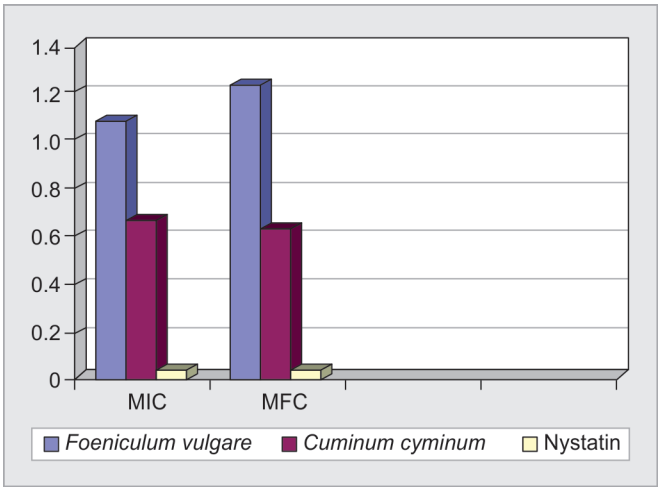

Fig. 1: Comparison of MIC and MFC values for *Foeniculum vulgare*, *Cuminum cyminum,* and Nystatin (µg/mL)

Conclusion

In conclusion, although Nystatin mediates the most effective inhibition of fungi at its lowest concentration, it also has the harmful side effects of other synthetic drugs, including drug resistance.

Plant-derived drugs present a promising alternative that may avoid the issue of drug resistance.

In this study, we evaluated the antifungal effects of essential oils of *Foeniculum vulgare* and *Cuminum cyminum* plants on *Candida*. Our results showed that essential oil from mediated much more effective inhibition of *Candida* than that extracted from *Foeniculum vulgare*. Thus, our findings indicate that essential oils derived from fennel and cumin are promising alternatives that may avoid the disadvantageous side effects associated with conventional drugs as anti-candidiasis treatments; however, this requires further investigation.

The strength of the present study is that we cultured the clinical *Candida* strains from the oral cavity of edentulous patients but the limitation is that we only investigate the *in vitro* effects of the essential oils. Also another limitation of the present study is that we did not investigate the effective constituents of each essential oil that could be the subject of future studies.

Clinical Significance

Cumin and fennel essential oils could be used as alternatives to conventional antifungal drugs for treating edentulous patients with oral candidiasis.

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