Original Article

Antifungal Effect of Foeniculum Vulgare Mill Essential Oil on Strains of Candida Albicans in Vitro

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Abstract

Aim: To evaluate the in vitro antifungal effect of the essential oil of Foeniculum Vulgare Mill "fennel" against two strains of Candida albicans i.e. ATCC 90028 and native strain. **Materials and Methods:** This was a comparative, prospective and experimental study which was carried out in the Microbiology Laboratories of the Catholic University of Santa María and the National University of San Agustín. The method of direct suspension of colonies in broth with tubes for the strains was used in this study. In vitro antifungal susceptibility to both strains of Candida albicans was evaluated using the Kirby-Bauer (hole) technique. Whereas, growth inhibition halos were measured using the Durafford scale. **Results:** The highest inhibition halo (50mm) was found at concentration of 100% essential oil. When measured according to the Durafford scale, an extremely sensitive (+++) inhibitory effect was achieved for both strains of Candida albicans. **Conclusions:** The essential oil of fennel at 100% showed a greater antifungal effect on both the native and ATCC 90028 certified strains of Candida albicans.

Keywords: Foeniculum Vulgare Mill, Candida albicans, Inhibition Halo

Introduction

Pathogens that produce fungal infections, such as Candida albicans, are common and may impact the skin and mucosal surface causing systemic illness. Species of Candida are found in as many as 400,000 systemic fungal infections.[1] Of all the species, Candida albicans is the most prevalent causal agent of mucosal and systemic infections, and is responsible for around 70 percent of fungal infections over the globe.[2] It has been found as the primary cause of life-threatening invasive infections over the last many decades. Despite therapy, the death rate is near to 40 percent, particularly under hospital circumstances.[1,3] The current study overviews, the virulence features of Candida albicans and its clinical manifestations in the oral cavity, intestinal mucosa, skin and invasive infections.

Candida albicans is a unicellular, gram-positive microorganism[3] which occurs naturally on the human body.[1] Candida is a yeast-like fungus that is often present in tiny quantities on the lips, skin and intestines. The equilibrium of microorganism is controlled by the healthy microorganisms in the body called microbiome. When Candida is out of whack, the yeast often overgrows and causes illness i.e. candidiasis.[1]

Candidiasis is an infection caused by Candida, a form of yeast (fungus). It generally resides on the skin and throughout the body, including the mouth, throat, intestines and vagina, without causing any health issues. Scientists believe that about 20% of women have Candida in their vagina without experiencing any symptoms. Vulvovaginal candidiasis (VVC) is a vaginal fungal infection caused by Candida species., It is an acute inflammatory disease in women and a frequent reason for their gynecological consultation. It affects 75% of women of reproductive age at least once in their lives. It is the second most prevalent kind of vaginal infection in the United States, followed by vaginal infections caused by bacteria. Annually, there are around 1,400,000 outpatient visits for vaginal candidiasis.[2] This disease

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is worrisome due to the recurrence of symptoms and its refractory nature towards conventional treatments.[1]

Candida may cause an infection if vaginal circumstances shift to favour its development. Hormones, medications, or changes in the immune system might increase the likelihood of infection. Other names for VVC are vaginal yeast infection vaginal candidiasis and candida vaginitis.[2]

Essential oils are frequently used in aromatherapy, a type of complementary medicine in which aromatic molecules are believed to have therapeutic effects. The use of aromatherapy to promote relaxation may be beneficial, however there is insufficient proof that essential oils may successfully treat any condition. Essential oils can be harmful if used improperly causing allergic responses, inflammation and skin irritation. Children may be more vulnerable to the harmful effects of their inappropriate use. Essential oils and their components have a therapeutic potential and are associated with fascinating biological functions. For instance, these oils show antibacterial and antiviral properties. Hence they can be utilised as an effective therapy for a variety of diseases. Crucial oil usage is essential to both the beauty and food industries because they can serve a variety of purposes.[4]

The chemical structure of essential oils are made up of hydrocarbon complexes and their oxygenated derivatives. The presence of these components determines its physical properties, such as being liquid at room temperature, soluble in organic solvents and insoluble in water. In addition, they also determine their biological properties such as: antimicrobial, antioxidant, anti-inflammatory and anticancer.[5] The pH is a defense mechanism of the vagina and is clearly correlated with its microbiological state; It varies depending on the moment and the stage of life.[6] Maintaining an acidic pH in the stratum corneum is a crucial component of the skin's protective barrier and produces a hostile environment for pathogenic microbe invasion. This barrier is nonetheless surmountable on healthy and especially weakened skin.

Foeniculum vulgare (fennel) belongs to an apiacea species of plants and comes under the umbelliferous family. Its leaves, seeds and even the root have medicinal value and are used in the pharmaceutical, food and cosmetic industries. The name of the plant derives from the Latin word "foenum" which means "hay straw" in reference to the fineness of its leaves. Regarding its composition, the seeds contain up to 12% of essential oil.[7] The essential oil of sweet fennel generally contains 80% or more of E-anethole, 10% of methyl-chavicol (estragole) and less than 5% of fenchone. Anethole is the principal chemical responsible for its distinctive flavour and odour. On the other hand, essential oil from bitter fennel contains 50-80% E-anethol, 3-20% estragole and up to 24% fenchone. [8] Various studies have demonstrated its antifungal and antimycotic effect.[9,10]

Traditionally, fennel is utilised for medicinal and culinary purposes. For example, its expanded base is used as a vegetable, its leaves are used in cooking and its seeds are used both as a spice and for the extraction of essential oil. Additionally, the blooms and leaves of this plant are used to produce yellow and brown colours.[9] With respect to the medicinal properties, this plant has antibacterial, antifungal and antiviral properties. The essential oil extracted from this plant relieves stomach discomfort and bloating by relaxing smooth muscles. This antispasmodic action is also beneficial in the musculoskeletal and reproductive systems, therefore alleviating muscle spasms and discomfort. Anti-inflammatory effects of fennel have been linked to cyclooxygenase-2, the enzyme inhibited by nonsteroidal anti-inflammatory drugs. Therefore, the objective of this study was to evaluate the in vitro antifungal effect of fennel essential oil on two different strains of Candida albicans i.e. native strain and ATCC 90028 certified strain, using Clotrimazole as a control. It is an antifungal used in the syndromic management of sexually transmitted infections in the MINSA Standards.[11]

Material and Methods

This was a comparative, prospective and experimental study carried out in the microbiology laboratories of the Catholic University of Santa María and the National University of San Agustín. The Candida albicans strain was isolated from clinical samples of patients with Candidiasis and was identified morphologically by means of the germ tube test. In this test, one of the characteristic colonies of each pure strain was suspended in 0.5 ml of human serum and incubated at 35-37 °C for 2 hours and 30 min in an oven. After that, 2 to 3 drops of the emulsion were put under a microscope. The formation of the germ tube, which is an elongated structure that originates from yeast confirms the presence of Candida albicans .[12]

The sterility of the oil was checked by seeding 0.1 ul. of Foeniculum Vulgare Mill essential oil on a tryptose agar plate, for 24 hours at 37° in an oven. The petri dish swab method was used to see any contamination in the fennel essential oil.

Petri dishes were prepared with Sabouraud Agar culture medium, allowed to solidify and a suspension of Candida albicans (150,000,000 CFU/ml) was prepared.[13]

For the quantification of the diameter of the inhibitory halo for the native and certified strain, the diffusion agar method was used.[14,15] Five sabouraud agar culture plates were prepared, and a hole was made in the center of the plate with a sterile pipette. Then a suspension of Candida albicans corresponding to the Mc Farland tube No. 0.5 (150,000,000 CFU/ml) was prepared. This was obtained from the GenLab laboratory of PERU SAC. Lima Peru and derived from ATCC® 90028 C. albicans

[LOT.264-33-6, REF.0264P]. Once the surface evaporated, essential oil was added to each plate at concentrations 25 %, 50%, 75% and 100%. The plates were incubated at 37° for 24 hours. Similarly, a control plate was used for Clotrimazole at 100% and a concentration of 100ul was added.[16]

The inhibitory effect in vitro was determined by using the Duraffourd Scale i.e according to diameter of inhibition.

- ✓ Null (-) diameter less than 8 mm.
- ✓ Limit sensitivity (sensitive +) diameter between 8 to 14 mm.
- ✓ Medium (very sensitive ++) diameter between 14 and 20 mm.
- ✓ Extremely sensitive (+++) diameter greater than 20 mm.

Results

Identification of Candida albicans

The presence of elongated germ tube of Candida albicans was observed through an optical microscope with a magnification of 100x.

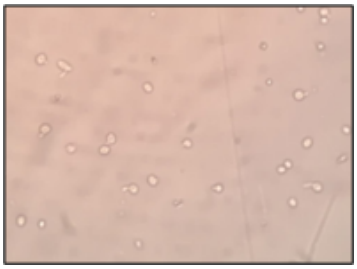


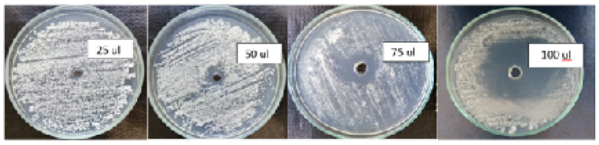
Figure 1: Candida albicans germ tube

2. Antifungal activity of Foeniculum vulgare Mill on Candida albicans strain of ATCC 90028 and native strain

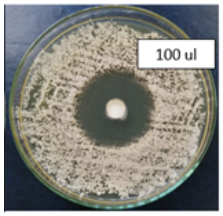
Antifungal effect of essential oil of fennel was evaluated at different concentrations i.e. 25%, 50%, 75% and 100%. Their effect was observed through the growth of inhibition halo (Figure 2.a) in the strains of candida albicans ATCC 90028. The results showed that the inhibitory halo with 100% fennel essential oil was 50mm whereas with Clotrimazole, it was 35mm. Likewise, the antifungal activity was found to be increased (Table 1) as the concentrations of Foeniculum Vulgare Mill was increased.

Table 1: Antifungal activity of fennel essential oil and clotrimazole on the inhibition halo of candida albicans in the ATCC 90028 strain

	Inhibition halo in mm				
	Aceite esencial de Foeniculum vulgare Mill				Clotrimazol
CONCENTRATION	25%	50%	75%	100%	100%
Candida albicans ATCC 90028	0.80mm	11mm	27mm	50mm	38



a) Growth inhibition halos of candida albicans in ATCC 90028 strains with Foeniculum vulgare Mill



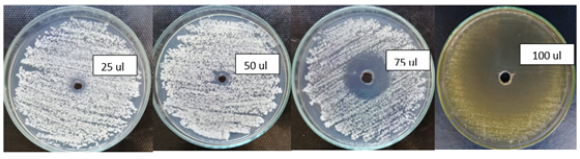
b) Growth inhibition halos of candida albicans in ATCC 90028 strains with Clotrimazole

Figure 2: Antifungal effect on the strain Candida albicans ATCC 90028

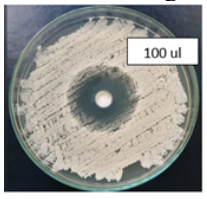
Similarly, antifungal effect of fennel essential oil was also observed on native strain of Candida albicans in the same way as was observed with the strain of ATCC 90028. The results showed that the inhibitory halo with 100% fennel essential oil was 50mm and with Clotrimazole, it was 38 mm. Likewise, it was observed that the antifungal activity increased (Table 2) as the concentrations of Foeniculum Vulgare Mill increased.

Table 2: Antifungal activity of fennel essential oil and clotrimazole on the halo of inhibition of native candida albicans

	Inhibition halo in mm				
	Aceite esencial de Foeniculum vulgare Mill				Clotrimazol
CONCENTRATION	25%	50%	75%	100%	100%
Candida albicans Nativa	0.90mm	12mm	30mm	50mm	35mm



a) Growth inhibition halos of native candida albicans with Foeniculum vulgare Mill.



b) Growth inhibition halos of Native candida albicans with Clotrimazole

Figure 3: Antifungal effect on the native Candida

albicans strain

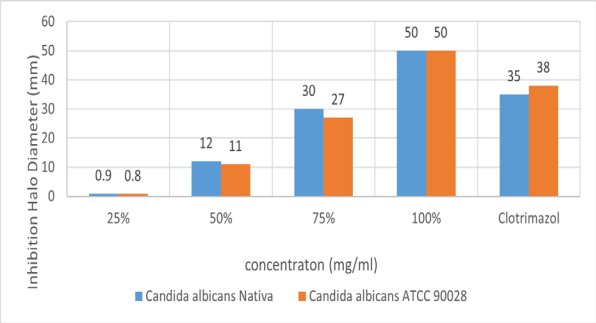


Figure 4: Diameter of the inhibitory halo in the ATCC 90028 and Native Strain

Comparison of the antifungal activity of Foeniculum Vulgare Mill and Clotrimazole on Candida albicans ATCC 90028 and native strains can be seen in Figure 4.

Discussion

Infections caused by yeasts of the genus Candida have increased dramatically in recent decades. Parallel to this increase, the appearance of resistance to antimycotics has been noted. In addition, the selection of species other than Candida albicans has made it necessary to standardize in vitro susceptibility tests.[6,7] Among the various tests available is the agar diffusion test with discs impregnated with antifungals. Its reading is done electronically (BIOMIC), which provides objective indications of the minimum inhibitory concentration (MIC). Until a few years ago, fungi were considered to be regularly susceptible to antifungals. It is now essential to classify all the yeasts of the genus Candida at the species level. Equally important is the performance of antifungal susceptibility tests.[12]

With the exception of its anti-flatulent and anti-colic benefits, there are relatively few randomised, double-blind, placebo-controlled clinical studies on fennel to make scientific conclusions on its numerous medical uses.[1,12] It is now proven that cellular damage caused by oxidative stress is a component of the pathophysiology of a variety of illnesses.[2]

In this study, the antifungal activity of the essential oil of fennel was observed when a concentration of 100 ul was applied on ATCC 90028 strain of Candida albicans. The results showed higher inhibitory halo of 50 mm (Table 1) as compared to that of Clotrimazole, which showed inhibitory halo of 38 mm (Table 1). Similar findings were depicted by a study[17] which investigated the antifungal activities of nine essential oils against clinical isolates which were resistant to fluconazole or susceptible to dependent doses and Candida albicans ATCC 10231 by means of a diffusion method.[8,14,16] Their results showed significantly higher antifungal activity of fennel oil against C. albicans as compared to other oils tested (P = 0.000).

The present study also evaluated the antifungal activity of the essential oil of fennel on native strains of Candida albicans. The results revealed that the essential oil at concentration of 100ul showed an inhibitory halo of 50 mm (Table 2). On the other hand, inhibitory halo of 35 mm (Table 2) was found when essential oil at same concentration was applied on Clotrimazole. A study evaluated the antifungal potency of Foeniculum Vulgare Mill by using methanolic extract from its seed. They found it to be less effective against test fungi as compared to its aqueous extract.[12] However, in case of Candida species, both types of extracts were found to be effective.[9]

Another study evaluated the antimicrobial activity of five species of medicinal plants which are commonly used in folk medicine. They are: Nigella Sativa, Foeniculum Vulgare Mill, Black Piper, Loranthus Capitellatus, Cassia Alata and Drynaria Qurshiforia. In this study, the antibacterial and antifungal effects of these plants were investigated on fungal isolates including Candida albicans, Candida tropicalis, Candida glabrata, Aspergillus niger, Aspergillus flavus, Aspergillus terrus, Alternaria sp., Trichophyton mentagrophytes and Microsporum gypseum. They found that plant extracts were effective in inhibiting the growth of bacteria and fungi.[13]

All these findings ratify what was found in our study and corroborate the virtues of Foeniculum Vulgare Mill in different latitudes. The present study showed that with the increase in the concentration of Foeniculum Vulgare Mill, its antifungal activity also increases.

Conclusions

Foeniculum vulgare is a valuable traditional medicinal source and offers a significant role in the development and formulation of novel medications and potential clinical applications. Numerous studies have shown that the phenolic compounds found in fennel exhibit powerful antioxidant properties. The results of this study suggest that these bioactive compounds in fennel may be developed as new pharmacological agents which might be of significance to human health.

The essential oil of Foeniculum Vulgare Mill. (fennel) at 100% showed a greater antifungal effect on both the native and ATCC 90028 certified strain of Candida albicans. Moreover, Clotrimazole had a lower inhibitory effect as compared to Foeniculum vulgare Mill showing a lower inhibitory halo to 100%.

Acknowledgment

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