In vitro EVALUATION OF THE ANTIFUNGAL ACTIVITY OF THREE ESSENTIAL OILS AGAINST Candida albicans

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A B S T R A C T

AIM: To evaluate the antifungal activity of Three essential oils against Candida albicans

BACKGROUND: Oral candidiasis is a condition in which fungus Candida albicans accumulates on the lining of mouth. Candida is a normal commensal in oral cavity, but sometimes it can overgrow and cause disease. Oral candidiasis occurs when the C. albicans begins to grow out of control. Normally immune system uses good microorganisms to keep C. albicans and other bad microorganisms under control when this balance is disrupted, harmful fungi and bacteria begin to multiply.

MATERIAL AND METHOD: Essential oils of Thymus vulgaris, Trachyspermum ammi and Citrus sinensis L was subjected to Antifungal assay to determine its activity against Candida albicans by agar well diffusion assay.

RESULTS: Thyme and Ajwain oil shows significant antifungal activity against Candida albicans at different concentration such as 40, 80, 100µl/ml. While orange oil did show antifungal activity but comparatively less.

CONCLUSION: Natural products are important source of new drugs which are having importance in modern medicine. This study shows the anti fungal activity of the essential oils – thyme oil, ajwain oil and orange oil against Candida albicans. Thus Oral candidiasis may be treated with thyme oil and ajwain oil after formulating them in to a proper dosage form with optimum concentration.

INTRODUCTION

Candidiasis is the most common yeast infection that range from superficial mucosal infections, such as oral thrush, systemic and potentially life-threatening diseases, such as disseminated candidiasis. In the last two decades, it has been observed a considerable increase in the incidence of deep fungal infections, in both immunocompromised and in healthy subjects [1]. The most commonly used classes of antifungal agents to treat Candida infections are the azoles, polyenes, and echinocandins; however, the management of Candida infections faces many problems, such as toxicity, resistance of Candida to commonly used antifungal drugs, relapse of Candida infections, and the high cost of antifungal drugs [2, 3]. To elude these problems, investigators are exploiting alternative therapeutic strategies, such as the use of natural products, especially essential oils (EOs) [4–7]. The essential oil from locally grown T. vulgaris also contains high concentration of thymol about 20%-54%. Except thymol, the essential oil from this plant also contains some other additional active chemical constituents such as p-cymene, myrcene, borneol and linalool.

Therapeutically, thymol is widely used as an antiseptic. The other active chemical ingredient is listerine, commercially used to produce mouthwashes [8]. Thyme oil was also used to medicate bandages before the discovery of modern pharmaceutical medicine antibiotics [9]. The result for phytochemical screening of hexane, ethyl acetate, chloroform, butanol and methanol extracts were from the leaves of T. vulgaris showed the presence of flavonoids, saponins and steroids, but alkaloids, tannins, and triterpenoids not present in the crude extract [10]. Trachyspermumammi yield 2% to 4% brownish essential oil, with thymol as the major constituent (35%-60%) [11]. The nonthymol fraction (thymene) contains para-cymene, γ terpene, α and β pinenes, dipentene, α terpinene and cravacrol [12]. Minute amounts of camphene, myrcene, and α-3-carene also have been found in the plant. Alcoholic extracts contain a highly hygroscopic saponin. From the fruits, an yellow, crystalline flavone and a steroid-like substance has been isolated and it also contains 6-O-β-glucopyranosylthymol,[13] glucoside and yields 25% oleoresin containing 12% volatile oil (thymol, γ-terpinene, para-cymene, and α- and β-pinene).[14] The principal oil constituents of T. ammi are carvone (46%), limonene (38%), and dillapiol (9%).[15]

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The essential oil of orange fruit extracted has dominantly been by limonene which presented a 85.35%. The rest of compounds were weakly represented with low levels of monoterpenes β-pinene, camphor and bornyl acetate of 1.80, 4.81, and 4.21%, respectively. Concerning sesquiterpenes, α-humulene (0.16–0.34%) followed by germacrene-D (tr-o-12%) were found to be the most represented compounds [16].

MATERIALS AND METHOD

MATERIALS

Fungal strain: Candida albicans (from Department of microbiology, Saveetha Dental college and Hospitals).

Oils Used: Thyme, Ajwain and Orange oil.

METHOD

Anti mycotic Assay

Agar well Diffusion Technique:

The essential oils at different concentrations were screened for their antifungal activity against Candida albicans by Agar well diffusion method. The fungal culture was grown on Sabouraud dextrose agar [Hi media M063]. The colony forming units (CFU/ml) of suspension of the fungus was determined and test inoculum was adjusted to 0.5 Mc Farland’s standard and used for antifungal assay. 18-21 100µl of the test inoculum was applied on the surface of the Sabouraud dextrose agar plate and spread using sterile glass spreader. Wells were cut on the agar plates using sterile cork borer for different concentration of the extracts. Essential oils at different concentrations [ 40, 80, 100 µl] were loaded in to the wells and incubated for 48 h at 28°C. As a positive control, fluconazole (10 mcg /disc) was used. Zone of inhibition in mm diameter were determined after 48 h. The test was performed in triplicate to minimize test error. It is a method which shows the movement of the molecules through the matrix that is formed by the gelling of agar. When this method is performed under controlled conditions, the degree of movement of the molecules can be related to the concentration of the molecule. This phenomenon forms the basis of agar diffusion assay that is used to determine the susceptibility of the resistance of the fungal strain to an antifungal agent. Antifungal activity was carried out using disc diffusion method. Petri plates were then prepared with 20 ml of sterile MHA . The test culture was swabbed on the top of the solidified media and allowed to dry for 10 min. Wells were made on the media using a well borer. Different concentrations of the sample (40, 80 and 100 µL per well) of essential oils diluted in dimethyl sulfoxide (DMSO). Chlorhexidine (0.2% µg/ml) was used as a positive control. These plates were incubated for 48 hrs at 37 °C. Zone of inhibition was recorded in millimeters.

RESULTS

Thyme and Ajwain oil shows significant antifungal activity against Candida albicans at different concentration such as 40,80,100µg/ml. Table 1 and 2) While orange oil (Table 3) did show antifungal activity but comparatively very less. Maximum Zone of inhibition was observed in highest concentration in 3 oils (100µg/ml).

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Concentration µg/ml</th>
<th>Zone Of Inhibition mm</th>
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<tbody>
<tr>
<td>THYME</td>
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<tr>
<td>40</td>
<td>55</td>
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<td>80</td>
<td>60</td>
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<td>100</td>
<td>65</td>
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<tr>
<td>Chlorhexidine (Control)</td>
<td>0.20%</td>
<td>30</td>
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</table>

DiSCUSSION

The traditional use of plants as medicine provides the basis for indicating specific medical conditions. It is important to scientifically analyse these plants which have been used in traditional medicines.

The method that is used in extracting oil are Agar well Diffusion method. This study determines the antifungal
activity of 3 essential oil against Candida albicans, as it is the most common species that affects Candidiasis. The essential oil caused a significant decrease in the activity of the above mentioned fungi and it causes suppression in their growth at concentrations 40μL, 80μL, and 100μL. Chlorhexidine 0.2% was taken as a positive control in well diffusion method.

Petri plates were prepared with 20ml of sterile MHA

Then the culture was layered down on the top of the solidified media and allowed to dry for 10min. These plates were then incubated for 48hrs at 37 degree Celsius and the zone of inhibition was identified and recorded. The Thyme and ajwain oil shows effective reaction against the fungi at 100μL concentration.

CONCLUSION

This study was conducted to evaluate the anti fungal activity of 3 essential oils namely thyme, ajwain and orange. Natural products are important source of new drugs which are having importance in modern medicine. Thus Oral candidiasis can be also treated with thyme and ajwain oil giving its maximum effect.

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Reference


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