International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319 – 6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 3; March 2017; Page No. 2948-2950
DOI: http://dx.doi.org/10.24327/ijcar.2017.2950.0150

Research Article

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

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A R T I C L E I N F O

Article History:

Received 18th December, 2016
Received in revised form 16thJanuary, 2017
Accepted 26th February, 2017
Published online 28th March, 2017

Key words:

Antifungal activity, *Candida albicans*, thyme, ajwain, orange, Candidiasis

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.

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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 terpenine, α and ß 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-glucopyranosyloxythymol,[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 dillapiole (9%).[15]

The essential oil of orange fruit extracted has mainlydominated bylimonene 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-0.12%) 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

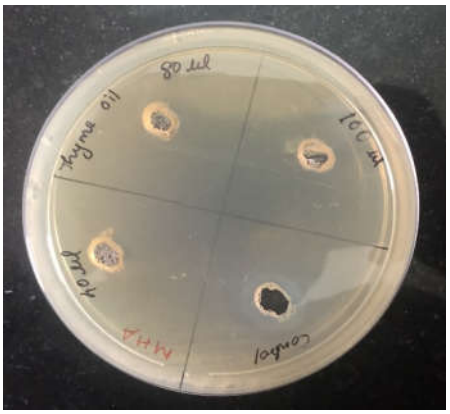
Agar well Diffusion Technique:

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 Sabouraudsdestrose 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 Sabouraudsdestrose 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 28ºC. 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 anitifungal activity against *Candida albicans* at different concentration such as 40,80,100µl/mg. 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 (100µl/ml).

Essential Oil	Concentration µG/ML	Zone Of Inhibition Mm
THYME	40	55
	80	60
	100	65
Chlorhexidine (Control)	0.20%	30



Essential Oil	Concentration µg/ml	Zone Of Inhibition mm
ORANGE	40	22
	80	26
	100	32
Chlorhexidine (Control)	0.20%	23

Essential Oil	Concentration µg/ml	Zone Of Inhibition mm
AJWAIN	40	51
	80	55
	100	60
Chlorhexidine (Control)	0.20%	25

DISSCUSSION

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 40muL, 80muL, and 100muL. 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 100muL 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.

Acknowledgement

2. Dr. Geetha, Professor, Department of Microbiology, Saveetha Dental College and Hospital, Chennai, Tamil Nadu, for their assistance in microbiological procedures during the study.
3. Dr. Anitha, Professor and Head, Department of Phamacology,Saveetha Dental College, Chennai, Tamil Nadu, for their assistance in the preparation of the extract.

Refference

1. Polke M, Hube B, Jacobsen ID. Candida survival strategies. *AdvApplMicrobiol*. 2015; 91:139-235.
2. Tyagi AK, Malik A. Liquid and vapour-phase antifungal activities of selected essential oils against Candida albicans: microscopic observations and chemical characterization of Cymbopogoncitratus. BMC Complement *Altern Med*. 2010; 10:1-11.
3. Martins N, Ferreira IC, Barros L, Silva S, Henriques M. Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. *Mycopathologia.* 2014; 177:223-40.
4. Khan MS, Malik A, Ahmad I. Anti-candidal activity of essential oils alone and in combination with amphotericin B or fluconazole against multi-drug resistant isolates of Candida albicans. *Med Mycol.* 2012; 50:33-42.
5. Devkatte AN, Zore BG, Karuppayil SM. Potential of plant oils as inhibitors of Candida albicans growth. *FEMS Yeast Res*. 2005; 5:867-73.
6. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils - a review. *Food ChemToxicol*. 2008; 46:446-75.
7. Fontenelle RO, Morais SM, Brito EH, Brilhante RS, Cordeiro RA, Lima YC, Brasil NV, Monteiro AJ, Sidrim JJ, Rocha MF. Alkylphenol activity against Candida spp. and Microsporumcanis: a focus on the antifungal activity of thymol, eugenol and o-methyl derivatives. *Molecules*. 2011; 29:6422-31.
8. Ozguven M, Tansi S. Drug yield and essential oil of Thymus vulgaris L. as in influenced by ecological and ontogenetical variation. *Turk J Agr Forest* 1998; 22: 537-542.
9. Baydar H, Sağdiç O, Özkan G, Karadoğan T. Antibacterial activity and composition of essential oils from Origanum, Thymbra and Satureja species with commercial importance in Turkey. *Food Control* 2004; 15: 169-172.
10. Mohammad Amzad Hossain*, Khulood Ahmed Salim AL-Raqmi, ZawanHamood AL-Mijizy, Afaf Mohammed Weli, Qasim Al-Riyami. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. *Asian Pac J Trop Biomed* 2013; 3(9): 705-710
11. Ishikawah T, Sega Y, Kitajima J. Water-soluble constituents of ajowan. *Chem Pharm Bull*. 2001; 49:840-4. [PubMed]
12. Chopra RN. Chopra's Indigenous Drug of India. 2nd ed. Calcutta: *Academic Publishers*; 1982. pp. 93-4.
13. Garg SN, Kumar S. A new glucoside from *Trachyspermumammi*. Fitoterapia. 1998; 6:511-2.
14. Nagalakshmi S, Shankaracharya NB, Naik JP, Rao LJM. Studies on chemical and technological aspects of ajowan (*Trachyspermumammi* syn. Carumcopticum) *J Food Sci Technol.* 2000; 37:277-81.
15. Choudhury S. Composition of the seed oil of *Trachyspermumammi* (L.) Sprague from northeast India. *J Essent Oil Res*. 1998; 10:588-90.
16. J. Degenhardt, T. G. K¨ollner, and J. Gershenzon, "Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants," *Phytochemistry*, vol. 70, no. 15-16, pp. 1621-1637, 2009.

Please cite this article in press as:
Anitha Roy (2017), '*In vitro* Evaluation Of The Antifungal Activity Of Three Essential Oils Against *Candida albicans* ', *International Journal of Current Advanced Research,* 6(3), pp. 2948-2950.
http://dx.doi.org/10.24327/ijcar.2017. 2950.0150

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