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RESEARCH ARTICLE

Isolate, Diagnosis and Treatment of Yeast *Candida Albicans* accompanying the human body

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Abstract

In order to study the effect of oils of thyme, peppermint, basil and cinnamon on the growth of the yeast *Candida albicans* isolated from some regions of the human body,This experiment was conducted in the Department of Life Sciences - College of Sciences - University of Diyala, for the period from December 2012 until April 2013,The results of the examination showed 90 samples (vaginal and oral swabs) implant on the medium of dextrose Sabouraud agar with chloramphenicol that 60 were positive by smear examination and 30 negative smear examination and by 66% and 34% respectively.The tests proved the diagnosis of genus and the presence of yeast *C.albicans*,The results showed that the lowest percentages as well as the inhibition of the growth of the yeast was the concentration at 20 mg / ml (16.6, 39.9, 28.5 and 23.3%. And the highest concentration in the 100 mg / ml (90.0, 93.9, 92.8 and 93.3%, respectively, And significantly differences between the concentrations of oil, thyme, peppermint, basil, cinnamon, And Did not show significant differences between the concentration of plant oils 100 mg / ml and anti-fungal Nystatine 2 mg / ml compared to the treatment of comparison 0.0.

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Introduction

The yeast Candida albicans species that causes of President Disease infecting Candidaisis followed by other species (Satana et al, 2010). Available several remedial products for the treatment of fungal diseases at the present time, including the treatment of candidiasis in the form of ointments skin surface only because of their high toxicity to the liver and the pancreas (Kwon-chung and Bennett, 1992, So there is a need to use natural materials and anti-fungal (Al-Saeed et al, 2003) , And that's where candidiasis of opportunistic diseases and common spreading in the world, including oral infections and skin and systemic urinary tract and genital infections (Maza, 2002 and Saporiti, 2001) The study aimed to:

1- Isolate yeast *C. albicans* associated with the human body .

2- Determine the effectiveness of oil, thyme, peppermint, basil and cinnamon in inhibiting the growth of yeast.

Materials and Methods

Collection of samples

93samples were collected from the cities of Baquba (Diyala University) and Jalawla (Jalawla General Hospital) in the province of Diyala, for the period from December 2012 until April 2013, Medical Swabs mediated from clinical cases and non-clinical people infected and not infected with disease Candida from different body regions, including: mouth 19, skin 20, nose, 15 ear,16 nails,13 vagina 10, And samples are placed in the test tubes sterile container on the physiological salt solution, and kept in the refrigerator until use.

Plant Oils

Were obtained on four plant oils are thyme, peppermint, basil and cinnamon, from local markets in Diyala province (Pakistan produce 0.2012).

Sabouraud Dextrose Ager with Chloramphenicol (SDAC)

Attended the medium dissolving 65 g of sabouraud dextrose Agar in 1 liter of distilled water With 0.05 g of anti-bacterial chloramphenicol, and then placed on a plot of magnetic heater to mix it well, distilled medium in Autoclave at a temperature of 121 Cº and pressure of 1.5 bar / cm ² for 20 minutes.Use medium to isolate and diagnose skin fungus (Emmons et al, 1977)

Corn Meal Ager (CMA)

Attended the medium dissolving 30 grams of maize flour in distilled water in 1glassy flask and preheat until boiling in a water bath, Move the mixture for an hour and then was nominated by pieces consist of two layers of gauze and after that added a 20 g agar and he finished size to 1000 ml of distilled water, then Sterile the medium in the autoclave at temperature 121 Cº under the pressure of 1.5 bar/cm² for 20 minutes (Booth, 1971).

Sabouraud Sucrose Broth (SSB)

Attended medium by following the way (Booth, 1971), dissolving 40 grams peptone with 10 g sucrose in 1000 ml of distilled water in a water bath until boiling, sterility in autoclave. And follow the same method for the preparation of the medium of Sabouraud Dextrose Broth (SDB)

Diagnose yeast *C. albicans*

Yeast *C. albicans* diagnosed by method Celetus and Jack (1998) the adoption of phenotypic characteristics and planting.

Planting Test

Test of Germ Tube Formation

Put 2 ml of egg whites in sterile test tubes, pipes inoculated in a part of developing a yeast colony on the medium sabouraud dextrose liquid, and incubated at a temperature 30 ° C for 3-2 hours (AL-Hamadan, 1997). Then I took a drop on a glass slide and examined under an optical microscope to observe the formation of tube germination.

Test of Chlamydospores formation

Was conducted the test planning extract medium corn along three parallel lines, each 10-mm and a 45 ° angle, Inoculated middle in yeast to be diagnosed and put it cover slice sterilized, incubated at a temperature 37 º C for 48 hours, thereafter was light microscope examination of the spores Note blastospore and chlamidospore as well as the fungal pseudo mycelium (Konemam et al, 1979)

Test of Surface Growth

Followed the method of Van Derwatt (1970) to test the growth surface and that inoculating test tubes contain the medium sabouraud sucrose liquid (SSB) a part of the colony of yeast, tubes were incubated at a temperature of 25-30 ºC for 24 hours .

Test the effectiveness of plant oils in the inhibition of the growth of the yeast *C. albicans*

Followed the method of El-Kady et al (1993) to make sure no contamination of plant oils thyme, peppermint, basil, cinnamon and 0.01 in a planting of oil on the medium SDAC and incubated for 3-7 days to make sure no contamination, Mix the plant oils in concentrations 20, 40, 60.80 and 100 mg / ml with medium SDA in Plastic dishes (diameter 9 cm) and in the two replicates for each concentration, Inoculated medium in disk (diameter 6 mm) from the colony of yeast developing on the SDA for 7 days in the center of the dish, And used two types of comparison, positive comparison with anti-fungal Nystatine in concentration 2 mg / ml to the medium SDA, And The negative comparison included only medium SDA, the dishes were incubated at a temperature 28-30 ºC for a week, measure the diameter of the colony was developing (rate diagonals orthogonal) and calculated percentage of inhibition using the following equation:

$$\text{Inhibition percentage} = \frac{\text{diameter of fungi incomparing plates} - \text{diameter of fungi in treatment plates}}{\text{Diameter of fungi in comparing plates}} \times 100$$

Statistical Analysis

Results analyzed according to factorial experiment using the Completely Randomized Design (CRD), and identified differences between the averages of treatments using the test least significant differences LSD ($P < 0.05$) using statistical program (SPSS) (Al-Rawi ,1984).

RESULT AND DISSECTION

Testing samples

The results of the examination of samples of vaginal and the oral for those infected and non-infected with Candidiasis Table 1, That the 60 swab positive in planting on medium SDAC at a percentage of 66% and 30 swab negative test at a percentage of 34%, and The negative results are attributable to the in planting insufficient sample collected or may be a fungus non fungi that cause candidiasis (Milne, 1996), Or that the reason to the use of topical treatments Random without consulting you're a specialist doctor because of the discomfort caused by this infection (Collee et al, 1996).

Table 1. Examination samples using medium SDAC

planting on SDAC	Number of samples	Percentage
Pos.(+)	60	%66
Neg.(−)	30	%34
Total	90	100%

The results are consistent with the findings of the Danbos (2011)where the 75 cases were positive when planting, and by 77.32%, As well as with the findings of the Gravine et al (2007) The infection percentage of disease Candidiasis 69.35% isolated from of oral infections to children with cancer, And with as noted byal- Sadik (2006) that infection disease Candida was 66.6%, as well as what was said by Al-Albiad (2004)who mentioned that the infection rate of people with cancer, 76.6%, And is attributable the cause of these differences in the results to geographical locations and methods of sampling (Campisi et al, 2002)

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Diagnosis of yeast *C. albicans*

Diagnosed yeast *C. albicans* depending on the phenotypic characteristics of the genus Candida and the rest of the agronomic characteristics,This appeared genus species colony to milky white color, smooth shiny and a convex when you development on the medium SDAC for 7-10 days at a temperature of 25 ± 2 م°, And examined microscopically colony after staining at cram and blue lactophenol pigment, were observed cells globular to oval or a longitudinal single and the existence of fungal Pseudohyphae sometimes (Cletus and Jack 1998) .

Susceptibility yeast *C. albicans* to form tube germination.

Table 2 shows that the yeast *C. albicans* the ability to form a tube germination.

Table 2. Some biological manifestations of yeast C. *albicans*

Fungus (yeast)	Biological characters		
C.albicans	**Germ tube**	**Chlamydospore**	**Growth surface**
	+	+	−

Susceptibility of *C. albicans* to form chlamydospores

Table 2 shows that the yeast *C. albicans*, colonies of creamy white color with a sticky appearance, take the form of dendrite off on agar, indicating its ability to formation chlamidospore .

Susceptibility of yeast *C.albicans* on growth surface

The results showed in Table 2 the inability of the yeast *C.albicans* on growth surface ,Where the back of growth in the bottom of the tube at the medium of the implant sabouraud liquid (SDB), Approaching these results with the findings of the Alsaidy et al (2012) that the percentage of yeast *C.albicans* isolated from the human body in Diyala province, 69.3%, These results also approached with the findings of Satana et al (2010) that the percentage of yeast C.albicans isolated from the oral cavity for people suffer from oral infection 73.1%, The examination showed the implant at the medium of PDA the presence of other fungi facilities to the human body such as *Aspergullis sp*. and *Trychophtone sp*., The reason may be due to contamination of the samples collected at or when planting on medium, is attributed to the superiority of the yeast *C.albicans* possess several virulence factors such as the shape of the

bilateral, who can switch from yeast to form a filamentous form, Where you start weaved growth and colonization of the surface of the mucous membranes (Erkose Erturan, 2007), as well as the ability to adhesion membranes epithelial cells with a high degree compared to other species, The reason for this goes back to the role of surface receptors in increasing the capacity of *C.albicans* yeast cell adhesion to the epithelium of the host's body, As well as their ability to excrete the digestive enzymes of protein and most important Aspartic Proteinase responsible for the analysis of protein, which helps to increase the speed of entry into force of the yeast cells into the tissues of the host and cause infection as well as secreted enzymes fat Phospholipase responsible for the analysis of phospholipids, which are the main component of cell membranes (Gary and Kevin, 2000) .

Effectiveness of inhibition of the oils of thyme, peppermint, basil and cinnamon to the yeast *C. albicans*

Table 3 shows the adoption of Effectiveness of inhibition of the oils of thyme, peppermint, basil and cinnamon to the yeast *C. albicans* on the type of oil and the concentration and type of fungus, as well as the type of plant, The relationship between the growth of the colonies and the concentration of extract counterproductive, as less than the growth rates in the increased concentration on the contrary, the percentage of inhibition where increases with concentration.

Table 3.Inhibition susceptibility for thyme, peppermint, basil and Sinnamon oils at yeast *C.albicans*

Fungus	**Conc. Mg/ml**		**Plant oils**							**LSD**
			Growth(mm)/percentage %							
		Thyme	%	peppermint	%	Basil	%	Cinnamon	%	
c.albicans	0.0	30	0.0	33.0	0.0	28.0	0.0	30.0	0.0	1.7
	20	25	16.6	20.0	39.3	20.0	28.5	23.0	23.3	1.7
	40	20	33.3	27.0	18.1	25.0	10.7	17.0	56.6	1.4
	60	15	50.0	13.0	60.6	13.0	53.5	12.0	60.0	2.8
	80	7.0	76.6	6.0	81.8	10.0	64.2	7.0	76.6	2.4
	100	3.0	90.0	2.0	93.9	2.0	92.8	2.0	93.3	2.2
	LSD	3.1		4.5		2.7		3.7		

Extracts oil of thyme, peppermint, basil, cinnamon gave lowest growth rates in the concentration 100 mg / ml diameters growth of 3, 2, 2 and 2 mm and the highest inhibition percentages 90.0, 93.9, 92.8 and 93.3%, And The highest growth rates in concentration 20 mg / ml growth diameters 25, 20, 20 and 23 mm and minimum inhibition percentages of 16.6, 39.3, 28.5 and 23.3%, respectively, with significant differences between the concentrations ($p < 0.05$) ,And Not appeared to concentration 100 mg / ml of plant oils were significant differences for Effectiveness of inhibition of the anti-fungal Nystatine in concentration 2 mg / ml, which gave a percentage of 100% inhibition of the growth of the yeast *C. albicans* compared to the comparison treatment of 0.0, And Outperformed These results are reached Bandar (2013) that the rate of growth of yeast *C.albicans* in concentration 40 mg / ml was 22 mm, and conform results with what was said by Wenzhang et al (2009) that the basil oil extracted from the leaves contain many active substances Cyclohexene 44.41%, Linalool 29.68%, Cinnamic acid methyl ester 21.49%, Guan-1 10%, a-Candiol 3.99%, 3,5-Pyridine-dicarboxylic acid 2.01%, 11-Diene 1.58%, Cadinene 1.41%, and Ciannamic acid methyl ester 1.36 % of inhibitory effect of the growth of some plant pathogenic fungi, And with Ababs (2010) that the clove oil in concentration 100 mg / ml gave the percentage of inhibition of 100% of the fungi *Alternaria alternate* 1 and *A.alternata* 2 and *A.citri* and *A.phfragmospora* and *A.denissii* and isolated from the roots of a cabbage plant The reason for Effectiveness of oils to oil materials such as effective Flavones which owns the properties of high toxicity towards fungi through the inhibition of the action of enzymes for metabolic reactions basic non-specialist to interfere with the proteins, leading to their inability to continue Mills et al (2006).

REFERENCES

1. Abbas, F.A.2010. The effect of essential oil from two plants (syzygium Aromatic and Eucalyptus camaldulensis) against some species of Alternaria isolated from Cabbage roots.*J.of Basrah Research (Scientific)* 6(36):133-142.
2. AL-Abeid, H.M.; K.H. Abu-Elteen; A.Z. Elkarmi & M.A. Hamad. .2004. Isolation and Characterization of *Candida* spp. in

Jordanian Cancer patients: prevalence, pathogenic determinants, and antifungal sensitivity. *Jpn. J. Infect. Dis*., 57: 279-284.

3. Al-Hamadani, A.H.A. 1997. Enzyme activity, purification of keratinase And proteinase and their roles in the.Pathogenicity and Immunogenicity of clinical isolates of dermatophytes and yeast.Ph.D.thesis, COLLEGE of EDUCATION, UNIV. of Basrah.
4. Al-Rawi, k.S.1984. Vital Statistics, Univ. of Mosul, the Press, the Ministry of Higher Education and Scientific Research.
5. Al-Sadik, s. m. 2006. The effect of some effective of the compounds extracted From lemon grass and thyme in the types of Candidaisis. M.Sc. thesis. College of Science. Univ. of Baghdad.
6. Alsaidy, H.A., N.G. Alzebedee and I.K.M. Danbuss. 2012. Inhibition activity Of plant extract *thymus vulgaris* and *Mentha pipertia* against *Candida Albicans.J.of* Diyala *Agric. Sci.* 4(1): 128-139.
7. Bander, K.L.2013. Study the inhibitory efficiency of clove oil and thyme and Extract of *Pseudomonas aeroginosa* and the fungus *Aspergillus niger* on the Growth of some dermatophyte fungi. *J. of Tikrit Univ. for Agric. Sci*. 13(1):33-38.
8. Booth. (1971).Method in Microbiology, Vol.4.Academic Press, London, New York.
9. Campisi, G; G. Pizzo; M.E. Milici; S. Mancuso and V. Margiotta. 2002. Subjects. *Oral Surg*. *Oral Med. Oral pathol .oral Radoil .Endod* ,93:281-286.
10. Cletus P. Kurtzman , Jack W. Fell . 1998. The Yeasts, a Taxonomic Study, Fourth edition. New York. Oxfo
11. Collee, J. C., Fraser, A. G., Mamain, B. P. and Simmons, A. 1996. Mackie And Mc Cartney. Practical Medical Microbial. 14th ed. Churchill Livingstone Inc., USA.
12. Danbos, I. Q. M. 2012. Evaluation efficiency of *Thymus vulgaris* and *Mantha pipertia* plant extract and antibiotic against of *Candida spp*. Isolated for human in Diyala Province. M.Sc. thesis Al- Razi College Of Education. Univ. of Diyala
13. El-kady, J.A.; S.S. El-Maraghy & E.M. Mohamed .1993. Antibacterial And antidermatophyte activity of some essential oils from spices. *Qatar Univ. Sc. J*., 13(1): 63-69.
14. Erkose, G. and Z. Erturan. 2007 .Oral candida colonization of human Immunodeficiency virus infected subjects in Turkey and its Relation with viral load and CD4 T-lymphocyte count. *Mycoses* 50:485-490.
15. Gary, C. & K. Kevin. 2000. Adherence Mechanisms of *Candida albicans*. *Brit. J. of Biomed. Sci*., P.1- 4.
16. Gravina, H. G., E. G. de Moran, O. Zambrano, M. L. Chourio, S. R. de Valero, S. Robertis and L. Mesa .2007. "Oral Candidiasis in children And adolescents with cancer Identification of *candida spp* ", Med Oral Patol Oral Cir Bucal.
17. Konemon, E.W.; G.D. Roberts and S.E. Wright. 1979. Practical Laboratory Mycology. 2nd ed. Williams and Wilkins Company, Baltimore
18. Kwon- Chung, K.J. and J. E. Bennett. 1992. Medical Mycology. Lea And Fibiger, Philadelphia, London.
19. Maze J. L., N.Elguezabal, C.Prado, J. Ellacuria, I. Soler and J.Ponton .2002.Candida albicans adherence to resin-composite restorative dental material: Influence of whole human saliva. *Oral Sirg Oral Med Oral Pathol Oral Radiol Endod*.94:589-92.
20. Milne, L.J.R. 1996. Fungi in: Mackie and McCartney Practical Medical Microbial. Collee, J.G., A.G. Fraser, B.P.Marmion And A. Simmons. (eds). 14th ed. Churchill Livingstone, London
21. Mills, E., J. J. Duguoa, D. Perri and G. Koren. 2006. Herbal Medicines in

Pregnancy and Lactation .An Evidence-Based Approach, London and New York.
22. Saporiti, A.M, Gomez D. and Levalle S. 2001.Vaginal candidiasis: etiology And sensitivity profile to antifungal agents in clinical use. *Rev Argent Microbial*, 33:217-22.
23. Satana, D., G. E. Genc and Z. Erturan. 2010. "The antifungal Susceptibilities of oral *candida spp*. isolates from HIV-infected patents" *African J. of Microbial. Res.*, Vol. 4(6): 466-470.
24. Van Der Watt, J. P. 1970. Criteria and method used in classification In: *J. Lodder* (ed). The yeast a taxonomic study. Second revised and Enlarged edition North Hell and Publishing Company. Amsterdam London. pp3213.