

Study Effect of Some Pakistan Oil Isolation of Yeast *Candida albicans* causing oral thrush children Who Visit Women Hospital and Children in Diwaniya

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Abstract

The aim of the study was to evaluate some of the essential oils of the *candida albicans* yeast causing candidiasis in children referred to the women hospital and children. The study included 108 oral swabs from infants with candidiasis, all samples were diagnosed by diagnostic methods and after initial isolation of *candida albicans* 37.5% followed by species *C.tropicalis* 30%, *C.parapsilosis* 12.38% as for *C.krusei* and *C.glabrata* they were 9.4%. The results of the agar well diffusion method showed that the *nigella sativa* oil was the most inhibited followed by cinnamon oil, while garlic oil was the least inhibited compared with the nystatin.

Key words: *Candida albicans*, *Nigella sativa* oil, Cinnamon oil, Garlic oil, Nystatin

Introduction

Oral disease is one of the most common opportunistic fungal diseases, especially in the case of people with immunocompromised individuals, *candida albicans* is one of the most common types of candida and has a very high to cause diseases and injuries to the oral cavity¹, at present other species have been identified as the main causes of oral thrush after *candida albicans* such as *C.tropicalis*, *C.parapsilosis*, *C.krusei*². Aromatic oils and spices extracted from plants are also highly effective as microbial antiperspirants and are extracted from either steam, water, or organic solvents, and can be extracted by cold pressing. The antimicrobial reaction to microbes is that it destroys many enzymatic systems of microorganism³.

Materials & Methods

Collection of samples

108 oral swabs were collected from children who visited the women hospital and children's education through sterile cotton swabs and then transferred to the laboratory for development, diagnosis and testing.

Isolation

Samples were streaked on the Sabouraud dextrose agar and incubated at 37 °C for 24 hours.

Diagnosis of candida

Phenotypic diagnosis

Special tests were conducted on the isolation of the genus candida according to what he described⁴⁻⁸ which included:

a. Growth on Sabouraud dextrose agar

Cultured of the samples on the Sabouraud dextrose agar according to method (Ellis et al., 2007).

b. Growth on chrom agar candida

The samples were streaked on chrom agar candida and incubated at 37 °C for 24-48 hours. The color of isolation that has grown on this medium is observed. Use this test to confirm the diagnosis of Candida.

c. Detection of presence of the Capsule

The presence of the capsule was discovered by using India ink in addition to a drop of India ink according to

the method ⁵.

d. Growth at 37 °C

The SDA medium was inoculated with yeast tested and incubated at 37 °C and observed daily growth for 2 weeks according to method ⁵.

Biochemical tests

a. Sugars fermentation test : Perform this test according to a method ⁹ .

b. Sugars assimilation test : in this test method adopted.

Virulent Factors

a. Germ formation : Perform this test according to a method (Ellis , 1994)

b. Chlamyospores formation : Perform this test according to a method (kangogo *et al.*,2011) .

c. Cycloheximide sensitivity: Perform this test according to a method (Ellis , 1994).

d. Growth at 45 °C: Perform this test according to a method ¹⁰.

e. Urase test: This test was conducted to investigate the ability of yeasts to produce urease enzyme and depending on the method (Fadhy and Ajello ,1977).

f. Lipolytic activity: Perform this test according

to a method ¹¹.

g. Phospholipase production: Perform this test according to a method ¹².

Test the efficacy of some types of Pakistan oil on candida albicans yeast on the SDA medium compared to the nystatin antibiotic

This test was carried out to determine the biologic effect of the oils used in the study using the method of agar well diffusion method contained in ¹³.

Statistical analysis

The data were statistically analyzed at a significant level of 0.05 and using the ANOVA table to confirm that there were significant differences between the tested parameters Al-rawyi (2000).

Results and Discussion

Isolation and Diagnosis

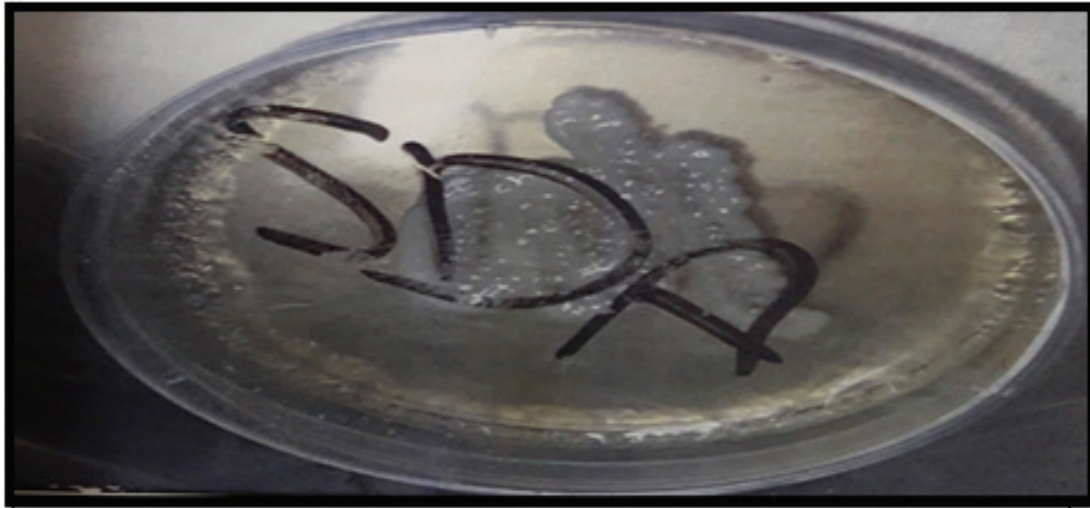
Five types of candida were isolated based on culture ,microscopic ,biochemical and virulence factors and these species are *Candida albicans* by 37.55% followed by species *C.tropicalis* 30% *C.parapsilosis* 12.38% as for *C.krusei* and *C. glabrata* they were 9.4%. these results are consistent with Hussein, (2011) ,who isolated five types of candida species from the oral cavity the type *candida albicans* came in the foreforent followed by the following species *C. tropicalis* , *C. parapsilosis*, *C. krusei*, *C. rugosa*..

Table (1) Percentage of isolated species

Number	Isolates species	Percentage
1	Candida albicans	37.5%
2	C. tropicalis	30%
3	C. parapsilosis	12.38%
4	C. krusi	9.4%
5	C. glabrata	9.4
LSD(0.05)	10.015	

Phenotypic diagnosis

The results showed that all isolated species were grown on the SDA at a temperature of 37 C in white colonies circular and these results were consistent with(Ellis et al ., 2007)



Picture (1) growth *Candida albicans* on SDA at 37 °C

Microscopic examination

The results of the microscopic examination showed that *Candida* yeast was positive for G+ stain and negative for the dye in India ink (negative to test composition of the capsule) these results were consistent with ¹⁴.

Growth on Chrom agar candida medium

Results showed the grown of *C .albicans* on the Chrom agar candida medium as it appeared green color and these results agree with Rajaa *et al.*,(2015) ¹⁵.

Biochemical tests

Sugar fermentation test

The results of the sugary fermentation test showed that the *Candida albicans* type has the ability to ferment the sugars of glucose ,maltose, galactose but lactose and sucrose sugar cannot ferment. these results agree with Ellis *et al .*;(2007) which showed that the species *C.albicans* have a high efficiency in the representation of sugars, which shows that one of the reasons for the frequent isolation of tissues infected with candidosis, these results are consistent with Rajaa *et al.*; (2015) ¹⁵, as they found that *C.albicans* has the ability to ferment

sugars :glucose, maltose, galactos but lactose and sucrose sugar cannot ferment.

Table (2) Sugar fermentation test at 37 °C

Number	Used sugars	<i>C. albicans</i>
1	Glucose	+
2	Maltose	+
3	Galactose	+
4	Lactose	-
5	Sucrose	-

+ sugar fermented , - Cannot fermented

Sugars assimilation test

Cultured isolates species of *Candida albicans* on the SDA medium and the saturated sugars were added from the following sugars: Glucose , Maltose , Galactose , Lactose Sucrose, as in table (3) as all isolates of *Candida*

albicans assimilate all sugar except lactose sugar, these results are consistent with AL-Edamy,(2012)¹⁶.which is a very important cause of the repeated isolation of tissue infected with candidiasis (Ellis et al .;2007)

Table (3) Sugars assimilation test

Number	Used sugars	C. albicans
1	Glucose	+
2	Maltose	+
3	Glactose	+
4	Lactose	-
5	Sucrose	+

+ positive -negative

Virulence Factors

Germ tube formation

The results of the study showed that all isolates belonging to species *C. albicans* had formed the germ tube when incubated for 2-3 hours in serum blood at 37 °C , these results are consistent with Boon *et al* .,(2013) which showed that the species of *C.albicans* has the ability to form a germ tube and the presence of the catalyst(serum) is formed around the yeast cell and that the germ tube plays an important role in the process of penetrating the layer of epithelial cell of the body and tissues and access to the blood stream as well as thought necessary to feed the yeast¹⁷.

Chlamydia spores Formation

The results of the study showed that all the isolates of the type *C.albicans* had formed the Chlamydia spores when cultured in the Corn meal agar medium and incubated at 25 °C for 24 hours .these results agree with¹⁸,whose results showed that the type *C.albicans* has the ability to form Chlamydia spores on the Corn meal agar medium, the Chlamydia spores are round with thick walls at the end of hypha which may be single or clustered when cultured on the CMA medium as a result of starving the yeast for lack of food sources where they are formed when the conditions are not appropriate to

them .this medium is described as starving for yeast.

Resistance to Cycloheximide

The results of the study showed that isolates of the species *C.albicans* had resistant to cycloheximide as it growth on the SDA medium added 0.5 g/ l of the cycloheximide .these results are consistent with Hussein, 2011 and al- Ubaidy.,2012; Takaku *et al*. 2004 .the results of their study showed that species *C.albicans* only was resistant to the cycloheximide and gave a positive results for this test .

Growth at 45 °C

C. albicans was cultured on the SDA and incubated at 45 °C .the growth was monitored daily for 10 days .the results showed that *C.albicans* had the ability to grow at this level. these results are consistent with AL-Obeidi,(2012) which showed that the *C.albicans* species managed to grow at 45 °C while the rest of the species *C. tropicalis* , *C. krusei* , *C. glabrata* could not .

Urease Enzyme Production

Results of the growth of *C.albicans* on the agar urea showed that *C. albicans* did not have the ability to produce the urease enzyme .these results came consistent with Jasim and Nasir ,(2013) which showed that the result *C. albican* *C. parapsilosis* , *C. Tropicalis* were unable to produce urease under the same conditions.

Lipase Enzyme Production

The results of the study showed that the species *C. albicans* has the ability to produce the Lipase and these results were consistent with Hussein ,(2011) whose results showed that the species *C. parapsilosis* , *C. tropicalis* , *C. albicans* have the ability to produce lipase.

Phospholipase Enzyme Production

The results of the study showed that the species *C. albicans* has the ability to produce the Posoholipase ,this explains the ability of the species to cause the disease more than of the species and these results are consistent with (Hussein, 2011).

Table (3) Virulence Factors of *Candida albicans*

Number	Virulence factors	C. albicans
1	Germ tube	+
2	Chlamyospores	+
3	Resistance to cycloheximide	+
4	Growth at 45C	+
5	Lipase	+
6	Posoholipase	+
7	Urease	-

+ positive , -negative

Test the effect of some types of Pakistan oil on *Candida albicans* yeast compared to the nystatin antibiotic.

The results showed that *C.albicans* was sensitive to the oil used in the study .the nigella sativa oil was one of the most influential oils on the growth of *C.albicans* yeast it also showed its superiority over the antibiotic nystatin used as a control agent with the rate of inhibition zoon diameter 31mm, these results are consistent with Khuder,(2012) finding that nigella sativa oil have a good antibacterial activity on all bacterial types used in study, the inhibition effect of the nigella sativa oil due to chemical compounds Thymol , Thymohydroquinone, Thymoquinone in the essential oils of the nigella sativa oil which according to previous studies ,showed its antimicrobial effect. Salman *et al.*,(2008), a while the comparison treatment (nystatin) 30mm,cinnamon oil was the average diameter of the inhibition zoon 22mm ,these results are consistent with Abdullaha,(2013) finding that cinnamon oil has an active role in inhibiting the growth of certain bacterial isolates causing diarrhea ,which were better than antibiotics used as a control groups. as for garlic oil was the least effect on the growth of yeast under study as showed in the table the rate of inhibition zoon diameter 15mm.

Table (4) effect of some types of Pakistan oil on *Candida albicans* yeast compared to the nystatin antibiotic

Number	Oils (Concentration) 100%	Inhibition zone diameter(mm)
1	nigella sativa oil	31
2	Cinnamon oil	22
3	Garlic oil	15
4	Nystatin	30
LSD(0.05)	4.016	

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Furat Al-Awsat Technical University and all experiments were carried out in accordance with approved guidelines.

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