

Study the effect of ethanolic extract of leaf (*Eucalybtus austral*) on *Candida albicans* isolated from suparingival plaques gum of different age, sex and smoking state

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Key word: Candida, Eucalybtus, gum, extract

Summary

The present study was designed to evaluate the inhibitory effect of the different concentration (200, 400, 800) mg/ml from local plant ethanolic extract leaf (*Eucalybtus austral*) against the growth of *Candida albicans* in culture media by using of agar well diffusion method, it which take from suparingival plaques gum and effect of age, sex and smoker state.

The result showed that the extract (*Eucalybtus austral*) was more effective concentration 800 mg/ml, comparative with effect other concentration from of(Nystatin and Clotrimazole).

The statistical analysis by using Chi- squared test at level (P<0.05) showed that the sex and smoker state were a significantly preeminence.

دراسة بتأثير المستخلص الايثانولي لأوراق الكالبتوز على المبيضات المعزولة من

المصابين بالتهاب اللثة بمختلف الاعمار والاجناس وحالات التدخين

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الكلمات الافتتاحية: المبيضات، الكالبتوز، اللثة، المستخلص

الخلاصة

صممت هذه الدراسة لتقييم الفعل المثبط وتأثيره لمختلف التراكيز (٢٠٠, ٤٠٠, ٨٠٠) ملغم/ مل من المستخلص الايثانولي لأوراق نبات الكالبتوز ضد خميرة المبيضات البيضاء في الاوساط الزراعية وبطريقة الانتشار بالاكار ، والتي اخذت من حالات التهاب اللثة وبتأثير العمر والجنس وحالات التدخين. حيث اظهرت النتائج ان مستخلص الكالبتوز اكثر فعالية في تركيز ٨٠٠ ملغم/مل مقارنة مع الادوية المضادة للفطريات (النستاتين والكلوترايمازول).
تم اجراء التحليل الاحصائي باستخدام اختبار مربع كاي مع اقل فرق معنوي تحت مستوى احتمالية (٠.٠٥) حيث لوحظ ان للجنس وحالة التدخين تأثيراً معنوياً .

Introduction

The medicinal importance of plant produce is well known to man from the ancient times. Many plants possess substances of fungi static or fungicidal nature. There are 50,000 valid species of fungi but only 50 to 100 are generally recognized as known to cause pathogenic disease to human beings.⁽¹⁾

Candida albicans is frequently carried in the oral cavity without causing disease ⁽²⁾ but symptomatic carriage may place some individuals a

higher risk of complications through yeast infections if they become immunosuppressed ^{(3),(4)}.

Among subject initially asymptomatic for *C.albicans* infection, clinical thrush developed only in those patient who are permanent carrier of *C.albicans* prior to developing symptoms ^{(5),(6)}.

The yeast candida is the dominant opportunistic pathogen, and it accounts for the majority of invasive infections ⁽⁴⁾.

Although *C. albicans* most frequently infects the skin and mucosal surfaces, it can cause systemic infections manifesting as pneumonia, septicaemia or endocarditis in severely immunocompromised patients, *C.albicans* also can grows vigorously in the vagina and pregnant woman transmit the infection to the babies during birth ⁽⁷⁾.*Candida albicans* to investigate the anticandida effect of eucalybtus these factors necessitate the search for new antifungal agent so this study was investigate the antifungal activity of ethanolic extract of leaf *Eucalybtus austral*.

Martials and method

1.Plant collection and preparation:

In this experiment we used local medicinal plant leaf *Eucalybtus austral*. these plants included were obtained from the local market and identified by the national Iraqi institute for herb's, we take the leaf *Eucalybtus austral* plants were subjected to aerial drying for two weeks, after drying of these parts we grinded it very well until it became as fine powder. The ethanolic extraction of the plants were done by Harboren method ⁽⁸⁾ by using of ethanol of concentration (96%).

2. *Candida albicans* isolated:

The samples for mycological study were collected from the supargingival plaques gum of patient from 50 patient, aged 19-70 years from both sexes and see effected of smoker. Collected from patient attended to Al-Diwanyiah teaching hospital in Al-Diwanyiah governorate.

The period from March 2012 to April 2012. All patient clinical signs included painful and redness in gum (gingivitis) and patient suffering from bleeding during cleaning of the teeth, this signs diagnosis by specialist clinician. All samples were cultured directly on Sabouraud Dextrose Agar with antibiotic supported (chloromphenicol) to prevent bacteria growth, incubation at 30-37 C° to 3 day. all positive result from primary cultured, subculture on the Sabouraud Dextrose Agar medium without (chloromphenicol) used to tested inhibition growth *Candida albicans* by used different dilutions of eucalybtus.

3. Antifungales

In this study used two antifungal agents Nystatin drops (Nystaser) (Pharmasyer, Dmascus, SYRIA) 100000 IU\ml, and the second antifungal was Clotrimazole solution (Candistan) (Arab drug company, Cario, A.R.E.) 10 mg\ml.

4. Serial dilutions:

For each of the tested medicinal plants we had been made a serial dilution to study the effect of the plants in inhibition the growth of *Candida albicans* at a different concentrations of the plant extract depending on the concentration of inhibition of growth that been given by each concentration, we started with a concentration(800 mg\ml) (prepared by add 10 ml from ethanol 96% to 8 gm of the plant extract) and second dilution is(400mg\ml)(prepared by add 8 ml first dilution and we add 8ml of ethanol 96% to first dilution) and third dilution is(200mg\ml) (prepared by add 4 ml from ethanol 96% to 4ml from second dilution).

5. Sensitivity test study:

After preparation and ethanolic extraction of the studied medicinal plants and activation of *Candida albicans* in the nutrient broth were prepared a (SDA) (HIMEDIA laboratories, Mumbai, India) 65gm\1000ml D.W by adding the agar powder to distal water in a flask and after complete

dissolving of the agar by using of magnetic stirrer we used the autoclave for antiseptic the agar at 15 IB for 15 minutes, then the agar were empty into Petri plates (9 Petri plates for each test agents) and then after the media is solidified in all the plants we made (4) pores in the (SDA) in the Petri plates to put the tested agents dilutions in it and a central pore for the control (ethanol 96%), these pores were made by using a pasture pipette in a diameter of (5mm) and put (0.1) ml form each concentration of the tested agents in these pores by using of micropipette. For each one of the tested agents (medicinal plants: *Eucalyptol* made (9) plates and each plates contain (4) pores put in these pores the serial dilution of the tested medicinal plants and the fourth pore for control (ethanol 96%). *Candida albicans* were plates that contain the (SDA) before putting the tested agents and the we added the serial dilution of each on the medicinal plants (800,400,200) mg/ml and put the Petri plates in incubator (48) hour at 37 C° following overnight incubation, the culture was examined for areas of no growth around the pores (zone of inhibition) in millimeter (mm) ⁽⁹⁾.

Results and discussion

The genus *Candida* is composed of an extremely heterogeneous group of organisms that grow as yeasts. Most members of the genus also produce a filamentous type of growth (pseudohyphae), In addition pseudohyphae, *Candida albicans* from true hyphae (germ tubes) and thickwalled cell referred to as chlamydospores, both of which are used by mycology diagnostic laboratories in identifying these species *Candida albicans* species are now emerging as major agents of gingivitis, *C. albicans* many virulence factors , such as germ tube formation , exoenzyme production, and phenotypic switching ⁽¹⁰⁾. *Candida albicans* is a diploid fungus (a form of yeast) and is a causal agent of opportunistic oral and genital infection ⁽¹¹⁾.

Candida has been known to infect every organ of the body, but its ability to cause infection depend upon the presence of sufficient amount of fungal

organism or generally reduced resistance or both ⁽¹²⁾. In our study we try to experiment the effect of selected medicinal plant in comparison to antifungal drug on *Candida albicans* growing in vitro in a Sabouraud Dextrose Agar by using of agar diffusion method.

Agar-based method are attractive because of their simplicity and low cost, in addition to that it may help to detect if their any resistance from the yeast or the bacteria to any drug, medicinal plants or agent that may be used study it is effect ⁽¹³⁾.

In view of this, there is need to develop more effective and less toxic agent for the treatment of common, as well as drug resistant fungal infection. This has led to a search for therapeutic alternatives, particularly among medicinal plants and compounds isolated from them used for their empirically antifungal properties. In these natural source, a series of molecules with antifungal activity against different strains of fungus have been found, which are of great importance to humans and plants ⁽¹⁴⁾.

In vitro *anticandida albicans* effect of local plant ethanolic extract (*Eucalybtus austral*) and two standard antifungal drugs (Nystatin, Clotrimazole) were studied in this experiment (table 1 and 2). The active ingredient include: volatile oil 1,8-cineol (cineole), alkaloids, phenols and tannins ⁽¹⁵⁾.

The ethanolic extract of the medicinal plant showed a various antifungal activities against the isolated of *Candida albicans* according to the type of the medicinal plants and the concentration which had been used. The most active extract were that obtained from (*Eucalybtus austral*) that gave a zone of inhibition zone with Standard Error as follow:

(12.6±0.14, 16.3±0.13, 21.75±0.22) mm at concentration 200, 400, 800 mg/ml respectively. Our study showed that there was a proportional relationship between the concentration of the plant extract that had been used and the zone of inhibition of *Candida albicans* growth in the media of Sabouraud Dextrose Agar at the petri plates at a confidence interval

($P < 0.05$), when the concentration increase this lead to increase the zone of inhibition of the growth.

The antifungal drugs produce an inhibition zones as follow: Nystatin (1000 IU/ml)(15.5 ± 0.26 mm) Clotrimazol (1mg/ml)(18.8 ± 0.61 mm) and ethanol 96% (0 ± 0).

Clotrimazol (10mg/ml) had a significant differences ($P < 0.05$) in the zone of inhibition than that for Nystatin (100000 IU) and ethanol 96% (15.5 ± 0.26 versus 18.8 ± 0.61 and 0 ± 0) respectively. The result of *Eucalybtus austral* ethanolic extract was close to those obtained by oil of the *Eucalybtus austral*, the yeast *Candida albicans* was sensitive to 8gm/ml exhibited inhibitory activity against *Candida albicans* at high concentration ⁽¹⁶⁾.

The oil and leaves of Eucalyptus are used for medicinal purposes. Eucalyptose oil is used as an anti-microbial element in different kinds of cream, soap and toothpaste ⁽¹⁷⁾.

⁽¹⁸⁾ showed in his study that the volatile oil of the medicinal plant *Eucalybtus austral* have antifungal effect against *Candida albicans* and refer to the content of this volatile oil that it is composed mainly of oxygenated monoterpenes.

⁽¹⁹⁾ Either Nystatin suspension or the Clotrimazole douches is the drug of choice in Candidiasis for non-immunosuppressed adults.⁽²⁰⁾ The *Anticandidal albicans* effect of Nystatin had been approved in many researches ^(21,22) .

⁽²³⁾ indicate the inhibition effect of this drug against *Candida albicans* , and against other fungal infection than *Candida albicans* Nystatin is less stronger than clotrimazol⁽²³⁾.

Table (1): anticandida albicans activity of *Eucalybtus austral* alcoholic extract.

<i>Eucalybtus austral</i>	Extract concentrations (mg/ml)		
	200	400	800
Zone of inhibition (mm)	12.6 ± 0.14	16.3±0.13	21.75±0.22

- Values were expressed as means± standard error

Table (2): anticandida albicans activity of some antifungal drugs (positive control and ethanol (negative control)).

Name of antifungal drug	Inhibition zone (mm)
Nystatin (1000 IU)	15.5 ± 0.26
Clotrimazole (1 mg/ ml)	18.8 ± 0.61
Ethanol (96%)	0 ± 0

A total of 24 *C. albicans* strains were isolated in primary culture in which (48%) derived from superagingival plaques.

1.Relationship between gingivitis and age :

Out of 15 (5%) cases from candidiasis that gave positive on culture media, have (19-35) years age, while 5(31.25)% cases from candiasis that gave positive have age (35-50) years. While 4(100%) cases from candidiasis that gave positive have age (50-70) years.

There were non-significant differences (P-value >0.05) between the gingivitis and age of patient Table (3).

Table (3).Relationship between the gingivitis and age in patient.

Age	Gingivitis				Total	X ² and p-value
	+Ve	%	-Ve	%		
19-35y.	15	50	15	50	30	X ² =6.18 DF=2 P>0.05 Non Significant
35-50y	5	31.25	11	68.75	16	
50-70y.	4	100	0	0	4	
Total	24	100	26	100	50	

The study indicates that host age is a determining factor in yeast carriage⁽²⁴⁾. The above presented results indicate that the frequency of oral yeast, mainly *Candida albicans* species, is different in different aged.(2).

2.Relationship between gingivitis and sex:

Out of 13 (54%) male cases from candidiasis that gave positive on culture media, while 11 (57.7)% female cases from candiasis that gave positive on culture media.

There were significant differences (P-value >0.05) between the gingivitis and sex of patient Table (4).

Table (4).Relationship between the gingivitis and sex in patient.

Sex	Gingivitis				Total	X ² and p-value
	+Ve	%	-Ve	%		
Male	13	54	11	46	24	X=0.703 DF=1 P>0.05 Significant
Female	11	42	15	57.7	26	
Total	24	100	26	100	50	

1.Relationship between gingivitis and smoking :

Out of 6 (54.5%) male cases from candidiasis that gave positive on culture media have smoker state, while 18 (46)% cases from candidiasis that gave positive on culture media have non-smoker state .

There were significant differences (P-value >0.05) between the gingivitis and smoke and non-smoker state of patient Table (5).

Table (5).Relationship between the gingivitis and smoking state in patient.

Smoke	Gingivitis				Total	X ² and p-value
	+Ve	%	-Ve	%		
Smoker	6	54.5	5	46	11	X ² =0.703 DF=1 P>0.05 Significant
Non-smoker	18	46	21	54	39	
Total	24	100	26	100	50	

Candida albicans is the most important commensal organism in the oral cavity, and the yeast carriage frequency varies not only by the age but also according to geographic area. It is therefore a reasonable possibility that these changes in frequency may be due to physiological changes and mucosal surfaces, changes related to natural barriers against yeast colonization, and changes in the living environment and habits of the individual and to the ecological environment of the oral cavity⁽⁶⁾.

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