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Study the Effect of *Quercus infectoria* Galls Extracts on Growth of *Candida albicans* and *Candida glabrata* In Vitro Which Isolated from Vaginal Swabs

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Summary

The results of isolation in this research showed prevalence of *Candida albicans* at 35.7% which isolated from 70 samples of infected women who suffered from vaginal thrush compared with other infected agent. In vitro antifungal activities of ethanolic and aqueous(hot and cold distilled water) extracts of Quercus infectoria galls were tested against growth of *C. albicans* and *C.glabrata* in different concentration , showed that ethanolic extracts of Quercus infectoria was more effective against *C. albicans* at concentration 700mg/ml while the aqueous(hot and cold distilled water) extracts were more effective against *C.glabrata* compared with growth of *C. albicans* at same extracts .

Keywords : *Quercus infectoria* , *C. albicans* , *C.glabrata* , vaginal thrush , vaginitis , in vitro

دراسة تأثير مستخلصات كرات العفص على نمو *C. albicans* و*C.glabrata* خارج الجسم والمعزولة من المسحات المهبلية

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الخلاصة

اظهرت نتائج العزل في هذا البحث تغلب الاصابة بخميرة*C. albicans* المعزولة من (70 عينة) من المسحات المهبلية لنساء مصابات التهاب المهبلي الفطري بنسبة 37.5% مقارنة بالعزلات الاخرىواختبرت دراسة تأثير للمستخلص الكحولي والمائي (الحار والبارد) لكرات العفص كمضاد للفطريات ضد نمو خميرة *C. albicans* و*C.glabrata* خارج الجسم (في الاطباق) بمختلف التراكيز حيث اظهرت النتائج ان المستخلص الكحولي لكرات العفص كان لة تأثير فعال ضد نمو خميرة *C. albicans* عند تركيز 700ملغم /مل بينما لوحظ ان المستخلص المائي لكرات العفص (الحار والبارد) كان اكثر فعالية ضد نمو خميرة *C.glabrata* عند نفس التركيز مقارنة بتأثيرة ضد نمو خميرة *C.albicans* عند نفس التركيز.

Introduction

Candidiasis or thrush is a fungal or yeast infection of any of the *Candida* species (all yeasts), of which *Candida albicans* is the most common (1). *Candida albicans* is a fungus that is normally present on the skin and in mucous membranes such as the vagina, mouth, or rectum. The fungus also can travel through the blood stream and affect the throat, intestine, and heart valves. *Candida albicans* becomes an infectious agent when there is some change in the body environment that allows it to grow out of control and imbalances in body system initiated by exposure to the use of antibiotics and sulfa drugs , steroid hormones , immunosuppressant drugs , diabetes mellitus , in pregnant women. *Candida albicans* and *Candida glabrata* are most common cause of vaginal candidiasis. Vaginal yeast infection, which is the most common form of vaginitis . Symptoms of candidiasis may vary depending on the area affected. Infection of the vagina or vulva may cause severe itching, burning, soreness, irritation, and a whitish or whitish-gray cottage cheese-like discharge, often with a curd-like appearance. Yeast infection can be difficult to treat as it is resistant to common anti-fungal drugs Recent studies have revealed that medicinal plants from various parts of the world could be rich sources of antibacterial and antimicrobial activities.(2 and 3). In this research, *Quercus infectoria* Olivier (Fagaceae) was studied in order to investigate its antifungal properties. *Quercus infectoria* is a round-shaped abnormal growth found arising on young branches of the oak tree (4). The galls are locally known as manjakani in Malaysia, and are used in combination with other herbs as drinking remedy by women after childbirth to restore the elasticity of the uterine wall (5). Galls of *Quercus infectoria* are widely known in Indian traditional medicine have been used as dental powder and in the treatment of toothache and gingivitis.(6and7) . The galls of *Q. infectoria* have also been pharmacologically documented to possess astringent, antidiabetic, antitremorine, local anaesthetic antiviral, antibacterial, antifungal, larvicidal, and anti-inflammatory activities(8). The main constituents found in the galls of *Q. infectoria* are tannin (50-70%) and small amount of free gallic acid and ellagic acid (9) and vitamins A and C, calcium, iron, fiber, protein and carbohydrates (10) and has the ability to be an antibacterial (12) and antifungal agent(13) .

This study was planned to investigate: The occurrance of Vaginal yeast infection from infected women. To study the antifungal activities of ethanolic and aqueous(hot and cold distilled water) extracts of Quercus infectoria galls in vitro against the growth of *C. albicans* and *C.glabrata* .

Materials and methods

Collection of the samples : 70 vaginal swabs were taken from infected women (vaginal thrush and vaginitis). these swabs were cultured on Sabouraud Dextrose Agar (SDA) and differentiated by use (CHROM candida agar), Germ tube test and use of API candida .

Collection of the medicinal plant : *Quercus infectoria* was brought from the local market The oak galls were washed with distilled water, left for drying at room temperature then the samples were crushed into pieces and grinded into powder using a grinder before being sieved to get only a fine powder the powder was kept in the dry container for using in another time (13).

Preparation of the plant extracts : Eighteen **(**18) gm of powder of *Quercus infectoria* in 3 flask then add 150 ml of (distilled water at room temperature 25C˚, boil distilled water for aqueous extracts and ethanol at 95% for ethanolic extracts for each flask) then left on magnetic stirrer for two days. Suspension was filtrated by Whattman No.2. filter paper and the filtrate was dried in oven at 37 C˚ for(48-72)hr until fully dried and kept in the refrigerator for usage in another time (14). this powder which used to proper different concentration for different plant extracts .

Alcoholic (ethanol) of *Quercus infectoria* extract: Take 1-10g of powder and solvent with 10ml of ethanol at 95% to proper the concentration (100-1000 mg/ml).

Aqueous (boil distilled water) of *Quercus infectoria* extract: Take 1-10g of powder and solvent with 10ml of disitilled water at room temperature 25C° to proper the concentration (100-1000 mg/ml).

Aqueous (distilled water at 25C˚) of *Quercus infectoria* extract: Take 5-10g of powder and solvent with 10ml of disitilled water at room temperature 25C° to proper the concentration (500-1000 mg/ml).

Preparation of antifungal susceptibility test of *Quercus infectoria* extracts:

0.1ml was taken of Candida suspension (10^6 cells / ml) was put on surface of (SDA) media , spread by using glass spreader L-shaped , left to dry for (10-20) minutes, then by using sterilized cork borer (5mm) diameters , wells were made in the SDA medium then add 0.1ml for each concentrations of the *Quercus infectoria* extracts in three repeated Petri dishes for each treatment and incubated at 37C° for (24-48) hr .

The activity of each concentration of the Quercus infectoria extracts was determined by measuring of diameter of the inhibition zone around each well using the ruler(15) .

Statistical Analysis :the significant differences determined under probability level 5% by using (LSD) Least Significant Difference.

Results and Discussion

The infection ratio for the prevalence of *C. albicans* at 35.7% whereas *C.glabrata* at 28.5% , *C.tropicalis* 5.7% and others such as bacterial infection at 30% (Table 1**)**

Table 1: number of fungal and bacterial isolated and percentage from the vaginal swabs .

Type of microbial isolates	Number	Percentage %
C. albicans	**25**	**35.7**
C.glabrata	**20**	**28.5**
C.tropicalis	**4**	**5.7**
bacterial infection	**21**	**30**
Total	**70**	

The result of Inhibitory effects of Alcoholic (ethanol) of *Quercus infectoria* extract on growth of *C. albicans* and *C.glabrata* in different concentration of *Quercus infectoria* extracts, showed inhibition on growth of *C. albicans* which was the higest inhibition compared with zone inhibition of growth of *G.glabrata* table 2 and figure (1-8) .

The concentration 700mg/ml of *Quercus infectoria* extracts gave 29mm zone of inhibition on growth of *C. albicans* compared with 23.3mm of zone inhibition on growth of *C.glabrata* in same concentration which explain that C.glabrata is more resistant than *C. albicans* , and the *C.glabrata* was difficult treated and resistant to commen antifungal drugs more than *C. albicans* (16). the use of alcoholic solvents have been commonly employed to extract phenolics from natural sources even though alcoholic solvents are not highly selective for phenols because it is able to yield high quantities of total extract compared to other types of solvent (17). The result of inhibitory effects of Aqueous (boil distilled water) of *Quercus infectoria* extract on growth of *C. albicans* and *C.glabrata* at different concentration of Quercus infectoria extracts , showed more inhibition on growth of *C.glabrata* compared with inhibition on growth of C.albicans showed in table 3 and figure (9-16) and showed that the increase of concentration of aqueous *Quercus infectoria* gave more zone of inhibition on growth of *C. albicans* and *C.glabrata* because the used of boiled distilled water was need to dissolve out all these these extracts may have high total tannin contents as tannin is a major compound in *Q. infectoria* which is soluble in water (8and10). Tannin concentration is high in gall oak; a typical analysis is: gallotannin 53.1%, gallic acid 9.5%, ellagitannin 6.9%. Tannins have structural diversity and are divided into two basic groups: hydrolysable type and condensed type. Hydrolyzable tannins include gallic acid and ellagic acid. Both types of tannins have been used in disease treatment, but hydrolysable tannins

have been used more medicinally used to be antifungal and antibacterial activity (13). While the result of Inhibitory effects of Aqueous (distilled water at 25C˚) of *Quercus infectoria* on growth of *C. albicans* and *C.glabrata* were gave inhibition at high concentration of Quercus infectoria extracts from (500-1000 mg/ml) and increase of zone inhibition of growth of *C.glabrata* which increase by increase of concentration of *Quercus infectoria* extracts compared with of zone of inhibition of growth of *C. albicans* in same concentration of *Quercus infectoria* extracts as in table 4 and figure (17-20) . and the used high concentration of *Quercus infectoria* extracts intiated from (500-1000 mg/ml) compared with Aqueous (boil distilled water) and Alcoholic (ethanol) of *Quercus infectoria* because the used distilled water at room temperature 25C˚ at most not dissolved all amount of tannine which explain that using of alcohol and water mixture gave better phenolic constituents extraction than other solvent systems (18).

Table 2: .The effects of Alcoholic (ethanol) of *Quercus infectoria* extract on *Candida albicans* and *C.glabrata* in vitro

Type of extract	Concentration mg /ml	Zone of inhibition mean± SE *Candida albicans*	Zone of inhibition mean± SE *C.glabrata*
Ethanol 95%	100	e 11±0.57	c 15.3±0.33
	200	d 16 ±1.15	bc 16.3±0.66
	300	d 19.3 ±0.66	b 19±0.57
	400	c 20.3 ±0.88	b 20.3±0.33
	500	cd 22.6±0.33	a b 23±1.15
	600	b 25± 0.57	a b 26.6±0.66
	700	a 29±0.57	a b 23.3±0.33
	800	bc 23±1.2	a b 24.3±0.66
	900	b 26±1	a b 25.6±0.33
	1000	a b 27.3 ±0.55	a 27±0.57
		L.S.D= 2.1	L.S.D= 1.6

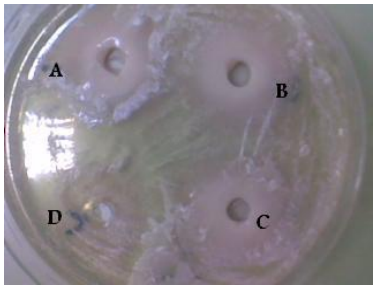

Fig. (1)
A= 400mg/ml , B= 500mg/ml
C= 600mg/ml , D= solvent

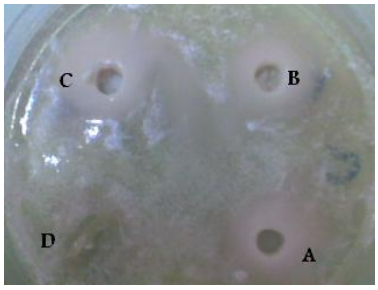

Fig. (2)
A= 100mg/ml , B= 200mg/ml
C= 300mg/ml , D= solvent

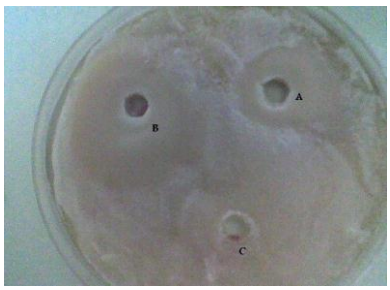

Fig. (3)
A= 900mg/ml , B= 1000mg/ml
D= solvent

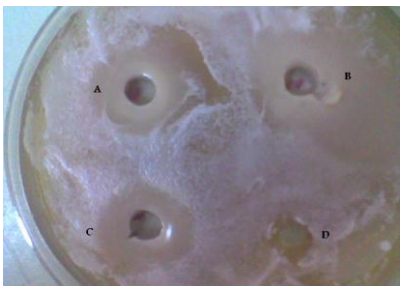

Fig. (4)
A= 900mg/ml , B= 700mg/ml
C= 800mg/ml , D= solvent

Figures (1, 2, 3, 4) : the effects of of Alcoholic (ethanol) of Quercus infectoria extract on *Candida albicans* in vitro at different concentration.

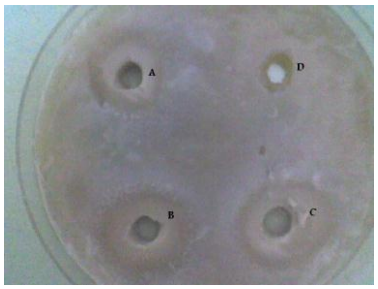

Fig. (5)
A= 400mg/ml , B= 500mg/ml
C= 600mg/ml , D= solvent

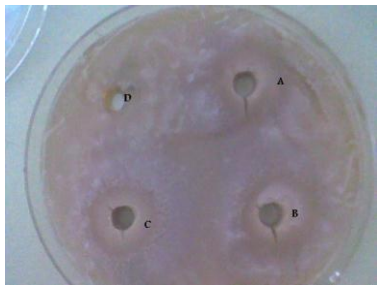

Fig. (6)
A= 100mg/ml, B= 200mg/ml
C= 300mg/ml , D= solvent

Fig. (7)
A= 1000mg/ml , D= solvent

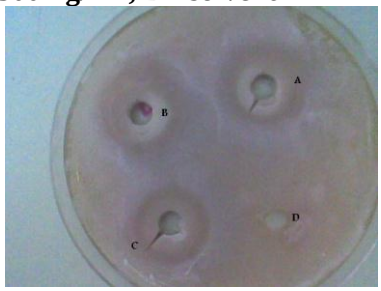

Fig. (8)
A= 900mg/ml, B=800mg/ml
C= 700mg/ml , D= solvent

Figures (5, 6, 7, 8,): the effects of of Alcoholic (ethanol) of Quercus infectoria extract on *C.glabrata* in vitro at differents concentration.

Table 3: The effects of Aqueous (boil distilled water) of *Quercus infectoria* on *Candida albicans* and *C.glabrata* in vitro

Type of extract	Concentration mg/ml	Zone of inhibition mean± SE *Candida albicans*	Zone of inhibition mean± SE *C.glabrata*
Boil distilled water	100	d 7.3±0.66	c 16.6±1.66
	200	c 14±1.15	c 19.3±0.52
	300	c 17±1	bc 21.3±0.88
	400	c 18.3±0.66	bc 21±1.15
	500	bc 20.3±0.33	bc 21.6±0.88
	600	bc 19.6±0.66	b 24±0.57
	700	b 22±0.57	b 24±1.15
	800	b 21±0.57	ab 27.6±1.45
	900	a 27±1	ab 30.3±0.33
	1000	a 27.3±1.2	a 31±0.91
		L.S.D.=2.2	L.S.D=2.7

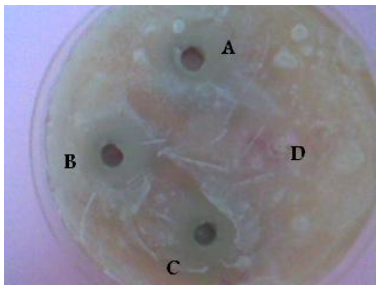

Fig. (9)
A= 400mg/ml , B= 500mg/ml
C= 600mg/ml , D= solvent

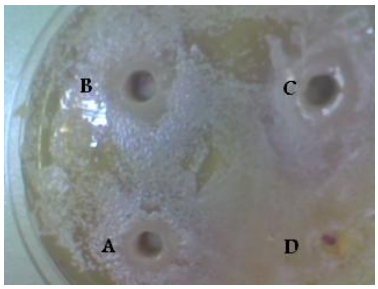

Fig. (10)
A= 100mg/ml , B= 200mg/ml
C= 300mg/ml, D= solvent

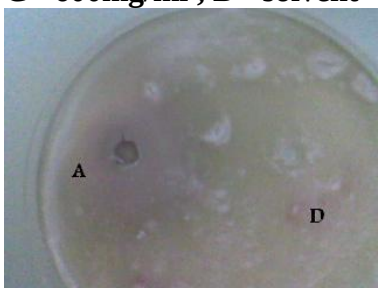

Fig. (11)
A= 1000mg/ml , D= solvent

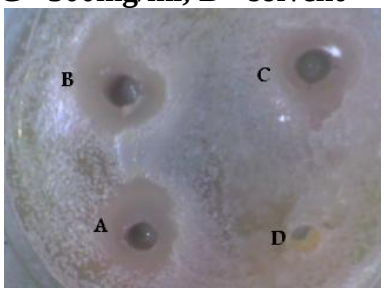

Fig. (12)
A= 700mg/ml , B= 900mg/ml
C= 800mg/ml ,D= solvent

Figures (9 ,10,11,12) : the effects of the Aqueous (boil distilled water) of Quercus infectoria on *Candida albicans* in vitro at differents concentration.

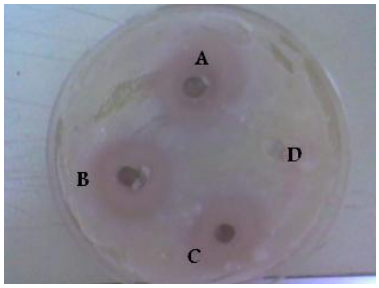

Fig. (13)
A= 600mg/ml , B= 500mg/ml
C= 400mg/ml , D= solvent

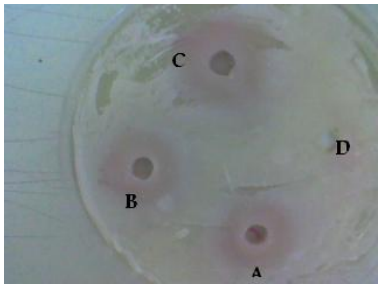

Fig. (14)
A= 100mg/ml , B= 200mg/ml
C= 300mg/ml , D= solvent

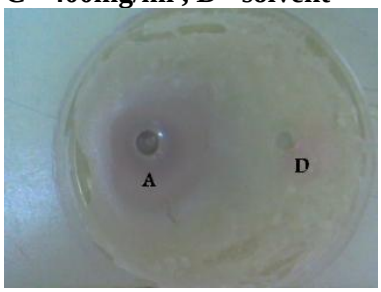

Fig. (15)
A= 1000mg/ml , D= solvent

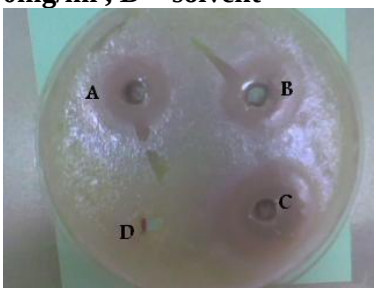

Fig. (16)
A= 700mg/ml , B= 800mg/ml
C= 900mg/ml , D= solvent

Figures (13, 14, 15, 16) : the effects of Aqueous (boil distilled water) of Quercus infectoria on *C.glabrata* in vitro at differents concentration.

Table 4: The effects of Aqueous (distilled water at room temperature 25C) of *Quercus infectoria* on *Candida albicans* and *C.glabrata* in vitro

Type of extract	Concentration mg/ml	Zone of inhibition mean± SE *Candida albicans*	Zone of inhibition mean± SE *C.glabrata*
distilled water at room temperature 25C	500	c 8.6±0.88	d 11.6±1.2
	600	b 15.3±0.66	c 15.3±0.33
	700	b 16±1.15	bc 18.6±0.66
	800	ab 17.3±1.45	b 21±1
	900	ab 18.6±0.62	ab 23.3±1.66
	1000	a 21.6±0.88	a 27.6±0.99
		L.S.D.=2.7	L.S.D.=2.7

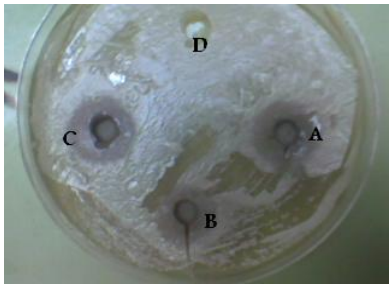

Fig. (18)
A= 800mg/ml , B= 900mg/ml
C= 1000mg/ml , D= solvent

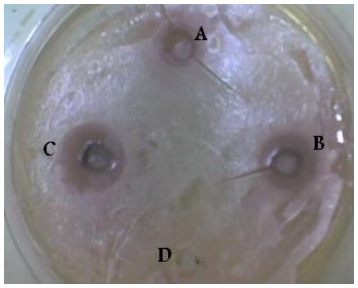

Fig.(17)
A=500mg/ml , B=600mg/ml
C=700mg/ml , D=solvent

Figures (17, 18) : the effects of Aqueous (distilled water at room temperature 25C) of Quercus infectoria on *Candida albicans* in vitro at differents concentration.

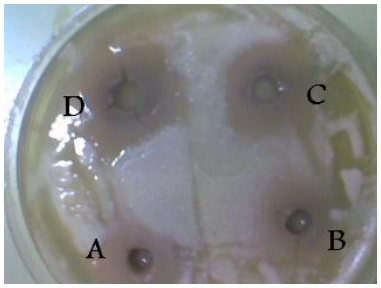

Fig. (20)
A= 700mg/ml , B=800mg/ml
C =900mg/ml , D=1000mg/ml

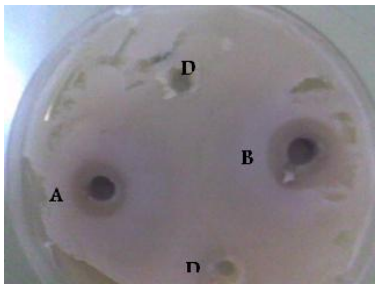

Fig. (19)
A= 500mg/ml , B= 600mg/ml
D= solvent , D= solvent

Figures (19, 20) : the effects of Aqueous (distilled water at room temperature 25C) of Quercus infectoria on *C.glabrata* in vitro at differents concentration.

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