

EFFECT OF CINNAMOM EXTRACT ON REDIAL GROWTH OF *Aspergillus ochraceus* AND CELLULAR GROWTH OF *Candida albicans*

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ABSTRACT :

The experimental was conducted to study effect of Cinnamom ethanol extracted at concentration (20 , 40 , 60 , 80 and 100 mg/ml) on *Aspergillus ochraceus* and *Candida albicans* growth by agar disc diffusion method. Ethanolic extracts of Cinnamon barks showed a significantly inhibition of mycelial growth of *Aspergillus ochraceus* and cellular growth of *Candida albicans*. The highest antifungal inhibition zone of Cinnamon bark extract against *Aspergillus ochraceus* and *Candida albicans* was showed at 100mg/ml (2.79 and 2.48 mm respectively) while lowest antifungal inhibition zone for previous fungi was showed at 20mg/ml, (0.81 and 0.51 mm respectively).

تأثير مستخلص القرفة في النمو الفطري للفطر *Aspergillus ochraceus* و النمو الخلوي للفطر
Candida albican

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الخلاصة :

أجريت التجربة لدراسة تأثير مستخلص الكحول الايثيلي للحاء القرفة عند التركيز (20 و 40 و 60 و 80 و 100 مل/مل) على نمو الفطرين *Aspergillus ochraceus* و *Candida albicans* بطريقة الانتشار على الوسط الصلب .

أظهر مستخلص لحاء القرفة الايثيلي تثبيطاً "معنوياً" لنمو الغزل الفطري لفطر *Aspergillus ochraceus* و النمو الفطري الخلوي لفطر *Candida albicans*. أذ بلغ أعلى قطر تثبيطي مضاد للفطريات لمستخلص الكحول الايثيلي للحاء القرفة ضد *Aspergillus ochraceus* و *Candida albican* لوحظ بتركيز 100 ملغم /مل (2.79 و 2.48 ملغم على التوالي) بينما اقل قطر تثبيطي مضاد للفطريات السابقة لوحظ بتركيز 20 ملغم / مل (0.81 و 0.51 ملغم على التوالي) .

Introduction :

Herbal medicine is still the mainstay about 75 - 80% of the world population,

mainly in the developing countries, for primary health care (2000,Kamboj). Because of the general belief that herbal

drugs are without any side effects besides being cheap and locally available (1998,Gupta and Raina). According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times (2003, Sanjoy and Yogeshwer). The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Many conventional drugs originated from plant sources: a century ago, most of the few effective drugs were plant origin include aspirin (willow bark) , digoxin (from foxglove),quinine(from cinchonabark), and morphine (from the opium poppy) (1999,Vickers and Zollman). The genus Cinnamon is an aromatic tree belong the family Lauraceae, and is one of the most widely studied flowering families, comprising about 250 species. Members of this family are evergreen trees, up to 10-17m high, that grow in south-eastern Asia, Australia, and South America. Traditional uses of Cinnamon throughout Asia, Africa, and Europe have been recorded, where it has been used as a medicine for diarrhea, nausea and chill, or as a spice for seasoning meats. Cinnamon bark is an important source for these purposes, since it contains a great amount of the function-bearing essential oil (2005,Chericoni et al(.

The aim of this study was to test the effect of Cinnamon (Cinnamon zeylanicum) on *Aspergillus ochraceus* and *Candida albicans* growth.

Materials and method :

Plant material

Dried Cinnamon barks were bought from local market in Hilla. Samples were crushed and ground into fine powder by electrical blander machine (2000, Okogun.(

Extraction method :

The extraction method using Soxhlet apparatus. Fifty gram of Cinnamon powdered was put inside the thimble and 500 ml of 85% ethanol was added in the Soxhlet apparatus flask (2014 ,Snehata et al.). The extraction was carried out for 24 hours by heating at 50-60°C until a clear and colorless solvent appeared in the extracting unit of Soxhlet apparatus. After that, the extract was dried by using an incubator at 40°C (2014, Hadi et al .(.

Determinations of weight of water remove :

Weight of water remove was determined by oven drying method. 2gm of yield extract well-mixed sample was accurately weighted in clean, dried crucible (W1). The crucible was allowed in an oven at $135 \pm 5^\circ\text{C}$ for one hour until a weight constant. The crucible was placed in the desiccators for 30 min to cool. After cooling it was weighted again (W2). Weight of water remove was calculated by following formula (2014, Advanced Technology Tackle-:(

Weight of water remove= (W1+Tare)-(W2+Tare(

The samples were kept by wrapping in aluminum foil at -20 °C till used.

The goal of this step is to identify the real weight of the dry matter of the

extract of cinnamon and at the same time to identify the water content found in this extract even taken into consideration for the preparation of different concentrations.

Isolates and identification of

C. albicans and A. ochraceus

C. albicans strain isolated from mid stream of woman urine patients with vulvovaginal candidiasis. The strains were stored in medical Microbiology laboratory, Community health Dept. , Babil technical institute. C. albicans were transferred onto fresh Sabouraud's dextrose agar, (SDA) plates and incubated at 37°C for 24h. Then isolates were re-identified by germ tube test (C.albicans were inoculated into 0.5 ml of human serum and incubated at 37°C for 2.5 hour) (2005 , Samaranayake et al.) and biochemically identified isolates of C. albicans were used (1990, Wang) While A. ochraceus were isolated from samples of the Iraqi rice, inoculated on SDA for 5-7 days at 25 °C and different species of fungi growth were appeared. A. ochraceus identified macroscopically by shape and colour (powdery and yellow like cream) and microscopically appearance as a sun shine depend on the shape and arrangement of metulae and phialides around the vesicle (1988, Samson and Van Reenen-Hokstra.(

Stock solution preparation :

Different concentrations were prepared by tacking 1000 mg extract and dissolved in the 10 ml absolute ethanol

to give 100 mg/ml stock solution and other concentration (80, 60, 40 and 20 mg/ml) were prepared from previous stock solution according to $V1.C1=V2.C2$ equation (2005, Mary et al. (.

Antifungal test :

Fungal cultures of A. ochraceus were incubated at $28^{\circ}\pm 2^{\circ}$ C for five days (1972,Ciegler) , while C. albicans were incubated at 37°C for 24 hours on SDA (2013, Boon et al.(.

A. ochraceus spores suspension was used to inoculate the PDA medium. The method used to prepare spore suspension was used by (1990, Faraj). While C. albicans cells suspension was used to inoculate the Potato's dextrose agar (PDA) medium .Inoculums was prepared in saline solution. Its turbidity was adjusted accordance to the absorbance of 0.08-0.10 at 625nm corresponding to 5×10^6 CFU/ml (2013, Ricardo and Edeltrudes.(

Agar disc diffusion method (2006, Maidment et al .) was used to test for the inhibition activity of the extracts against A. ochraceus and C. albicans . Both fungi were cultured on 20 ml SDA in petri-dishes. An inoculum of 0.1ml fungal spores and yeast cells suspension were spread uniformly over this medium by using the spreader. Fifty μ l from each concentration of Cinnamon Extract (100, 80, 60, 40 and 20 mg/ml) was added into five mm diameter disc made from sterilization filter paper(Whatman No.1) by autoclave 121°C , 15 Ib for 15 minutes (2011, Zohreh,et al.) and dried

by using electrical drier then put on the SDA medium and allowed to stand on the bench for one hour for proper diffusion. Number of petri-dish for each concentration repeated 5 times and each petri-dish contained about four discs .

Inhibition activities of the extracts were determined by measuring the inhibition zones formed around the discs in millimeter. The plates were observed for presence of zones of inhibition around the discs from day five for *A. ochraceus* and 24 hours for *C. albicans* .

Samples of 50 µl of absolute ethanol and normal saline were used in the same manner as negative control. The controls were the solvents used for preparation of plant stock alcoholic extracts and they showed no inhibitions in preliminary studies.

Statistical analysis

Statistical analysis of the experimental results were conducted according to Statistical Package for the Social Sciences (SPSS) version 13.00 where

one way ANOVA was used to assess the significance of changes between experimental groups . The data were expressed as Mean±Standard Errors (SE) and P-value<0.05 was considered statistically significance. LSD was carried out to test the significance levels among means of treatments (2008, Joda.(

Results and discussion :

The present study to evaluate antifungal activity of ethanolic Cinnamon bark extract against *A. ochraceus* by using different concentrations (100, 80, 60, 40, 20 mg/ml) in comparison with normal saline and absolute ethanol , where the results showed significant increase in inhibition zone diameter when increasing the concentration of Cinnamon extract at (P<0.05) . Maximum antifungal inhibition zone was detected at 100mg/ml while minimum antifungal inhibition zone was 20mg/ml, as table and figure (1 .(

Table (1) Inhibition zone diameter of Cinnamon extract against *A. ochraceus* .

Concentration of Cinnamon extract	Inhibition Zone (mm) M±SE
100 mg/ml	2.79±0.14 A
80 mg/ml	2.54±0.20 A
60 mg/ml	2.48±0.12 A
40 mg/ml	1.38±0.11 B
20 mg/ml	0.81±0.03 C
Normal saline	0.00±0.00 D
Absolute ethanol	0.00±0.00 D

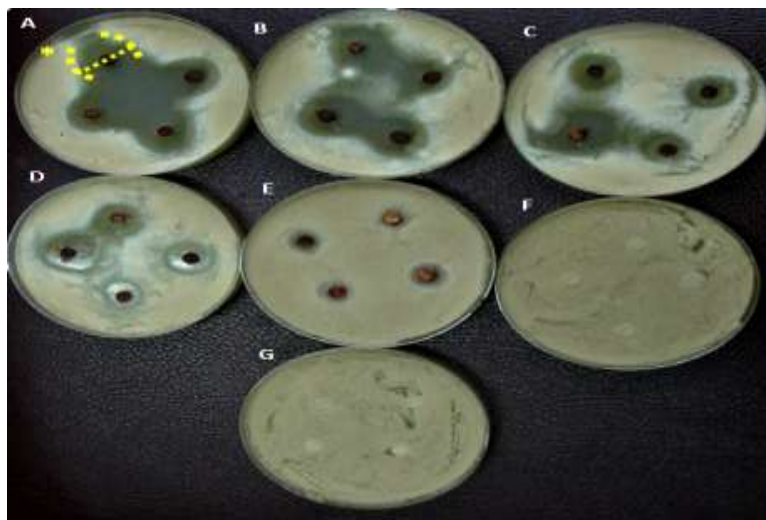


Figure (1):- Inhibition Zone of Cinnamon extract against *Aspergillus ochraceus* inoculated on SDA for 5-7 days at 25 °C (A=100mg/ml , B=80mg/ml, C=60mg/ml , D=40mg/ml , E=20mg/ml , F=Normal saline and G= Absolute ethanol) .

The results of this study were agreement with (2012,Sameer) who showed that the alcoholic extracts of Cinnamon barks have antifungal activity against *Penicillium digitatum* and *Aspergillus niger* and he explain antifungal activity of Cinnamon crude extracts can be resulted from phytochemical constituents (flavonoids, tannins, alkaloids, saponins, terpens and steroids) (1984, Harborne.(Antimicrobial activity of Cinnamon bark may be resulted from volatile gas phase of Cinnamon oil and clove oil which have a good potential to inhibit growth of spoilage fungi, yeast and bacteria normally found on Intermediate Moisture Foods (IMF) (2006, Matan, et al.). Also Cinnamon bark is an important source for medical uses, since it contains a great amount of the function-bearing essential oil. The bark- derived Cinnamon (termed Cinnamon hereafter) contains 45% ~

65% cinnamaldehyde, 12% ~ 18% eugenol (2005,Chericoni et al.). In other study showed the synergistic effect of Cinnamon extract components (Cinnamaldehyde with eugenol) could be attributed to the integrated actions of cell wall alteration and interference of cell wall synthesis of fungi (2008,Yen and Chang.(

In the current study antifungal activity of Cinnamon extract at different concentrations may be resulted from cinnamaldehyde and eugenol which causes stopped cell wall synthesis of mycelia cells of *A. ochraceus*.

The results showed significant increase ($p<0.05$) in zone inhibition diameter when increasing the concentration of ethanolic Cinnamon bark extract (100, 80, 60, 40, 20 mg/ml) against *C. albicans* by using plate diffusion method in comparison with distilled water and absolute ethanol.

Maximum antifungal inhibition zone was detected at 100mg/ml while minimum antifungal inhibition zone was 20mg/ml, as table and figure (2).*

Table (2) Inhibition zone diameter of Cinnamon extract against *C. albicans*.

Concentration of Cinnamon extract	Inhibition Zone (mm) M±SE
20 mg/ml	2.48±0.22 A
40 mg/ml	1.77±0.08 B
60 mg/ml	1.13±0.09 C
80 mg/ml	0.84±0.06 C
100 mg/ml	0.51±0.81 D
Distilled water	0.00±0.00 E
Absolute ethanol	0.00±0.00 E

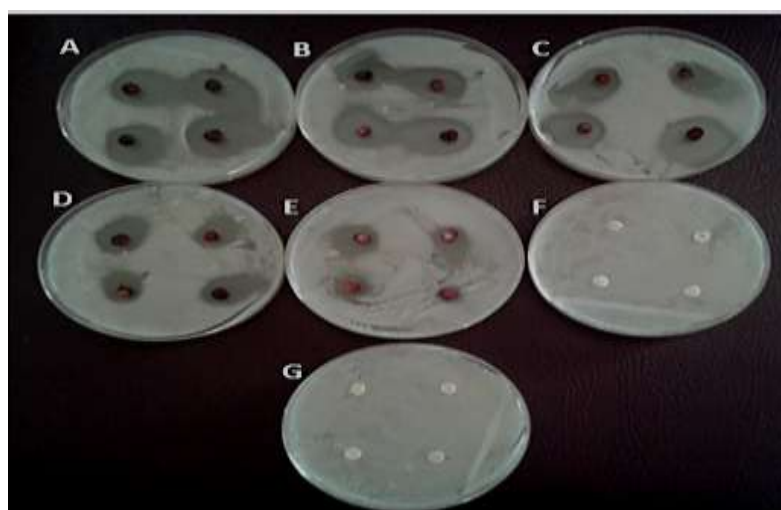


Figure (2):- Inhibition Zone of Cinnamon extract against *Candida albicans* inoculated on SDA for 24 hours at 37 °C (A= 100mg/ml, B=80mg/ml, C= 60mg/ml , D= 40mg/ml , E= 20mg/ml , F= distilled water and G=Absolute ethanol.)

The results obtained in this study are in agreement of (2011,Ibtisam) who showed alcoholic Cinnamon extract was strongly inhibited the growth of *C. albicans* . Also these results were similar to those described by (2013, Ricardo, and Edeltrudes) which evaluated the antifungal activity of essential oil of *C. zeylanicum* on candida strains resistant to fluconazole .

The zones of inhibition against *C. albicans* strain resulting from the exposure to different Cinnamon extract concentration which contains phytochemical component (phenols, alkaloids, steroids and tannins in varying concentration as well as cinnamaldehyde, p-methoxy cinnamaldehyde, cis-2-methoxy cinnamic acid and cinnamic acid)

(2013,Ahmad et al .) caused *C. albicans* inhibition growth which resulted from irregular hollows appeared on the surfaces, inside organelles were destroyed and the cells burst after treatment , cell walls were damaged, organelles were destroyed and most cytoplasm became empty bubbles (2012,Wang et al .).

In other study showed antifungal effects of Cinnamon extract by using scanning electron microscopy suggest that this potential bioactive extract has distinct influence on *Candida* cell by causing breakage in the cell membrane and leakage of cellular content (2013,Ahmad et al.).

Conclusions

Cinnamon extract have significant antifungal activity against mold (*A. ochraceus*) and yeast (*C.albicans*).

Recommendations :

Study of molecular effects of the Cinnamon extract on the moulds and yeasts by the electron microscope technique.

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