

Evaluation of the activity of chemical components of *Calvatia craniformis* mushroom in treatment of Candidal vaginitis in Khalis city women in vitro .

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Abstract

Candidal vaginitis is a fungal disease caused by yeast fungi resulting in vaginal discharge , irritation , and itching .

This study aims to evaluate the efficacy of the chemical components of *Calvatia craniformis* mushroom powder in treatment of Candidal vaginitis in women in vitro .

Three different concentrations of ethanolic extract were prepared from *C. craniformis* mushroom powder (1 , 0.8 , 0.6) % and used as comparison with common antifungal drugs as fluconazole and nystatin . Statistical analysis of values is revealed significant effect ($p < 0.05$) of two concentrations (1 , 0.8) % ; this result reflected by values of the diameter of zone of inhibition were be (2 , 1.6) centimeter when were compared with the effect of fluconazole and nystatin which be (0.9 , 0.8) centimeter respectively .

تقويم فاعلية المكونات الكيميائية للفطر *Calvatia craniformis*

في علاج التهاب المهبل المتسبب عن الكانديدا في نساء مدينة الخالص في الزجاج *In vitro*

وسيلة عبد الرضا عبد الرزاق

الخلاصة

يعد التهاب المهبل بالكانديدا من الامراض الفطرية المتسببة عن الخميرة الفطرية ، ينتج عن ذلك نزول سوائل التهابية من المهبل ، تهيج للانسجة الداخلية وحكة .
تهدف الدراسة الى تقويم كفاءة مكونات الفطر *C. craniformis* الكيميائية في علاج التهاب المهبل المتسبب عن الكانديدا البيضاء في النساء ، في الزجاج .

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تم تحضير ثلاثة تراكيز مختلفة من المستخلص الكحولي لمسحوق الفطر *C. craniformis* (0.6 , 0.8 , 1) % بالمقارنة مع الادوية شائعة الاستعمال مثل fluconazole و nystatine . أظهر التحليل الاحصائي للقيم وجود تأثير معنوي ($p < 0.05$) للتركيزين (0.8 , 1) % ؛ وعكست هذه النتيجة بتقييم اقطار التثبيط التي كانت (1.6 , 2) سنتيمتراً بعد مقارنتها مع تأثير الادوية المستخدمة مثل fluconazole و nystatine إذ كانت اقطار التثبيط (0.8 , 0.9) على التوالي .

الكلمات المفتاحية: - فطريات النانيد , التهاب المهبل .

Introduction

Vaginitis is an inflammation of the vagina , and is often associated with an irritation or infection of the vulva ⁽¹⁾ . The three main kinds of vaginitis are , bacterial vaginosis , vaginal candidiasis and trichomoniasis . A woman may have any combination of vaginal infections at one time ⁽²⁾ .

Candidiasis is a polyorganic disease , caused by yeast fungi (*Candida albicans* , *C. glabrata* , *C. tropicalis*) .

The predisposing factors were be divided to ; endogenous long lasting diseases such as diabetes mellitus , avitaminosis , and exogenous factors , that predispose fungal colonization and decrease the general reactivity of the organism (long treatment with antibiotics) and local immunity in vaginal mucosa high virulence of candidas ⁽³⁾ . The symptoms that arise vary with infection , and it must be noted that infected women may also be asymptomatic .

Vaginal candidiasis present as itching of the genital area , inflammation (irritation , redness and swelling caused by the presence of extra immune cells) of the labia majora , labia minora , or perineal area , vaginal discharge with foul odor and pain , irritation with sexual intercourse ⁽⁴⁾ . *Candida albicans* appears as large , round , white or cream colonies on agar plates ⁽⁵⁾ .

A diagnosis of *Candida vulvovaginitis* is made after finding a normal vaginal PH (4 to 4.5) and the presence of many yeast cells in the sample of vaginal discharge or growth yeast on laboratory media ⁽⁶⁾ . *Candida albicans* may be found at rate 30%

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in women vagina without any pathological signs , but under certain conditions is become pathogenic and cause vaginal candidiasis and vulvovaginitis especially in women at age of reproduction ⁽⁷⁾ .

Vaginal infections is treated topically with nystatin or fluconazole ⁽⁴⁾ . *Candida* vulvovaginitis is most often treated by the application of medicated gels , creams , or suppositories applied directly to the vagina . The antifungal drugs used to treat *Candida* vulvovaginitis include oral fluconazole , butoconazole , clotrimazole , miconazole and ticonazole . Most require only one or a few days of therapy to be effective . Women who have recurrent *Candida* infections may receive treatment for several weeks and then some form of a long-term preventative treatment ⁽⁸⁾ .

The mushroom used in this study is puffball mushroom, belongs to Basidiomycota division, Lycoperdaceae Family, *Calvatia* genus, *craniformis* species (figure -1a,b)⁽⁹⁾.



(Smith, 1951)

Figure- 1a: represent the mushroom in the world.

b : represent the longitudinal section .

The study aims to evaluate the efficacy of the ethanolic extract of *Calvatia craniformis* mushroom in treatment of candidal vaginitis in vitro.

The figure (2a,b) are represents the discovered mushroom in Jadidat Al-Shat Village in Hibhib city and Bany Saad city-Diyala province for first time in Iraq according to the

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diagnosis of laboratory of fungus researches and plant diseases in the college of Agriculture-Baghdad University with certification of professor Kamil Salman Jabor as a taxonomist.



**Figure- 2a: represent the mushroom in the Iraq .
b: represent the longitudinal section.**

Materials and Methods

1- Preparation of antifungal agent :

- a. Fruiting body of *Calvatia craniformis* mushroom is dried and crushed in sterile Petri dish to obtain a yellow – brown powder .
- b. By using a balance weigh 10 gm from the powder .
- c. The weigh is dissolved in 10 ml ethanol and mixed perfectly . The concentration become 1 gm / 1 ml and considered as stock solution .
- d. By disposable syringe pull (0.1 , 0.08 , 0.06, 0.04) ml from stock solution and diluted by (10 , 9.2 , 9.4,9.6) ml distill water to obtain (1 , 0.8 , 0.6, 0.4) % concentrations respectively .
- e. Circular pieces from filter paper are prepared and sterilized by autoclave at 121 degree of temperature for 15 minutes and rinsed in each concentration , then conserved in refrigerator in Petri dish till be used .

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2- Vaginal swabs were taken from sixty women who suffered from vaginal secretion after menstruation. Most of them were non pregnant.

The swabs were taken by sterile speculum under observation of a specialist in gynecology and obstetrics in Khalis hospital and special clinic. The swabs were cultured on specific media of fungal growth as Sabouraud dextrose agar at (25 ± 2) degree of temperature in incubator for 5-7 days in laboratory of biology department - college of education for pure science – Diyala university.

Antifungal drugs were used as fluconazole and nystatin as comparative treatment in culture media. Some diagnostic tests were done to diagnose the fungal species as formation of implantation tube, formation of disappearing spores, surface growth character, ability of sugar fermentation and the ability of carbohydrates metabolism.

Statistical Analysis

The differences are compared by using (F-test) at $p < 0.05^{(10)}$.

Result and Discussion

Twenty seven samples were revealed fungal growth in Sabouraud agar and the rate of infection was 45% according to the number of the total samples.

The thirty three were given negative result. After using laboratorial diagnostic examination of fungal infection, the causative agent in all the samples were *Candida albicans*, and the table-1 reveals the biochemical tests which were done.

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Table – 1

Represent the results of biochemical tests which were done in diagnosis of candidal infection

| Type of isolate | Formation of disappear spores | Formation of implantation tube | Surface growth character | C. albicans ability in sugar fermentation | | | | C. albicans ability in carbohydrate metabolism | | | | |
|-----------------|-------------------------------|--------------------------------|--------------------------|---|-----------|---------|---------|--|-----------|---------|---------|--------|
| | | | | Glucose | Galactose | Sucrose | Maltose | Glucose | Galactose | Sucrose | Maltose | Starch |
| C. albicans | + | + | - | + | - | - | + | + | - | - | + | - |

These results are coming near to the results of Alazawi study who is found the rate of the candidal infection of women in Baghdad was be 53% ⁽¹¹⁾, while the rate of infection is differ in other study which be 22.1% ⁽¹²⁾. Also other study referred to 17.4% rate of infection ⁽⁷⁾. Study was done on pregnant women to diagnose the causes of inflammation of the cervix, vagina, and abortion or intrauterine embryonic death and the rapture of amniotic membrane, the *Candida albicans* represents 44% from the total swabs which are taken from 1425 pregnant women ⁽¹³⁾.

The Petri dish number-1 reveals the effect of the four concentrations of ethanolic extract of mushroom powder on candidal colonies. The (1, 0.8, 0.6)% were be active and, the 0.4% was be inactive.

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Figure-3: Represent Petri dish number-1 which reveals the effect of the four concentrations which were prepared from the mushroom on candidial colonies.

The results in table – 2 are represent the sensitivity of the causative fungus to the common antifungal drugs which are used in treatment of the candidal vaginitis and also reveals the effect of the three concentrations which were prepared from the *C. carniformis* mushroom powder in addition to the diameters of zone of inhibition which is showed in Petri dish number -2.

Table – 2

Represent the effect of common antifungal drugs and mushroom extracts on *Candida albicans*

| The drugs | <i>Candida albicans</i> | Zone of inhibition / cm |
|---------------|-------------------------|-------------------------|
| Fluconazole | + | 0.9 |
| Nystatine | + | 0.8 |
| 1% Alcohol | + | 2 * |
| 0.8% solution | + | 1.6* |
| 0.6% | + | 1.2 |

($p < 0.05$) *

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Figure -4: Represent the Petri dish number-2 which reveals the effect of the three concentrations which are prepared from the mushroom and some antifungal drugs .

The media culture in figure -4 revealed the effect of the antifungal drugs as fluconazole and nystatine and the three concentrations of *C. carniformis* extract (0.1 , 0.08 , 0.06) % . The zones of inhibition were be (0.9 , 0.8 , 2 , 1.6 and 1.2) centimeter respectively . All the drugs and preparations were be active against the *C. albicans* .

The statistical analysis reveals significant effect ($p < 0.05$) of 1% and 0.8% concentrations of the extract on fungal colonies when compared with other drugs as fluconazole and nystatine which is shown in table - 2 .

The medical analysis of this mushroom proved the presence of three components; the first is calvatic acid which has chemical formation P-carboxyphenyl-azoxycarbonitrile (14) . This calvatic acid reveals strong antimicrobial activity against the Gram- positive bacteria, and weak action against the Gram- negative bacteria and against the yeast and fungi like *Saccharomyces cerevisiae* and some *Candida* species and *Trichophyton* asteroids (15) .

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The second components from chemical analysis and spectroscopic means of the mushroom is hydroxyphenylazoformamide derivatives which has three chemical compounds, 4-hydroxyphenyl-1-azoformamid, 4-hydroxyphenyl-ONN-azoformamid and 2-methylsulfonyl-4-hydroxy-6-methylthiophenyl-1-azoformamid, which we named it craniformin (phenolic tautomer of rubroflavin), and also three components known steroids, ergosta-4,6,8 (14), 22-tetraene- 3-one, ergosta-7,22-diene-3-01 and ergosterol peroxide (16) . The hydroxyphenylazoformamide derivatives or craniformin have phenolics in its formation which are endowed with interesting biological activities as a broad spectrum bactericidal and fungicidal effect represented by *Candida albicans*, *Aspergillus niger*. Also the craniformin has azol compound which acts as antifungal azol derivatives for example Fluconazol and Itrakanzol (17) . They inhibit the synthesis of ergosterol by blocking the action of 14- α -demethylase and stop proliferation of the fungus (18) . The action of azol compounds reveals inhibition fungal mRNA transcription and treating fungal infections in human and animal subjects and fungal infestations in plants (19) . The third component which resulted from chemical analysis is three steroid compounds, and these are lipophilic and this character facilitates entry into the cells. Also the specific binding proteins which are present in any animal cells may facilitate steroids entry into target tissues (20) .

Foiani et al., (1994) proved that the B subunit of the DNA polymerase α - primase complex in *Saccharomyces cerevisiae* has essential function at initial stage of DNA replication and this should be inhibited by ergosterol peroxidase which results in inhibition of the proliferation of the yeasts and fungus (21) .

Also the chemical analysis of mushroom powder which is done in White Fields Company for Chemical and Engineering Studies and Consultations in Baghdad – Iraq proved the presence of different materials as β -glucans, , ergothioneine and gallic acid.

The β - glucans are polymers of β - (1,3)-D -glucose (with or without β -(1,6)-D-glucose side chains) found in the cell walls of many bacteria ,plants and yeasts (22) . β - glucan bind to glucan receptors on phagocytic cells (23) ,and cause these cells to become "activated " (24) . Other material is ergothioneine (ET) which is an unusual sulfur-containing derivative of the

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amino acid, histidine. It may be represent a new vitamin whose physiologic roles include antioxidant cytoprotectant (25) .

The last material termed Gallic acid ; it is a trihydroxybenzoic acid, a type of phenolic acid. Gallic acid is found both free and as part of tannins .Salts and esters of Gallic acid are termed "gallates". Gallic acid seems to have anti-fungal and anti-viral properties. Gallic acid act as an antioxidant and help to protect human cells against oxidative damage(26). Gallic acid was found to show cytotoxicity against cancer cells, without harming healthy cells(27) .

β -glucans , ergothioneine and Gallic acid are displaying immunological , antimicrobial and physiologic cytoprotection effect respectively.

These effects may be led to enhance the immunity of patients ,and improve the infected tissue status result in a rise in possibility of using these preparations in vivo.

There is other studies on plant extracts and it's effects on *Candida* species in vitro as the study which proof the ability of oil extracts from lemon fruit to inhibit the growth of candidal vaginitis (28) . Other study showed the high ability of thyme oil to inhibit *Candida albicans* growth , while the pennyroyal and lemon oils haven't any inhibitor effect on *Candida* species (29) .

Because of the strong anti-candidal effects of ethanolic extract of *Calvatia craniformis* mushroom which was proved through in vitro tests in our study , it is proposed to do further research in treatment of candidiasis with mushroom extract experimentally specially in rabbits .

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