

Antifungal Activity of Acetonic Extracts of *Syzygium aromaticum*' Flowers and *Mentha longifolia*'s Leaves Against Clinical Isolates of *Candida albicans*

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Yasser Muafaq Al-qertani¹ and Shaymaa Majeed Mohammed²

Biology Department- College of Sciences- Diyala University -Iraq

[¹alqertaniyasser@gmail.com](mailto:alqertaniyasser@gmail.com)

Received: 14 December 2018

Accepted: 28 February 2018

Abstract

The current study was performed to evaluate antifungal potential of acetonic extracts of *Syzygium aromaticum*'s flowers (*S. aromaticum*) and *Mentha longifolia*'s Leaves (*M. longifolia*) on the growth of pathogenic *Candida albicans*. The results illustrated that the growth of the yeast was inhibited in both extracts when acetonic extract (20, 40, 60) mg/ml of *S. aromaticum*, Inhibition percentage of *C. albicans* were 56.22%, 67.22% and 100% respectively, compared to the same concentrations of leaves extract *M. longifolia* the inhibition percentage of *C. albicans* were 4.4%, 14.59% and 24.52%, respectively. Also, the results showed that t increasing the concentrations of acetonic extract led to increase the percentage of inhibition of *C. albicans*. Our study concluded that extract of *S. aromaticum*) could possibly be used for future pharmaceutical applications as anti-candidiasis better than *M. longifolia*.

Key words: Antifungal, acetonic extracts, *Syzygium aromaticum*, *Mentha longifolia*, *Candida albicans*

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الفعالية التضادية للمستخلصات الاستوائية لزهرة نبات القرنفل واوراق النعناع ضد بعض عزلات خميرة

Candida albicans

ياسر موفق الكرطاني و شيماء مجيد محمد

قسم علوم الحياة - كلية العلوم - جامعة ديالى-العراق

الخلاصة

أجري هذا البحث لتقييم الفعالية المضادة للفطريات للمستخلصات الاستوائية لأزهار نبات القرنفل واوراق النعناع على نمو خميرة الـ *Candida albicans* الممرضة. اوضحت نتائج ان نمو الخميرة الممرضة قد تم تثبيطه عندما تم استخدام كل من المستخلصين المذكورين وبتركيزهما المختلف. باستخدام المستخلص الاستوائي لأزهار القرنفل و بتركيز (20، 40، 60) ملغم/مل، كانت النسبة المئوية لتثبيط الـ *Candida albicans* (56.22%، 67.22%، 100%) وعلى التوالي، مقارنة بـ (4.40%، 14.59%، 24.52%) من تثبيط نمو الخميرة الملاحظ عند استخدام مستخلص اوراق النعناع الاستوائي وبنفس التراكيز (20، 40 و 60) ملغم/مل. اظهرت نتائج البحث انه بزيادة تركيز المستخلص الاستوائي، تزداد النسبة المئوية لتثبيط الخميرة. هذه الدراسة اشارت الى امكانية الاستفادة من الفعالية المضادة للفطريات لمستخلص ازهار القرنفل في الصناعات العلاجية المستقبلية ضد خميرة الـ *Candida* المعزولة كونها الافضل فعالية مقارنة بمستخلص اوراق النعناع.

الكلمات المفتاحية: الفعالية التضادية، المتخلص الاستوائي، ازهار القرنفل، اوراق النعناع، خميرة المبيضات

Introduction

The recent antibiotic resistance and reoccurrence of fungal infections attacked the attention of scientists to perform intensive studies about such problematic issue. It has been suggested that immune deficiency disease (AIDs), diabetes, and leukemia are related to fungal re-infection [10]. Candidiasis is a common opportunistic diseases of skin and oral cavity caused by *Candida* species. The most common and worldwide distributed one is *C. albicans*, in addition to *C. guilliermondii*, *C. dubliniensis*, *C. tropicalis*, and *C. krusei* [19]. These pathogenic yeasts

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posses several virulence factors including; adherence, germ tube formation and enzymes production, such as phospholipase [22]. Candidiasis is a common recurrent infection due to the existence of the causative agent, *C. albicans* mainly, as a commensal fungi in oral cavity, vaginal and digestive system[11], Getting infected with human immunodeficiency virus (HIV), diabetes, Leukemia or administration of broad spectrum antibiotics may enhance converting the commensal species to opportunistic microbes [20& 7]. Currently, several plant pharmaceuticals have been used as natural anti-fungal formulations instead of synthetic anti-fungi which are used as skin ointment due to their toxicity on liver and pancreases when used at high concentrations [13]. So, an alternative antimicrobial substances are urgently required instead of using toxic treatment in order to challenge the fungal re-infection. The beneficial and antimicrobial effect of natural extracts from medical plant have been studied recently and evaluated since they are free of synthetic phytochemicals, the chemicals that posses a negative health impact. In addition, new methods and preservatives techniques, in capsules and dry tablets, have been innovated to facilitate using of these plants and maintain their antimicrobial activity (3). Furthermore, these plants regard as a repository of many effective ingredients, such as essential and volatile oils, ketons, and tannins which are produced as secondary metabolites (16). The study of (18) referred to extracts' antifungal activity of thymus, pennyroyal and lemon against *Candida* spp. The oil extract of thymus showed a higher activity through inhibiting and/or killing *Candida albicans* in comparison to pennyroyal and lemon. Moreover, the oil extracts of thyme and clove was evaluated against *Candida albicans* by (17) who reported that minimum inhibitory concentration (MIC) of thymus oil extract is much lower than the MIC of clove against studied fungi. In addition, the growth of fungi in the presence of black cumin, clove, cinnamon was previously evaluated by (1) who found that oils of these spices inhibited the growth of the tested fungi. This study comes to identify the minimum inhibitory concentrations (MICs) of studied extracts that could be used to inhibit or kill the clinical isolates of *C. albicans*.

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Materials and Methods

Samples Collection and Isolation of *C. albicans*

Samples were collected using sterile swabs from patients showed a clinical sings of Candidiasis. A one hundred samples were collected from vagina, mouth, middle ear, skin, nails and urine from patients who were suffers of fungal infections in oral cavity, skin (superficial mycosis) and urinary tract inflammation (UTI). Samples were collected at Educational Hospital of Ba'qubah and inoculated directly in a test tubes containing phosphate buffer saline (PBS). All samples were kept at refrigerator (4°C) until the time of inoculation and identification.

Direct Examination

Each sample was directly examined microscopically and stained using Gram staining method to identify the Gram-positive yeast [8&13]. For direct examination and identification of yeast cells and pseudomycilium, light microscope was used at (40X, 100X). For direct examination method, the sensitivity percentage was measured by [14] according to following equation:

$$\text{Sensitivity\%} = \frac{\text{No. of positive samples}}{\text{No. of (Negative samples + positive results)}} \times 100$$

Culture Media and Growth Conditions

Swabs from infected area were inoculated onto sabouraud dextrose agar. All agar plates were incubated for 2-4 days at 25-30°C. The morphology and features of yeast cells, including color, texture and shape of colonies, on culture media were monitored and recorded daily.

Staining of Fungal Colonies

After incubation and fungal colonies have grown on sabouraud dextrose agar with Chloramphenicol, Gram staining techniques was used for yeast buds identification. Lactophenol cotton blue stain was also used for observing budding and psedomycilium.

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Germ Tube Formation

The method of germ tube formation was performed according to [4] with minor modifications. Briefly, 2 ml of egg yolk was inoculated with a part of fungal colony growing on sabouraud dextrose agar. The mixture was incubated for 2-3 h at 30 °C. After incubation, a drop was taken and placed on glass slide for microscopic identification of germ tube formation.

Preparation of Acetonic Extracts

Acetonic extracts of *S. aromaticum* and *M. longifolia* were prepared following [21] with some modifications. Briefly, the powder of plants 20 gm were mixed with 250 ml of acetone 75%. The mixture was incubated with shaking for 48 h at 35°C. A gauze was used for initial separation of the leaked which was centrifuged (3000 rpm, for 10 min) and the supernatants were filtered by filter papers 0.22 µm. Rotary evaporator was used for complete evaporation of acetone and getting a crude extract of plant. The extracts were then kept into test tubes which sealed tightly and kept at refrigerator at 8°C for further evaluation.

Antifungal efficiency of extracts against pathogenic *C. albicans*

The sterility of plant extracts was evaluated and confirmed by inoculating 0.01 ml of each extract onto sabouraud dextrose agar and then incubating them for 3-7 days at 37°C. The antifungal activity assay was conducted following [12] for assessing antifungal effect of acetonic extracts on the growth of *C. albicans*. Briefly, extracts at various concentrations (20, 40, 60 mg/ml) were prepared by mixing them well with sabouraud dextrose agar at 45°C. After solidifying of Agar, A disc of 6 mm in diameter was cut from the margin of colony *C. albicans* and placed on the center of the culture medium for 7 days. A control groups (culture medium only and culture medium with yeast growth) were included in this experiment. The treatments and control plates, were incubated at 28-30°C for one week. After incubation, the diameters of colony were measured. The following equation was used to calculate the percentage of yeast growth inhibition:

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$$\text{Inhibition \%} = \frac{\text{Diameter of fungal growth of control group} - \text{Diameter of fungal growth of treated group}}{\text{Diameter of fungal growth of control group}} \times 100$$

Statistical analysis

SPSS program was used for data analysis.

Results and Discussion

In our study, ninety-seven swabs were collected from oral cavity, skin, vagina, middle ear, and nails. The samples were directly examined under microscope and cultivated on sabouraud dextrose agar with chloramphenicol. The results showed that 40 (41.24%) of the total samples were positive at both microscopic and cultivation examination. In addition, 35 (36.08%) were positive at fungal cultivation but negative at microscopic examination while 10 (10.3%) samples were positive at microscopic but negative at cultivation tests. The rest, 12 (12.38%) samples were negative to both tests. The reasons behind negative results of microscopic and cultivation tests are suggested to be the Insufficient numbers of the collected samples or the infection caused by other etiologies rather than fungi [15]. In the same regard, Collee et al. [8] stated that the abuse and overuse of local treatment without physician's consultancy may be an additional reason behind negative results. In comparison to control group in table 1, there was no significant differences in fungal growth inhibition% at $P \geq 0.05$ when 20mg/ml of acetonic extracts of *M. longifolia* was applied. However, A significant differences were identified in fungal growth inhibition% between the extract concentrations 40mg/ml, 60mg/ml and control group at $P \geq 0.05$. In table2, the differences of fungal growth inhibition% were not statistically significant when 20gm/ml, 40gm/ml of acetonic extracts of *S. aromaticom* were applied compared to the control group, but a significant difference was found between 60gm/ml of extract and control group at $P \geq 0.05$.

Our data sheds the light on the relationship between the concentrations of the tested extracts and the diameter of the fungus colony. A reduction in the diameter of the fungal colony was greater when the concentrations of the acetonic extract increases. The antimicrobial potential of the extract is proposed to be due to its active composition, such as flavons, alkaloids and tannins. The findings of [5] study proposed that active ingredients of plant extracts are highly

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toxic compounds inhibiting the growth of fungi. Non-specialized interaction of extracts' active molecules with succinate dehydrogenase and NADH will cause an inhibition of enzymes and the cofactors that responsible for essential metabolic processes, leading to cellular death [23]. Moreover, our results is in agreement with [2,6 & 9]. who reported that inhibition of pathogenic fungi, *Melina azedarach*, *Fusarium monili forme*, *Aspergillus flavus*, and *Candida albicans* were due to an interaction of these extracts with NADH. The authors [2,6 &9] found that alcoholic extract and watery extract of *M. longifolia* as antifungal agents against the tested fungi. Anitha and Kannan [6] used different types from *S.aromaticum* extract and another plants against *Aspergillus flavus* and its toxin (aflatoxin B1) The authors concluded that plant extracts could be used as the better choices and alternatives treatment for fungal infections and re-infections compared to the usage of synthetic antibiotics. This conclusion comes from the results of the current report in which we found that acetonic extracts of *S.aromaticum* posses a potential inhibitory effect on clinical isolates of *C. albicans* growth. The findings of this work are interesting in regards of future pharmaceutical applications.

Table 1: Inhibition % of *C. albicans* treated with acetonic extracts of *M.longifolia*

Concentrations of extracts	0mg\ml	20 mg\ml	4 mg\ml	6 mg\ml
Diameter of fungal colony	7.95	7.60	6.79	6
Inhibition %	0%	4.40%	14.59%	24.52%

Table 2: Inhibition % of pathogenic yeast treated with acetonic extracts of *S. aromaticum*

Concentrations of extracts	0 mg\ml	20 mg\ml	40 mg\ml	60 mg\ml
Diameter of fungal colony	7.95	7.60	6.79	6
Inhibition %	0%	56.22%	67.22%	100%

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