# Evaluation of Antimicrobial Activity of *Aspergillus* Spp. Secondary Metabolite Against Some of Clinical Bacteria and Fungi

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## Abstract

The present study investigates the inhibitory effect of secondary metabolite of fungus *Aspergillus paraciticus, Aspergillus flavus,* and *Aspergillus oryzae* against four pathogenic bacteria (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermids,* and *Staphylococcus aureus*), and three clinical candida species (C. *albicans*, *C.famata* and *C. guillermondii* ) by well diffusion method . The results showed that the secondary metabolite of species *Aspergillus oryzae* has clear inhibitory activity against subjected bacterial isolates and candida species in the concentrations of( 50 ,100 ,150 )mg / ml, as compared with the antibiotics amoxicillin and nystatin. There is reverse relationship between the concentration of secondary metabolite and microbial growth. Bacterial pathogens are more susceptible to fungal secondary metabolite than candida species. *Aspergillus paraciticus* and Aspergillus *flavus* secondary metabolite have slight inhibitory effect on pathogenic bacteria in the concentration more than 200mg/ml, but they don't have any inhibitory effects against subjected *Candida.* In conclusion, present study showed that secondary metabolite of *Aspergillus oryzae* have antimicrobial activity against various pathogenic bacteria and fungi.

**Key words:** *Aspergillus* spp., secondary metabolites, Antimicrobial activity.

# تقييم الفعالية الحيوية لمركبات الايض الثانوي لـ .Aspergillus Spp
# ضد بعض البكتيريا والفطريات السريرية

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**الخلاصة**

اظهرت الدراسة الحالية التأثير المثبط لمركبات الايض الثانوي لأنواع الفطريات Aspergillus paraciticus و Aspergillus flavus و Aspergillus oryzae ضد أربعة أنواع من البكتيريا المسببة للأمراض (*Escherichia coli* و *Pseudomonas aeruginosa* و *Staphylococcus epidermids* و*Staphylococcus aureus* ) وضد ثلاثة أنواع المبيضات السريرية (C. *albicans* و *C.famata* و *C. guillermondii* ) باستخدام طريقة الانتشار بالحفر . أظهرت النتائج أن لمركبات الايض الثانوي للفطر Aspergillus oryzae نشاط مثبط واضح ضد العزلات البكتيرية وأنواع المبيضات المستخدمة في الدراسة بتركيز (50، 100، 150) ملغم / مل، مقارنة مع مضادات حيوية الأموكسيسيلين والنيستاتين.كما لوحظ وجود علاقة عكسية بين تركيز مركبات الايض الثانوي والنمو الميكروبي، وان البكتيريا المسببة للأمراض أكثر حساسية لمركبات أيض الفطريات الثانوية من أنواع المبيضات. كما وجد ان مركبات الايض الثانوي لانواع الفطريات Aspergillus paraciticus و Aspergillus flavus ذات تأثير مثبط طفيف على البكتيريا المسببة للأمراض في تركيز أكثر من 200 ملغم / مل ، ولكن ليس لديهم أي تأثير مثبط ضد المبيضات. في الختام، أظهرت الدراسة الحالية أن مركبات الايض الثانوي لفطر Aspergillus oryzae ذا فعالية حيوية ضد عدة أنواع من البكتيريا والفطريات المسببة للأمراض.

**الكلمات المفتاحية:** .*Aspergillus* spp ، مركبات الايض الثانوي , الفعالية الحيوية.

## Introduction

Filamentous fungi have long been known for their ability to produce an enormous range of unusual chemical compounds known as secondary metabolites [1]. In contrast to the primary metabolism, secondary metabolism are often not necessary for fungus natural growth, as they are produced after they have completed its initial growth phase and is beginning a stage of development represented by the formation of spores[2]. Many of secondary metabolites have potentially useful antibiotic or pharmacological properties. Some of them are toxic [3].

*Aspergillus* spp. (Aspergillus *paraciticus, Aspergillus flavus,* and *Aspergillus oryzae*) produce a wide range of important secondary metabolites that have industrial and therapeutic significance [4]. A few of the many metabolites synthesized by *Aspergillus* spp. that have been great importance in human application; a few examples include the cholesterol reducing drug( lovastin), the antibiotic (penicillin), and the pathogenic human toxin (aflatoxin) [5]. Chemical studies of such isolates have led to the discovery of a variety of new bioactive metabolites, many of which display significant antibacterial and/or antifungal activities [6].

*Aspergillus* species are known for their ability to produce enzymes, that have biological control of many diseases, especially plant diseases [7]. The objective of the present study is to investigate whether the secondary metabolites of *Aspergillus* spp. (Aspergillus *paraciticus, Aspergillus flavus,* and *Aspergillus oryzae* ) have *in vitro* curative effect toward some pathogenic bacteria and fungi.

## Material and Methods

**Preparation of *Aspergillus Spp*. secondary metabolite:** The isolates *Aspergillus paraciticus, Aspergillus flavus,* and *Aspergillus oryzae were* obtained from soil, and identified depending on morphological and cultural characteristics. Fungi were inoculated onto malt secondary metabolite agar (MEA), potato dextrose agar (PDA), and yeast secondary metabolite sucrose agar (YES). After allowing the fungi to grow for 7 to 14 days at 25 °C, typical prolific growth of fungal colonies was observed on the surface of the media. Samples of fungal hyphae, together with underlying culture media, were taken by vertically cutting two plugs of 6mm diameter using a cork borer. The plugs were transferred to 5mL disposable screw-cap bottles. The first plug was secondary metabolized twice with 2 ml ethyl acetate, 1% formic acid and then 2 ml isopropanol, while the

second plug was secondary metabolized twice with 2 ml ethyl acetate, 1% formic acid and then

2 ml acetonitrile , followed by 1 min vortexing and 30 min total ultra-sonication[8]. The secondary

metabolites were filtered and evaporated gently under a nitrogen stream. The residues in both cases were dissolved in 1 ml methanol, ultrasonicated for 10 min and passed through Whatman No.2 filter paper and Stored the extracts at 4°C for while in use for various antimicrobial bioassays [9].

**Microbial Strains:** Four different isolates of clinical bacteria (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermids,* and *Staphylococcus aureus*), and three clinical candida species ( *C. albicans, C.famata* and *C. guillermondii* ) obtained from ministry of health, central library, microbiology department .

**Preparation of different concentrations of fungal secondary metabolite:** Different concentrations of (50 ,100 ,150, 200) mg /ml were prepared by mixing known volume from the stock solution with dimethyl sulfoxide (DMSO) as diluent solution [10].

**Antimicrobial assay:** Antimicrobial activity of *Aspergillus Spp*. secondary metabolite was tested using an agar well diffusion method [10]. For bacteria and candida species growth, Mueller-Hinton agar (MH) and Sabouraud Dextrose agar (SDA) medium were respectively used. Each plate was inoculated by one species of bacteria or fungi by spreading method and leave almost 30 minutes. Wells of 5 mm diameter were made into the agar medium and filled with 100 µl of secondary metabolite and leave the dishes until the fluid is diffuse into the medium for 1 h at room temperature. Plates of test organisms were incubated at 37°C for 24 h for bacteria and at 30°C for 72 h for candida species. Antimicrobial activity of each concentration was estimated by measuring the inhibition zone diameter against the test organisms [10].

**Statistical analysis :**To analysis the results of agar-well diffusion method statically .The significant differences are determined in rate of probability value $\geq 5\%$ [11].

## Results and Discussion

Inhibitory effect of *Aspergillus paraciticus, Aspergillus flavus,* and *Aspergillus oryzae* secondary metabolites against clinical pathogenic bacteria selected for antibacterial activity,

(*E.coli , Pseudomonas aeruginosa, Staphylococcus epidermids, and Staphylococcus aureus*), compared with amoxicillin, and clinical pathogenic Candida selected for antifungal activity ( *C. albicans*, *C. famata* and *C. guillermondii* ) compared with nystatine, subjected during the present study by well diffusion method[10].

Results in table (1) shown the inhibitory activity of different concentrations of *Aspergillus oryzae* secondary metabolite against selected bacterial isolates, compared with the antibiotics amoxicillin. The statistical analysis results revealed that there is a significant difference between concentration and the growth of bacteria .The secondary metabolites of the species *Aspergillus paraciticus* and *Aspergillus flavus* shows no inhibitory effects against selected bacterial isolates on the concentrations ( 50 , 100 , 150 ) mg /ml, and they have slight inhibitory effect in the concentration more than 200mg/ml.

Table (1): The inhibitory effect of *Aspergillus oryzae* secondary metabolite against clinical bacterial isolates

| bacterial isolates | Diameter of inhibition zone of different concentration of secondary metabolite and antibiotic ( mm) | | | |
|---|---|---|---|---|
| | 50 mg /mL | 100 mg /mL | 150 mg /mL | Amoxicillin (25 µg / ml) |
| *E.coli* | 9.5 ± 0.2 A | 11±1.5 B | 14.5 ± 0.5 C | 19±0.5 D |
| *Pseudomonas aeruginosa* | 11.5 ± 0.57 A | 13.5 ± 0.28 B | 15.5 ± 0.28 C | 20 ± 0.5 D |
| *Staphylococcus epidermids* | 12 ± 0.57 A | 15.5 ± 0.28 B | 18 ± 0.28 C | 19.5 ± 0.5 D |
| *Staphylococcus aureus* | 11.5 ± 0.57 A | 14.5 ± 0.57 B | 18 ± 0.76 C | 21.5 ± 0.76 C |

• Different letters within a column imply a significant difference ($P < 0.05$).

On the other hand, the antifungal activity of *Aspergillus oryzae* secondary metabolite shows high inhibitory on the yeast subjected in the study, compared with the antibiotics nystatine, as represented in table (2). The data appears that there is reverse relationship between secondary metabolite concentration and fungal growth, and there is not clear inhibitory effect of secondary metabolites prepared from the species *Aspergillus paraciticus, Aspergillus flavus* against

subjected *candida.* Figure (1) shows the inhibitory effect of *Aspergillus oryzae* secondary metabolite against C. *albicans*, grown on SDA medium at 30°C for 72 h.

Table (2): The inhibitory effect of *Aspergillus oryzae* secondary metabolite against clinical Candida isolates

| Fungal isolates | Diameter of inhibition zone of different concentration of secondary metabolite and antibiotic ( mm) | | | | |
|---|---|---|---|---|---|
| | 50 mg /mL | 100 mg/mL | 150 mg /mL | 200 mg /mL | Nystatin (32 µg / ml) |
| *C. albicans* | 11.5 ± 0.57 A | 14.5 ± 0.57 B | 16.5 ± 0.76 C | 19 ± 0 D | 23.5 ± 0 D |
| *C. famata* | 16 ± 0.57 A | 9 ± 0.57 B | 19 ± 1 C | 20.5 ± 0 D | 22 ± 0 D |
| *C. guillermondii* | 15 ± 0.5 A | 19.5 ± 0.2 B | 6 ± 1 C | 22 ± 0 D | 19 ± 0 D |

• Different letters within a column imply a significant difference (P <0.05).

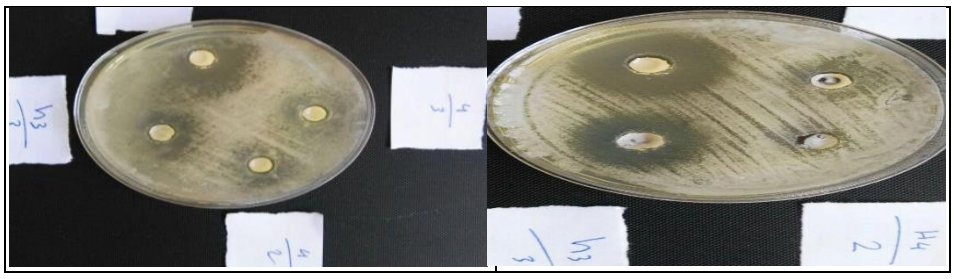

Figure (2): The effect of *Aspergillus oryzae* secondary metabolite activity on *Candida albicans*

It was observed that bacterial pathogens are more susceptible to fungal secondary metabolite than candida species. This can be attributed to the ability of these secondary metabolites to break down the peptide bonds of prokaryotic proteins [12]. Number of previous studies confirms that *A. oryza* is effective in the inhibition of a number of positive and negative bacterial strains as well as yeast [13]. This activity was attributed to the synthesis of chitinase, B-glucanase, proteinase that break down the bacterial cell membrane [14].

As a conclusion, secondary metabolite of *Aspergillus oryzae* have clear antimicrobial activity against pathogenic bacteria and fungi. So, it can be used in the production of therapeutic agents, thus reducing the incidence of resistance among pathogens that appear as a result of the misuse and overuse of antibiotics.

## References


1- Keller, N.P and Turner G. (2012). Fungal Secondary Metabolism .Methods and Protocols. Springer protocols.

2- Nielsen, K. F.; Mogensen, J. M.; Johansen, M.; Larsen, T. O. and Frisvad, J. C. (2009). Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. Anal Bioanal Chem., 395:1225-1242.

3- Keller, N.P., Turner, G. and Bennett, J.W. (2005) Fungal Secondary Metabolism-From Biochemistry to Genomics. Nature Reviews Microbiology, 3: 937-947.

4- Faulkner D. J. (2000). Marine natural products. Natural Product Reports 17: 7-55.

5- Gibbons, J.G and Rokas, A. (2013). The function and Evolution of the Aspergillus genome. Trends in microbiology, 1:14-22.

6- Abdel-Hady, H.; Abdel-Wareth, M. T.; El-Wakil, E. A. and Helmy, E. A. (2016). Identification and evaluation of antimicrobial and cytotoxic activity of *Penicillium islandicum* and *Aspergillus tamarll* ethyle acetate extracts. World Journal of Pharmacy and Pharmaceutical Sciences, 5: 2022- 2039.

7- Su, H.N.; Chen, Z.H.; Song, X.Y.; Chen, X.L. and Shi. M. (2012). Antimicrobial Peptide trichokonin vi-induced alterations in the morphological and nanomechanical properties of *Bacillus subtilis*. PLoS, 7(9): 45818.

8- Laatsch, H. (2006). Marine Bacterial Metabolites, in: Frontiers in Marine Biotechnology. Horizon Bioscience, 18(1): 225-288.

9- McMullin D. Roderick (2014) Structural characterization of secondary metabolites produced by fungi obtained from damp Canadian bulildind. A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of PhD. Ottawa-Carleton Institute of Chemistry .Carleton University.Ottawa, ON, Canada.

10- Perez,L., Pauli,M. and Bazequre.P.(1990). Antibiotic assay by the agar-well diffusion method. Journal of Actabiology. 15:113-115.

11- John, W. (1988). Downstream Processes: Equipment and Techniques, R. Liss, Inc., 5: 315-351.

12- Song, X.Y.; Shen, Q.T.; Xie, S.T.; Chen, X.L.; Sun, C.Y. and Zhang, Y.Z. (2006). Broad-spectrum antimicrobial activity and high stability of trichokonins from*Trichoderma koningii* SMF2 against plant pathogens. FEMS Microbiol Lett, 260: 119–125.

13- Schirmbock, M.; Lorito, M.; Wang, Y.L.; Hayes, C.K.; Harman, G.E.and Kubicek, C.P. (1994).Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of Trichoderma harzianum against phytopathogenic fungi. Applied and Environmental Microbiology , 60: 4364-4370.

14- Jadulco, R.; Proksch, P.; Wray, V.; Sudarsono, A.and Gräfe, U. (2001) New macrolides and furan carboxylic acid derivative from the sponge-derived fungus Cladosporium herbarum. J Nat Prod., 64(4) :527-30.