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ORIGINAL ARTICLE

# Antimicrobial agents production by fungi isolates from the whisperers

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**ABSTRACT**

**Objective:** The current study aimed to screen of local fungi isolates for antimicrobial bioactive compounds production.

**Methods:** Fifteen samples of whisperers soil were analyzed for antimicrobial producing by fungi. Seven species of fungi were obtained from these samples, these fungi were *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus flavus*, *Penicillium notatum*, *Bacillomyces sp.*, *Trichoderma* sp. and *Cladosporium* sp. and the filtrate of each fungal isolate given special label as AA, AB, AC, AD, AE, AF and AG, respectively. These fungal filtrates were tested their antimicrobial activity against three Gram positive bacteria (*Staphylococcus* sp. , *Streptococcus agalicae* and *Bacillus* sp.) also three Gram negative bacteria (*Enterobacter* sp. , *Pseudomonas* sp. and *Klebsiella* sp.) by disc technique.

**Results:** *Bacillus* sp. was the most sensitive to all fungal filtrations, especially for AC (*Aspergillus flavus*) and AE (*Bacillomyces* sp.) where the inhibition zone were the largest compared to the antibiotic which used, the inhibition zones were (6 and 5.33)mm, respectively. *Streptococcus agalicae* was affected only by AD (*Penicillinum notatum*) and AG (*Cladosporium* sp.) with inhibition zone ( 4 and 3.33)mm, respectively. In Gram negative bacteria, *Enterobacter* sp. is highly sensitive to AG (*Cladosporium* sp.) with inhibition zone 7 mm.

**Conclusion:** Some species of fungi which have antibacterial activity can be isolated from the soil of whisperers.



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## INTRODUCTION

Infections have been the major cause of disease throughout the history of human population especially bacterial infections which is major threat microorganism to human health that cause disease some acute other fatal . Every year millions of people suffering of these organism. Major Poor people, especially from developing countries who are exposed to unhygienic conditions in their daily activities, are the worst sufferers of infectious diseases. Their sufferings have been increased many-fold due to prolonged illness caused by widespread drug resistant pathogens and cost of treatment. It has been estimated that more than 70% of pathogenic bacteria are resistant to at least one existing antibiotic as a result of a steady increase in drug-resistant pathogens every year, there have been

demands for the development of new and effective antimicrobial drugs [1].

Fungi are one of the largest group of organisms, worldwide distributed and play a vital role in ecosystems and as one of the most important tool in biotechnology. Fungi can be used in many applications in (industry, agriculture, medicine, and environment). The production of secondary metabolites such as antimicrobial agents is the most important use of fungi, these metabolites can be beneficial for medical therapy[2]. Naturally, Antibiotics occurring antimicrobials of secondary metabolic processes of some microorganisms, species of Actinomycetes, fungi, and bacteria, which are deleterious to other microorganisms and can inhibit or even destroy microorganisms completely. It used to treat infections caused by bacteria and other organism that can cause illness to both humans and animals by different modes of action, due to the nature of their structure and degree of affinity to certain target sites within bacterial its might be undergo to the following groups: Inhibitors of cell wall synthesis (Penicillin's, Bacitracin and Vancomycin), Inhibitors of cell membrane function (Polymixin B and Colistin ), Inhibitors of protein synthesis(Aminoglycosides, Chloramphenicol, Tetracyclines) , Inhibitors of nucleic acid synthesis (Quinolones, Metronidazole, and Rifampin). There are so many different potential sources where antibiotics can be discovered, such as medicinal herbs and soil. However, soil, which is a naturally occurring loose mixture of mineral and organic particles, still remains the most important target for most researchers in their efforts to discover novel antibiotics which have pharmaceutical values [3, 4, 5].

Secondary metabolites are a small organic molecules produced by an organism that are not essential for their growth , development and reproduction although. They play a role in competition ,antagonism and self-defense mechanisms against other living organisms to allow the organism to occupy the niche and utilize the food. Four major classes (alkaloids, glycosides, phenolic, terpenoids), microbial secondary metabolites have been an important resource for drug development. It was the discovery of penicillin that led to later discoveries of potent antibiotics isolated from microbial broths. There is a continuous need to search for novel drug compounds as the numbers of drug resistant microorganisms are continuously increasing [4].

## MATERIALS AND METHODS

**Samples collection:** Fifteen soil samples were collected from Karbala city, Al-Hindia at different elevations from different sites at10 cm, Spatula was used to collect the soil samples into 200 ml sterile glass containers. Soil sample was labeled and transported to Microbiology research laboratory of Clinical Laboratory department, Applied Medical Sciences College, Karbala university, Iraq.

**Isolation and identification of fungal isolates:** Isolation of fungi was carried out using the pour plate technique after serial dilution, 1 g soil sample was suspended in 9 mL sterile distilled water and the mixture vortexed vigorously. Ten-fold serial dilution was carried out, 0.1ml of third and fourth dilution were planted out in duplicate on PDA, using Potato Dextrose Agar medium supplemented with 50µg/mL Streptomycin to inhibit the growth of bacteria also the pH of the medium was adjusted to 5.8 to encourage the growth of the fungi. The plates were incubated at room temperature 28°C for 96 hour individual fungal colonies were removed and repeatedly sub-cultured until pure cultures were obtained[6].

The isolated fungi were identified to the genus level and to the species when possible on the basis of macro morphological (The colonies were examined for slow or for rapid growth, topography (flat, heaped, regularly or irregularly folded), texture (yeast like, powdery, granular, velvety or cottony), surface pigmentation and reverse pigmentation) and micro morphological (Hyphae, macro conidia, micro conidia, chlamydospores and other special fungal structure) characteristics using suitable media, slide cultures and the most updated keys for identifications.The identified fungi confirmed with microbial expert [7, 8].

**Inoculation of media with fungal isolate:** Brain heart infusion broth was sterilized by autoclaving at 121 ºC for 60 min.10 ml of the Sterilized media inoculated under aseptic conditions with a single isolated fungal colony selected from the pure cultured fungi on PDA. Inoculated media was kept at room temperature (27-28) ºC with pH 5.8 for 7 days at the end of the incubation, the cultures were washed by centrifugation at 4000g for 30 mins after which the crude supernatant were assayed using the agar well diffusion technique [9].

**Activation of bacteria:** Six types of bacteria were obtained from the Laboratory of Medical Microbiology, Department of Clinical Laboratories, College of Applied Medical Sciences, University of Kerbala, three bacterial species were Gram positive: *Staphylococcus* sp. , *Streptococcus agalicae* and *Bacillus* sp. also three bacterial species were Gram negative: *Enterobacter* sp. , *Pseudomonas* sp. and *Klebsiella* sp.

The activation of the test bacteria on Muller Hinton broth before a half hour of culturing.

**Antimicrobial bioactivity assay:** After the activation of the bacteria on Muller Hinton broth , Filter paper discs (0.6 mm) are being sterilized by autoclave and socked in each fungal crude extract solution for 5 min., Filter paper discs with extracts are placed on the surface of agar medium(Mueller-Hinton ) in Petri-dishes streaked with 0.2 ml of bacterial suspension of pathogenic bacteria Plates are incubated at 37 ◦C for 24 hr , an presence of inhibition zones around the filter paper disc indicating the bioactivity of crude metabolites of the tested fungal isolates [9].

**Statistical Analysis:** Statistical analysis included factorial experiences analysis 8 × 3 with 6 replicates. The factors analyzed are the fungal filtrate and types of bacteria. P value of 0.05 is the level of probability that was used to identify a significant difference. The significant differences between the averages were also

tested by using less significant difference (LSD) test at the level of probability of 0.05 [10].

## RESULTS

Seven fungal species were identified from all soil samples, these species were *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus flavus*, *Penicillium notatum*, *Bacillomyces sp.*, *Trichoderma* sp. and *Cladosporium* sp. and the filtrate of each fungal isolate given special label so as Table 1. This study assessed the antimicrobial activities against pathogenic strains of Gram positive bacteria: *Staphylococous aureus* , *Streptococcus agalicae* and *Bacillus* sp. So Gram negative bacteria: *Enterobacter* sp. , *Pseudomonas* sp. and *Klebsiella* sp. with expectation for developing a therapeutic antibiotic from the soil fungi extracts.

Table 1: **Fungi isolated from soil and the label of their filtrates.**

| Fungi Isolates | Label of filtrate |
|---|---|
| *Aspergillus niger* | AA |
| *Aspergillus ochraceaus* | AB |
| *Aspergillus flavus* | AC |
| *Penicillinum notatum* | AD |
| *Bacillomyces* sp. | AE |
| *Trichoderma* sp. | AF |
| *Cladosporium* sp. | AG |

The fungal filtrates show a great variety of activity versus both gram negative and positive bacteria. The results in Table 2 show the sensitivity of gram negative bacteria to the filtrate AE (*Bacillomyces* sp.) and AG (*Cladosporium* sp.) with inhibition zone (2.56 ; 2.33) mm, respectively. Comparison with control (antibiotic) and other filtrates with a significance difference ($P< 0.05$).

*Pseudomonas* sp. is resistance, *Enterobacter* sp. is highly sensitive to AG (*Cladosporium* sp.) with inhibition zone 7 mm , followed by AA (*Aspergillus niger*) and AE (*Bacillomyces* sp.) with inhibition zone (4 ; 3.7) mm, respectively. *Klebsilla* sp. is sensitive to AC (*Aspergillus flavus*), AD (*Penicillinum notatum*), AE (*Bacillomyces* sp.) with inhibition zone (4 ; 4 ; 4) mm, respectively.

Results in Table 3 showed that *Bacillus* sp. was the most sensitive to all fungal filtrations, especially for AC (*Aspergillus flavus*) and AE (*Bacillomyces* sp.) where the inhibition zone were the largest compared to the antibiotic which used, the inhibition zones were (6 and 5.33) mm, respectively.

*Streptococcus agalicae* was affected only by AD (*Penicillinum notatum*) and AG (*Cladosporium* sp.) with inhibition zone ( 4 and 3.33)mm, respectively. While *Staphylococcus aureus* was resistant to all the fungal filtrates which used in this study.

Table 2: **Inhibition zone(mm)of fungal filtrates against Gram-negative bacteria.**

| Fungal Filtrates | Bacterial Isolates | | | Mean of Fungal Filtrate | $LSD_{0.05}$ Filtrate |
|---|---|---|---|---|---|
| | ***Enterobacter*** | ***Pseudomonas*** | ***Klebsiella*** | | |
| Antibiotic | 4 ± 0.57 b | 7 ± 0.57 a | 4 ± 0.57 b | 5.00 A | |
| AA | 4 ± 0.57 b | 0 ± 0.0 c | 0 ± 0.0 c | 1.33 C | |
| AB | 0 ± 0.0 c | 0 ± 0.0 c | 0 ± 0.0 c | 0.00 D | |
| AC | 0 ± 0.0 c | 0 ± 0.0 c | 4 ± 0.57 b | 1.33 C | **0.559** |
| AD | 0 ± 0.0 c | 0 ± 0.0 c | 4 ± 0.57 b | 1.33 C | |
| AE | 3.7 ± 0.33 b | 0 ± 0.0 c | 4 ± 0.57 b | 2.56 B | |
| AF | 0 ± 0.0 c | 0 ± 0.0 c | 0 ± 0.0 c | 0.00 D | |
| AG | 7 ± 0.57 a | 0 ± 0.0 c | 0 ± 0.0 c | 2.33 B | |
| Mean of Isolate | **2.333 A** | **0.875 C** | **1.875 B** | **$LSD_{0.05}$ Interference** | **0.968** |
| **$LSD_{0.05}$ Bacterial Isolates** | | **0.342** | | | |

Table 3: **Inhibition zone(mm)of fungal filtrates against Gram-positive bacteria.**

| Fungal Filtrates | Bacterial Isolates | | | Mean of Fungal Filtrate | $LSD_{0.05}$ Filtrate |
|---|---|---|---|---|---|
| | *Staphylococcus* sp. | *Streptococcus agalicae* | *Bacillus* sp. | | |
| Antibiotic | 5 ± 0.57 abc | 6.33 ± 0.33 a | 4.33 ± 0.33 bcd | 5.22 A | |
| AA | 0 ± 0.0 e | 0 ± 0.0 e | 4 ± 0.57 c | 1.33 E | |
| AB | 0 ± 0.0 e | 0 ± 0.0 e | 4.66 ± 0.88 bc | 1.55 DE | |
| AC | 0 ± 0.0 e | 0 ± 0.0 e | 6± 0.57 a | 2.00 CD | **0.661** |
| AD | 0 ± 0.0 e | 4 ± 0.57 cd | 4.33 ± 0.33 bcd | 2.77 B | |
| AE | 0 ± 0.0 e | 0 ± 0.0 e | 5.33 ± 0.66 ab | 1.77 DE | |
| AF | 0 ± 0.0 e | 0 ± 0.0 e | 3.33 ± 0.33 d | 1.11 E | |
| AG | 0 ± 0.0 e | 3.33 ± 0.33 d | 4.33 ± 0.88 bcd | 2.55 BC | |
| Mean of Isolate | **0.625 C** | **1.708 B** | **4.541 A** | **$LSD_{0.05}$ Interference** | **1.145** |
| **$LSD_{0.05}$ Bacterial Isolates** | | **0.405** | | | |

## Discussion

The results from this study revealed the presence of microbes capable of producing antimicrobial metabolites in soil habitats. A number of seven isolates exhibited inhibitory action against the test bacteria and the high proportion of antibiotic producers may be associated

with an ecological role (salinity , PH, temperature ), serving as a defensive mechanism to maintain their niches, or enabling the invasion of an established microbial community.

The *Bacillus* sp. show a great susceptibility to all the filtrates with a verity of inhibition zones , while *Pseudomonas* sp. and *Staphylococcus* sp. show a highly resistant against the filtrate.

Some inhibitory screening investigations have recorded values closed to what was obtained in this study while other recorded different values either higher or lower than this study. In a study, carried out by Adelaide (2011) reported that out of 119 isolate from soil source 23% of the isolates were active against test organisms [11]. Ivanova *et al.* (1998) reported that out of the 491 bacteria isolated from different marine sources, 26% of the isolates shows activity on the test bacteria [12]. Zheng *et al.* (2005) also reported that eight out of twenty-nine strains, representing 28 % of the microbes considered in their study were able to inhibit the growth of at least one of the target microorganisms [13].

The results of this investigation have further confirmed that soil whisperers antibiotic producing fungi are mostly in the genus *Aspergillus*. Therefore the antibiotic producing isolate from the soil environment of karbala city can be harnessed for the production of novel antibiotics.

## Conclusions

Some species of fungi which have antibacterial activity can be isolated from the soil of whisperers and we can carry of many experments to screen the ablity of these microorganism. In the soil where most antimicrobial substance producing microorganisms are found, life is competitive and changes in composition of microbial community due to secondary metabolites. We recommend in secondary screening for the filtrate using a different concentration and competition with other material.

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## REFERENCES

1. Mondol M.A.M. and Hee Jae Shin H.J. "Antibacterial and Antiyeast Compounds from Marine-Derived Bacteria". Marine drugs. 2014; 12(5): 2913-2921. doi: 10.3390/md12052913.
2. Farjana A., Zerin N. and Kabir M.S." Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria". Asian Pacific Journal of Tropical Disease. 2014; 4(Suppl 2): S920-S923. doi.org/10.1016/S2222-1808(14)60758-1.
3. Bbosa G.S., Mwebaza N., Odda J., Kyegombe D.B. and Ntale M. "Antibiotics/antibacterial drug use, their marketing and promotion during the post-antibiotic golden age and their role in emergence of bacterial resistance". 2014; 6(5): 410-425. DOI: 10.4236/health.2014.65059. DOI: 10.4236/health.2014.65059.
4. Ramesha A., Sunitha V.H. and Srinivas C. "Antimicrobial activity of secondary metabolites from endophytic fungi isolated from Nerium oleander L.". International Journal of Pharma and Bio Sciences. 2013; 4(1): 683-693.
5. Yahaya S., Idris B., Yusuf I., and Rabiu M.K. " Screening and identification of antibacterial agents produced by Aspergillus species from the soil of Bayero University Kano". Bayero Journal of Pure and Applied Sciences. 2017; 10(1): 498 - 502.
6. Raja M., Praveena G. and John William S. " Isolation and Identification of Fungi from Soil in Loyola College Campus, Chennai, India". International Journal of Current Microbiology and Applied Sciences. 2017; 6(2): 1789-1795. doi: http://dx.doi.org/10.20546/ijcmas.2017.602.200.
7. Takahashi J.A., Castro M.C.M., Souza G.G., Lucas E.M.F., Bracarense A.A.P., Abreu L.M., Marriel I.E., Oliveira M.S., Floreano M.B. and Oliveira T.S. "Isolation and screening of fungal species isolated from Brazilian cerrado soil for antibacterial activity against Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Streptococcus pyogenes and Listeria monocytogenes". Journal de Mycologie Médicale. 2008; 18(4): 198-204. doi.org/10.1016/j.mycmed.2008.08.001.
8. Pitt J.I. "A laboratory guide to common penicillium species". 3rd Ed. Australia; Food Science Australia Publishers. 2000; 197 p.
9. Ogbonna O.J., Ekpete W.B., Onyekpe P.I., Udenze E.C.C. and Ogbeihe G.O. "Antimicrobial agent production by fungi isolates from petroleum product contaminated soil". Archives of Applied Science Research. 2013; 5(3):1-6.
10. Dequ G. and Tessema F. "Biostatistics". University Gondar, Funded under USAID from the American people, Cooperative Agreement No. 663-A-00-00-0358-00. 2005.
11. Adelaide A.T. "Screening of Aquatic microorganisms for antimicrobial metabolites production". Thesis, Faculty of pharmacy Kwame Nkrumah, University of Science and Technology, Kumasi, Ghana. 2011, 58-72.
12. Ivanova E.P., Nicolau D.V., Yumoto N. and Taguchi T. "Impact of conditions of cultivation and adsorption on antimicrobial activity of marine bacteria". Marine Biology. 1989; 130(3): 545–551.
13. Zheng L., Chen H., Han X., Lin W. and Yan X. "Antimicrobial screening and active compound isolation from marine bacterium NJ6-3-1 associated with the sponge Hymeniacidon perleve". World J. Microbiol. Biotechnol. 2005; 21(2): 201–206.