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# Preliminary screening of bioactive metabolites from three fungal species of *Drechslera* isolated from soil in Basrah, Iraq

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## Abstract

Screening of secondary metabolites from three fungal species of the genus *Drechslera* was made. Fungal extraction was carried out by ethyl acetate solvent. Bioactivity of fungal extracts against *E. coli and S. aureus* by using a disc diffusion technique was examined. All fungal extracts exhibited an inhibitory action against both bacterial isolates.The inhibition zones exhibited by fungal extracts ranged between 22-28 mm diam. MIC test revealed that the extract of *D. australiensis* exhibited a minimal inhibition values (12.5 and 6.25 ug/L) against *E. coli and S. aureus*, respectively. The bioactivities of extracts after being stored for six months at 8◦C were slightly decreased as compared with initial freshly prepared extracts. A comparison between the inhibitory action of five commercial drugs and the fungal extracts showed that the extracts rendered a greater bioactivity than the examined drugs, with an exception of chloramphenicol. Chemical analysis showed that extract of *D. ausraliensis* contains Tannin compounds while *D. halodes* and *D. hawaiinsis* contain alkaloids.

**Keywords**: Antibiotics, alkaloids, bacteria, bioactive metabolites, drugs, fungi, growth inhibition zones, tannin

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## Introduction

Fungi are common in nature and considered as good natural sources for antimicrobial agents (Lindequist et al., 2005; Abad et al,. 2007; Muhsin et al., 2011). So far, several antibiotics have been discovered from the secondary metabolites produced by fungi and actinomycetes (Hugo and Rusell, 1987; Ankee, 1989; Pohanka, 2006). Nevertheless, during the last decade the research interests were focused on the bioactive compounds derived from mushrooms (Basidiomycotina) (Suay et al., 2000; Gardiner et al., 2005; Lindequist et al., 2005). This is mainly due to an increase of microbial resistance to the available drugs, hence, more fungi needs to be examined for new antimicrobial agents (Lindquist et al., 2005). However, less information regarding the antimicrobial bioactive metabolites produced by the hyphomycetous fungi is available. Nevertheless, among these fungi, the genus *Drechslera* (anamorphs) is a world widely distributed fungus inhabiting soils (Domsch et al., 1980) and causes various plant diseases (Ellis 1971, 1976; Shotwell and Ellis 1976). It has been reported that some species of this genus produce phytotoxins and mycotoxins (Shotwell and Ellis, 1976; Evidente et al., 2005), although a species produce a potential mycoherbicide known as ophiobolin (Evidente et al., 2006). In this report an attempt was made to investigate some fungal isolates of the genus *Drechslera* isolated from soils for their bioactive metabolites production in culture media as a preliminary step for a further investigation for a specific antimicrobial compounds to be tested against human pathogenic bacteria.

## Materials and methods

### Fungal isolates

Soil samples were collected from different cultivated localities in Basrah (southern Iraq) during the year 2010, brought to the laboratory and processed for fungal isolation. Soil dilution plating technique was applied for the fungal isolation from soil samples using Potato Dextrose Agar (PDA). Incubation was conducted at 25°C for 7 days. Axenic fungal cultures were made for each fungal isolate and the identification of fungi was confirmed according to the available taxonomic literature (Ellis 1971, 1976; Domsch et al., 1980).

### Fungal culture extraction

Five discs (0.5 mm diam.) were cut from the axenic fungal culture of each isolate by using a cork borer were amended into PD liquid medium in 500 ml flasks (with triplicates) and incubated at 26°C for 2 weeks on a rotary shaker. Fungal cultures were filtered on Whatman No 1 filter paper and the pH was adjusted at 3 by drops of HCl for each fungal filtrate. Filtrate was extracted in ethyl acetate (1:1 vol:vol) by using separating funnel. The organic layer was collected by dehydration of water by using $Na_2SO_4$. The filtrate was filtered again and placed in Petri dishes then left to be dried at room temperature. 100 ug of the dried extract was dissolved in 1 ml ethanol as stock extract solution to be used for further experiments.

### Antimicrobial bioactivity assay

Filter paper discs (0.6 mm) after being sterilized by autoclave were socked in each fungal crude extract solution for 5 min., filter paper discs with extracts were placed on the surface of Muller-Hinton agar medium in Petri-dishes streaked with 0.2 ml of bacterial suspension of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) provided by Microbiology Laboratory at Biology Department, College

of Science, Basrah University. Plates were incubated at 37◦C for 24 hr, an appearance of inhibition zones around the filter paper disc indicating the bioactivity of crude metabolites of the tested fungal isolates (Casals, 1979). The diameters of the clear zones were measured and compared with control agar plates containing discs with solvent only (control), triplicates were made for each of the fungal extract.

**Bioactivity of fungal mycelia crude extract**

Mycelium of each fungal culture was harvested on filter paper, dried in oven at 40 ◦C and grounded in a mortar with ethyl acetate solvent (1:1 vol). The bioactivity of the mycelium extract of each fungal species was examined against the two selected bacterial isolates by using a disc diffusion technique as mentioned above.

**Minimal inhibitory concentration test**

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were determined by the standard serial dilution assay (McGinnis, 198o). The MFC value in this assay was indicated by the absence of bacterial growth at the minimal concentration of the extract. Crude extracts of three fungal isolates (*D. australiensis*, *D. halodes* and *D. hawaiinsis*) were selected for this test. The inhibitory test was carried out on Muller-Hinton agar medium.

**Effect of storage period on the fungal extract bioactivity**

Fungal extracts of three selected species of *Drechslera* were kept in screwed vials at 8 ◦C for six months storage period to examine the bioactive longevity against both bacteria. Disc diffusion technique was applied and the inhibition zone diameters were measured.

**Sensitivity of bacteria isolates against commercial antibiotics**

Six commercial antibiotics (Carbencillin, Cefalotaxime, Chloramphenicol, Gentamycin, Penicillin and Tetracyclin) were selected and the sensitivity of the two bacterial isolates was tested against each of these antibiotics by using a disc diffusion method at concentration of 30 ug.

**Cytotoxicity test**

Cytotoxicity of the fungal crud extracts was examined by using human RBC following a previously described method ( Xian- guo and Ursula, 1994).

**Chemical analysis of fungal crude extracts**

Fungal culture extracts of D. *australienses*, *D. halodes* and *D. hawaiinsis* were chemically analyzed for alkaloids, phenols, amino acids, flavenoides and Tannins according to following the method described by Harbone (1984).

**Statistical Analysis**

SPSS 11 statistical program by using ANOVA for data analysis was applied.

**Results**

The preliminary screening of three species of the genus *Drechslera* for their bioactivity against both bacteria *E. coli* and *S. aureus* revealed that the crude filtrate extracts of the examined species exhibited a growth inhibitory action by the appearance of clear zones ranged between 22 and 28 mm diam (Table 1) (Figs. 1-2 ). The extracted mycelia of the examined fungi revealed a much lower inhibition zones against both bacterial isolates as compared with fungal filtrate extracts (Table 2), however, *D. australienses* mycelium showed a relatively higher inhibition zones in comparison with the other fungal mycelia extracts.

The MIC and MBC tests indicated that the extract of *D. australienses* exhibited the minimal values of MIC (12.5 and 6.5 ug/L) and MBC (25 and 12.5 ug/L) against *E. coli* and *S. aureus,* respectively, as compared with the other two fungal species (Table 3).

The bioactivity of the fungal extracts kept at 8 ◦C for six months storage period showed insignificant difference compared to the initial (fresh) fungal extracts (Table 4). Nonetheless, slightly lower values of growth inhibition zones were noticed for all the fungal extracts after being stored for six months. However, amongst the screened fungal species, *D. australiensis* extract exhibited highest inhibition zones diam before and after the storage period (Tables 1,4).

The growth sensitivity of both *E. coli* and *S. aureus* against the selected commercial antibiotics showed that both bacterial isolates were more sensitive to chloramphenicol with an inhibition zones reached 24 and 26 mm diam, respectively (Table 5). All other antibiotics showed no /or less inhibition zones against the examined bacterial isolates. Comparatively, the fungal extracts exhibited a more inhibitory effect on the growth of both bacteria than the tested commercial drugs (Tables 1, 5).

The chemical analysis of the fungal crude extracts showed that *D. australiensis* extract contains Tannins group only, *D. halodes* extract contains Tannins and Alkaloids groups while *D. hawaiinsis* extract contains alkaloids. However, non of theses extracts showed amino acid, flavenoide and phenol compounds (Table 6).

**Discussion**

Fungi in genera l are a good source for antimicrobial agents (Lindequist et al., 2005; Janes et al., 2007). Among fungi, *Drechselra* is a common genus with several well recognized species (Ellis 1971, 1976) and being described as anamorph (conidial state). The members of this genus are mostly saprophytic inhabiting soils or as plant pathogens (Shotwell and Ellis, 1976; Domsch et al., 1980). Some reports regarding the secondary metabolites produced by *Drechslera* species are available. It has been investigated that some species of this genus are sources for a potential secondary metabolites such as phytotoxins, in particular sesterterpenoid compounds (Sugawara et al., 2011), and also as a herbicidal bioactive compounds known as ophiobolin (Evidente et al., 2005; Evidente et al., 2006). Nevertheless, the production of metabolic substances by fungi, in general, is often affected by various growth conditional factors mainly the fermentation medium (Jonathan and Fasidi, 2003; Vahidi et al., 2004). In the present study a liquid state fermentation medium used is efficient for a mass production of bioactive metabolites by the selected fungi. These findings are in concomitant with other studies (Stamets, 2002; Roberts, 2004; Janes et al., 2007) examined some higher fungal species.

The crude filtrate extract of *t*he examined species of *Drechslera* exhibited an inhibitory action against both bacterial strains *E. coli* and *S. aureus.* However, a variation in the inhibitory action, based on the inhibition zones diameter, among the examined fungi was noticed. Extracts of *D. ausraliensis* and D. *hawaiinsis* revealed higher inhibition zones against *S. aureus* while the extract of *D. halodes* exhibited highest inhibitory action against *E. colli* (Table 1). Such variations may be attributed to the differences between the chemical constituents the fungal extracts.

The Chemical analysis of the crude extracts indicated that *D. australiensis* possesses tannin compounds only. While the extracts of *D. halodes and D. hawaiinsis* contained alkaloids. The antimicrobial inhibitory impact of these extracts can be related to the bioactivity of these compounds. It has been reported that several phenolic compounds including tannin are potent inhibitors of microbial enzymes like protease (Kamba

and Hassan, 2010). Other studies showed that tannin inhibits the growth of both *E. coli and S. aureu*s and has been attributed to the mechanism of tannin binding with the protein of the bacterial cell walls (Marjorie, 1999; Shihabudeen et al., 2010). On the other hand, the inhibitory action of alkaloides against G-negative and G-positive bacteria has also been demonstrated (Navarro and Delgado, 1999; Sawer et al., 2005). Nonetheless, the inhibitory mechanism has been related to the inhibition of DNA synthesis by a specific alkaloid compounds. Therefore, a further purification of the crude extracts and chemical analysis of tannins and alkaloids that have been detected in the present study are required. Growth sensitivity of the tested bacteria isolates against the selected commercial antibiotics demonstrated that both bacteria are highly sensitive to chloramphenicol and less sensitive to other drugs. This is due to the wide spectra of inhibition exhibited by chloramphenicol against different strains of pathogenic bacteria.

Comparatively with the examined drugs, the fungal extracts in the present study rendered a similar inhibitory action of chloramphenicol, or in some cases a slightly higher inhibition action was observed. A verification of non toxicity of the fungal extracts against human blood revealed a negative test. Evidently, the effect of storage for six months did not significantly alter the bioactivity of these extracts against the tested bacteria. However, a slight decrease in the bioactivities of extracts was detected. It can be recommended here that the extract should be kept in capsulated polymeric substances so it may last longer and maintains its bioactivity. A conclusion can be derived from this preliminary screening of *Drechslera* species that this fungus possessing a potential secondary chemical compounds that can be of significance and a promising as antimicrobial agents. However, a further investigation is needed to examine the bioactivity of these extracts against more pathogenic bacteria and pathogenic fungi as well.

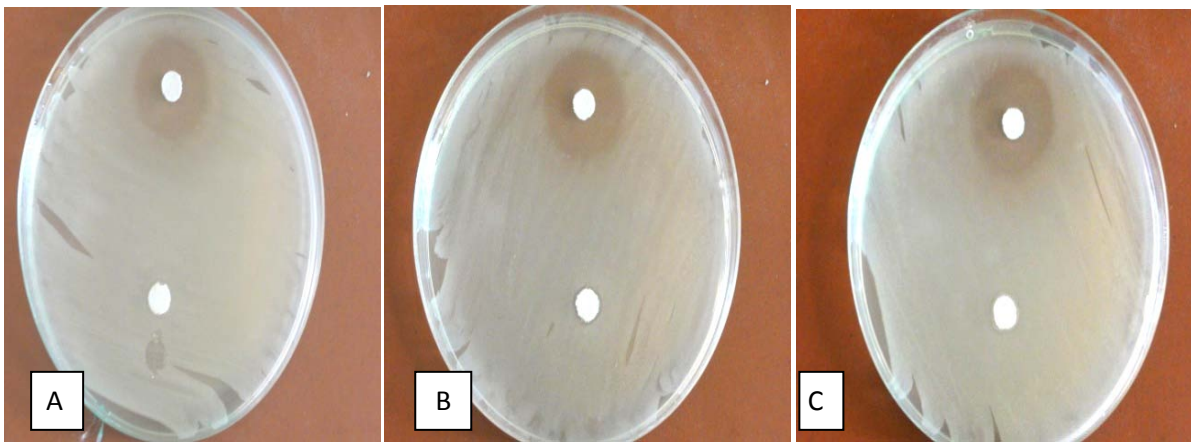


**Fig. 1. (A-C): Inhibition zones exhibited by fungal crude extracts against *E. coli.* (A. *Drechslera austrlaiensis* B. *D. halodes*, C. *D. hawaiensis*).**

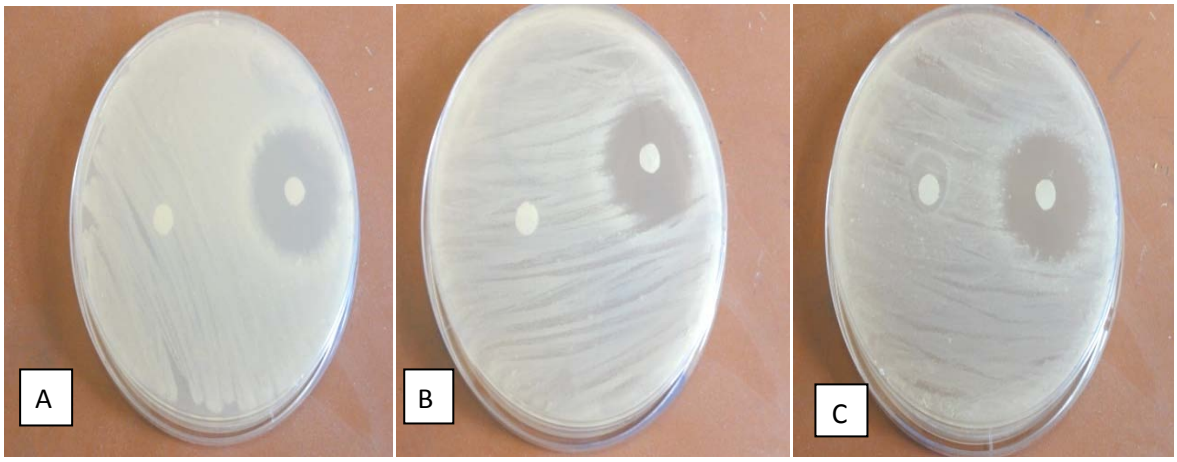


**Fig.2. (A-C): Inhibition zones exhibited by fungal crude extracts against *S. aureus*. (A. *Drechslera austrlaiensis* B. *D. halodes*, C. *D. hawaiensis*).**

**Table 1. Growth inhibition zones (mm diam) exhibited by the fungal culture filtrate extracts against two isolates of bacteria.**

| Fungal species | *E. coli* | *S. aureus* |
|---|---|---|
| *Drechslera australiensis* | 24 | 28 |
| *D. halodes* | 28 | 25 |
| *D.hawaiiansis* | 23 | 28 |
| *RLSD* | 2.977 | 0.987 |

Numbers represent average of three replicates
$P \leq 0.05$

**Table 2. Growth inhibition zones (mm diam) exhibited by fungal mycelia extract against two isolates of bacteria.**

| Fungal species | *E. coli* | *S. aureus* |
|---|---|---|
| *Drechslera australiensis* | 16 | 10 |
| *D. halodes* | 7 | 5 |
| *D.hawaiiansis* | 7 | 9 |
| *RLSD* | 6.175 | 5.442 |

Numbers represent average of three replicates
$P \leq 0.05$

**Table 3. The minimal inhibitory concentrations (MIC) and minimal Bactericidal concentrations (MBC) of fungal crude extracts against two isolates of bacteria.**

| Fungal species | MIC (ug/L) | | MBC (ug/L) | |
|---|---|---|---|---|
| | *E. coli* | *S. aureus* | *E. coli* | *S. aureus* |
| *Drechslera autralienses* | 12.5 | 6.25 | 25 | 12.5 |
| *D. halodes* | 50 | 25 | 100 | 50 |
| *D. hawaiinsis* | 25 | 12.5 | 50 | 25 |

Numbers represent average of three replicates

**Table 4. Bioactivity (inhibition zones mm diam) of the fungal extracts stored at 8 ◦C for six months against two isolates of bacteria.**

| Fungal species | *E. coli* | *S. aureus* |
|---|---|---|
| *Drechslera australiensis* | 26 | 22 |
| *D. halodes* | 20 | 19 |
| *D.hawaiiansis* | 22 | 23 |

Numbers represent average of three replicates

**Table 5. Growth inhibition zones (mm diam) exhibited by different Commercial antibiotics (30 ug/100 ml conc.) and fungal extracts against two isolates of bacteria**.

| Commercial Antibiotics<br><br>(Bioanalyse Comp., Turkey) | *E. coli* | *S. aureus* |
|---|---|---|
| Carbenicillin | 0 | 7 |
| Cefalotaxime | 17 | 0 |
| Chloramphenicol | 25 | 24 |
| Gentamycin | 17 | 19 |
| Penicillin | 6 | 14 |
| Tetracycline | 7 | 17 |
| *D. australensis* | 22 | 28 |
| *D. halodes* | 27.5 | 24 |
| *D. hawaiinsis* | 23.5 | 25.5 |
| RLSD | 6.634 | 3.0791 |

Numbers represent average of three replicates
P≤ 0.06

**Table 6. Chemical compound groups of the fungal crude extracts**

| Fungal species | Alkaloides | Amino acids | Flavenoides | Phenols | Tanins |
|---|---|---|---|---|---|
| *Drechslera autralienses* | - | - | - | - | + |
| *D. halodes* | + | - | - | - | + |
| *D. hawaiinsis* | + | - | - | - | - |

**+ present - absent**

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