

## ANTIBACTERIAL BIOACTIVE COMPOUND FROM THE FUNGUS *DRECHSLERA HALODES* (DRECHSLER) SUBRAM. & JAIN ISOLATED FROM SOIL OF BASRAH, IRAQ

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### ABSTRACT

The fungus *Drechslera halodes* was isolated from soil samples of Basrah, Iraq, and examined for its ability to produce antimicrobial bioactive compounds after being cultured in two types of liquid media for a production of secondary metabolites. The fungal culture filtrate and mycelia extracts were tested against two strains of bacteria *E. coli* and *S. aureus* using a disc diffusion method. The fungal filtrate exhibited a growth inhibition against both bacteria *E. coli* and *S. aureus* with inhibition zones of 25 mm and 30 mm diam., respectively. The minimal inhibitory concentrations (MIC) of the purified compound was examined and revealed that 50 ug/ml was against *E. coli* and 25 ug/ml for *S. aureus*. Toxicity of the fungal extract using human blood indicating that no toxic effect was detected. The fungal extract revealed three separated compounds by using thin layer chromatography (TLC). Solubility, melting points and functional chemical groups were also determined. A bioactive compound was isolated, purified and identified by using Gas Chromatography (GC-mass) and Infra Red (IR). The bioactive compound is identified as 6-allyl-5,6-dihydro-5-hydroxypyran-2-one, with a molecular weight of 154 kd and the chemical formula is  $C_8H_{10}O_3$  which belongs to aromatic esterase group.

**Keywords:** Bacteria, bioactive compound, *Drechslera*, secondary metabolites, soil fungi.

### INTRODUCTION

Fungi are good natural producers for secondary metabolites which contain different pharmaceutical agents including antibiotics, antitumors and antioxidants (Zähner *et al.*, 1983; Abad *et al.*, 2007). As only a small part of mycota is known for a production of bioactive compounds, however, fungi are still one of the most promising sources for antimicrobial agents production (Srivastava and Sharma, 2011). So far, many new biologically active metabolites have been isolated and characterized from Basidiomycota and Ascomycota (Anke, 1989; 1997; Lindquist *et al.*, 2005; Abad *et al.*, 2007). However, less information is available regarding the antimicrobial bioactive metabolites produced by the anamorphic fungi (Deuteromycota). Among these fungi, the genus *Drechslera* is widely distributed over the world with many species are common in soil and as plant pathogens (Ellis, 1971). Nonetheless, some reports indicated that some species of *Drechslera* are capable of producing phytotoxins and mycotoxins (Shotwell and Ellis, 1976; Evidente *et al.*, 2005) as well as potential mycoherbicide known as ophiobolin (Shen *et al.*, 1999; Osterhage *et al.*, 2002; Evidente *et al.*, 2006). In this report an attempt was made to investigate the fungus *Drechslera halodes* isolated from soils in Basra,

Iraq, for its potentiality for a production of antibacterial bioactive compounds.

### MATERIALS AND METHODS

#### Fungal isolate:

Soil samples were collected from different localities in Basrah (southern Iraq) during the year 2010. 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 day. Five discs (0.5 mm diam ) were cut from fungal culture and inoculated in submerged cultures of 500 ml flasks containing 300 ml Potato Glucose Broth (PGB) ( 500 g potato and 20 g glucose) and other flasks containing Yeast Glucose Broth (YEB) (20 g glucose, 10 g Peptone and 5 g Yeast extract) and incubated at 25°C for 2 weeks in a rotary shaker at 120 rpm (Kim *et al.*, 1999).

#### Extraction of fungal bioactive metabolite:

Fungal cultures were filtered on Whatman No.1 filter paper. The filtrate was extracted three times with Ethyl acetate (1:1 vol) using a separating funnel. The organic layer was collected then placed in Petri dishes and dried at room temperature (Kim *et al.*, 1999).

#### Antibacterial fungal filtrate bioactivity assay:

Filter paper discs diffusion method was used to examine the antimicrobial activity of the fungal filtrate and mycelia extracts against two

strains of bacteria, *Escherichia coli* (ATCC 25922 ) and *Staphylococcus aureus* (NCTC 6571). Each of extract was dissolved in ethanol at a concentration of 100 µg, then sterilized filter paper discs (0.6 mm diam) were soaked in fungal extract (Casals, 1979). The filter paper discs saturated with extracts were placed on the surface of Muller-Hinton agar medium in Petri dishes streaked with 0.2 ml of bacterial suspension of *E. coli* and *S. aureus* separately. Plates were incubated at 37°C for 24 hr, an appearance of inhibition zones around the filter paper disc indicating for an antibacterial bioactivity of the fungal extract (Casals, 1979).

#### **Bioactivity of fungal mycelia extract assay:**

Mycelium of the fungal culture was harvested on filter paper, dried in oven at 40 °C for 24 hr and grounded in a mortar with ethyl acetate solvent (1:1 vol) (Petit *et al.*, 2009). The bioactivity of the mycelium extract was examined against the two selected bacterial isolates by using a disc diffusion technique as mentioned above.

#### **The minimum inhibitory concentration (MIC) test:**

The MIC values were determined by the standard serial dilution assay (NCCLS, 2002) using serial dilutions of the fungal filtrate extract (200, 100, 50, 25, 12.5 , 6.5 , 3.1 , 1.5, 0.7 , 0.3 µg/ml ). The MIC values in this assay were indicated by the absence of bacterial growth at the minimal concentration of the fungal extracts.

#### **Cytotoxic test:**

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

#### **Chemical analysis of fungal crude extract:**

Fungal filtrate extract was chemically analyzed for alkaloids, phenols, amino acids, flavenoides, Tannins, Triterpenes, Steroids, Glycosides and Carbohydrates according to the described method (Harborne, 1984).

#### **Solubility test:**

Different solvents were used to examine the solubility of the fungal filtrate extract.

#### **Melting point determination:**

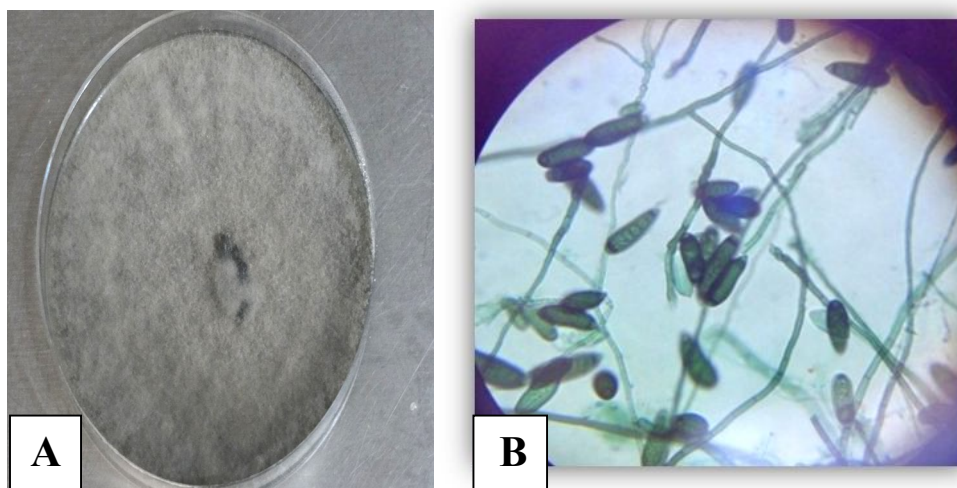
Melting point was determined for the fungal filtrate extract .

#### **Isolation of bioactive compound:**

Thin Layer Chromatography (TLC) was applied for the isolation of the fungal filtrate extract using silica gel of 6 x 1.5 cm (silica gel GF243, Merck, Germany) by Ethyl acetate: Hexane (2:1 vol) and the Rf values were measured. Purification of the extracted compound was made on silica gel column chromatography (silica gel G-60, Merck, Germany).

#### **Identification of the bioactive compound:**

Infra-red spectrum (IR) and Gas chromatography Mass (GC-mass) methods were applied for the identification and determination of the molecular weights, chemical formula and structure of the purified bioactive compound.



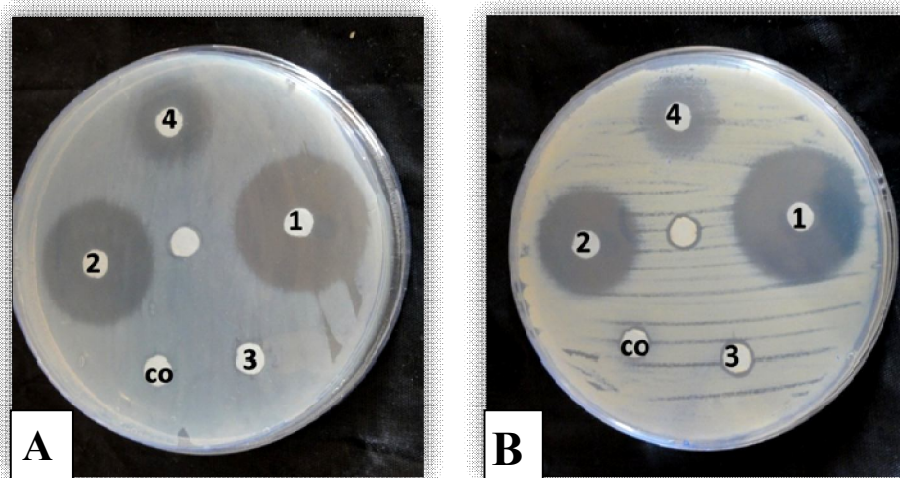
**Figs .1.** *Drechslera halodes* A. Fungal culture growth on PDA medium for 7 days at 25°C. B. Conidia and conidiophores (40 x)

Filtrate extracts from the fungal cultures grown in PGB and YEB media exhibited a growth inhibitory action against both strains of bacteria (Table 1, Fig.2). However, the fungal filtrate from YEB medium revealed a higher

inhibition zones compared with PGB medium. On other hand, fungal mycelia extract exhibited a lower inhibitory action against the tested bacteria with a significant variation ( $p < 0.001$ ) compared with the fungal filtrate extract.

**Table 1.** Growth inhibition zones (mm diam) exhibited by the fungal culture filtrate (\*\*) and mycelia (\*) extracts from PGB and YEB media against two isolates of bacteria. (Numbers represent average of three replicates)

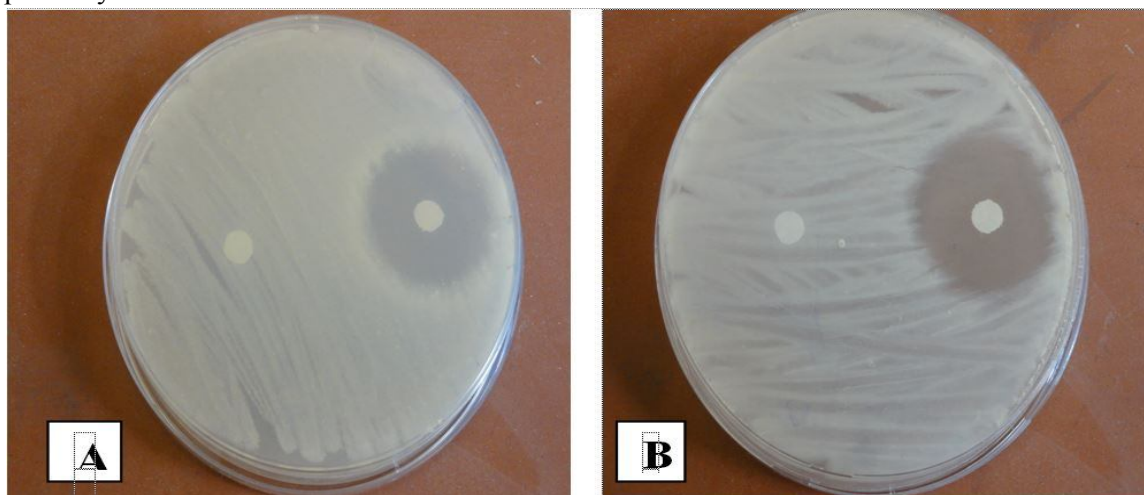
Bacteria	Broth media (inhibition zone mm diam)			
	PGB		YEB	
<i>E. coli</i>	27.6**	7.0*	29.6**	3.0*
<i>S. aureus</i>	25.3	17.3	30.0	5.3



**Fig.2.** Inhibition zones exhibited by the fungal filtrate and mycelia extracts against *E. coli* (A) and *S. aureus* (B): 1. Fungal filtrate extract from YEB medium, 2. Fungal filtrate extract from PGB medium, 3. Fungal mycelia extract from YEB medium, 4. Fungal mycelia extract from PGB medium, and Co: Control (with no extract).

TLC test showed that the fungal filtrate extract contained three spots indicating that there are three compounds (referred as A, B, and C) with Rf values of 0.56, 0.95, and 0.65, respectively.

Among the three isolated compounds, only one compound (B) showed an inhibition zone of 12 mm and 15 mm diam against *E. coli* and *S. aureus*, respectively (Fig.3).



**Fig. 3.** Inhibition zones exhibited by the purified compound (B) against *E. coli* (A) and *S. aureus* (B)

MIC value of the fungal filtrate extract was 25  $\mu\text{g/ml}$  against *E. coli* and 12.5  $\mu\text{g/ml}$  against *S. aureus*. The fungal filtrate extract from both media (PGB and YEB) did not show any toxicity by using human RBC test.

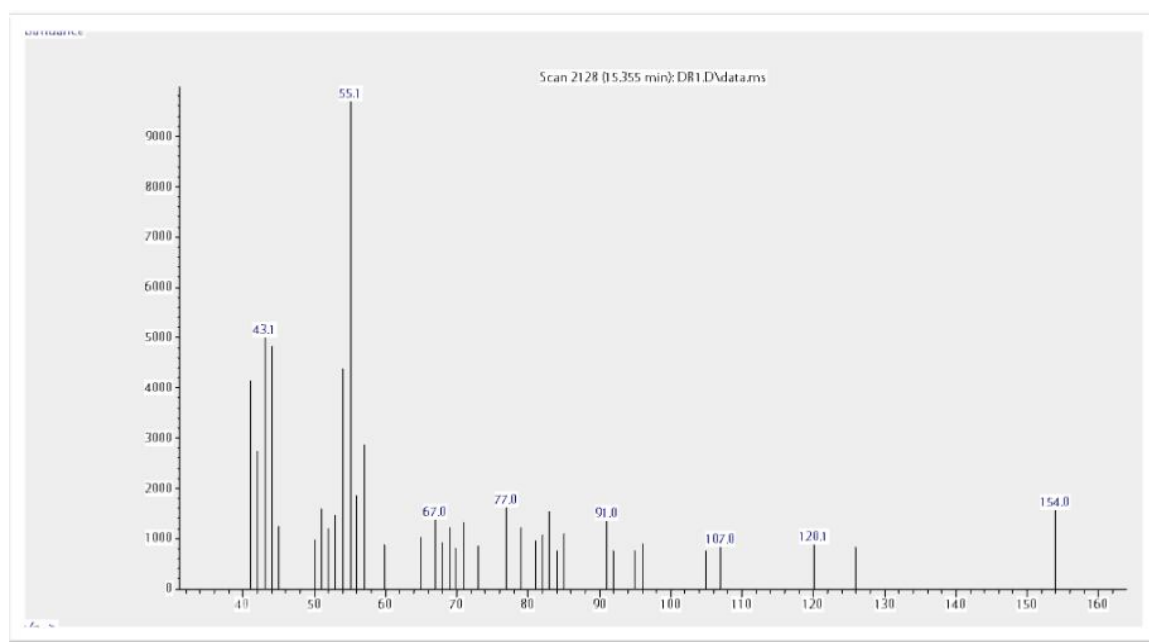
The Chemical analysis of the fungal filtrate extract revealed that *D. halodes* metabolite contains Tannins, Triterpens, Alkaloids and Glycosides.

Solubility test indicated that fungal filtrate extract was insoluble in water and either soluble or partially soluble in the other examined solvents (Table 2)

**Table 2.** Solubility test of fungal filtrate extract.

Solvents	Solubility
Methanol	soluble
Ethanol	soluble
Ethyl acetate	soluble
Chlorophorm	partially soluble
Hexane	partially soluble
DMSO	soluble
Water	insoluble

The melting point of the fungal filtrate extract was 70 – 90 °C. Based on GC-mass (Fig.4) and IR methods (Fig.5), apparently the molecular formula of the bioactive compound (B) is  $\text{C}_8\text{H}_{10}\text{O}_3$  and its chemical structure is 6-allyl-5,6-dihydro-5-hydroxypyran-2-one, with a molecular weight of 154 kd . This indicated that this compound is more related to aromatic Estrase group by comparing its spectroscopic data with available literature (Fig. 6).



**Fig.4.** GC-mass of the purified compound (B) isolated from *D. halodes*

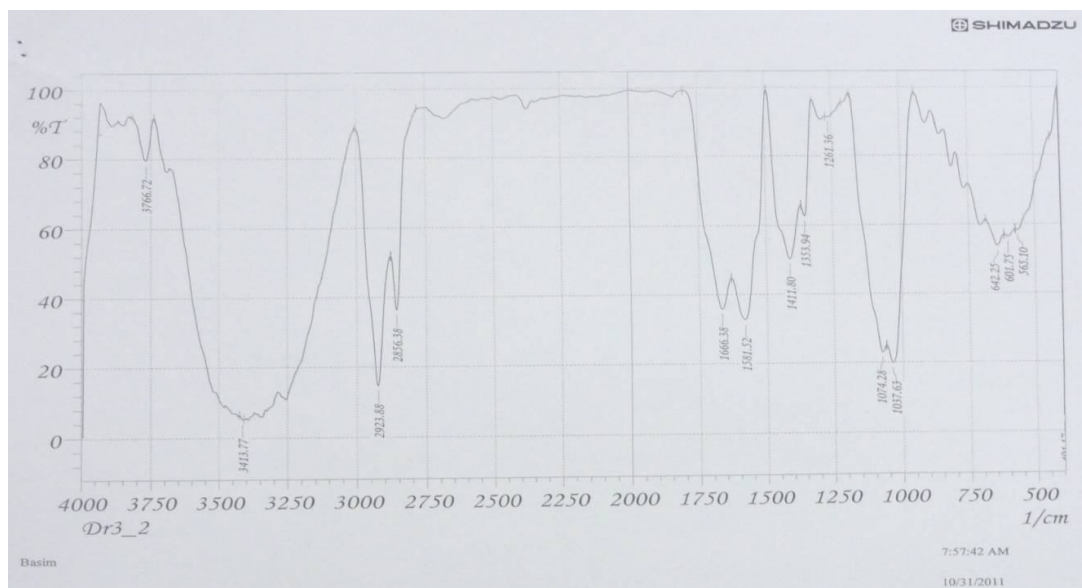


Fig.5. Infra Red spectra of the purified compound(B) isolated from *D. halodes*

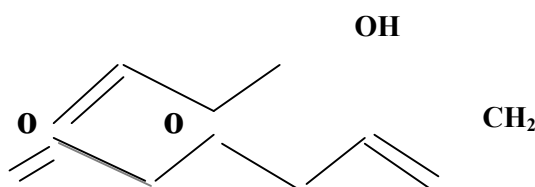


Fig. 6. Chemical structure of the bioactive compound (B) isolated from *D. halodes*.

## DISCUSSION

Fungi have been shown to be one the major natural sources for a production of variety of useful products (Janes et al., 2007; Davis et al., 2008) and with a diversity of chemical compounds (Schulz et al., 2002; Stroble and Daisy, 2003). Nonetheless, natural products are more successful in providing novel bioactive substances than industrial products (Schulz et al., 2002; Newman et al., 2003; Stroble and Daisy, 2003). It has been reported that some biologically active compounds are produced by species of *Drechslera* such as ophiobolins being isolated from *D. oryzae* (Shotwell and Ellis, 1976). Also, triticones A and B were obtained from the plant pathogenic species *D. tritici-repentis* and eremophilane sesquiterpene was isolated from *D. gigantea* (Sugawara et al., 1988). More recently, it has been found that some species of this genus produce potential secondary metabolites such as phytotoxins, in particular sesterterpenoid compounds from *D. maydis* and *D. sorghicola* (Sugawara et al., 2011). Nevertheless, the production of metabolic

substances by fungi is often affected by various growth conditional factors mainly the fermentation medium composition (Jonathan and Fasidi, 2003; Vahidi et al., 2004). In the present study two liquid media (PDB and YEB) were used and both were efficient for a production of bioactive metabolites by the selected fungus. However, the fungal filtrate extract from YEB medium gave a slightly higher antibacterial bioactivity than that derived from PGB cultures. These findings are in concomitant with other studies (Stamets, 2002; Roberts, 2004; Janes et al., 2007) which examined some Basidiomycetous fungi.

The results indicated that the filtrate extract of *D. halodes* exhibited a higher inhibitory action than the mycelia extract against both bacterial strains *E. coli* and *S. aureus*. This can be attributed to the excretion of metabolites by the fungus into the culture medium during the stationary growth phase. In a previous investigation (Muhsin and Mohammad, 2012) found that crude extracts of *D. halodes* exhibited a higher inhibitory action than *D. ausraliensis*



and *D. hawaiiensis* against *E. coli*. Such variations may be attributed to the differences between the chemical constituents the fungal filtrate which may contain various secondary metabolites. 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. aureus* 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 alkaloids against G-negative and G-positive bacteria has also been demonstrated (Navarro and Delgado, 1999; Sawyer *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.

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. A conclusion can be derived from this study that *D. halodes* 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.

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الفعالية الحيوية ضد مايكروبيه للمركب المستخلص من الفطر **Drechslera halodes** المعزول من ترب البصرة

العراق

الملخص

عزل الفطر من التربة في البصرة، جنوب العراق. وتم اختبار الفعالية الحيوية للمركب المعزول منه اتجاه عزلتين من البكتريا باستخدام طريقة النشر بالاقراص. تم عزل ثلاثة مركبت من الراشح الفطري باستخدام تقنية كروموتكرافيا الطبقة الرقيقة. أظهرت النتائج امتلاك المركب فعالية جيدة ضد البكتريا حيث كانت مناطق التثبيط 25 و 20 ملم ضد *E. coli*, *S. aureus* على التوالي.

تم اختبار السمية للمركب اتجاه دم الأنسان ولوحظ انه غير سام. حدد التركيب الكيميائي والوزن الجزيئي والصيغة التركيبية له بواسطة طيف الرنين المغناطيسي وطيف الكتلة. ان الوزن الجزيئي له هو 154kd والصيغة الجزيئية هي  $C_8H_{10}O_3$  والتركيب الكيميائي هو allyl-5,6-dihydro-5-hydroxypyran-2-one