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STUDIES ON THE BIOACTIVE FUNGAL SECONDARY METABOLITES OF SOME FUNGI ISOLATED FROM AL-KHARJ SOIL

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ASTRACT

The present study aimedto investigate the chemical constituents and biological activity of certain soil microorganisms isolated from Al-Kharj Soil, Saudi Arabia.The study includes the investigating the antimicrobial activity as well as searching for new compounds from these soils isolated fungi.Theextra-cellular extracts of nine fungal isolates didnot show any activity against all tested fungi except one fungal isolate who showed slight activity against some tested fungi.On the other hand, the extra-cellular extract of *Alternaria alternata* soil fungal isolate was the only extract that showed an activity against all the tested Gram-negative and Gram-positive bacteria.Consequently, this fungus was chosen forconducting further study. Both extra- and intra-cellularextracts of *Alternaria alternata*were subjected to biological study.Chloroform extract of *A. alternata*fungal broth culturegave the best antibacterial activity against *Proteus vulgaris* and *Salmonella typhi*.Three compounds were isolated from that extract and were identified as hydroxy-2, 5 dimethyl chromen-4-one, chryasophanol, and emodin. The structure elucidation was based on ^{1}H and ^{13}C NMR, proton- proton correlation spectroscopy.

Keywords: Fungal Secondary Metabolites, *Alternaria alternata,* Gram-negative and Gram-Positive Bacteria

INTRODUCTION

Soil is considered one of the most suitable environments for microbial growth.It is consider an extensively explored ecological niche for microorganisms that produce useful biologically active natural products including antibiotics **[1, 2]**. The majority of antibiotics, so far isolated, were produced by Streptomycetes and fungi, which are common inhabitants of the soil **[3]**. The fungi are prolific producers of secondary metabolites. Turner determined almost 1000 fungal products but by 1983 this had more than doubled **[4, 5]** and today it is realistic to anticipate in excess of 6000 different metabolites produced by fungi **[6]**. However, fungal secondary metabolites have a wide range of chemical structure and biological activities; they are derived from many different intermediates by special enzymatic pathways encoded by specific genus **[7]**. The fungal secondary metabolites, or biochemical indicators of fungal development, are of intense interest to humankind due to their pharmaceutical and/or toxic properties **[8]**.The need to search for newer antibiotics has increased several folds due to the development of drug resistance in pathogenic microbes, as well as the increasing frequency of infectious diseases in immunocompromised individuals. Screening of fungal metabolites led to the discovery of novel as well as rediscovery of high numbers of previously described metabolites **[9]**. For this reason, this study is an attempt to investigate the antimicrobial activity of certain soil microorganisms isolated from Al-Kharj soil, Saudi Arabiaand search for new isolated active compounds.

MATERIALS AND METHODS

Soil Sampling and Fungal Isolations

The investigated fungi were obtained from soil samples collected from Al-Kharj, Saudi Arabiaduring 2012. The soil samples were collected in clean plastic bags, at the depth of 5-20 cm, from different sites with various habitats including rocky, clay, and sandy soils. The direct inoculation method was used for sampling and isolation of fungal isolates **[10]**.

Media and Culture Conditions

For isolation, culturing, maintenance of stock cultures, and experimental studies, the following range of media were used: Czapek-Dox Agar (Sucrose, 30 g; Sodium nitrate, 2 g; Potassium hydrogen orthophosphate, 1 g; Potassium chloride, 0.5 g; Magnesium sulphate, 0.5 g; Ferrous sulphate, 0.002 g; Agar, 20 g; Distilled water, 1 L), Malt Extract Agar (Malt extract, 20 g; Peptone, 1 g; Glucose, 20 g; Agar, 20 g; Distilled water, 1 L), Potato Dextrose Agar (Potato extract, 4 g; Glucose, 20 g; Agar, 20 g; Distilled water, 1 L), Sabouraud dextrose agar (Peptone, 10 g; Dextrose, 40 g; Agar, 20 g; Distilled water, 1 L), Yeast Extract Sucrose Agar (Yeast extract, 20 g; Sucrose, 150 g; Agar, 20 g; Distilled water, 1 L), Yeast Malt Agar (Yeast extract, 3 g; Malt extract, 3 g; Peptone , 5 g; Glucose, 10 g; Agar, 20 g; Distilled water, 1 L).The bacterial test organisms were cultured on nutrient agar (Pepton, 5 g; Beef extract, 3 g; Sodium chloride, 3 g; Agar, 20 g; Distilled water, 1 L) and for antibacterial activity were cultured on Mueller hinton agar (Beef Extract, 2 g; Acid Hydrolysate of Casein, 17.5; Starch, 1.5; Agar, 20 g, Distilled water, 1 L). The liquid medium was prepared by using the same ingredients without agar.

For isolated fungi, the petridishes were incubated at 28°C for 4-7 days until fungal growth of the colonies was fully formed. The purified fungal isolateswere maintained on Potato Dextrose Agar slants and stored at 4°C. The investigated soil fungal isolates were incubated at 28°C for 14 days for production of both extra- and intra-cellular metabolites.

Production and Extraction of Fungal Metabolites

The investigated soil fungal isolates were grown on four different media; Malt Extract, Sabouraud Dextrose, Yeast Extract Sucrose and Yeast Malt broth media for the production of both extra- and intra-cellular metabolites. After incubation, the culture broth media werefiltered through filter paper to remove the fungal cell mass (mat) and the cell-free supernatant (broth) was stored in vials at 4°C until further use. The resulting filtrates were used to evaluate antimicrobial activity.

The Extra-cellular extracts (broth) of nine fungal isolates (1 L from each) were extracted by n-butanol (3 L). This step was repeated until complete extraction. The butanol extract was filtered on anhydrous sodium sulphate. The combined filtrates were subjected to concentration using reduced pressure at temperature not exceeding 35°C.

Antimicrobial Activity

Representatives of Gram-negative bacteria; namely, *E. coli* ATCC 025922, *Enterobacter aerogenes*ATCC 013048, *Klebsiella pneumoniae* ATCC 700603, *Proteus vulgaris*

ATCC 006899, *Pseudomonas aeruginosa* ATCC 027853, *Salmonella typhi* ATCC 002202,Gram- positive bacteria;namely, *Bacillus subtilis* ATCC 006633, *Staphylococcus aureus* ATCC 029213,and unicellular fungi; namely, *Candida albicans* ATCC 010231, *Candida tropicalis* ATCC 000665 and filamentous fungi; namely, *Aspergillus niger*RCMB 002007, *Mucor rouxii* RCMB 015002and *Syncephalastrum racemosum* RCMB 005922 were used as test organisms. The fungal strains obtained from the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

The agar well diffusion method (well diameter 9 mm) was used for antimicrobial activity screening of both extra- and intra-cellular extracts of the investigated fungal isolates determination of the minimum inhibitory concentration (MIC). The fungal mat and broth filtrate were extracted as before, left to evaporate till dryness, and then dissolved in 3 ml distilled water.

The test organisms were separately seeded in the agar medium. The wells were cut from the agar and 100 µl of each sample was transferred into them (under a septic condition). After incubation, the plates were examined and the inhibition zones were determined **[11]**.

Extraction and Isolation of Active Compounds

The Extra-cellular extracts (broth) of Kh2 fungal isolate (5 L) was extracted by n-butanol (10 L). This step was repeated until complete extraction. The butanol extract was filtered on anhydrous sodium sulphate. The combined filtrates were subjected to concentration using reduced pressure at temperature not exceeding 35°C.

The obtained residue of Kh2 fungal isolate (55.5 g) was kept for investigation and symbolized as AL1.

The Kh2 fungal mat (500 g)was harvested, washed with distilled water andthen extracted by refluxing in boiled ethanol (2 liter) for 2 hours and then filtered off,this step was repeated until complete extraction. The extracts were filtered on anhydrous sodium sulphate. The combined filtrates were subjected to concentration using reduced pressure at temperature not exceeding 35°C. The obtained residue of Kh2 fungal mat (38g) was kept for investigation and symbolized as AL2.

Both AL 1 and AL 2 were subjected to Antimicrobial activity against selected bacterial test organisms (Gram-negative and Gram-positive bacteria).

The obtained residue of fungal broth extract (55.5 g) was diluted with distilled water and extracted successively by diethyl ether, chloroform, ethyl acetate and n-butanol. Each extract was dried over anhydrous sodium sulphate and concentrated using reduced pressure. The obtained residues were 4, 13, 17 and 20 g, respectively.

Isolation of the active compounds was carried out using ethyl acetate and chloroform extracts because they showed higher antimicrobial activity.

Accordingly, 30 g from the combined extracts (ethyl acetate and chloroform extracts) was applied on silica gel column (450 g, 5 cm x 120 cm), and gradiently eluted with chloroform containing increasingproportion of methanol. One hundred fractions (50 ml each) were collected.Similar fractions were pooled together to afford 4 groups offractions, each groupwas concentrated under reduced pressure to yield (3.5, 5.5 and 1.7 g. respectively). The residue of each fraction group was separately reapplied on Silica gel columns (60, 100 and 40 g. respectively) and gradiently eluted with $CHCl_3$-methanol mixtures to afford three compounds (**1-3**).

Identification of Kh 2 Fungal Isolate

Fungal spores were cultivated on Potato Dextrose Agar media (9 cm diameter Petri dishes) at isolated temperature for 6 days. The germination and growth of mycelium were observed daily under a light microscope SLM (LABO AMERICA, U.S.A.).

Fungal isolate Kh 2 was subjected for certain morphological studies by an Image Analysis System using Soft-Imaging GmbH software (pro ver. 3.0) as well as using RCMB Database Management System for fungalidentification at the Regional Center for Mycology and Biotechnology, Al-Azhar University using documented keys in fungal identification **[12- 19]**.

RESULTS AND DISCUSSION

Nine fungal isolates were obtained from soil samples collected from Al-Kharj, Saudi Arabia during 2012. Also Makut and Owolewa were isolate *Alternaria alternata* and other nine fungal soil isolates from samples and these included *Absidia corymbifera, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium herbarum, Curvularia lunata, Penicillium* sp., *Rhizopus stolonifer* and *Trichoderma viride* **[20]**. The fungal isolates from Al-Kharj soil were subjected to Antimicrobial activities.

Biologicalactivity

a) Screening of Soil Fungal Isolates

The investigated soil fungal isolates were grown on four different medium; Malt Extract, Sabouraud Dextrose, Yeast Extract Sucrose and Yeast Malt broth media for production of both extra- and intra-cellular metabolites.

Extra-cellular extracts of nine fungal isolates were screened for their antimicrobial activities against test organisms (Gram-negative and Gram-positive bacteria; unicellular and filamentous fungi) using agar well diffusion technique. The tested samples (100 µl) were placed in well (9 mm in diameter), inhibition zones around each well were measured in millimeter **(Tables 1)**. Screening of the antimicrobial activities of nine fungal strains, isolated from soil samples, collected from different localities in Al-kharj, showed variable effects **(Tables 1)**.

Allextra-cellular extracts of nine fungal isolates didnot show any activity against all tested fungi except Kh3 fungal isolate was showed slight activity against some tested fungi. The Kh3 soil fungal isolate showed the highest activity (13, 12, 11 mm) grown on yeast Malt, Yeast Extract and Sabouraud Dextrose Broth media respectively against *Syncephalastrum racemosum.*

However, there was a significance activity against Gram-negative and Gram-positive bacteria. The extra-cellular extract of fungal isolate symbolized Kh 2 was the only extract that showed an activity against all the tested Gram-negative and Gram-positive bacteria. The highest activities (29, 27, 26 mm) of Kh 2 grown on Yeast Extract broth medium were determined against *Salmonella typhi, Proteus vulgaris*and *Enterobacter aerogenes* respectively.Among the tested fungal extracts, the *Alternaria alternata* extract inhibited the growth of three tested pathogenic microorganisms presenting an inhibition zone with diameters measuring between 16 mm for *C. albicans* and 27 mm for *S. aureus* **[20]**.The results obtained in this screening showed that *Alternaria alternata* is a promising source of bioactive natural products. Also in the same study showed *Alternaria alternata* is a promising source of bioactive natural products **[21]**. The structural differences presented in the cellular walls of different types of bacteria and fungi are likely to affect the performance of the crude extract **[20]**.

b) Extra- and Intra-Cellular Extracts of Kh 2 Fungus

The Kh 2 soil fungal isolate was grown on four different medium; Malt Extract, Sabouraud dextrose, Yeast Extract Sucrose and Yeast Malt broth media for production of extra- and intra-cellular metabolites. Extra- and intra-cellular extracts (AL 1 and AL 2) of Kh 2 fungal isolate were screened for their antimicrobial activities against selected bacterial test organisms (Gram-negative and Gram-positive bacteria) using well diffusion agar technique. The tested samples (100 µl) were placed in well (9 mm in diameter), inhibition zones around each well were measured in millimeter **(Tables 2)**.

The antibacterial activity of Kh 2 extra-cellular extract (AL 1) was higher than intra-cellular extract (AL 2) against the selected bacteria test organisms at all events (Tables 2).The extracellular extracts of both *Aspergillus flavus* and *Aspergillusniger* showed their activity against Gram-negative bacteria only while the intracellular extracts did not show any antibacterial activity **[22]**.

c) The MICs of Kh 2 Fungal Extract Fractions

The minimum inhibitory concentrations activity of extra-cellular Kh 2 extract fractions by successive solvents were tested against selected test organisms *Proteus vulgaris* and*Salmonella typhi* using agar well dilution agar technique. The tested sample (100 µl) were placed in well (9 mm in diameter), inhibition zones around each well were measured in millimeter. The lowest concentration at which it has inhibition zones was recorded as the minimum inhibitory concentration (MIC) and it was measured in mg/ml of sample **(Tables 3)**.

The lowest minimum inhibitory concentration (3.125, 6.25 and 12.50 mg/ml) were detected by Chloroform, Diethyl ether followed by Ethyl acetate fractions respectively against *Salmonella typhi*. While, the lowest minimum inhibitory concentration (6.25, 12.50 and 25.00 mg/ml) were achieved by Chloroform, Diethyl ether and Ethyl acetate fractions respectively against *Proteus vulgaris* **(Tables 3)**.

Table 1: Antimicrobial activity of the soil fungal isolates grown on different media

Tested organisms / Isolated fungi		Gram –ve bacteria						Gram +ve		Unicellular fungi		Filamentous fungi		
		E. co.	*En. ae.*	*Kl. Pn.*	*Pr. Vu.*	*Ps. ae.*	*Sa. ty.*	*Ba. Su.*	*St. au.*	*Ca. al.*	*Ca. tr.*	*As. ni.*	*Mu. ro.*	*Sy. ra.*
		Diameter of inhibition zone (mm)												
Kh 1 grown on	**Me**	10	00	11	11	00	13	12	00	00	00	00	00	00
	Sa	10	00	00	11	00	12	11	00	00	00	00	00	00
	Ye	12	11	14	12	13	15	13	10	00	00	00	00	00
	Ym	11	00	12	00	10	14	00	00	00	00	00	00	00
Kh 2 grown on	**Me**	11	18	19	15	11	22	18	00	00	00	00	00	00
	Sa	10	18	18	15	10	18	14	10	00	00	00	00	00
	Ye	18	26	25	27	18	29	20	14	00	00	00	00	00
	Ym	14	17	17	21	17	20	19	00	00	00	00	00	00
Kh 3 grown on	**Me**	00	12	00	10	11	12	18	00	00	00	00	00	10
	Sa	10	12	11	12	11	12	15	00	00	00	00	00	11
	Ye	00	14	20	13	14	00	10	00	00	00	00	10	12
	Ym	00	12	13	11	11	13	15	10	00	10	10	10	13
Kh 4 grown on	**Me**	10	11	11	00	00	11	10	00	00	00	00	00	00
	Sa	00	10	12	10	00	00	00	00	00	00	00	00	00
	Ye	11	12	12	10	10	12	11	00	00	00	00	00	00
	Ym	12	14	12	00	10	11	14	00	00	00	00	00	00
Kh 5 grown on	**Me**	00	10	13	10	00	10	11	00	00	00	00	00	00
	Sa	00	14	10	00	00	11	10	00	00	00	00	00	00
	Ye	11	13	00	10	00	17	12	10	00	00	00	00	00
	Ym	10	11	10	10	00	12	15	00	00	00	00	00	00
Kh 6 grown on	**Me**	10	11	12	11	10	12	00	10	00	00	00	00	00
	Sa	00	10	10	11	00	11	10	00	00	00	00	00	00
	Ye	12	10	00	10	00	10	00	00	00	00	00	00	00
	Ym	00	10	00	00	00	11	00	00	00	00	00	00	00
Kh 7 grown on	**Me**	00	00	00	00	00	10	10	00	00	00	00	00	00
	Sa	00	10	00	00	00	11	11	00	00	00	00	00	00
	Ye	10	11	10	10	00	14	10	10	00	00	00	00	00
	Ym	00	10	11	00	00	10	00	00	00	00	00	00	00
Kh 8 grown on	**Me**	00	00	10	10	10	10	00	10	00	00	00	00	00
	Sa	00	10	10	11	00	00	00	00	00	00	00	00	00
	Ye	10	00	12	14	00	11	10	10	00	00	00	00	00
	Ym	00	00	10	00	00	00	00	00	00	00	00	00	00
Kh 9 grown on	**Me**	10	11	10	00	00	12	18	12	00	00	00	00	00
	Sa	00	11	12	15	10	12	14	11	00	00	00	00	00
	Ye	10	12	00	00	13	00	21	13	00	00	00	00	00
	Ym	10	00	00	10	00	11	17	14	00	00	00	00	00

E. co., Escherichia coli; En. ae., Enterobacter aerogenes; Kl. Pn., Klebsiella pneumonia; Pr. vu., Proteus vulgaris; Ps. ae., Pseudomonas aeruginosa; Sa. ty., Salmonella typhi; Ba. Su., Bacillus subtilis; St. au.,Staphylococcus aureus; Ca. al., Candida albicans; Ca. tr., Candida tropicalis; As. ni., Aspergillus niger; Mu. ro., Mucor rouxii; Sy. ra., Syncephalastrum racemosum; **Me, Malt Extract broth; Sa, Sabouraud Dextrose Broth; Ye, Yeast Extract Broth; Ym, Yeast Malt broth; well diameter (9 mm) and tested sample 100 µl.**

Table 2: Antimicrobial activity of fungal Kh 2extra- and intra-cellular extracts grown on different media

Tested organisms / Kh 2 fungal extracts		Gram –ve bacteria						Gram +ve	
		E. co.	*En. ae.*	*Kl. Pn.*	*Pr. Vu.*	*Ps. ae.*	*Sa. ty.*	*Ba. Su.*	*St. au.*
		Diameter of inhibition zone (mm)							
AL 1 of	Me	11	18	19	15	11	22	18	00
	Sa	10	18	18	15	10	18	14	10
	Ye	18	26	25	27	18	29	20	14
	Ym	14	17	17	21	17	20	19	00
AL 2 of	Me	00	16	16	12	00	19	12	00
	Sa	00	15	13	10	00	14	00	00
	Ye	15	19	18	20	13	20	12	11
	Ym	13	13	12	16	10	14	12	00

E. co., Escherichia coli; En. ae., Enterobacter aerogenes; Kl. Pn., Klebsiella pneumonia; Pr. vu., Proteus vulgaris; Ps. ae., Pseudomonas aeruginosa; Sa. ty., Salmonella typhi; Ba. Su., Bacillus subtilis; St. au.,Staphylococcus aureus; Ca. al., Candida albicans; Ca. tr., Candida tropicalis; As. ni., Aspergillus niger; Mu. ro., Mucor rouxii; Sy. ra., Syncephalastrum racemosum; **Me, Malt Extract broth; Sa, Sabouraud Dextrose Broth; Ye, Yeast Extract Broth; Ym, Yeast Malt broth; AL 1, Extra-cellular extract of Kh 2 medium; AL 2, intra-cellular extract of Kh 2 medium; well diameter (9 mm) and tested sample 100 µl.**

Table 3: Determination of MIC value of fractions of extra-cellular Kh 2 fungal extract

Fractions / Test organisms	Diethyl ether	Chloroform	Ethyl acetate	n-butanol
	Minimum Inhibitory Concentration (mg/ml)			
Proteus vulgaris	12.50	06.25	25.00	50.00
Salmonella typhi	06.25	3.125	12.50	25.00

(a) (b)

Figure 1: Light microscopy; Conidia were produced in chains (a) and showed transverse and longitudinal septa (b) of the fungal isolate Kh 2 (× 800)

Identification of Kh 2 Soil Fungal Isolate

The isolated soil fungus Kh 2 was identified based onmacro morphological and micro morphological characteristics using Potato Dextrose Agar medium, fungal wet mount, slide cultures and the most updated keys for identifications. The isolated fungus was cultivated on Potato Dextrose Agar and incubated for 5 days at 28±2°C. Colonies grew moderately well, attained 3-4 cm diameter. The isolate Kh 2 has greyish black colonies with a very thin white margin, and cottony texture. Conidiophores of isolate Kh 2 were brown, straight, septate, bearing light brown conidia with a short obovate beak at the tip. Conidia were produced in chains and showed 3-7 transverse and longitudinal septa **(Figure 1a, 1b)**. The main descriptive features of the isolate Kh 2 indicated that the isolate belongs to the genus *Alternaria*. However, the specific description and diameter of the diagnostic features of the isolate matched the diagnostic features of species *alternata*.

Isolated Compounds

Compound (1): colorless needles, ^{1}H-NMR (500 MHz, DMSO d_6) δ: 2.33 (3H,s, CH3-11), 2.70 (3H, s, CH3-12), 6.04 (1H, s, H-3), 6.67 (1H, d, *J* = 0.9 Hz, H-6), 6.69 (1H, d, *J* = 0.9 Hz, H-8), 10.65 (1H, s, OH); ^{13}C-NMR (DMSO d_6): 178.26 (C-4), 163.83 (C-2), 160.88 (C-7), 159.09 (C-9), 141.40 (C-5), 116.46 (C-6), 114.0 (C-10), 110.64 (C-3),), 100.47 (C-8), 19.3 (C-11).

Compound (2): obtained as an orange crystal; (6 mg) ^{1}H-NMR (DMSO, 500 MHz); δ (ppm) 12.04 (1H, s, OH-8), 11.97 (1H, s, OH-1), 7.80 (1H, dd, *J* = 7.2 , 2.5 Hz, H-5), 7.73 (1H, dd, *J* = 7.2, 7.5 Hz,H-6), 7.56 (1H, d, 2.2 Hz, H-4), 7.40 (1H, d, *J* = 8.4 Hz, H-7), 7.23 (1H, s, H-2), 2.44 (3H, s, CH3).^{13}C-NMR:δ (ppm);192.42(C-9), 181.85 (C-10),162.65(C-1), 162.36(C-8),149.32 (C-3),136.91(C-6), 133.56(C-12), 133.19 (C-14), 124.51124.32(C-2),121.31 (C-4),119.89 (C-5),(C-7), 115.80 (C-13),113.66(C-11),22.24(CH_3).

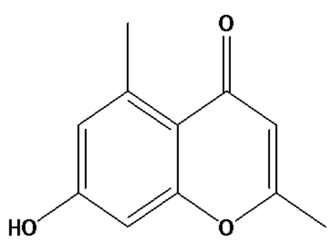
Compound 3: Orange- yellow needle, ^{1}H NMR (125 MHz, DMSO): δ 7.49 (1H, d, *J* = 2.4 H-4), 7.22 (1H, d, *J* = 2.5, H-7), 7.18 (1H, d, *J* = 2.4, H-2), 6.75 (1H, *J* = 2.5, H-5), 2.40 (3H, s, CH3)., protons at δ 12.04 and 12.06 ppm are assignabletohydroxyprotons atthepositions 1 and 8respectively. ^{13}C NMR (125 MHz, DMSO): 186.3 (C-9), 181.9 (C-10), 163.8 (C-1), 161.2 (C-8), 147.9 (C-3), 137.2 (C-12), 132.5 (C-6), 121.1 (C-11), 119.2(C-2), 115.6 (C-5), 115.4 (C-4), 106.4 (C-7).

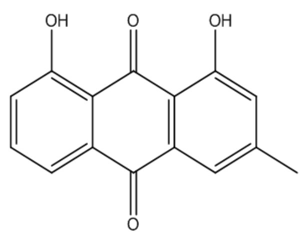
The isolated compounds were identified examining their ^{1}H-NMR, ^{13}C-NMR and as well comparison with the published data **[23]**.

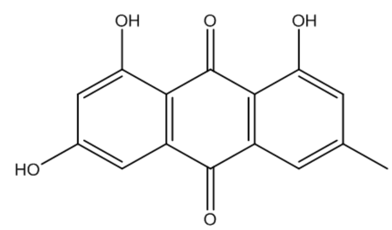
Compound 1from the given data of ^{1}H-NMR, ^{13}C-NMR, COSY, HMQC and HMBC (Fig 1) this compound identified as 7-hydroxy-2,5-dimethyl-chromen-4-one, also the spectral data were in agreement with the literature reported **[24-26]**. This compound previously misidentified as Altechromone A **[27]**.

Compound 2 is identified as chrysophanol as it gave positive test for anthraquinones **[28- 30]**. Its ^{1}H-NMR spectrum displayed two sharp singlets at δ11.97 and 12.04 ppm, assigned for two chelated hydroxyl groups, five signals assigned for aromatic protons at δ 7.23 (1H, s, H-2), δ 7.56 (1H, s, H-4), δ 7.40 (1H, d, *J*= 8.4 Hz, H-7), δ 7.80 (1H, d, *J*= 7.5 Hz, H-5), δ 7.73 (1H, m, H-6). Besides, the methyl singlet at δ 2.44 ppm was assigned to the methyl group at C-3. ^{13}C-NMR spectrum and DEPT experiment data are almost identical with the reported data for chrysophanol **[26, 31, 32]**.

Compound 3 From the given data ^{1}H NMR, ^{13}C NMR, HMBC and HMQC spectra, compound (3) was identified as emodin (1,6,8-trihydroxy-3-methylanthracene-9,10(4aH,9aH)-dione) **[33]**.







[1] 7-hydroxy-2,5-dimethyl-chromen-4-one; [2] Chrysophanol; [3] Emodin

CONCLUSION

In conclusion, most of the extra-cellular extracts of nine fungal isolates didnot show activity against all tested fungi while,the extra-cellular extract of *Alternaria alternata* soil fungal isolate was the only extract that showed an activity against all the tested Gram-negative and Gram-positive bacteria. Three pure compounds isolated from *Alternaria alternata* broth culture. Further researches needed for studying the biological activity of these isolated compounds from *Alternaria alternata.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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