Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp. 1077-1091.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

GC-MS ANALYSIS OF SOME ENDOPHYTIC FUNGAL EXTRACTS

Abdullah H. Al-Saadoon[1*], Najwa M. Jameel [2], Eman H. Bishara [2]

[1]*Department of Pathological Analyses, College of Science, University of Basrah, Iraq*
[2]*Department of Biology, College of Science, University of Basrah, Iraq*

Background : *Endophytic fungi have a wealth of bioactive compounds that are specific to their plant hosts. These compounds benefit a variety of purposes and include antioxidants, immunosuppressant.* ***Aim of study:*** *Investigation to examine the bioactive component of the dichloromethane extract of three endophytic fungi that were isolated from three distinct host plants. The current study used Gas Chromatography-Mass Spectrometry (GC-MS) to analyze the crude extract from secondary metabolites produced from endophytic fungi.* ***Methods:*** *The endophytic fungi Aspergillus fumigatus, Alternaria tenuissima, and Nigrospora zimmermanii were isolated from twigs and leaves of Tamarix aphylla, Trigonella foenum-graecum, and Nerium oleander, respectively. The isolated fungi were identified phenotypically and molecularly using universal and specific primers. The fungi were grown in a liquid fermentation medium Potato Dextrose Broth at 25°C for 30 days. The organic solvent dichloromethane was used in this test, and these extracts were analyzed by Thin Layer Chromatography (TLC) Gas Chromatography-Mass Spectrometry (GCMS).* ***Results:*** *The results showed presence of many Active chemical compounds in the crude extracts produced from the three tested fungi and identified by (TLC) and (GC-MS) analysis.* ***Conclusion :*** *The findings of this study indicate that a broad range of endophytic fungi are capable of producing bioactive compounds that can be used to cure a number of diseases*

Keywords*: Endophytic fungi, Secondary metabolites, (TLC), GC-MS Analysis*

INTRODUCTION

De Bary (1886) was the first to use the term 'endophytes' (Greek: endon-within; phyton-plant). Endophytes are microorganisms that infect living plant tissues without creating obvious disease symptoms and live in mutualistic connection with plants for at least part of their life cycle[1] . Endophytes include all forms of microorganisms (fungi, bacteria, and actinomycetes). Fungi are the most commonly found endophytes [2]. Endophytes are abundant and diverse, having been discovered in every plant species studied to date. It is worth noting that, among the over 3,000,000 plant species that exist on the planet, each individual plant is unique one or more endophytes[3] . Endophytes produce a diverse range of bioactive compounds with distinct structural characteristics. These abundant natural compounds represent a massive reservoir with immense potential for medical, agricultural, and industrial applications [4,5]. Endophytes have produced a diverse spectrum of distinct structures, including alkaloids, benzopyranones, quinones, flavonoids, phenolic acids, and quinines[6]. Fungal secondary metabolites divided to classes, which include nonribosomal peptides such as penicillins, polyketides such as aflatoxin, hybrids of these two classes, and terpenes, indole alkaloids, or oxylipins [7-9] . Secondary metabolites have a wide range of functions and are used as antibiotics, immunosuppressants, antioxidants, antivirals, antidiabetics, and anticancer drugs[10]. In recent years, Gas chromatography-Mass spectrometry (GC-MS) has been employed as one of the technical platforms for fingerprint identification of secondary metabolites in both plant and non-plant species. The current study demonstrates the separation and characterization of

Received : 28/8/2024 & Accepted : 20/10/2024

*Corresponding author: Abdullah H. Al-Saadoon, E-mail: noor.a.ali.748209@gmail.com

metabolites from three species of fungi, using GC-MS.

MATERIALS AND METHODS

Plant samples

Three healthy plant materials including twigs and mature leaves were taken from various locations of Basrah province (Abu-khasib, Al-Zubayr, and Al-Karmah). These plant species were *Tamarix aphylla*, *Nerium oleander*, and *Trigonella foenum-graecum* **(Fig.1)**. Healthy plant parts were collected with a sterile sharp blade, placed in clean sterile plastic bags, and promptly transported to the laboratory.

Isolation of endophytic fungi

Endophytic fungi were isolated from plant parts using the method reported by (Arnold et al . 2000) [11] with some modifications. Plant samples were sliced into small bits (about 5 x 5 mm) with a sterilized sharp scalpel. To reduce the possibility of isolating epiphytic bacteria during sampling, plant pieces were surface sterilized by immersing them in 5% sodium hypochlorite for 3 minutes followed by 70% ethanol for 3 minutes, washed twice with sterile distilled water, and dried on sterile dry filter paper. The fragments were cut up into small pieces transversely and longitudinally and inoculated on to Petri dishes containing potato dextrose agar (PDA) medium supplemented with chloramphenicol (10 µg/mL).The plates were then incubated at 25 °C under dark condition for 7 days and observed for growth of fungal hyphae. The hypha tips were harvested and sub-cultured on PDA plates, incubated at 25 °C to obtain a pure cultures .

Morphological Identification

Taxonomic keys were used to perform morphological identification based on macroscopic and microscopic characteristics, according to the relevant references (de Hoog et al 2000 ؛ Watanabe ،2002 ؛Sheifert et al ، 2011 ؛ Guarro et al ،2012) [12-15] .

Molecular identification

Molecular identification involved several steps:

DNA extraction

Using a sterile needle, the mycelia of pure culture isolates were scraped off in order to extract DNA, and they were then floated in 400–600 µL of lysis buffer (2% Triton X-100, 1% SDS, 1 ml Tris–HCl, 2 ml NaCl, and 2 ml EDTA). 400–600 µL of phenol, chloroform, and isopropanol (25:24:1) were introduced to a 2 mL Eppendrof tube together with 0.11mg glass beads measuring 4 mm in diameter. The samples were vortexed ferociously for 15 minutes. Centrifugation was then used to separate the cellular debris for ten minutes at 4°C and 10,000 rpm. Centrifugation was then used to separate the cellular debris for ten minutes at 4°C and 10,000 rpm. A pipet was used to remove the aqueous phase, and a fresh 1.5 ml Eppendrof tube was filled with 1000 µL of 100% ethanol to precipitate the nucleic acid. After combining the suspensions, they were centrifuged at 4 °C for 10 minutes at 10,000 rpm. They were reconstituted by gradually incorporating 1000 µL of 100% ethanol, 10 µL of ammonium acetate, and 400 µL of Tris-EDTA pH 8 into the mixture. The mixture was centrifuged one again, and the supernatant was thrown away. Until they were needed, the DNA pellets were resuspended in 75–100 µL of TE buffer and kept at -20 °C. Utilizing the UV transilluminator, DNA bands were found and examined [16] .

Amplification of DNA In accordance with White et al. (1990) [17], the Polymerase Chain Reaction assay was carried out. The universal primers ITS1–ITS4 (F-5~-CTT GGT CAT TTA GAG GAA GTAA-3}, R-5{-CGC TAT CAA GCG GAG GAA AAG GC -3}) and NL1–NL4 (F-5{-GCA TAT CAA TAA GCG GAG GAA AAG -3}, R-5~GGT CCG TGT TTC AAG ACGG – 3) are described by Friggens et al. (2017)[18] . To do DNA amplification, each of the items listed in **(Table 1)** was combined in a 100 microliter Eppendorf tube, resulting in a reaction volume of 50 microliters per sample. Following that, a centrifuge was used to mix each component for 10 seconds at 10,000 rpm/min. All samples were processed and centrifuged before being utilized in the sprint thermal cycler PCR, two hours and thirty minutes after turning it on, let the device run, the polymerase chain reaction-amplified DNA was then detected using the electrophoresis method.

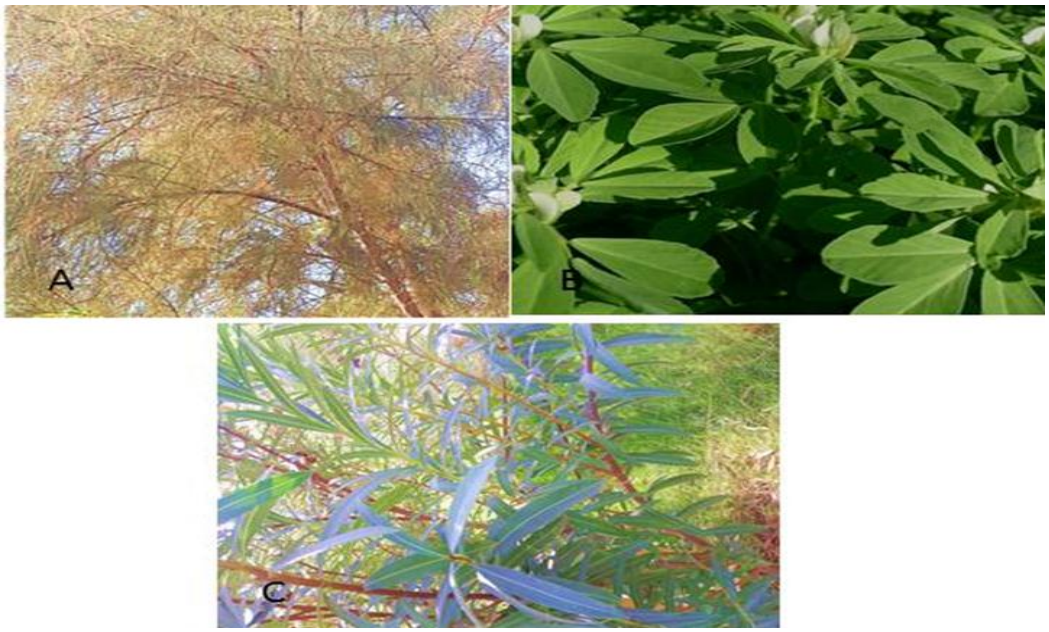

Fig. 1: Sources isolation of endophytic fungi from plants A. *Tamarix aphylla* B. T*rigonella foenum-graecum* C. *Nerium oleander.*

Table 1: Chemicals for the PCR reaction , concentration of DNA.

No.	**materials**	Volume/ µl
1	DNA template(50-70ng)	2
2	Master Mix	25
3	Nuclease free water	19
4	Forward Primer (20 Pmol)	2
5	Reverse Primer (20 Pmol)	2
6	Total vole	50

Production of fungal extracts

The fermentation process was carried out for three fungal isolates by placing, three agar discs of pure culture (6 mm diameter) in 250 ml of potato dextrose broth (PDB), and the pH of the medium was adjusted to 6 by HCl , NaOH before being incubated on a rotating shaker at 120 rpm, 25°C for 30 days. After incubation, the fungal cultures were taken out for production of fungal extracts. The dichloromethane extract of the three endophytic fungi were prepared according to Strobel et al. (1996) [19] and add 0.025% of Na_2CO_3 with solvent, water was desiccated using Na_2SO_4 to produce the organic layer. the extracted filtrated was again prior to being put in Petri dishes and given time to dry at room temperature. One milliliter of methanol was used to dissolve a portion of the dried crude extraction as a stock secondary metabolites for use in a later experiment.

Analysis of crude fungal extract

Thin layer chromatography (TLC)

The crude fungal extract was placed on a TLC plate using capillary tubes and then placed in the solution chloroform/methanol (7:1,v/v) for 10 minutes [19]. After using an iodine detector-which, when heated, evaporates to a violet color and causes spots to appear that indicate the presence of chemical compounds in the crud fungal extract [20] .

Gas chromatography mass spectrometry (GC-MS)

GC-MS was performed at the quality control laboratories in Nahran Omar of Basrah Oil Company, which are equipped with a column the DB-MS5 capillary have dimensions (30 mm in length, 0.32 mm in diameter), to diagnose the fungal extracts using gas chromatography-mass (spectrograph GC-MS) using a Shimadzu GC-MS 2010 gas

chromatograph device. The fixed phase thickness of the column is 0.25 m. As a carrier gas, absolute helium with a concentration of 99.99% was employed. After acquiring the mass spectra of each component included in the extract, the column temperature was raised from its initial temperature of 40 °C for five minutes to a final temperature of 290 °C for one minute A GC-MS software package was used to process the data, and peaks separated curves were defined using the data already included in the 2008NIST Library.

RESULTS AND DISCUSSION

Results

In the current study, *Aspergillus fumigatus*, *Alternaria tenuissima*, *Nigrospora zimmermanii* were isolated from the twigs and leaves of selected plants *Tamarix aphylla*, *Trigonella foenum-graecum*, and *Nerium oleander* respectively **(Fig. 1)**. Morphologically identification was made for the three fungi **(Fig. 2)** and it was found that they belong to Ascomycota, and molecular identification was done for the two species, *Alternaria tenuissima* and *Nigrospora zimmermanii*, using both universal and specific primers. *Alternaria tenuissima* was assigned the code KY315929.1, and *Nigrospora zimmermanii* was assigned the code KY806275.1, and these numbers and symbols represent the fungal species tested in this study and registered in the Japanese Gene Bank **(Fig. 3)** .

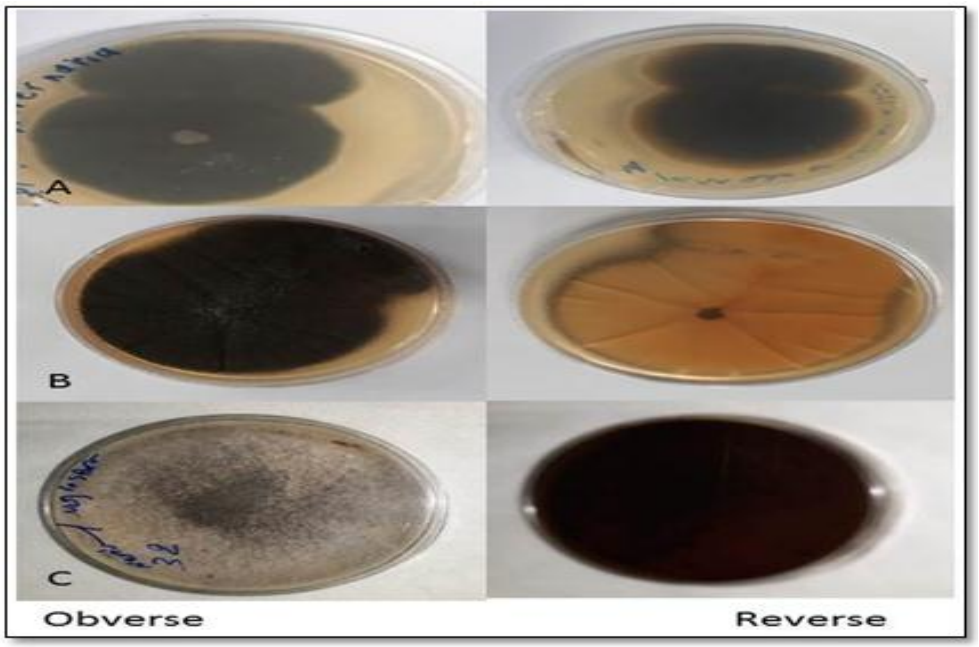

Fig. 2: Obverse and reverse morphology of fungal colonies on potato dextrose agar A . *Alternaria tenuissima* . B *Aspergillus fumigatus* C. *Nigrospora zimmermanii.*

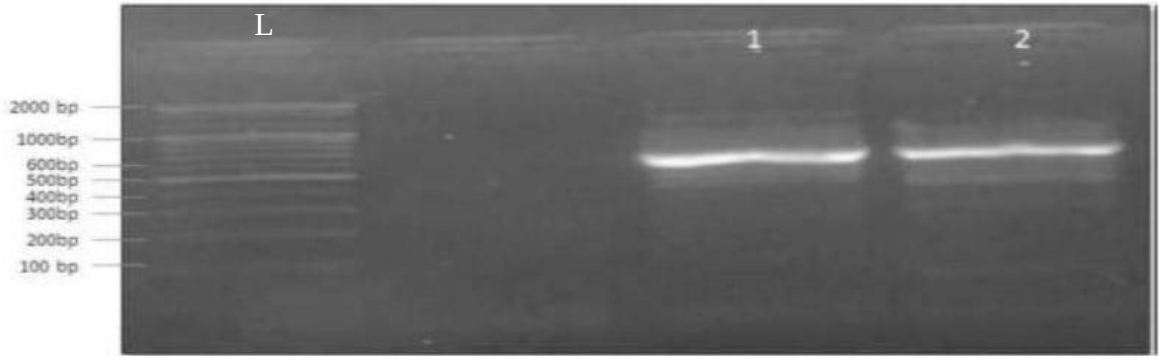

Fig. 3 : Agarose gel electrophoresis 2% of PCR product , L : (100 bp)DNA ladder , 1- *Nigrospora zimmermanii* (578 bp) , 2- *Alternaria tenuissima* (627pb).

Then, a fermentation process was conducted for the three selected fungal species for the production of secondary metabolites. Secondary metabolites, which are generally produced by microorganisms and plants and are often associated with certain genera, species, or strains, are chemically distinct, naturally occurring compounds with relatively low molecular weights [21,22] . Endophytic fungi are becoming more well-known as sources of new bioactive chemicals and secondary metabolites drugs [3]. According to Fox & Howlett (2008) [23], SMs are compounds that neither contribute to nor are required for normal growth or development. Numerous endophytic fungi have been found to create new metabolites with biological relevance, including compounds with antibacterial, antifungal, antiviral, anti-inflammatory, and anticancer properties that are derivatives of alkaloids, steroids, flavanoids, and terpenoids [24,25]. The fermentation results of the fungal species tested in this study showed the presence of various chemical compounds that were detected using the thin layer chromatography as a preliminary detection of the compounds present in the crude extract ,and this method depended on Retention factor (Rf) is the main metric that can be extracted from a TLC investigation and the migration solution was used in the thin layer chromatography chloroform/methanol according to[19]. This may be attributed to the fact that the chemical compounds to be extracted from the secondary metabolism of fungi are insoluble in water and the best solvent to separate these compounds is chloroform. This metric, which is the ratio of the distances traveled by the solvent front and the center of a spot, is used to describe the migration of components over a TLC plate. Both distances are measured from the beginning point [26] **(Fig. 4)** .

These multiple spots represent chemical compounds resulting from secondary metabolites of fungal species. After that, the chemical compounds were detected by GC-MS chromatography. The GC-MS analysis revealed numerous chemicals with varied retention times (RTs) that were formed during the fermentation process of *Nigrospora zimmermanii*, *Alternaria tenuissima*, and *Aspergillus fumigatus* **(Tables 2,3,4)**.

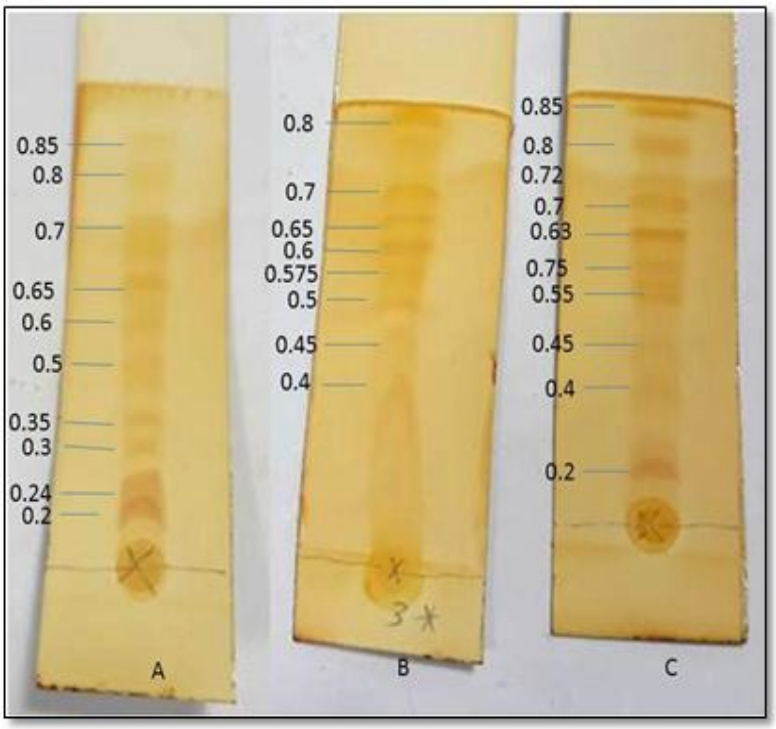

Fig. 4: Thin layer chromatography to detection of chemical compounds present in crude extract of **A.** *Alternaria tenuissima* ,**B**. *Nigrospora zimmermanii* ,**C**. *Aspergillus fumigatus* , Nnmbers = Rf.

Table 2: List of identified compounds of *Aspergillus fumigatus* crude extract by GC-MS analysis.

Peak	RT	Name	Formula	Area	Area%
1	12.844	Azulene	$C_{10}H_8$	807305	0.95
2	16.886	3-(N,N-Dimethyllaurylammonio)propanesulfonate	$C_{17}H_{37}NO_3S$	3500174	4.13
3	17.94	5-Hexadecenoic acid, 2-methoxy-, methyl ester	$C_{18}H_{34}O_3$	272343	0.32
4	19.002	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	308408	0.36
5	19.192	1-Ethynyl-3,trans(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol	$C_{13}H_{22}O_2$	657007	0.78
6	19.463	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	2277980	2.69
7	19.946	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	$C_7H_{10}N_2O_2$	5799073	6.85
8	20.4	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	8962146	10.59
9	20.598	1,18-Nonadecadien-7,10-dione	$C_{19}H_{32}O_2$	237797	0.28
10	20.7	1,9-Dioxacyclohexadeca-4,13-diene-2-10-dione, 7,8,15,16-tetramethyl-	$C_{18}H_{28}O_4$	1501052	1.77
11	21.367	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	10791767	12.75
12	21.579	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	18027827	21.29
13	21.682	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	9854516	11.64
14	22.099	12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one	$C_{14}H_{24}O_3$	202746	0.24
15	23.095	6-Octadecanoic acid	$C_{18}H_{34}O_2$	399345	0.47
16	23.212	1,2-Ethanediamine, N,N,N',N'-tetramethyl-1,2-diphenyl-	$C_{18}H_{24}N_2$	883066	1.04
18	23.776	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	342580	0.40
19	23.878	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	$C_{12}H_{22}N_2O_2$	1789355	2.11
20	24.793	Oleic Acid	$C_{18}H_{34}O_2$	399345	0.47
21	25.16	Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'.alpha.,10.alpha.)-	$C_{33}H_{37}N_5O_5$	5279132	6.24
22	25.496	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	$C_{14}H_{16}N_2O_2$	8417735	9.94
23	26.778	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	$C_{28}H_{25}NO_7$	514206	0.61
24	27.32	(4R,5R)-4-[(E)-6-(Benzyloxy)-hex-3-enyl]-2,2,5-trimethyl-1,3-dioxan	$C_{20}H_{30}O_3$	1809902	2.14
25	27.671	N,N'-Bis(Carbobenzyloxy)-lysine methyl(ester)	$C_{23}H_{28}N_2O_6$	471524	0.56
26	28.33	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	$C_{20}H_{13}N_5O_2$	196738	0.23
27	34.744	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	279425	0.33

Table 3 : List of identified compounds of Alternaria tenuissima crude extract by GC-MS analysis.

Peak	RT	Name	Formula	Area	Area%
1	12.661	Azulene	$C_{10}H_8$	3811567	1.61
2	16.871	3-(N,N-Dimethyllaurylammonio)propanesulfonate	$C_{17}H_{37}NO_3S$	4634986	1.95
3	17.362	5-Hexadecenoic acid, 2-methoxy-, methyl ester	$C_{18}H_{34}O_3$	479895	0.20
4	19.002	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	$C_{18}H_{30}O_2$	690299	0.29
5	19.185	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	$C_{18}H_{30}O_2$	1466368	0.62
6	19.463	2,4-Octadienoic acid, 3-methyl-, methyl ester	$C_{10}H_{16}O_2$	3540280	1.49
7	19.529	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	$C_{16}H_{26}O_2$	1322930	0.56
8	19.829	Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-	$C_{28}H_{48}O$	4996592	2.10
9	20.049	cis-1-Hydroxybicyclo[4.4.0]decane	$C_{10}H_{18}O$	11431989	4.81
10	20.444	2-Ethylcyclohexylamine, N-(2-chloropropylidene)-, N-oxide	$C_{11}H_{20}ClNO$	15869645	6.68
11	20.642	Corymbolone	$C_{15}H_{24}O_2$	700016	0.29
21	20.73	1,9-Dioxacyclohexadeca-4,13-diene-2-10-dione, 7,8,15,16-tetramethyl-	$C_{18}H_{28}O_4$	1292494	0.54
13	20.832	Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-	$C_{28}H_{48}O$	2032383	0.86
14	21.015	Corymbolone	$C_{15}H_{24}O_2$	1581067	0.67
15	21.096	9-t-Butyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	$C_{14}H_{24}O_2$	5684399	2.39
16	21.389	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	14078333	5.93
17	21.455	8-Quinolinol, 5-nitroso-	$C_9H_6N_2O_2$	5822126	2.45
18	21.63	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	32049043	13.50
19	21.733	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	14722254	6.20
20	22.011	Corymbolone	$C_{15}H_{24}O_2$	456016	0.19
21	22.209	1-Heptatriacotanol	$C_{37}H_{76}O$	422657	0.18
22	22.37	1-Heptatriacotanol	$C_{37}H_{76}O$	1131630	0.48
23	22.67	1-Heptatriacotanol	$C_{37}H_{76}O$	426438	0.18
24	23.212	1-Heptatriacotanol	$C_{37}H_{76}O$	1246340	0.52
25	23.886	4-Morpholineacetonitrile, .alpha.-styryl-	$C_{14}H_{16}N_2O$	6146247	2.59
26	24.025	Alloxazine	$C_{10}H_6N_4O_2$		11.18
27	24.881	5,8,11-Heptadecatriynoic acid, methyl ester	$C_{18}H_{24}O_2$		0.17
28	25.182	Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'.alpha.,10.alpha.)-	$C_{33}H_{37}N_5O_5$	413410	3.06
29	25.306	10,18-Bisnorabieta-8,11,13-triene	$C_{18}H_{26}$	885805	0.37
30	25.496	3H-Pyrido(3,4-b)indole, 4,9-dihydro-6-methoxy-1-methyl-	$C_{13}H_{14}N_2O$	36366953	15.32
31	27.122	Pyrimidine, 2,4-diamino-6-ethyl-5-phenyl-	$C_{12}H_{14}N_4$	8036473	3.38
32	27.298	Ethyl 11,11-diformylundeca-2,4,6,8,10-pentaenoate	$C_{15}H_{16}O_4$	1583158	0.67
33	27.62	N,N'-Bis(Carbobenzyloxy)-lysine methyl(ester)	$C_{23}H_{28}N_2O_6$	5907614	2.49
34	28.389	2,20-Cycloaspidospermidine-3-methanol, 6,7-didehydro-, (3.beta.,5.alpha.,12.beta.,19.alpha.,20R)-	$C_{20}H_{24}N_2O$	1400615	0.59
35	30.644	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1a.alpha.,1b.beta.,4a.beta.,5.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-	$C_{26}H_{36}O_8$	885788	0.37
36	31.178	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1a.alpha.,1b.beta.,4a.beta.,5.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-	$C_{26}H_{36}O_8$	72031	0.39

Table 4: List of identified compounds of *Nigrospora zimmermanii* crude extract by GC-MS analysis.

Peak	RT	Name	Formula	Area	Area%
1	12.507	Azulene	C10H8	7767593	1.27
2	16.864	1-Dodecanamine, N,N-dimethyl-	C14H31N	15423448	2.52
3	18.65	Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy-, (8S)-	C15H24O3	1529401	0.25
4	18.767	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol	C14H24O4	2199381	0.36
5	18.987	2(3H)-Benzofuranone, 6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-, [3S-(3.alpha.,3a.alpha.,6.alpha.,7.beta.,7a.beta.)]-	C15H22O2	2571041	0.42
6	19.031	Corymbolone	C15H24O2	1502861	0.25
7	19.156	1-Heptatriacotanol	C37H76O	7529022	1.23
8	19.273	Corymbolone	C15H24O2	1887648	0.31
0	19.47	Corymbolone	C15H24O2	2237078	0.37
10	19.544	Corymbolone	C15H24O2	1485331	0.24
11	19.844	3-Hydroxy-.alpha.-ionene	C13H20O2	12884879	2.11
12	20.056	Corymbolone	C15H24O2	4261685	0.70
13	20.107	Corymbolone	C15H24O2	1636714	0.27
14	20.188	Corymbolone	C15H24O2	3648796	0.60
15	20.32	Longifolenaldehyde	C15H24O	15482008	2.53
16	20.459	1-Heptatriacotanol	C37H76O	1224176	0.20
17	20.525	1-Heptatriacotanol	C37H76O	5118547	0.84
18	20.62	Corymbolone	C15H24O2	2968339	0.49
19	20.715	Corymbolone	C15H24O2	5547331	0.91
20	20.825	1-Heptatriacotanol	C37H76O	7736948	1.26
21	20.942	Corymbolone	C15H24O2	3562552	0.58
22	21.03	2(3H)-Benzofuranone, 6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-, [3S-(3.alpha.,3a.alpha.,6.alpha.,7.beta.,7a.beta.)]-	C15H22O2	6057648	0.99
23	21.169	2(3H)-Benzofuranone, 6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-, [3S-(3.alpha.,3a.alpha.,6.alpha.,7.beta.,7a.beta.)]-	C15H22O2	30980254	5.06
24	21.462	1-Heptatriacotanol	C37H76O	74077385	12.11
25	21.821	1-Heptatriacotanol	C37H76O	11953453	1.95
26	22.158	1-Heptatriacotanol	C37H76O	1963910	0.32
27	22.45	1-Heptatriacotanol	C37H76O	18750305	3.06
28	22.56	1-Heptatriacotanol	C37H76O	2229570	0.36
29	22.795	Methyl 5,9-heptadecadienoate	C18H32O2	122298629	19.99
30	22.846	1-Heptatriacotanol	C37H76O	9990383	1.63
31	23.227	1-Heptatriacotanol	C37H76O	12407009	2.03
32	23.461	1-Heptatriacotanol	C37H76O	6872833	1.12
33	23.578	1-Heptatriacotanol	C37H76O	2889602	0.47
34	24.01	1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol	C14H26O2	87418049	14.29
35	24.398	1-Heptatriacotanol	C37H76O	3946718	0.65
36	24.501	1-Heptatriacotanol	C37H76O	1641002	0.27
37	24.896	1-Heptatriacotanol	C37H76O	1476142	0.24
38	25.196	Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'.alpha.,10.alpha.)-	C33H37N5O5	10683689	1.75
39	25.511	1-Heptatriacotanol	C37H76O	8648788	1.41
40	26.785	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	C28H25NO7	1691540	0.28

According to Bills and Polishook (1991)[27], fungal endophytes are known to contain a variety of new secondary metabolites, some of which have beneficial biological properties. There have been numerous studies on the use of GC-MS for the identification of chemicals produced by various fungi, including [28-34]. Twenty-seven chemicals were found by GC-MS analysis of the *Aspergillus fumigatus* extract shown in **table 2**. Pyrrolo[1,2-a] was the main compound that was ostensibly found Pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-(21.29%), 6-Octadecanoic acid, Oleic acid, Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, 1,18-Nonadecadien-7,10-dione, (Abdel-Wareth et al., 2023) research on the extraction of raw extract from the same species bears similarities to this study[35].

The GC-MS analysis of *Alternaria tenuissima* extract provided in **table 3** resulted in the identification of 36 chemical compounds . The tentatively discovered compounds are, with the prominent ones being 3H-Pyrido(3,4-b)indole, 4,9-dihydro-6-methoxy-1-methyl-(15.32%), Alloxazine (11.18%), 1-Heptatriacotanol, Corymbolone, 8-Quinolinol, 5-nitroso-. GC-MS analysis was utilized to discover 40 compounds from the *Nigrospora zimmermanii* extract in **(Table 4).** The substances that have been provisionally identified are 1-Heptatriacotanol, Methyl 5,9-heptadecadienoate (19.99%), 1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol,

The fungal extracts of the tested species are similar in that they contain the compound Azulene, while the extracts of the *Alternaria tenuissima* and *Nigrospora zimmermanii* species are similar in that they contain the compound 1-Heptatriacotanol. 6-Octadecenoic acid. This item is a very pure unsaturated fatty acid that is perfect for use in biological systems and as a standard. The most prevalent naturally occurring monounsaturated fatty acid in both plants and mammals is oleic acid (6-Octadecanoic acid) **(Fig. 5 A,B)** , Oleic acid and linoleic acid have the ability to control some of the neutrophils functions, which in turn can have an impact on the inflammatory process [36]. It prevents myeloperoxidase from being released, protein kinase C activity in lymphocytes, and the chemotaxis of human neutrophils. It has been linked to a decrease in cardiovascular disease. Rheumatoid arthritis, and many malignancies. It can cause necrosis and death of human lymphocytes [37,38].

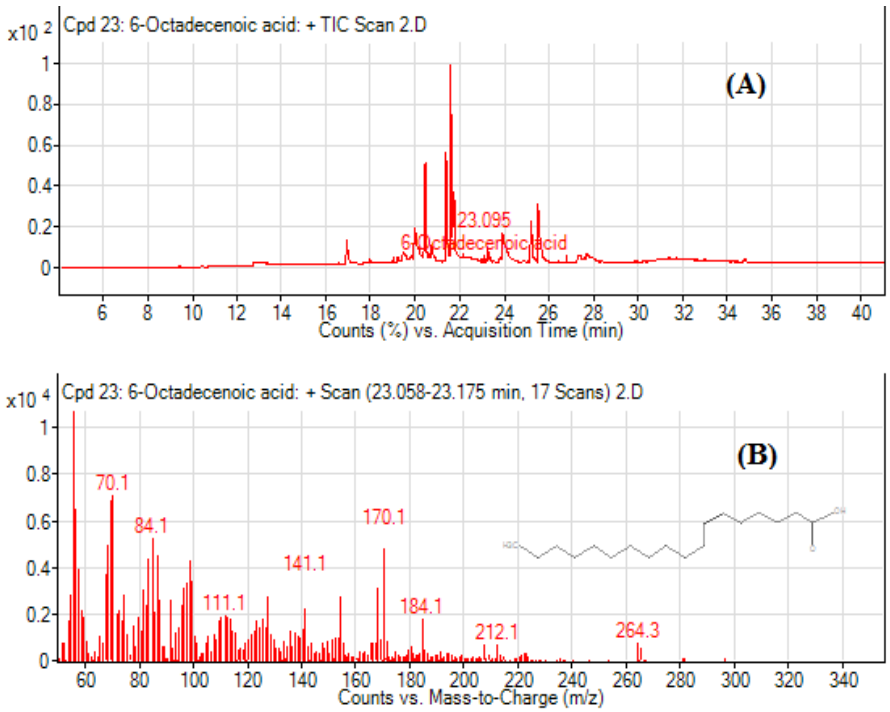

Fig. 5: GC-MS Chromatogram of all identified compounds 6-Octadecanoic acid in *Aspergillus fumigatus* extract, A. Rt 6-Octadecanoic acid =23.095 . B. MS Spectrum peak to 6-Octadecanoic acid.

Olive oil is known to have hypotensive (blood pressure-lowering) properties, which may be attributed to oleic acid [39] . therefor study of (Tutunchi, et al. 2020)[40] against limiting the intake of meals high in oleic acid in order to keep a healthy body weight . It is considered a nutritional supplement and useful in the prevention of metabolic diseases, carcinogens, and antioxidants to harden the arteries, even if it affects obesity and modulates the immune system. It is also valuable as an anti-seizure agent. It reduces cholesterol levels in the blood, and it is used as an anti-inflammatory and anti-arthritis agent and antimicrobial [41]. Monounsaturated omega-9 fatty acids include 6-octadecenoic acid (oleic acid). It has been linked to a possible increase in high density lipoprotein (HDL) cholesterol and a decrease in low density lipoprotein (LDL) cholesterol, when it comes to diabetes, octadecanoic acid, also known as stearic acid, has been demonstrated to reduce LDL cholesterol, be neutral toward HDL cholesterol, and decrease the ratio of total to HDL cholesterol [42,43] .

The compound (1-Heptatriacotanol) **(Fig. 6 A, B)** the Similar to the study (Baskaran et al , 2015; Junwei, 2018; Kalaimagal et al., 2019) [44-46] is the chemical compound 1-Heptatriacotanol. 1-Heptatriacotanol reduces hypercholesterolemia, created for the identical molecule and have significant medical implications .

Alloxazine as **(Fig. 7 A,B)** is used as a scaffold for the design of new and selective kinase inhibitors as antitumor actives. Alloxazine has Anti-inflammatory and Antinociception effects in vitro [47] .

The variations between GC-MS and TLC through the use of a solid stationary phase, such as silica, and a liquid mobile phase, thin layer chromatography used to identification both organic and inorganic materials. While method GS, it is utilized in the analysis and separation of volatile organic compounds and gases. Both the gaseous mobile phase and the liquid stationary phase are necessary for it to work. Compounds in complicated mixtures can be successfully separated and identified using GC-MS, a powerful tool in modern analytical chemistry [48-50] .

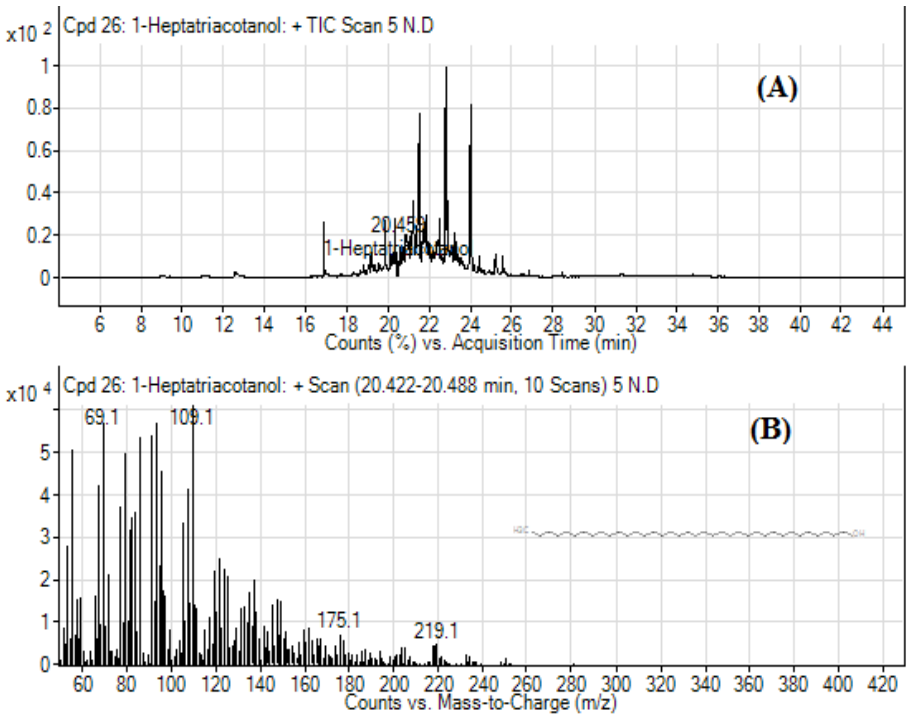

Fig. 6: GC-MS Chromatogram of all identified compounds 1-Heptatriacotanol in *Nigrospora zimmermanii* extract A.Rt 1-Heptatriacotanol =20.459 , B. MS Spectrum peak to 1-Heptatriacotanol.

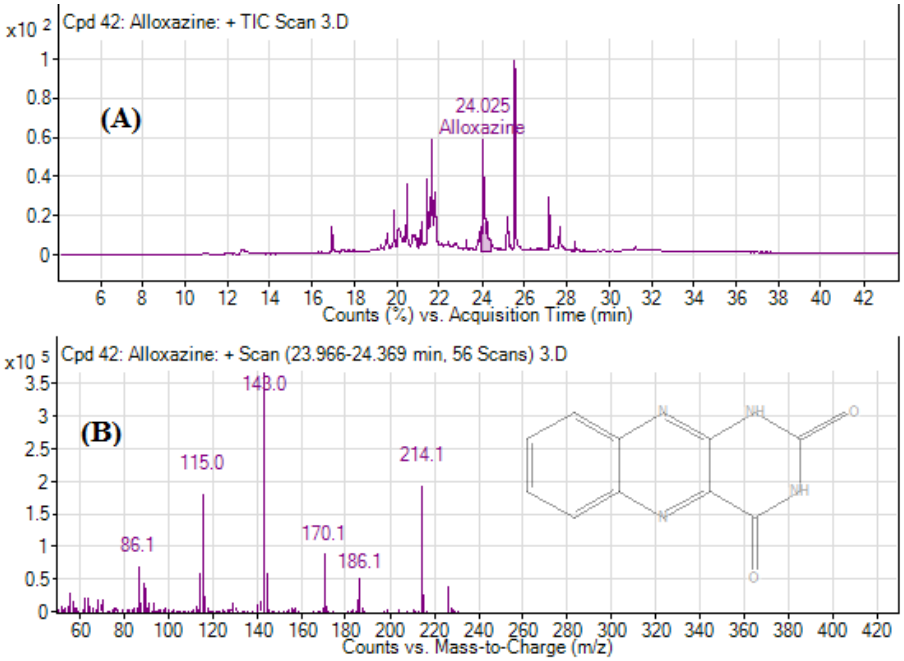

Fig. 7: GC-MS Chromatogram of all identified compounds Alloxazine in *Alternaria tenuissima* extract A. Rt Alloxazine = 24.025 , B . MS Spectrum peak to Alloxazine.

Because the capillary column has a strong resolution, the GC-MS approach, which is used to identify low-molecular-weight molecules, is exact. Although the TLC approach is less precise and has the drawback of not being able to separate the isomers, it can be used to screen for quickly detection in food, drink, and vegetable extracts, while GC-MS is more expensive but also more sensitive and yields significantly more information, therefore TLC is relatively the least expensive practical technology utilized in laboratories [51].

The current study showed that the identification of many chemical compounds in the tested fungal extracts , which were different from the compound recorded in previous studies [33,34,52]. Endophytes are the source of novel and distinctive secondary metabolites, which might be a wonderful source of medications with anti-inflammatory, antioxidant, anticancer, antibacterial, and anti-diabetic properties [53]. The creation of natural metabolites from endophytic fungi may also help to safeguard natural resources and meet drug demand by producing plant-derived natural metabolites .

Conclusion

Endophytic fungi are of great importance in the production of bioactive compounds. The crude extract of the three fungal species examined in this study contains a wide variety of chemical components. These substances are significant from a medical standpoint. In the lab, researchers may assess the potency of the substances found by GC-MS and determine whether they are efficient antibacterial and antifungal drugs. Research can be done to evaluate them and see how the pharmaceutical sector can use them. Additional work is required to separate and characterized the active compounds in these fungal extracts using other techniques such as HPLC and LC-MS evaluate their bioactivities .

Acknowledgement

The GC-MS instrumental facility provided by Nahran Omar of Basrah Oil Company and the University of Basrah, Science and Biology, are greatly appreciated by the authors.

REFERENCES

1. C.W. Bacon and J.F. White, "Microbial endophytes", ***Marcel Deker Inc***, New York, (2000).
2. A. Staniek, H.J. Woerdenbag and O. Kayser, "Endophytes, Exploiting Biodiversity for the Improvement of Natural Product-Based Drug Discovery", ***J of Plant Intera***, 3(2), 75-93 (2008).

3. G. Strobel, B. Daisy, "Bioprospecting for Microbial Endophytes and Their Natural Products", ***Micro and Mole Bio Rev,*** 67(4), 491-502 (2003).
4. R.X. Tan, W.X. Zou, "Endophytes, A Rich Source of Functional Metabolites (1987 to 2000)", ***Nat Pro Rep***, 18(4), 448-459 (2001).
5. H.W. Zhang, Y.C. Song, R.X. Tan, "Biology and Chemistry of Endophytes", ***Nat Pro Rep***, 23(5), 828-829 (2006).
6. M.R. Pimentel, G. Molina, A.P. Dionísio, *et al.*, "The Use of Endophytes to Obtain Bioactive Compounds and Their Application in Biotransformation Process", ***Biotechno Res Inter***, 2011, 1-11(2011).
7. N.P. Keller, G. Turner and J.W. Bennett, "Fungal Secondary Metabolism - from Biochemistry to Genomics", ***Nat Rev Micro***, 3(12), 937-947 (2005).
8. D. Hoffmeister, N.P. Keller, "Natural Products of Filamentous Fungi, Enzymes, Genes, and Their Regulation", ***Nat Prod Rep,*** 24(2), 393-416 (2007).
9. S.A. Christensen and M.V. Kolomiets, "The Lipid Language of Plant–Fungal Interactions", ***Fun Gen and Bio***, 48(1), 4-14 (2011).
10. A.A.L. Gunatilaka, "Natural Products from Plant-Associated Microorganisms, Distribution, Structural Diversity, Bioactivity, and Implications of Their Occurrence⊥", ***J of Nat Pro***, 69(3), 509-526 (2006).
11. A.E. Arnold, Z. Maynard, G.S. Gilbert, *et al.*, "Are Tropical Fungal Endophytes Hyperdiverse?", ***Eco Letters***, 3(4), 267-274 (2000).
12. G. S. De Hoog, J. Guarro, J. Gene, *et al.*, "Atlas of clinical fungi, Centraalbureau voor Schimmelcultures", 4th, ***Universitat Rovira***, Virgili, (2000).
13. T. Watanabe, "Pictorial atlas of soil and seed fungi morphologies of cultured fungi and key to species", ***CRC Press***, 486, (2002).
14. K.A. Seifert, W. Gams, "The Genera of Hyphomycetes - 2011 Update", ***Perso Mole Phylo and Evo of Fun***, 27(1), 119-129 (2011).
15. J. Guarro, J. Gene, A.M. Stachigel, *et al.*, "Atlas of soil Ascomycetes", ***CBS-KNAW Fungal Biodiversity Center Utrecht***, Netherland, (2012) P. 997.
16. J. Manjunathan, M. Kumar and V. Kaviyarasan, "Taxonomic studies, rapid and efficient protocol for DNA extraction, Purification, molecular characterstics of the basidiomycete lentinus tuberregium (FR) GQ292711", ***Asian J Pharma Clin Res,*** 4(2), 54-58 (2011).
17. TJ. White, T. Bruns, S. Lee, *et al.*, "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In, InnisMA, Gelfand DH, Sninsky JJ,White TJ (eds) PCR protocols, a guide to methods and applications", ***Academic Press***, San Diego, (1990), p. 315–322.
18. NL. Friggens, "Diversity and Community Composition of Aquatic Ascomycetes Varies between Freshwater, Estuarine and Marine Habitats in Western Scotland", ***Mycos***, 8(9), 1267-1287 (2017).
19. G. Strobel, X. Yang, J. Sears, *et al.*, "Taxol from Pestalotiopsis Microspora, an Endophytic Fungus of Taxus Wallachiana", ***Micro***, 142(2), 435–440 (1996).
20. L.S. Garcia, L.R. Ash, "Diagnostic parasitology clinical 2nd edition", ***The C V Mosby Company, Missouri***, U.S.A., (1979).
21. J.C. Frisvad, B. Andersen and U. Thrane, "The Use of Secondary Metabolite Profiling in Chemotaxonomy of Filamentous Fungi", ***Mycolo Res,*** 112(2), 231-240 (2008).
22. B. R. Herbert, "Biosynthesis of secondary metabolites 2nd", ***Champman and Hall London***, (1989)**,** p. 47-64.
23. E.M. Fox, B.J. Howlett, "Secondary Metabolism, Regulation and Role in Fungal Biology", ***Cur Opin in Micro***, 11(6), 481-487 (2008).
24. B. Guo, Y. Wang, X. Sun, *et al.*, "Bioactive Natural Products from

Endophytes, A Review", ***Appl Biochem and Micro***, 44(2), 136-142 (2008).
25. H. Yu, L. Zhang, L. Li, *et al.*, "Recent Developments and Future Prospects of Antimicrobial Metabolites Produced by Endophytes", ***Microbio Res***,165(6), 437-449 (2010).
26. J. Sherma, Thin layer chromatography, In Analytical Instrumentation Handbook, ***CRC Press***, 2004, p. 1021-1040.
27. G.F. Bills, J.D. Polishook, "Microfungi from *Carpinus Caroliniana",* ***Cana J of Bot***, 69(7), 1477-1482 (1991).
28. U. B, P. K, R. S, Y. Lakshmi, Sarayu,"Studies on GC/MS Spectroscopic Analysis of Some Bioactive Antimicrobial Compounds from *Cinnamomum Zeylanicum*", ***Bvsalud Org***, 8(31), 125-131 (2024).
29. N. N. Devi and M. S. Singh, "GC-MS Analysis of metabolites from endophytic fungus *Colletotrichum gloeosporioides* isolated from *Phlogacanthus thyrsiflorus* Nees", ***Int J Pharm Sci,*** 23(2), 392-395 (2013).
30. N. Senthilkumar, S. Murugesan, D. S. Babu, *et al.*, "GC-MS analysis of the extract of endophytic fungus, *Phomopsis* sp. isolated from tropical tree species of India, *Tectona grandis* L.", ***Int J Innov Res Sci Eng Tech***, 3, 10176-10179 (2014).
31. D. Sharma, A. Pramanik and P.K. Agrawal, "Evaluation of Bioactive Secondary Metabolites from Endophytic Fungus Pestalotiopsis Neglecta BAB-5510 Isolated from Leaves of Cupressus Torulosa D.Don", ***Biotech,*** 6(2), 210-218 (2016) .
32. K. Sahani and D. Thakur, "Gcms Analysis of Endophytic Fungi Curvularia Aeria Mtcc-12847 Isolated From Tribulus Terrestris L", ***Inter J of Pharma and Pharma Sci***, 11(4), 26–32 (2019).
33. R.R. Hateet, "GC-MS Analysis of Extract for Endophytic Fungus Acremonium Coenophialum and Its Antimicrobial and Antidiabetic", ***Res J of Pharma and Techno***, 13(1), 119-123 (2020).
34. S. Kumari, S. Kumari, C. Attri, *et al.*, "GC-MS Analysis, Antioxidant and Antifungal Studies of Different Extracts of Chaetomium Globosum Isolated from Urginea Indica", ***Bio Med Res Inter,*** 2022, 1–12 (2022).
35. TA, A. Eman, El-Shazly and Maha Awad, "Biological Activity and GC-MS/MS Analysis of Extracts of Endophytic Fungi Isolated from Eichhornia crassipes (Mart.) Solms", ***J of App Biotechno Rep,*** 10(1), 895-909 (2023).
36. H.G. Rodrigues, M.A.R. Vinolo, J. Magdalon, *et al.*, "Dietary Free Oleic and Linoleic Acid Enhances Neutrophil Function and Modulates the Inflammatory Response in Rats", ***Lipi***, 45(9), 809–819 (2010).
37. E. Waterman and B. Lockwood, "Active components and clinical applications of olive oil", ***Alter Med Rev,*** 12(4), 331-342 (2007).
38. L.G. Gillingham, S. Harris-Janz and P.J.H. Jones, "Dietary Monounsaturated Fatty Acids Are Protective against Metabolic Syndrome and Cardiovascular Disease Risk Factors", ***Lipi***, 46(3), 209–228 (2011).
39. H. Tutunchi, A. Ostadrahimi and M. Saghafi-Asl, "The Effects of Diets Enriched in Monounsaturated Oleic Acid on the Management and Prevention of Obesity, A Systematic Review of Human Intervention Studies", ***Adva in Nutr***, 11(4), 864–877 (2020).
40. S. Terés, G. Barceló-Coblijn, M. Benet, *et al.*, "Oleic Acid Content Is Responsible for the Reduction in Blood Pressure Induced by Olive Oil", ***Proce of the Nat Aca of Sci of the Uni Sta of Ame,*** 105(37), 13811-13816 (2008).
41. G. Sangeetha, S. Rajeshwari, R. Venckatesh, "Green Synthesis of Zinc Oxide Nanoparticles by Aloe Barbadensis Miller Leaf Extract, Structure and Optical Properties", ***Mat Res Bull***, 46(12), 2560-2566 (2011).
42. J.E. Hunter, J. Zhang and P.M. Kris-Etherton, "Cardiovascular Disease Risk of Dietary Stearic Acid Compared with Trans, Other Saturated, and

Unsaturated Fatty Acids, A Systematic Review", ***The Amer J of Clin Nutr***, 91(1), 46-63 (2009).

43. B. I. Mgbeje, J. Ofem-Out and F. O. Ugoanyanwu, "Phytochemical components of butanol fraction of *Vernonia amygdalina* leaf extract using GC-MS analysis", ***Mer Res J of Med & Med Sci***, 8(4), 74-79 (2020).
44. G. Baskaran, M.Y. Shukor, S. Salvamani, *et al.*, "HMG-CoA Reductase Inhibitory Activity and Phytocomponent Investigation of Basella Alba Leaf Extract as a Treatment for Hypercholesterolemia", ***Drug Des Devel and Ther***, 9, 509-517 (2015).
45. L. Junwei, C. Juntao, N. Changyu, *et al.*, "Molecules and Functions of Rosewood, Pterocarpus Cambodianus", ***Arab J of Chem***, 11(6), 763–770 (2018).
46. C. Kalaimagal, "Identification of Bioactive Compounds in Flower of Tabernaemontana Divaricata (L.) Using Gas Chromatography–Mass Spectrometry Analysis", ***Asian J of Pharma and Clin Res***,12, 129-132 (2019).
47. W.H. Malki, A.M. Gouda, H.E.A. Ali, *et al.*, "Structural-Based Design, Synthesis, and Antitumor Activity of Novel Alloxazine Analogues with Potential Selective Kinase Inhibition", ***Eur J of Med Chem***, 152, 31-52 (2018).
48. RA. Anderson, "Gas Chromatography–Mass Spectrometry", ***J of High Res Chrom,***12(5), 287-287 (1989).
49. M. Gallo and P. Ferranti, "The Evolution of Analytical Chemistry Methods in Foodomics", ***J of Chrom***, 1428 (January), 3-15 (2016).
50. J. Vázquez-Martínez and Mercedes Peláez López, "Chemometrics-based TLC and GC-MS for small molecule analysis, a practical guide", ***Chemo and Data Ana in Chrom***, 15-32 (2018).
51. R. Oprean, T. Martos and L. Roman, "Comparison of GC-MS and TLC Techniques for Asarone Isomers Determination", ***J of Pharma and Biomed Ana***, 18(1-2), 227-234 (1998).
52. H.H. Alfartossy, A.Q. Dawood, Dhurgham. *et al.*, "GC-MS Analysis of Fungal Filtrate Crude Extracts Produced by Two Species of Aspergillus", ***J of Edu for Pure Sci***,9(1), 271-283 (2019).
53. S.-S. Zhao, Y.-Y. Zhang, W. Yan, *et al.*, "Chaetomium Globosum CDW7, a Potential Biological Control Strain and Its Antifungal Metabolites", ***FEMS Micro Lette***, 364(3), 1-6 (2016).

Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp.1077-1091.

نشرة العلوم الصيدلية
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

تحليل بعض المستخلصات الفطرية الداخلية باستخدام التحليل الكروماتوجرافي الغازي و مطياف الكتلة

عبدالله حسين السعدون [١*] – نجوى محمد جميل [٢] – إيمان حسين بشارة [٢]

[١] قسم التحاليل المرضية، كلية العلوم، جامعة البصرة، العراق

[٢] قسم الأحياء، كلية العلوم، جامعة البصرة، العراق

تحتوي الفطريات الداخلية على ثروة من المركبات النشطة بيولوجيًا الخاصة بمضيفيها النباتيين. تفيد هذه المركبات مجموعة متنوعة من الأغراض وتشمل مضادات الأكسدة ومثبطات المناعة. الهدف من الدراسة: التحقيق في فحص المكون النشط بيولوجيا لمستخلص ثنائي كلورو ميثان لثلاثة فطريات داخلية تم عزلها من ثلاثة نباتات مضيفة متميزة. استخدمت الدراسة الحالية التحليل الكروماتوجرافي الغازي و مطياف الكتلة لتحليل المستخلص الخام من المستقلبات الثانوية المنتجة من الفطريات الداخلية. الطرق: تم عزل الفطريات الداخلية من أغصان وأوراق النباتات المختلف. تم تشخيص الفطريات المعزولة ظاهريا وجزيئيا باستخدام بادئات عامة ومحددة. تمت زراعة الفطريات في وسط تخمير سائل لمرق دكستروز البطاطس عند درجة حرارة ٢٥ درجة مئوية لمدة ٣٠ يومًا. تم استخدام المذيب العضوي ثنائي كلورو ميثان في هذا الاختبار، وتم تحليل هذه المستخلصات بواسطة كروماتوغرافيا الطبقة الرقيقة و التحليل الكروماتوجرافي الغازي و مطياف الكتلة . النتائج: أظهرت النتائج وجود العديد من المركبات الكيميائية النشطة في المستخلصات الخام المنتجة من الفطريات الثلاثة التي تم اختبارها والتي تم تشخيصها بواسطة تحليل كروماتوغرافيا الطبقة الرقيقة و التحليل الكروماتوجرافي الغازي و مطياف الكتلة. الاستنتاج: تشير نتائج هذه الدراسة إلى أن مجموعة واسعة من الفطريات الداخلية قادرة على إنتاج مركبات نشطة بيولوجيا يمكن استخدامها لعلاج عدد من الأمراض.