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Antioxidant, Antimicrobial, and Anticancer Cells Line of *Aspergillus flavus* ON764430 Extracts Isolated from Al Mudawara Mountain, El Fayum Governorate

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ABSTRACT

Fungal secondary metabolites possess powerful bioactive compounds formed by fungi isolated from diverse sources. In this study, a fungus was isolated from the sandy soil of Al Mudawara Mountain at El Fayum governorate to examine its antibacterial activity, cytotoxicity against cancer cells, and antioxidant effect. The fungus was identified using morphological and molecular methods as *Aspergillus flavus*, with a 99.29 percent similarity rate. The fungal secondary metabolites were extracted using different organic solvents (petroleum ether, ethyl acetate, and chloroform) in order. These extracts were tested for their antimicrobial activities against some pathogenic microorganisms using the well diffusion method. Furthermore, using the MTT viability assay, the antitumor properties of the active extracts were tested against HepG2 and PC-3, two different tumor cell lines. *Aspergillus flavus* petroleum ether extract had high inhibitory activity against HepG2 and PC-3 cells, according to the findings, However, lower inhibitory activity was detected with ethyl acetate extract against HepG2 and PC-3 cells, respectively. Also, the DPPH free radical scavenging assay was used to assess the antioxidant activity. The petroleum ether extract from *Aspergillus flavus* showed moderate antioxidant activity with IC_{50} of 272 ± 3.7µg/ml followed by ethyl acetate 901.3 ± 42.8µg/ml. GC-MS analysis of the petroleum ether extract showed the presence of different potent products. In conclusion, *Aspergillus flavus* ON764430 extracts made from ethyl acetate and petroleum ether had moderate antimicrobial, antioxidant, and antitumor properties. To find out how the activities work, additional pharmacological and *in vivo* studies were suggested.

1. Introduction

Antibiotic overuse has led to the emergence of multidrug-resistant (MDR) bacteria, which have significantly decreased the efficacy of important antimicrobials that are

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currently in widespread use, which is now a significant global problem [1]. In the pharmaceutical and agricultural industries, natural product studies have played a significant role in the creation of high-value products that can be used in human health care, nutrition, and therapeutics [2]. Fungi are one of the most prevalent sources of bioactive secondary metabolites [3], They are eukaryotic, heterotrophic microorganisms, and some of them symbiotically live in a variety of environments, spreading worldwide [4]. Fungi have been observed to have high metabolic rates, for instance, in desert soils [5]. Members of the genus *Aspergillus* are known to generate a range of beneficial secondary metabolites, which are mostly developed into therapeutic leads for human health [6,7]. Numerous studies have demonstrated that *Aspergillus* has potent antioxidant properties due to its abundant supply of phenolic compounds [8]. *Aspergillus* is a well-known genus of fungi that contains numerous species from which numerous metabolites of various classes, including alkaloids, steroids, polyketides, peptides, and terpenoids, were found to have remarkable biological effects, particularly against cancer and pathogenic microorganisms [9,10]. The main goal of this study is to investigate the bioactive metabolites of *Aspergillus flavus* ON764430 isolated from Al Mudawara Mountain, El Fayum Governorate, and Egypt, as antimicrobial, antioxidant, and anticancer agents, in addition to identifying the major bioactive metabolites of the fungus using GC-MS analysis.

2. Materials and Methods

2.1. Isolation of Used Fungal Strain

In this study, the used fungal strain was isolated from a El Mudawara mountain Fig.1, Fayoum government (Latitude 29.18836°N, Longitude 30.36106°E) [11]. It was purified and finally grown in slant of Malt agar media. It was morphological identified On Czapek's agar [12]. In addition to applying to the Data Base Identification Program of the Regional Center for Mycology and Biotechnology, it was identified using current universal keys [13,14].

2.2. Molecular Identification

The fungal isolate's 18S rRNA gene was amplified using polymerase chain reaction (PCR). For identification, the universal primers ITS1 (5′ - TCCGTAGGTGAACCTGCGG - 3′) and ITS4 (5′ - TCCTCCGCTTATTGATATGC - 3′) are utilized [15]. Using the Basic Local Alignment Search Tool (BLAST), the amplified gene sequences were aligned and compared to other fungal nucleotide sequences in the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic tree was constructed by Open Gene software Version 3.1 from Visible Genetics. After the gene sequence was added to the NCBI GenBank database, it was given an accession number [11] at The Regional Center for Mycology and Biotechnology.

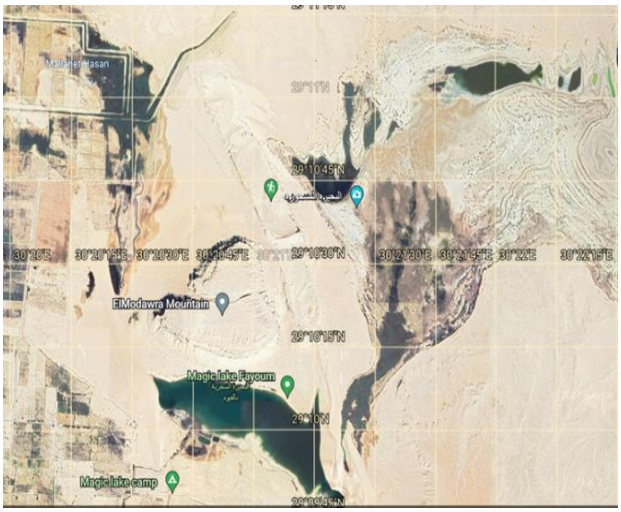


Fig. 1: Area guide of the review region by Google maps.

2.3. Cultivation and Extraction of extracellular Secondary Metabolites

Five liters of the yeast-extract sucrose (YES) broth medium were prepared and distributed into 250-ml Erlenmeyer flasks, each containing 100 ml. Further, the flasks were inoculated with the pure fungus and incubated for 21 days at 25˚C. The culture filtrate was obtained by filtration using Whatman No. 1 filter paper. The filtrate was extracted with different solvents in succession, beginning with petroleum ether, followed by ethyl acetate, and finally with chloroform. A rotary evaporator was then used to evaporate the solution, which was finally kept at 5˚C until the next analysis [5].

2.4. Antimicrobial assay

The *Aspergillus flavus* secondary metabolites were dissolved in 50µL Dimethyl Sulfoxide (DMSO) at concentrations of 10mg/ml of each extract. In 5-millimeter-diameter Petri dishes containing Malt Agar (MA) for fungi and Nutrient Agar (NA) for bacteria, dimethyl sulfoxide (DMSO) dissolved extract was added. The plates were cultured with test pathogenic microorganisms. Before being incubated at a temperature suitable for the growth of the test microorganisms, the cultures were kept at 2-8° C for 12 h for the purpose of antimicrobial metabolite diffusion. The inhibition zone was measured in mm [16]. All extracts were tested in contrast to: *Bacillus subtilis* (NRRL B-543), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium*

(NCIM 2501), *Proteus vulgaris* (ATCC 13315), *Candida albicans* (ATCC 10231) and *Aspergillus fumigatus* (RCMB 002008) [17]. The antibiotic gentamycin was used as a positive control for bacteria and amphotericin B for yeast and fungi. The RCMB culture unit at Al-Azhar University in Cairo, Egypt, housed all the microorganisms that were subjected to testing (Table 1).

2.5. MTT Cytotoxicity Assay

The MTT (3-(4, 5-dimethylthiazole-2yl)-2,5-diphenyl tetrazolium bromide) assay was used to investigate the *Aspergillus flavus* crude extracts' anticancer activity. The liver hepatocellular (HepG2) and human prostate cancer cell lines (PC-3) were obtained from the VACSERA Tissue Culture Unit. The cells were grown in Dulbecco's modified Eagle's medium (DMEM), containing 1% L-glutamine, 10% heat-inactivated fetal bovine serum, 50 mg/ml gentamycin, and HEPES buffer. All cells were sub-cultured twice per week and kept at 37ºC in a humidified environment with 5% CO_2. Crystal violet stain was used to investigate the cytotoxic effect of fungal extracts. It consisted of 0.5% (w/v) crystal violet and 50% methanol, was made up to volume with ddH_2O, and was completely filtered through Whatman No.1 filter paper. In addition, the relationship between absorbance at 490 nm and cell viability was evaluated [18].

The cell viability was calculated using the formula below:

$$\% \text{ cytotoxicity} = \frac{\mathbf{A} \text{ of control cells} - \mathbf{A} \text{ treated cells}}{\mathbf{A} \text{ of control cells}} \%$$

A= Absorbance

Linear regression was used to calculate the IC_{50} values [19].

2.6. Antioxidant Assay by DPPH Radical Scavenging Activity

The free radical scavenging activity of *Aspergillus flavus* extracts was measured using 2,2-diphenyl-1-picryl-hydroxyl (DPPH). Continuous measurements of the absorbance at 515 nm were taken at intervals of one minute until the absorbance stabilized (16 min). Ascorbic acid represents the positive control. Three r eplicates were used for the test. Using the following formula, inhibition of DPPH was determined as a percentage of radical scavenging activity:

Radical scavenging activity as a percentage = [(A control - A sample)/A control] × 100.

where **A** is the absorbance value. The IC_{50} value (µg/ml) was determined by comparing extract concentrations to the percentage of radical scavenging activity [20].

2.7. Identification of Bioactive Constituents by GC/MS

Analysis using Gas Chromatography-Mass Spectroscopy (GC-MS) was performed on the petroleum ether extract of *Aspergillus flavus*. Prior to detection, petroleum ether solvent (HPLC grade) was used to dissolve 5 mg of petroleum ether extract. Gas chromatography (GC) and a mass spectrometer selective detector (MSD) made by Thermo Scientific in California, USA, were used to identify the various chemicals [21].

2.8. Statistical Analysis

The student's unpaired t-test was used to determine whether the differences in mean values were statistically significant. Data were displayed as mean ± S.D. The data were deemed statistically significant at a significance level of $P< 0.05$. Both the IC_{50} estimates, and the graphing of the dosage response curve were performed using the STATA statistical analysis program.

3. Results

3.1 Isolation and Identification of the Fungal Strain

In this study, the fungal strain used was isolated from El Mudawara Mountain, Fayoum Government. The fungus was isolated and determined by its cultural and morphological characteristics. It was principally identified as *Aspergillus flavus* (Fig.2). The fungus was further identified molecularly, which confirmed the identification. The sequence was banked in NCBI GenBank and assigned an accession number; ON764430

3.2 Antimicrobial assay

It has been demonstrated that crude *Aspergillus flavus* extracts have a wide range of antimicrobial activity toward human pathogens. The results showed that the zone of inhibition of the tested pathogens in the tested crude fungal extracts ranged from 7.0 mm to 20.0 mm. A good diffusion assay of the extracts exhibits activity against Gram-positive and Gram-negative organisms (Table 1).

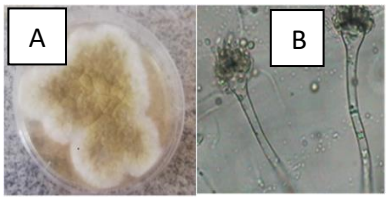


Fig. 2: Al Mudawara Mountain-isolated *Aspergillus flavus* fungus' morphological identification; (**A**) Culture characteristic on Malt agar plate, (**B**) Microscopic characteristic (40X).

Table (1): Antimicrobial activity of tested *A. flavus* extracts expressed in the mean zone of inhibition in mm ± standard deviation of the range of pathogenic microorganisms using the concentration of tested extracts (10 mg/ml).

Sample / Tested microorganisms	Petroleum Ether Extract	Ethyl Acetate Extract	Chloroform Extract	Control
FUNGI				Amphotericin B
Aspergillus fumigatus	7.1 ± 0.6*	-	-	23.7 ± 0.5
Aspergillus niger	-	-	-	26.1± 0.9
Cryptococcus neoformans	20.7 ± 1.1*	15.5± 1.2*	8.7± 0.7*	23.6± 1.2
Candida albicans	10.8 ± 0.7*	9.2 ± 0.8*	-	21.9 ± 1.3
Geotrichum candidum	9.1 ± 0.8*	-	-	26.4 ± 0.8
Penicillium expansum	-	-	-	22.9± 0.6
Syncephalastrum racemosum	8.3 ± 0.5*	-	-	25.4 ± 0.8
Gram Positive Bacteria:				Ampicillin
Bacillus subtilis	8.7 ± 0.6*	6.9 ± 0.7*	-	29.8 ± 1.5
Enterococcus faecalis	10.6± 0.8*	8.3± 0.5*	-	26.4± 1.4
Micrococcus sp.	14.3± 1.2*	9.5± 0.7*	7.5 ± 0.8*	21.8± 1.2
Staphylococcus aureus	11.4 ± 0.9*	10.4 ± 0.8*	-	28.9 ± 1.1
Staphylococcus aureus (MRSA)	-	-	-	-
Streptococcus mutans	16.2±0.9*	9.7 ± 0.8*	9.2 ± 0.6*	24.3± 0.9
Gram negative Bacteria:				Gentamycin
Klebsiella pneumoniae	10.9± 0.7*	7.8± 0.6*	-	22.4± 0.9
Escherichia coli	18.7± 1.1*	13.2 ± 0.8*	9.1 ± 0.6*	28.3± 1.8
Enterobacter cloacae	7.7 ± 0.5*	-	-	27.2± 1.7
Proteus vulgaris	14.9 ± 1.3*	12.4 ± 0.8*	8.2 ± 0.7*	21.7± 1.5
Pseudomonas aeruginosa	8.1 ± 0.5*	-	-	17.3± 1.2
Porphyromonas gingivalis	10.6± 0.8*	9.7± 0.7*	-	19.1± 0.9
Salmonella typhimurium	9.8± 0.9*	8.6± 0.7*	-	25.7± 1.5
Serratia marcenscens	7.2 ± 0.6*	-	-	23.9± 1.1

±Means standard deviations were used to represent the data. The Open Epi program was used to conduct the unpaired Student-t test. Standard drugs and the samples were compared, and differences between means were considered *significant at P values below 0.05. (-): No activity

Table (2): The petroleum ether extract of *Aspergillus flavus's in vitro* inhibition of the tested tumor cell lines.

Cell lines / extract conc. (µg/ml)	PC-3			HepG-2		
	Viability %	**Inhibitory %**	**S.D. (±)**	**Viability %**	**Inhibitory %**	**S.D. (±)**
500	7.94	92.06	0.92	3.86	96.14	0.48
250	23.18	76.82	0.84	9.74	90.26	0.62
125	36.87	63.13	1.47	20.95	79.05	1.31
62.5	45.26	54.74	2.92	38.12	61.88	1.49
31.25	64.03	35.97	2.85	48.37	51.63	2.95
15.6	76.19	23.81	1.43	65.94	34.06	2.74
7.8	88.42	11.58	1.68	78.69	21.31	1.97
3.9	95.81	4.19	0.97	89.04	10.96	0.82
0	100	0	0	100	0	0

3.3. MTT cytotoxicity assay

The cytotoxicity of *A. flavus* extracts was assessed using MTT assay against the proliferation of HepG-2 and PC-3 cell lines. The IC_{50} (half-maximal inhibitory concentration) of petroleum ether extract against PC-3 cells was 54.6 ± 1.2 µg/ml (Fig.3) while that of ethyl acetate extract was 175 ± 5.7 µg/ml (Fig.4). Conversely, the IC_{50} of the petroleum ether extract against HepG-2 cells was 29.8 ± 0.9 µg/ml (Fig.5). In Addition, ethyl acetate extract was 111 ± 4.2 µg/ml (Fig.6), (Tab.2&3).

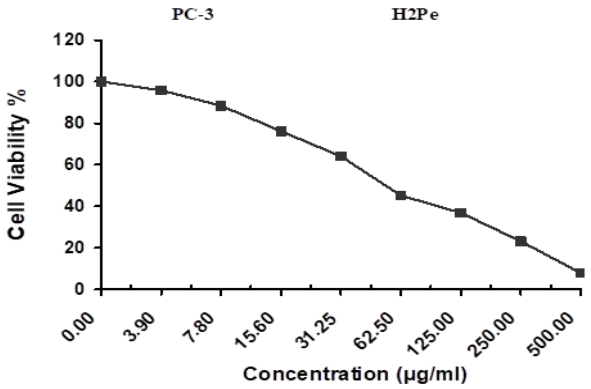


Fig. 3: Cytotoxicity of petroleum ether extract against the (PC-3) cell line *in vitro*.

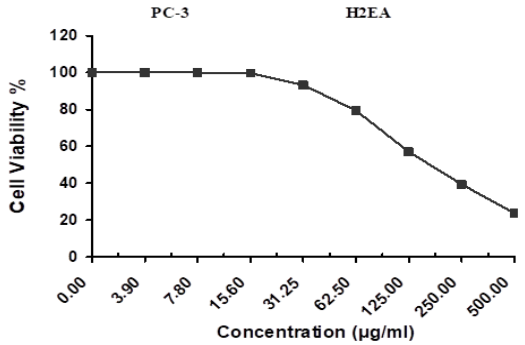


Fig.4: Cytotoxicity of ethyl acetate extract against the (PC-3) cell line *in vitro*

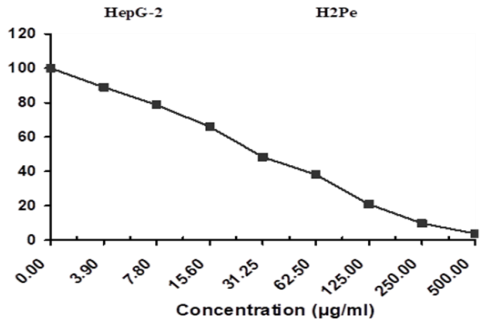


Fig.5: Cytotoxicity of petroleum ether extract against the HepG-2 cell line *in vitro*.

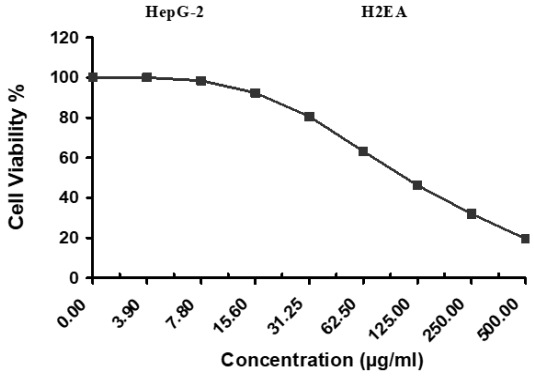


Fig.6: Cytotoxicity of ethyl acetate extract against the (PC-3) cell line *in vitro*

3.4. Antioxidant Assay by DPPH Radical Scavenging Activity

The stable free radical DPPH has been extensively utilized in evaluations of antioxidants' ability to neutralize free radicals. The findings confirmed that the investigated metabolites' DPPH scavenging capacities were dose dependent. The findings illustrated that the tested metabolites' DPPH scavenging activities were dose dependent. IC_{50} of petroleum ether extract from *Aspergillus flavus* showed antioxidant activity with 272± 3.7 µg/ml (Fig.7& Tab.4). In addition, *Aspergillus flavus* ethyl acetate extract's IC_{50} demonstrated antioxidant activity with 901.3 ± 42.8 µg/ml (Fig.8 & Table.5).

Table (3): The *in vitro* inhibitory activity of the ethyl acetate extract from *Aspergillus flavus*, toward the tested tumor cell lines.

Cell lines / extract conc. (µg/ml)	PC-3			HepG-2		
	Viability %	**Inhibitory %**	**S.D. (±)**	**Viability %**	**Inhibitory %**	**S.D. (±)**
500	7.94	92.06	0.92	19.54	80.46	2.72
250	23.18	76.82	0.84	31.98	68.02	1.87
125	36.87	63.13	1.47	46.27	53.73	2.89
62.5	45.26	54.74	2.92	63.19	36.81	3.45
31.25	64.03	35.97	2.85	80.65	19.35	2.41
15.6	76.19	23.81	1.43	92.43	7.57	0.75
7.8	88.42	11.58	1.68	98.51	1.49	1.32
3.9	95.81	4.19	0.97	100	0	0
0	100	0	0.92	100	0	0

Table (4): The DPPH radical scavenging activity of petroleum ether extracts from Aspergillus flavus at various concentrations (µg/ml) ±SD of three recreates.

Sample conc. (µg/ml)	DPPH scavenging %	(±)SD
1280	87.39	3.94
640	68.93	2.83
320	54.35	3.91
160	39.86	3.28
80	26.71	2.49
40	13.48	0.86
20	8.45	1.37
10	2.74	0.62
0	0	0

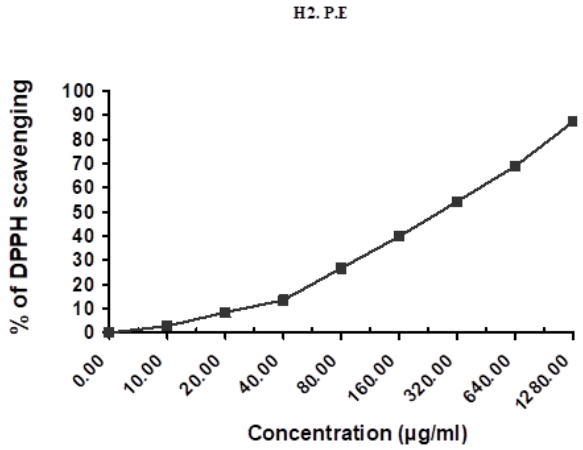


Fig. 7: Antioxidant activities of petroleum ether extract by DPPH radical scavenging activity *in vitro.*

Table (5): The DPPH radical scavenging activity of ethyl acetate extract from *Aspergillus flavus* at different fixations (µg/ml)±SD of three recreates.

Sample conc. (µg/ml)	DPPH scavenging %	(±)SD
1280	68.41	3.75
640	37.25	4.38
320	14.78	2.91
160	7.83	1.32
80	4.93	0.71
40	2.17	0.64
20	1.30	0.28
10	0.29	0.17
0	0	

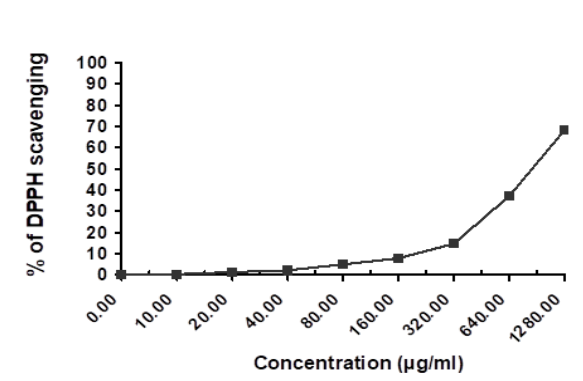


Fig. 8: Antioxidant activities of ethyl acetate extract by DPPH radical scavenging activity *in vitro.*

3.5 Identification of A. flavus Bioactive Constituents Using GC/MS

To investigate the potential biologically active components of the A. flavus petroleum ether extract, the constituent compounds were separated and detected using GC-MS (Table 6). Meanwhile, the GC-MS-detected metabolites of A. flavus are Hexadecenoic acid, oleic acid, octadecanoic acid, and 10,13-Octadecadiynoic acid, methyl ester,1-Dodecanamine, N, N-dimethyl as a major constituent (Fig.9).

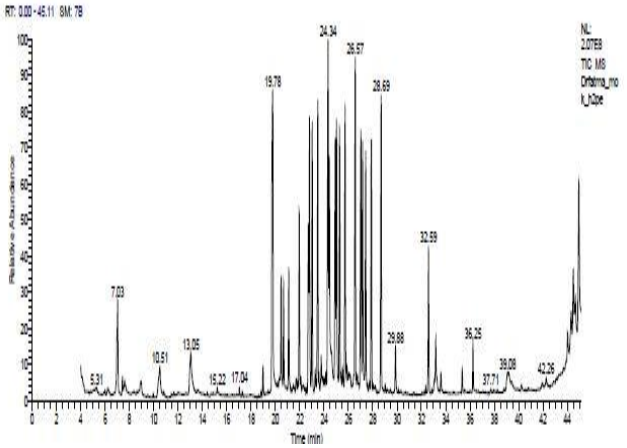
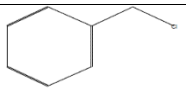
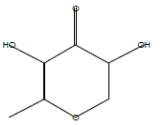
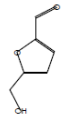
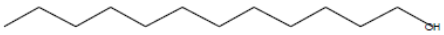
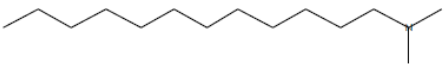
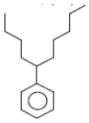
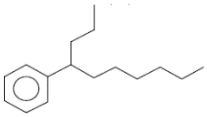
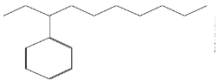
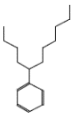
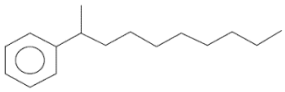
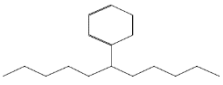
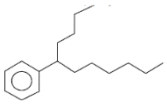
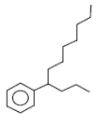
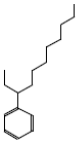
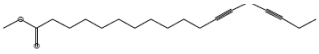
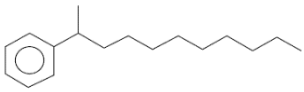
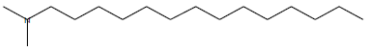
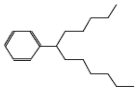
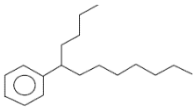
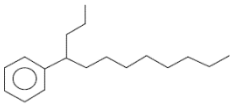
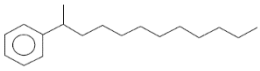
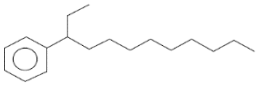
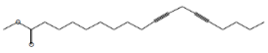
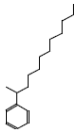
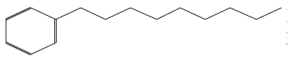
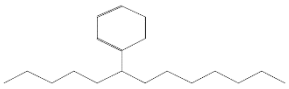
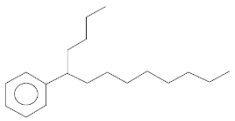
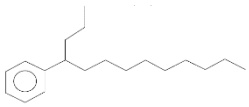
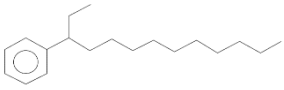
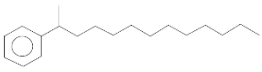
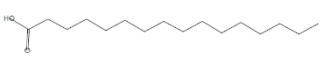
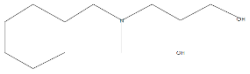
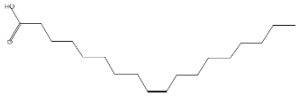
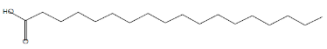
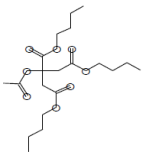
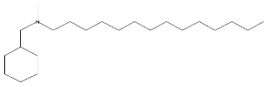
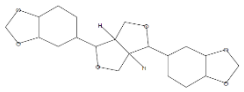
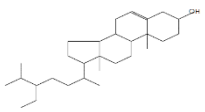
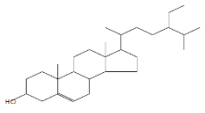


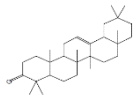
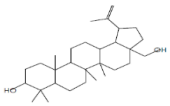
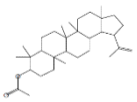
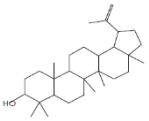
Fig. 9: GC-Mass of *A. flavus* petroleum ether extract.

Table (8): GC-MS Chromatographic separation technique for petroleum ether

Peak No.	Retention time	Compound name	Relative content %	Chemical formula	M.W	Chemical structure
1.	7.03	Chloromethyl)benzene	1.63	C7H7Cl	126	
2.	8.96	1H-Azonine, octahydro-1-nitroso-	0.30	C8H16N2O	156	
3.	10.51	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	1.27	C6H8O4	144	
4.	13.04	5-Hydroxymethylfurfural	0.97	C6H6O3	126	
5.	18.98	1-Dodecanol	0.42	C12H26O	186	
6.	19.77	1-Dodecanamine, N,N-dimethyl-	7.30	C14H31N	213	
7.	20.47	Benzene, (1-butylhexyl)-	1.51	C16H26	218	
8.	20.67	Benzene, (1-propylheptyl)-	1.35	C16H26	218	
9.	21.10	Benzene, (1-Ethyloctyl)-	1.73	C16H26	218	
10.	21.72	Benzene, (1-Butylheptyl)-	0.24	C17H28	232	
11.	21.96	Benzene, (1-methylnonyl)-	2.45	C16H26	218	
12.	22.71	Benzene, (1-pentylhexyl)-	2.22	C17H28	232	

13.	22.80	Benzene, (1-butylheptyl)-	3.83	C17H28	232	
14.	23.03	Benzene, (1-propyloctyl)-	4.04	C17H28	232	
15.	23.49	Benzene, (1-ethylnonyl)-	4.77	C17H28	232	
16.	23.77	12,15-Octadecadiynoic acid, methyl ester	0.58	C19H30O2	290	
17.	24.33	Benzene, (1-methyldecyl)-	5.95	C17H28	232	
18.	24.43	1-Tetradecanamine, N, N-Dimethyl	3.69	C16H35N	241	
19.	24.92	Benzene, (1-pentylheptyl)-	4.05	C18H30	246	
20.	25.03	Benzene, (1-butyloctyl)-	4.07	C18H30	246	
21.	25.28	Benzene, (1-propylnonyl)-	3.92	C18H30	246	
22.	25.53	Benzene, (1-methylundecyl)-	0.27	C18H30	246	
23.	25.74	Benzene, (1-ethyldecyl)-	4.47	C18H30	246	
24.	25.88	10,13-Octadecadiynoic acid, methyl ester	0.42	C19H30O2	290	
25.	26.57	Benzene, (1-methylundecyl)-	5.77	C18H30	246	
26.	26.74	Benzene, nonyl-	0.28	C15H24	204	

27.	27.03	Benzene, (1-pentyloctyl)-	4.71	C19H32	260	
28.	27.17	Benzene, (1-butylnonyl)-	3.45	C19H32	260	
29.	27.42	Benzene, (1-propyldecyl)-	3.44	C19H32	260	
30.	27.89	Benzene, (1-ethylundecyl)-	4.07	C19H32	260	
31.	28.69	Benzene, (1-methyldodecyl)-	5.00	C19H32	260	
32.	29.88	Hexadecanoic acid	0.77	C16H32O2	256	
33.	32.59	3-(N-Benzyl-N-methylamino)- 1,2-propanediol	2.23	C11H17NO2	195	
34.	33.18	Oleic Acid	1.29	C18H34O2	282	
35.	33.60	Octadecanoic acid	0.31	C18H36O2	284	
36.	35.36	Tributyl acetylcitrate	0.53	C20H34O8	402	
37.	36.25	N-Methyl-N-benzyltetradecanamine	0.67	C22H39N	317	
38.	39.08	3-hydroxy-terphenyllin	0.40	C20H18O6	354	
39.	39.18	Ergosta-4,6,22-trien-3.beta.-ol	0.75	C20H18O7	397	
40.	44.02	Stigmast-5-en-3-ol, (3á,24s)-	0.49	C29H50O	414	

41.	44.31	Beta-amyrenone	0.75	C30H48O	424	
42.	44.49	Erythrodiol	1.23	C30H50O2	442	
43.	44.67	Lup-20(29)-en-3-ol, acetate, (3á)-	1.09	C32H52O2	468	
44.	44.95	Lanosterol	2.78	C30H50O	426	

4. Discussion

With around 180 filamentous parasitic species, the variety *Aspergillus* is enormous and various. It is well-known to produce a variety of secondary metabolites that are valuable in therapeutics, including those that have anticancer, antimicrobial, cholesterol-lowering, and immunosuppressive properties [22, 23]. The genus *Aspergillus* has the highest innovation index among the fungal kingdom when it comes to the creation of biologically active secondary metabolites [24]. Human infections that can result in death are brought on by the emergence of pathogens that are resistant to multiple drugs. As a result, there are urgent demands for new antibiotics to be added to the existing groups [25]. *A. flavus'* antimicrobial activity was the goal of this study. In accordance with the findings of [26], the findings demonstrated that the ethyl acetate and petroleum ether extracts against *E. coli*, *S. typhimurium*, and *B. subtilis* had antimicrobial activity. who isolated fungi from local soil in El-Sharkia Governorate and detected that *A. flavus* methanol extracts have antibacterial activity against *Pseudomomas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes* and antifungal activity against *Fusarium oxysporum* and *Candida albicans.* Moreover, according to [27], many different secondary metabolites that have a lot of biological activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Shigella flexeneri*, *Escherichia coli*, *Klebsiella. pneumoniae*, *Salmonella typhi*, *Xanthomonas oryzae*, and *Bacillus subtilis* were found in the methanolic extract of *A. flavus*. [26] had previously demonstrated that *A. flavus* extract had anticancer activity against the HepG2 cell line, and the results presented here confirmed this. In addition, like the presented results in this study, the ethyl acetate extract of endophytic *A. flavus* exhibited notable cytotoxic effects toward breast cancer cells (MCF-7) [28]. Using the DPPH free radical scavenging assay, this study found that *A. flavus* had antioxidant activity, which was in accordance with the conclusions of [29], who demonstrated antioxidant activity using DPPH assay from the endophytic fungus *A. flavus* isolated from *Ocimum basilicum*. Additionally, the ethyl acetate extract of endophytic *Aspergillus unguis* SPMD-EGY from the *Sinularia* sp. showed antioxidant activity [30]. Moreover, *Aspergillus terreus* aqueous extract exhibited potent antioxidant activity using DPPH assay [31]. The GC-MS analysis detected the existence of hexadecenoic acid, oleic acid, octadecanoic acid, 1-Dodecanamine, N, N-dimethyl and 10,13-Octadecadiynoic acid, methyl ester, in the petroleum extract. In a similar vein, [32] stated that the chloroform extract ingredients of *A. flavus* from an Egyptian historical site contained most of the hexadecane in the GC-MS analysis. In addition, [33] informed that in the GC-MS analysis, the principal components of the acetone extract of *Paecilomyces lilacinus* were hexadecenoic acid, 10, 13 octadecadienoic acids, methyl ester as the major compounds. Experiments on the explained derivative of hexadecenoic acid have shown that it has anti-inflammatory and anti-cancer properties [34], which may account for the activity of our petroleum ether extract against PC-3 and HepG2 tumor cells. In addition, [35] reported that 9-octadecenoic acid (Z)-methyl ester had antioxidant, antimicrobial and cancer enzyme inhibitory properties.

Conclusions

In the current study, *Aspergillus flavus* demonstrated remarkable inhibition activities against the tested pathogenic microorganisms. Compared to ethyl acetate extract, the

inhibitory activity toward HepG2 and PC-3 tumor cells was higher in the petroleum ether extract. Additionally, the petroleum ether extract performed better than the ethyl acetate extract in terms of antioxidant activity. GC investigation uncovered the significant synthetic parts of the concentrates that would be liable for these moderate antimicrobial, cancer prevention agent, and antitumor properties. More pharmacological and *in vivo* research is recommended to learn how these activities work.

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