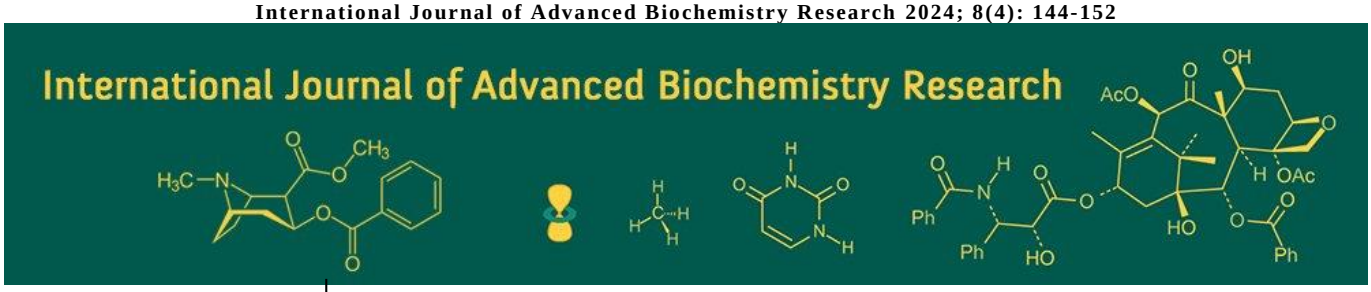

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(4): 144-152
www.biochemjournal.com
Received: 10-02-2024
Accepted: 15-03-2024
Published: 04-04-2024

Sanaa A Ghali
Department of Biology, College of Science, University of Basrah, Iraq

Furdos N Jafer
Department of Biology, College of Science, University of Basrah, Iraq

Areej HS Aldhaher
Department of Basic Science, College of Dentistry, Basrah University, Iraq

Corresponding Author:
Sanaa A Ghali
Department of Biology, College of Science, University of Basrah, Iraq

Evaluation secondary metabolite extract produced by *Aspergillus terreus* isolated from poultry droppings as antimicrobial agent

Sanaa A Ghali, Furdos N Jafer and Areej HS Aldhaher

DOI: https://doi.org/10.33545/26174693.2024.v8.i4b.940

Abstract
The purpose of this study: was to separate fungi that produce antibiotics from bird droppings.
Place and Time of Study: Between October 2022 and March 2023, poultry droppings were gathered from farms and marketplaces in Poultry fields in Basra / Karma Ali.
Approach: A total of 150 samples of bird droppings were gathered. Subsequently, 1 gram of freshly deposited chicken droppings were measured and diluted in a sequential manner. The resulting dilutions were then cultivated for a duration of five days on malt extract agar and potato dextrose agar. Microscopical and morphological description of *Aspergillus terreus*. The natural extract was analysed by GC-MS, and the minimum inhibitory concentration (MIC) against pathogenic bacteria was determined using the agar well diffusion technique. The extract's aggressive behavior was evaluated against a total of four bacterial isolates, encompassing both Gram-positive and Gram-negative strains.
Results: The results showed that the fungal extract isolated from *Aspergillus terreus* showed aggressive behavior against pathogenic bacteria, as extracts of *Aspergillus terreus* secondary metabolites were effective against *Klebsiella pneumonia*, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli* in a range of doses where the inhibitory concentration was the lowest for *Escherichia coli* and *Staphylococcus aureus* is 15 ug. /ml, while the minimum inhibitory concentration for *Bacillus cereus* and *Klebsiella pneumonia* was 33.5 ug/ml. When the extracts are refined, they can be used to create new medicinal molecules from natural sources.

Keywords: Metabolite, *Aspergillus terreus*, poultry droppings, antimicrobial agent, GC-MS

1. Introduction
The pharmaceutical and industrial sectors have long been interested in the eukaryotic organisms that make up the fungal kingdom because they provide important natural sources of biologically active secondary metabolites compared to other elements made from plants, and the biochemicals extracted from fungi are active agents with unexplored potential. (Hung and Lin 2017) [8]. According to a recent Food and Drug Administration (FDA) assessment, microorganisms were responsible for around 25% of the 38% of medications derived from natural materials. The importance of microbes as a long-term pipeline for the development of novel drugs has been highlighted by these findings (Newman and Cragg 2016) [14]. Chemicals derived from natural products provide substantial and uninterrupted resources for medicinal chemistry applications. As a result, most leading pharmaceutical firms have focused on evaluating microbial natural extracts to develop high throughput libraries. (Hung and Lin 2017) [8]. Secondary metabolites, which are organic molecules, are synthesized by organisms through a limited number of fundamental biosynthetic pathways that deviate from primary metabolic pathways. These compounds are often synthesized by certain families and genera of animals. (Pazouki & Panda, 2000) [18].
Since ancient times, people have been drawn to fungi because they provide a rich source of secondary metabolites. Secondary metabolism is typically employed for conflict, animosity, or self-preservation against other species. The functions of these secondary metabolites, which include defense against pathogens and pests, etc., are known to be important in helping an organism adapt to its environment. Moreover, they serve as a crucial source for high-quality pharmaceuticals (Devi, *et al* 2020) [5]. The filamentous fungi found in animal manure, which belong to the phylum Ascomycota, and the most important of which is the

genus Aspergillus, are considered important fungi in the pharmaceutical, industrial, and environmental fields due to their ability to produce important and diverse secondary metabolites (Bazouki). And Panda., 2000) [18]. An significant species of Aspergillus, *Aspergillus terreus* is used in the commercial manufacturing of lovastatin, a medication that lowers cholesterol, and itaconic acid, a biobased chemical. These industrial applications have demonstrated the superior fermentation capacity of this fungus. The genomic data has shown that more research is needed to fully understand the exceptional ability of *A. terreus* to synthesize natural products. Recent advancements in genome extraction technologies have facilitated the identification of the products derived from diverse biosynthetic gene clusters. Several metabolic engineering studies have been conducted on commercial strains of itaconic acid and lovastatin in order to improve production techniques. (Huang *et al*., 2021) [7].

2. Materials and Methods

2.1 Sample Collection

At the University of Basra/Karma Ali, samples of poultry waste were taken from domestic chickens and commercial poultry. We extracted fresh droppings from the chicken houses using disinfected plastic forks. Poultry litter that could not be collected with a plastic spoon was swabbed by passing a sterile swab over each sample until it darkened. Within an hour of collection, the samples were serially numbered and brought to the laboratory in plastic containers for processing.

2.2 Sample Processing

A 1 gram sample of poultry manure was subjected to homogenization in 10 milliliters of sterile water. The mixes were subjected to repeated dilution at a 10-fold ratio. The bacterial strains known as 10-5, 10-6, 10-7, and 10-8 were subsequently cultured on malt extract agar (MEA) and potato dextrose agar supplemented with chloramphenicol. The culture plates were incubated for a duration of five (5) days, during which daily monitoring was conducted till observation was made. Growth of fungal colonies. Maghraby *et al.*, 1991) [10].

2.3 Isolation of Fungi

The pure culture was established by growing the mixed fungal culture individually in fresh PDA media. The dishes were then subjected to another five (5) days of incubation at room temperature and after growth of the pure fungal colony they were kept tightly in the refrigerator. Until I use it in the second step of the study (Norhafizah, 2012) [16].

2.4 Identification and Classification of Fungal Isolate

2.4.1 Microscopic Examination

From each isolated fungal culture, a minute quantity of cell and agar was extracted and subsequently transferred onto a microscope slide. The slides were dyed with lactophenol blue and subsequently covered with a coverslip. The slides were then viewed using a light microscope at a low magnification of X40. The study observed several microscopic characteristics, such as the mycelial end, branching, hyphae structure, and the existence of spores. The physical and cultural properties of the fungal isolates were compared to those of other fungal isolates in order to identify them and classify them according to the established categorization in the fungal atlas. (Norhafizah, 2012 & Adegunloye and Adejumo, 2014) [16, 1].

2.6 Characterization of molecular genetics

The Prestomini gDNA yeast kit (Geneaid) was used to isolate fungal genomic DNA. For further study, genomic DNA was stored at -80°C; the conserved ITS region was amplified using the universal primer pair technique. The quantity, purity, and titer of genomic DNA products were evaluated using NanoDrop, electrophoresis, and polymerase chain reaction (PCR) (Mirhendi *et al.*, 2006) [11].

The PCR products were submitted to Macrogen, Korea, for sequencing (http://dna.macrogen.com). Using the Basic Local Alignment Search Tool (BLAST), the sequence was aligned with the public database to compare the homology in GenBank Figure (2).

2.7 Bacterial Organisms Used for the Screening

The effectiveness of the fungal extract was tested against four isolates of pathogenic bacteria, which are *Klebsiella pneumonia*, bacteria isolated from Wound swab, *Staphylococcus haemolyticus*, isolated from cesarean section infections, *Bacillus cereus* isolated from food, *Escherichia coli* isolated from urinary tract infections, *Staphylococcus aureus*. Isolated from Nasial swab, they were obtained from the microbiology laboratory and the applied microbiology laboratory in the College of Science / University of Basra.

2.8 Preliminary Screening of Fungi Isolates for Antagonism

The disk diffusion method was followed to test the effectiveness of the active substances of the crude extract, as the weight of 0.2 ml of the crude extract of each fungus was dissolved in 1ml of the organic solvent DMSO. The bacteria were activated on nutrient agar medium and after 24 hours of incubation at 36 degrees, a suspension of each strain was made. Petri shower dishes containing 0.1 of the bacterial suspension were injected for each strain of bacteria and the number of cells (10^6) was spread by sterile swabs on MHA medium and left for one hour, A cork drill was used to create a 6 mm diameter hole in the center of the dish. Subsequently, 100 ml of the extract was injected into the dish. The dishes were then placed in an incubator set at a temperature of 37 °C for a duration of 24 hours. The examination of the plates involved the evaluation of inhibitory activity by the measurement of the width of the inhibition zone in millimeters. (Balouiri *et al*., 2016) [3].

2.9 Extraction of Secondary Metabolite

Fungal cultures represented by fermentation media were filtered separately if the fermentation media was decanted, the media were placed in centrifuge tubes at 6000 rpm for 10 minutes, filtered using Whatman No1 filter papers, and then the pH was adjusted to 3 By adding drops of hydrochloric acid HCL at a concentration of 0.1, then the active ingredients were extracted from the filtrate by adding a volume of the filtrate to an equal volume of ethyl acetate. Then the extracts were collected and placed in sealed

containers for preservation in the refrigerator at a temperature of 4 ℃.

2.10 Minimum Inhibitory Concentration (MIC) Determination

The MIC was estimated using the agar diffusion method, as outlined by Ikegbunam *et al*. in 2018. The extract was prepared at different quantities (500, 250, 125, 65, 33.5, 15) and put to agar wells (6 mm in diameter) on culture plates of the test organisms. A positive control (Chromophenicol disks) and a negative control (DMSO) were also utilized. The wells were subsequently incubated at a temperature of 37 °C for a duration of 18 hours. The zone of inhibition was measured and recorded.

2.11 Characterization of the bioactive compound using GC-MAS

The crude extract of Aspergillus ***terreus*** SM was analysed by GC-MS at the Basrah Oil Company, Nahr Bin Omar laboratory. The analysis was performed using an Agilent Technologies 7890 B GC system coupled to an Agilent Technologies 5977A MSD with an EI ion source using HP-5MS 5% phenyl methyl siloxane (30 m x 250 Um × 0.25 mm). Helium gas was used as the carrier gas at a constant flow mode of 1 mL/min and a purge flow of 3 mL/min. The oven temperature was set at 40 °C, held for 5 min, raised to 1 °C/min to 280 °C for 1 min, and then held at 280 °C for the remaining 20 min. The injection mode was pulsed-split less with an injection temperature of 290 ºC, and the injection sample volume was 1 µl. The mass spectrometer used was Ion Source Temp, 230 °C, with a scan speed of 1562 (N2), and electron ionisation was obtained over a mass range of 35-650 m/z. The data were analysed using the NIST 2014 library database.

2.12. Statistical Analysis

The means ± standard deviation (SD) for all data were determined using SPSS Statistical Packages of Social Sciences (Version 26; USA) by the use of static analysis. The researchers employed the least significant difference test (LSD) to examine the disparity between averages. A statistical significance level of $p<0.05$ was deemed significant.

3. Results

3.1 Identification of Fungal Isolates from Poultry Droppings

The phenotypic diagnosis of the filamentous fungi isolated in the current study was based on the color of the colony, its height, and its ability to form reproductive spores. The differential dye ectophenol for fungi was used. It was based on the shape and type of conidia in diagnosing various fungi Figure (1).

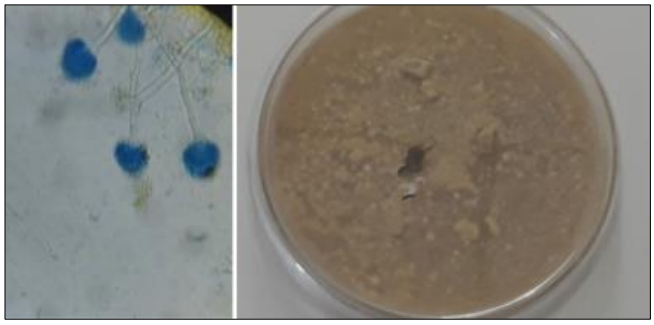

Fig 1: The phenotypic diagnosis of *A. terreus*

3.2 Molecular characterisation method

A. terreus isolate was identified using the advanced molecular characterisation method. The ITS region was amplified as a conserved region and sequenced. The ITS region sequences with a size of about 500-600 bp were compared with the sequences of the public database, which were deposited in the GeneBank (Fig 2). The homology was investigated using the BLAST. The identified *A. terreus* isolate was deposited at the GenBank with the accession number MT558939.1.

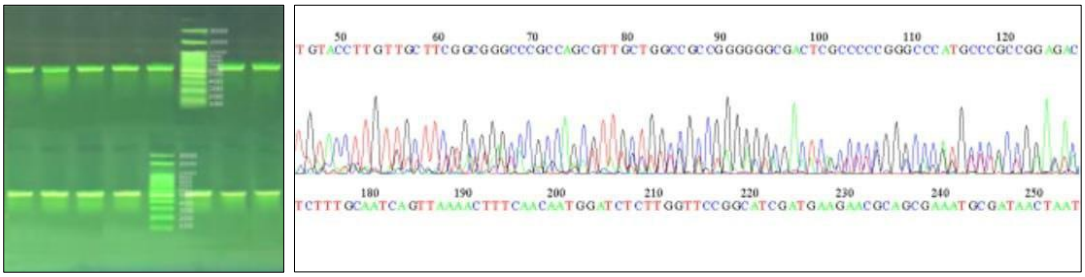

Fig 2: Identification of *A. terreus* species by molecular methods

3.3 Production and extraction of bioactive metabolites from *A. terreus:*

The secondary metabolites of the fungi produced in the fermentation medium were extracted after 30 days of incubation, and the organic acid ethyl acetate proved highly efficient in extraction, as shown in the following figure (3).

Fig 3: Growth of *the A. terreus* strain and preparation of bioactive metabolites extract. A: Dried extract of organic solvent at room temperature. B: Extraction of bioactive metabolite by ethyl acetate. C: Growth of *A. terreus* strain on PDB medium for 30 days at 27 °C.)

3.4 Biological Activity

The secondary metabolite extract of *A. terreus* exhibited significant antibacterial activity against the four unique bacterial isolates examined, yielding highly favorable outcomes. A notable disparity in inhibition rates was seen between *Staphylococcus aureus* II, *Klebsiella pneumoniae*, *Escherichia coli*, and *Bacillus cereus*, including both Gram-positive and Gram-negative bacteria. (Figure 4).

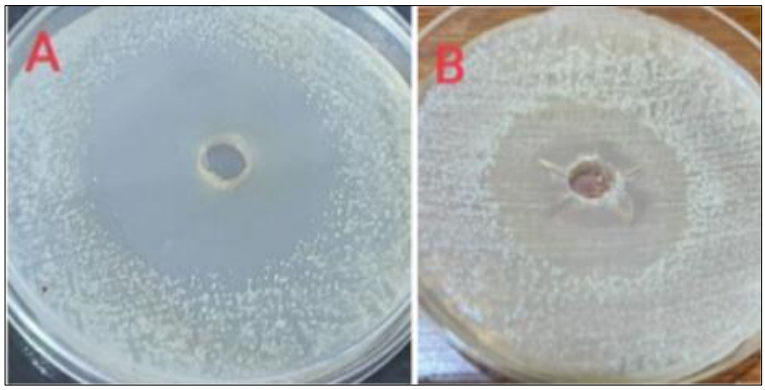

Fig 4: Showing the inhibitory activity of the fungal extract, (*Aspergillus terreus*) against pathogenic bacteria at a concentration of (1000 ug/ml), A: *Escherichia coli (*Gram-), B: *Staphylococcus aureus* (Gram+).

3.5 Measurement of the minimum inhibitory concentration of the fungal extract against pathogenic bacteria

Different concentrations of the fungal extract were used to determine the minimum inhibitory concentration for the growth of pathogenic bacteria. We used 6 concentrations of the extract, which are (500, 250, 125, 65, 3, 3.5, and µg /ml), The inhibitory concentration was measured as the inhibitory concentration was the lowest for *Escherichia coli* and *Staphylococcus aureus* is 15 mg. /ml, while the minimum inhibitory concentration for *Bacillus cereus* and *Klebsiella pneumonia* was 33.5 Table (1), Figure (5). Significant differences were also calculated for the inhibition rates of pathogenic bacteria Table (2).

Table 1: Minimum Inhibitory Concentration rate of *A. terreus* secondary metabolite extract against bacterial test organism µg /ml (Green color refers to MIC values)

Bacteria	500 µg/ml	250 µg/ml	125 µg/ml	65µg/ml	33,5 µg/ml	15 µg/ml
Staphylococcus aureus	+	+	+	+	+	+
Escherichia coli	+	+	+	+	+	+
Klebsiella pneumonia	+	+	+	+	+	_
Bacillus cereus	+	+	+	+	+	_

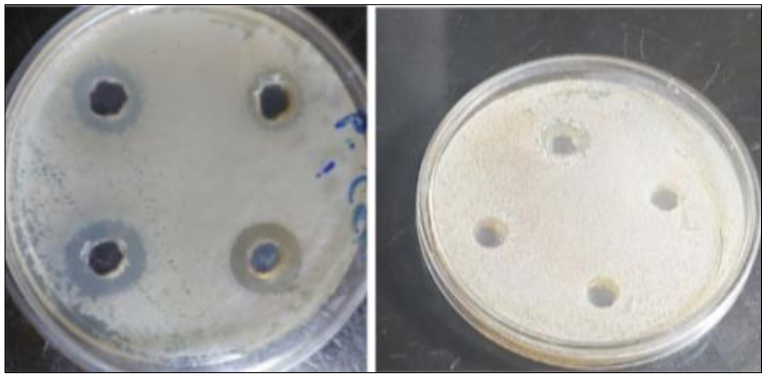

Fig 5: Varying zones of inhibition based on varying *A. terreus* extract doses. MIC values for *Bacillus cereus*

Table 2: Showing comparison between different pathogenic Bacteria using different Concentrations of the crude. (S: Significant difference between groups (p value <0.05), *groups with different letters are significantly different based on the p-values)

Concentration Bacteria		Inhibition zone mean								LSD p value
		500	250	125	65	33.5	15	Control	Mean Labels	
Types of Bacteria	*S. aureus*	18.5	9.5	8	5.5	3	1	18.5	9.75 A	3.292 0.044^{S}
	E. coli	19.5	10	7.5	4.5	2.5	1	22	9.57 A	
	B. cerseus	13	8	5	2.5	1.5	0	18.5	6.93 B	
	K. pneumonia	12.5	9.5	5	1.5	1	0	17	6.64 B	
Mean Labels		15.875 b	9.25 c	6.375 d	3.5 E	2 e	0.5 f	19 a		
p value		0.035^{S}								

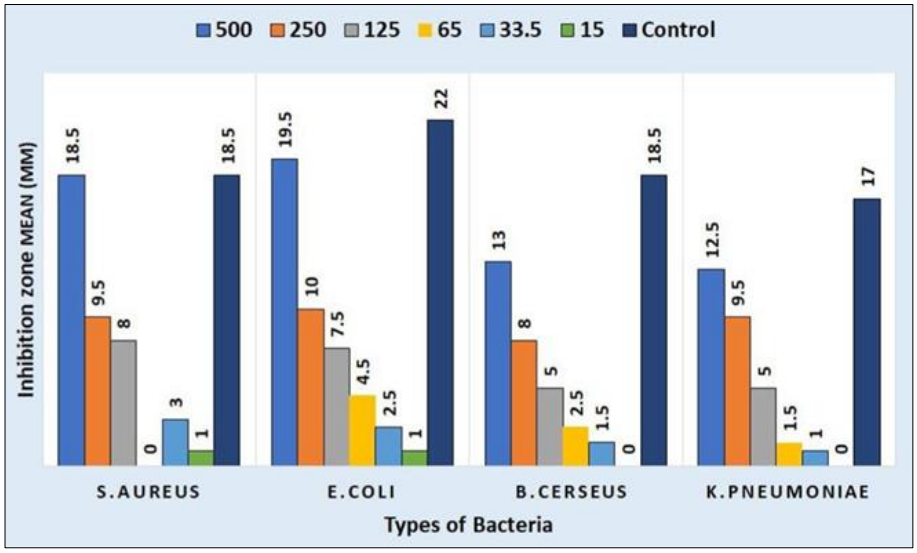

Fig 6: Showing of inhibition zone mean of *A. terreus* secondary metabolite extract against bacterial test organism

3.6 Fungal extract analysis by GC-MAS

60 compounds were identified from the extracts of Aspergillus *terreus* table **(3)** . Including 10 furan, ,9 hydrocarbons, 7 fatty acid, 6 ketone, 6 Chlorinated and Fluorinated aromatic substances , 5 phenols, 5 ester, 3 phthalate, 2 glycosides and 7 other types of compounds. Many of these compounds have been identified previously from plant, bacteria and fungus such as 5-methyl-2-Furancarboxaldehyde and 2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one identified in Saccharomyces cerevisiae. In addition, many of these compounds showed antibiological activity for example, the ketone compound Dehydroacetic Acid and hydrocarbon compound 2-Ethylacridine displayed a role as a fungicide and an antibacterial agent (Manju and Jegadeeshkumar, 2023) [21].. The phenol compound 4-Methoxy thiophenol showed an anticancer activity. 2-Chloro-5-methylbenzene-1,3-diol Antifungal activity against Bipolaris leersiae assessed as inhibition of conidial germination after 24 hrs at 500 ppm as recorded in Table (3).

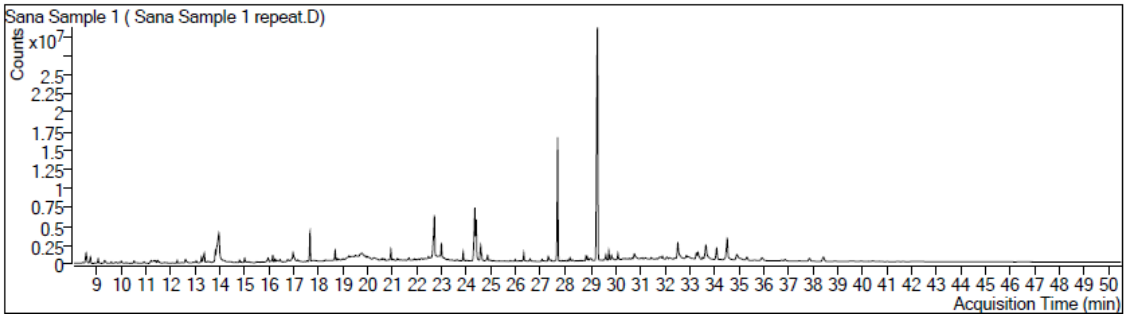

Fig 7: Gas chromatogram of *A. terreus* crude extract

Table 3: GC–MS analysis for *A. terreus* extract

No	Compound name	Rt.	Area %	Molecular Formula	*Record **
	Furan				
1	5-Methyl-2(3H)-furanone	8.763	0.594	$C_5H_6O_2$	*Picea abies*
2	3-methyl-2,5 Furandione	9.077	0.321	C_5H_4O3	*Coffea arabica* and *Schisandra chinensis*
3	5-methyl-2-Furancarboxaldehyde,	9.336	0.364	$C_6H_6O_2$	*Saccharomyces cerevisiae*.
4	Succinic anhydride	10.53	0.336	$C4H4O_3$	
5	3-Furancarboxylic acid	11.481	0.3979	$C_5H_4O_3$	*Penicillium herquei*,
6	5-Hydroxymethylfurfural	13.963	7.5962	$C_6H_6O_3$	some foods and alcoholic beverages
7	5-Acetoxymethyl-2-furaldehyde	15.008	0.3563	$C8H_8O_4$	
8	5-Hydroxymethylfurfural	15.102	0.2758		
9	Methyl 2-[[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]methyl]-3-(4-hydroxyphenyl)-4-methoxy-5-oxofuran-2-carboxylate	31.826	0.2909	$C_{25}H_{26}O_7$	
10	Furan-3,4-dicarboxylic acid, 2,5-diphenyl-, diethyl ester	32.541	2.3334	C18H12O5	
	Fatty acid				
11	4-Oxopentanoic acid	11.331	0.5371	C5H8O3	*Escherichia coli*
12	6-Pentadecenoic acid, 13-methyl-, (6Z)-	22.447	0.2751		
13	n-Hexadecanoic acid	22.682	5.3854	C16H32O2	*Escherichia coli*
14	(9Z,12Z)-octadeca-9,12-dienoic acid	24.324	8.5438	C18H32O2	*Calodendrum capense*, *Camellia sinensis*,
15	Octadecanoic acid	24.56	1.6247	C18H36O2	Various animal and plant fats, cocoa butter and shea butter.
16	9-Octadecenamide, (Z)-	26.304	0.6847	C18H35NO	*Desmos cochinchinensis*,
17	Decanedioic acid, bis(2-ethylhexyl) ester	29.626	0.4324	C26H50O4	
	Hydrocarbone				
18	Cyclododecane	13.256	0.4449	C12H24	*Terminalia chebula*
19	2-Tetradecene, (E)-	16.139	0.6112	C14H28	*Culcitium canescens*
20	Cetene	18.668	0.6885	C16H32	*Camellia sinensis*, Vanilla madagascariensis, and other organisms
21	1-Octadecene 1	20.923	1.0065	C18H36	*Vanilla madagascariensis*, *Daphne odora*
		23.853	0.6109		
22	3-Eicosene, (E)-	22.973	2.0705	C20H40	
23	1-Tetracosene	24.835	0.5277	C24H48	*Arctostaphylos uva-ursi*, *Matricaria chamomilla*
24	Tetracosane	28.173	0.2765	C24H50	*Vanilla madagascariensis*, *Magnolia officinalis*,
25	Squalene	29.744	0.5793	C30H50	plants, animals,
26	2-Ethylacridine 2	32.101	0.2893	C15H13N	
		32.886	0.3044		
		35.942	0.639		
	Ketone				
27	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	12.612	0.484	C6H8O4	Saccharomyces cerevisiae.
28	Benzophenone	19.226	0.6119	C13H10O	
29	Dehydroacetic Acid	20.239	0.6541	C8H8O4	
30	Tricyclo[4.2.2.2(2,5)]dodecan-3-one	27.293	0.5959	C12H18O	
31	4,5-Dihydro-5-oxo-3-(p-tolyl)isoxazole	29.854	0.5055	C10H9NO2	
32	(E)-2-bromobutyloxychalcone	35.337	0.4654	C19H19BrO2	

Phenol					
33	4-Methoxy thiophenol	16.312	0.2771	C7H8OS	as a screen for potential anti-cancer activity
34	2,4-Di-tert-butylphenol	17.655	2.0755	C14H22O	*Streptomyces, Bacillus subtilis,*
35	4',5-Dihydroxy-3',6,7-trimethoxyflavone, TMS (Fastigenin)	34.112	1.2626	C18H16O7	*Ajania fastigiata, Salvia officinalis*
36	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	36.885	0.3262	C14H22O2	
37	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	38.432	0.7409	C35H62O3	
Chlorinated and Fluorinated aromatic substances					
38	2-Chloro-5-methylbenzene-1,3-diol	16.744	0.4	C7H7ClO2	
39	4,6-Dichloro-2-methyl-1,3-benzenediol	16.964	1.5286	C7H6Cl2O2	
40	3,4-Dimethyl-6-(3-trifluoromethyl-phenylcarbamoyl)-cyclohex-3-enecarboxylic acid	27.042	0.2813	C17H18F3NO3	
41	3-Chloro-2,4-dimethyl-12-thia-1,5,6a,11-tetraaza-indeno[2,1-a]fluorine	28.817	0.7596	C17H11ClN4S	
42	2-(4-Chloro-phenyl)-4-naphthalen-2-yl-2,6-dihydro-pyrazolo[3,4-d]pyridazin-7-one	30.113	0.5647	C21H13ClN4O	
43	Pyrazole, 3,5-di(4-chlorophenyl)-1-(4-fluorophenyl)-	34.929	1.5227	C21H13Cl2FN2	
Ester					
44	Ethyl trans-3-isopropyl-2-oxiranecarboxylate	11.221	0.4397	C8H14O3	
45	Hydrazinecarboxylic acid, (2-ethoxy-1-methyl-2-oxoethylidene)-, ethyl ester	13.374	0.9388	C8H14N2O4	
46	Ethyl 2-hydroxy-4-methylbenzoate	16.233	0.2551	C10H12O3	
47	5-Hydroxymethyl-2-furancarboxylic acid, ethyl ester	16.437	0.282	C8H10O4	
48	Dimethyl 4-O-methylhexopyranosiduronate	19.752	1.205	C9H16O7	
Phthalate compounds					
49	Phthalic acid, monohexyl ester	21.661	0.3271	C14H18O4	
50	Phthalic acid, di(2-propylpentyl) ester	27.67	8.5672	C24H38O4	
51	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	29.289	25.1681	C24H38O4	
Glycosides					
52	methyl 2-acetamido-2-deoxy-alpha-D-galactopyranoside	17.16	0.3099	C9H17NO6	
53	beta.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl-	18.291	0.3778	C12H22O11	*Pseudostellaria heterophylla , Saccharomyces cerevisiae*
Others compounds					
54	Dimethyl sulfone	8.59	1.217	C2H6OS	*Vitis vinifera*
55	4N-Methylcytosine	15.95	0.6343	C5H7N3O	
56	1,1,1,2,2-Pentamethyl-2-[5-(thiophen-2-yl)thiophen-3-yl]disilane	30.781	0.8135	C18H12S4	
57	Silane, dimethylpropyl[(6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-yl)oxy]-, (6aR-trans)-	33.358	1.4714	C26H42O2Si	
58	1-(2-cyclohexylethyl)-2-(3,4-dimethoxyphenyl)benzimidazole	33.672	2.5152	C23H28N2O2	
59	,4-dimethyl-2,5-di(propan-2-yl)benzene	34.536	3.1944	C14H22	
60	Tris(2,4-di-tert-butylphenyl) phosphate	37.867	0.4966	C42H63O4P	

*https://pubchem.ncbi.nlm.nih.gov/

4. Discussion

The most significant bioactive substances for treating infectious disorders are antibiotics. However, the rise of microorganisms resistant to several drugs has created fundamental obstacles to the effective treatment of infectious diseases. Thus, there has been a growing interest in finding effective antibiotics due to the burden of highly frequent multidrug resistant infections worldwide. In a prior investigation, secondary metabolites and fungi that produce antibiotics were isolated using samples of chicken litter from several poultry farms in Ihiala that were chosen at random. According to earlier research, one of the most crucial steps in isolating several species of strong antibiotic-producing fungi was to choose various prospective locations, such as soil roots and chicken litter (Abo-Shadi *et al*., 2010) [2]. Because biotic and abiotic processes interact in poultry farms, they offer a favorable environment for microbial activities (Okoli 2006) [17].

The *Aspergillus* genus possesses a robust ability to synthesize most of the essential pharmaceutical compounds, including butenolide, aspergivones, aurasesperone, and Asperchondols H. (Wang *et al*., 2015, and C.H. Liu, 2015 & Li *et al*., 2018) [20, 19].

An significant species of *Aspergillus*, *Aspergillus terreus* is used in the commercial manufacturing of lovastatin, a medication that lowers cholesterol, and itaconic acid, a biobased chemical. These industrial applications have proved the superior fermentation capability. The genome data showed that more research is needed to fully understand *A. terreus*' exceptional ability to synthesize natural products. Recent developments in genome mining approach have facilitated the identification of compounds

derived from diverse cryptic biosynthetic gene clusters. Several metabolic engineering research have been conducted on commercial strains of itaconic acid and lovastatin in order to improve the production techniques (Huang *et al*., 2021) [7].

A. terreus has the capability to create an extra metabolite. This chemical demonstrated significant efficacy against clinical bacterial isolates in laboratory environments. The analysis of the extract revealed the presence of the phenolic compound 2-(4-hydroxyphenyl) tetrahydro-3, 4, which has significant antibacterial, antifungal, and antivirulence properties. -The compound furandiol was first identified in the species *A. terreus*. (Naser, *et al.*, 2023) [13].

In the current study, the ability of the isolated fungi to inhibit the growth of pathogenic bacteria has been revealed initially, and we have used two types of bacteria, gram-positive bacteria, which are *Staphylococcus aureus* bacteria. And bacteria negative for the gram stain, which are *Escherichia coli* bacteria, and both pathogenic bacteria isolated from patients with urinary tracts were obtained from the laboratory of applied microbiology in the Department of Life Sciences of the College of Science, University of Basrah. Very encouraging outcomes were obtained when the secondary metabolite extract of *A. terreus* was tested against the four different bacterial isolates that were the subject of the investigation. On the other hand, a substantial difference was observed in the rates of spike inhibition for both Gram-positive and Gram-negative bacteria in *Staphylococcus aureus* II, *Klebsiella pneumoniae*, *Escherichia coli*, and *Bacillus cereus*.

The creation of new drugs requires new sources. Given the importance of secondary fungal metabolites that have proven therapeutic efficacy, and given the rapid expansion of pathogenic bacteria and the emergence of drug-resistant strains, which represent serious clinical problems in the treatment of infectious diseases, the search for new antibiotics is required. (Cadelis, *et al.*, 2022 & Ng *et al.*, 2015) [4, 15].

Conclusion

In conclusion, the comprehensive study undertaken in this research elucidated the identification, molecular characterization, production, and biological activity of bioactive metabolites extracted from Aspergillus terreus. Through phenotypic diagnosis and advanced molecular methods, the fungal isolate was accurately identified and deposited in the GenBank. The efficacy of ethyl acetate in metabolite extraction was demonstrated, leading to the production of bioactive compounds exhibiting significant antibacterial activity against various pathogenic strains. The determination of minimum inhibitory concentrations further underscored the potency of the fungal extract against both Gram-positive and Gram-negative bacteria. Importantly, this study highlights the potential of *A. terreus* as a valuable source of secondary metabolites with therapeutic applications, particularly in combating multidrug-resistant bacterial infections. These findings underscore the importance of exploring fungal resources for novel antibiotic discovery, especially amidst the growing challenge of antibiotic resistance in clinical settings. Moving forward, further research into *A. terreus* and similar fungal species holds promise for addressing the pressing need for effective antimicrobial agents in the face of evolving infectious diseases.

5. References

1. Adegunloye DV, Adejumo FA. Microbial assessment of turkey (*Meleagris ocellata* L) and duck (*Anas platyrhynchos* L) faeces (droppings) in Akure metropolis. Advances in Microbiology. 2014;4:774-779.
2. Abo-Shadi M, Sidkey NM, Al-Mutrafy AM. Antimicrobial agent producing microbes from some soils' rhizosphere. Journal of America Science. 2010;6(10):915-925.
3. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016;6:71-79.
4. Cadelis M, Grey A, van de Pas S, Geese S, Weir BS, Copp B, *et al*. Terrien, a metabolite made by *Aspergillus terreus*, has activity against *Cryptococcus neoformans*. Peer J. 2022;10:e14239.
5. Devi R, Kaur T, Guleria G, Rana KL, Kour D, Yadav N, *et al*. Fungal secondary metabolites and their biotechnological applications for human health. In: New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier; c2020. p. 147-161.
6. Hostettmann K. Strategy for the biological and chemical evaluation of plant extracts. Pure and Applied Chemistry. 1998;70:1-8.
7. Huang X, Men P, Tang S, Lu X. *Aspergillus terreus* as an industrial filamentous fungus for pharmaceutical biotechnology. Curr Opin Biotechnol. 2021 Jun;69:273-280. DOI: 10.1016/j.copbio.2021.02.004. Epub 2021 Mar 10. PMID: 33713917.
8. Hung T, Lin S. Microbial natural products: A promising source for drug discovery. J Appl Microbiol Biochem. 2017;1:2-5.
9. Ikegbunam MN, Achugbu AN, Nwachukwu JC, Okoye AM, Uba CC. Combined activities of some selected Nigerian medicinal plants against ESBL producing strains of *Escherichia coli* and *Klebsiella pneumonia*. EC Microbiology. 2018;14(7):361-373.
10. Maghraby F, Curetti D, Cassinelli C, Bordese C. Keratinolytic fungi inhabiting floor dust of student houses at the South Valley University in Egypt. Journal of Aerobiology. 1991;24:99-106.
11. Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A one-enzyme PCR-RFLP assay for identification of six medically important *Candida* species. Jpn J Med Mycol. 2006;47:225-229.
12. Nair MG, Putnam AR, Mishra SK, Mulks MH, Taft WH, Keller JE, *et al*. Faeriefungin: A new broad-spectrum antibiotic from *Streptomyces griseus* var. *autotrophicus*. Journal of natural products. 1989;52(4):797-809.
13. Naser LA, Jafer FN, Aldhaher AH. Antibacterial Activities and Chemical Characterization of the Secondary Metabolites of *Aspergillus terreus*: Antibacterial Activities of the Secondary Metabolites of *Aspergillus terreus*. Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences. 2023;60(2):181-191.

14. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod. 2016;79:629-661.
15. Ng TB, Cheung RCF, Wong JH, Bekhit AA, Bekhit AED. Antibacterial products of marine organisms. Applied microbiology and biotechnology. 2015;99:4145-4173.
16. Norhafizah BS. Characterization of antibiotic-producing fungi from UNIMAS reserve forest and their antibiotics. Universiti Malaysia Sarawak UNIMAS Malaysia; c2012. p. 24.
17. Okoli IC, Nweke CG, Opara MN. Assessment of the mycoflora of commercial poultry feeds sold in the humid tropical environment of Imo State, Nigeria. International Journal of Environmental Science and Technology. 2006;3:9-14.
18. Pazouki M, Panda T. Understanding the morphology of fungi. Bioprocess Engineering. 2000;22(2):127-143.
19. Li WT, Luo D, Huang JN, Wang LL, Zhang FG, Xi T, *et al*. Antibacterial constituents from Antarctic fungus, *Aspergillus sydowii* SP-1. Natural product research. 2018;32(6):662-667.
20. Wang YT, Xue YR, Liu CH. A brief review of bioactive metabolites derived from deep-sea fungi. Marine drugs. 2015;13(8):4594-4616.
21. Manju S, Jegadeeshkumar D. Exploring the Antibacterial Potential of 2-Ethylacridine from Salacia chinensis: Insights into its Mechanism against Methicillin-Resistant Staphylococcus aureus (MRSA). Uttar Pradesh Journal of Zoology. 2023;44(24):100-108. https://doi.org/10.56557/upjoz/2023/v44i243815