# Antibacterial Activities and Chemical Characterization of the Secondary Metabolites of Aspergillus terreus

**Article** *in* Proceedings of the Pakistan Academy of Sciences B Life and Environmental Sciences · August 2024

DOI: 10.53560/PPASB(60-2)786

CITATIONS
3

READS
35

**3 authors:**

Areej Aldhaher
University of Basrah
**13** PUBLICATIONS **64** CITATIONS
SEE PROFILE

Lina Abbas Naser Al-Timimi
University of Basrah
**10** PUBLICATIONS **85** CITATIONS
SEE PROFILE

Furdos Noori Jafar
University of Basrah
**9** PUBLICATIONS **5** CITATIONS
SEE PROFILE

*Proceedings of the Pakistan Academy of Sciences: B*
*Life and Environmental Sciences* 60(2): 181-191 (2023)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(60-2)786

Pakistan Academy of Sciences

Research Article

# Antibacterial Activities and Chemical Characterization of the Secondary Metabolites of *Aspergillus terreus*

**Lina A. Naser[1], Furdos N. Jafar[1*], and Areej H. S. Aldhaher[2]**

[1]Department of Biology, College of Science, Basrah University, Iraq
[2]Department of Basic Science, College of Dentistry, Basrah University, Iraq

**Abstract**: The present study aims to assess the biological impact of secondary metabolites isolated from *Aspergillus terreus* that have been isolated from the soil, the fungus was grown on a fermentation medium to produce secondary metabolism, and the fungal extract extracted from the secondary metabolite was purified and chemically characterized. Antimicrobial activities of bioactive compounds extracted from the secondary metabolite of *Aspergillus terreus* isolate were tested against five types of human pathogenic bacteria*, Escherichia coli, Klebsiella* spp*., Staphylococcus aureus, Pseudomonas* spp*.,* and *Proteus* spp*.* The cytotoxicity was tested against a human blood solution. purification and chemical identification were carried out on a crude extract of *A. terreus* using TLC, GC–MS, and NMR data (1H proton and 13C carbon) analysis. The *A. terreus* secondary metabolite extract was effective against all isolated bacterial strains. The biocompatibility test showed no cytotoxic effect against a human blood solution used in different concentrations. One fraction was purified and identified as a novel compound: 2-(4-hydroxyphenyl) tetrahydro-3,4-furan diol. The results from a GC–MS analysis showed 18 peaks of the ethyl acetate extract of *A. terreus* metabolites, and the major compounds were bis(2-ethylhexyl) phthalate (47.60 %), n-hexadecanoic acid (16.41 %) and dodecamethyl-cyclohexasiloxane (9.79 %). According to the outcomes of this study, *A. terreus* can produce secondary metabolites that show potent activity against human pathogenic bacteria and are dependent sources for new secondary metabolites.

**Keywords:** *Aspergillus terreus* Secondary Metabolites, Antibacterial Activities, Chemical Identification

## 1. INTRODUCTION

Soil is a huge home of microbial types. Most of the bioactive secondary metabolites used today, including antimicrobial compounds, were isolated from soil microorganisms. Fungi are the most biotechnologically useful organisms. They are a critical source for producing enzymes, organic acids, and food additives besides antibiotics [1].

The genus *Aspergillus* is saprophytes spread in the natural environment [2]. Mycologists emphasize discovering *Aspergillus* species due to its simplicity of cultivation in media and economic application in addition to the notable ability in producing unique metabolites, recently about 315 compounds were documented [3, 4].

*A. terreus* highly contributes to the production of many novel chemical compounds as secondary metabolites, and these compounds are characterized by promising bioactivities, such as the anti-tumor compound beauvericin [5], and lovastatin, which has been found to lower cholesterol in humans [6]. Besides lovastatin, A. terreus produces many bioactive compounds, such as sulochrins, terretonines, asterriquinones, and butyrolactones, some of which have antimicrobial activities [7]. Also recently recorded the fungus production of new secondary metabolites including "terrein, aspulvinone E, flavipesolide C, butyrolactone II, and butyrolactone I, have been reported to exhibit a variety of bioactivities including anti-proliferative, anti-inflammatory, and antioxidant activities" the identified terrain.

The compound showed high antifungal activity against the yeast *Cryptococcus neoformancysteine* [8]. Secondary metabolites are necessary for the development of new pharmaceuticals. This is

Received: November 2022: Accepted: June 2023
*Corresponding Author: Furdos N. Jafar <furdos.jafer@uobasrah.edu.iq>

particularly urgent in the case of antibiotics due to the rapid spread of drug-resistant pathogenic bacteria, which pose dangerous clinical challenges in the treatment of infectious diseases [2, 9].

In 2019, more than 5 million deaths were recorded due to the generation of resistant bacterial strains [10]. This frightening number encouraged the discovery of a new antibiotic with a novel mode of action so the main purpose of this study is to evaluate the antibacterial activity of secondary metabolites extract of *A. terreus*, and isolate and identify the chemical compounds of crude extracts of *A. terreus.*

## 2. MATERIALS AND METHODS

### 2.1 Isolation of Fungus

Soil samples were collected in sterile polyethylene bags from different places in Basrah City, South Iraq. Samples were then transformed into the laboratories at Basrah University. A serial dilution method was used to isolate fungi. Potato carrot agar medium (PCA, 20 g potatoes, 20 g carrots, and 20 g Bacto agar (Difco) per L of distilled water containing 25 ppm chloramphenicol) was used for pre-isolation. Three serial dilutions of soil were used $10^{-1}$,$10^{-2}$ and $10^{-3.}$ PCA was poured into petri dishes containing 1 ml of each dilution. All cultured petri dishes were incubated at 25 °C for 7 days with periodic examination [11].

### 2.2 Identification of Fungus

Morphological characteristics of *Aspergillus* spp., including colony colour, conidial shape, length, width, and conidiophore shape and length were examined on malt extract agar medium (MEA, from Oxoid LTD company, the country producer in England ), where isolates were grown for 5-7 days at 12 h photoperiod; identification carry out under dissecting and compound microscopes [12]. Isolates were then cultured in a slant culture of MEA for 7 days at 25 °C and maintained at 4 °C for further experiments.

### 2.3 Fermentation Medium

A potato dextrose broth (PDB) from Oxoid LTD company, the country producer in England was used as a fermentation medium. The medium was sterilized in an autoclave for 15 min. Three discs of growing mycelia measuring 5 mm from activated 5 days ago *A. terreus* isolate colony were taken from the MEA medium and placed in PDB, the broth was incubated at 25 °C for 21 days [13].

### 2.4 Extraction

The fungal growth in PDB was filtered with Whatman Grade 1 filter papers from the supernatant remaining after centrifugation (6000 rpm/min for 10 min). Hydrochloric Acid (HCl) of 2N was used to regulate the pH to 3 to convert soluble compounds to crystallized form and facilitate the extraction of compounds The filtrate of the fermentation medium was extracted by organic solvent ethyl acetate with a ratio (1:1 vol). The organic layer was gathered and dried at room temperature. The dried extract was kept in a glass vial at 4 °C until use [13].

### 2.5 Antibacterial Activity

The antibacterial action of the extract was tested on 10 clinical bacterial strains: three strains of *Staphylococcus aureus*; two strains of *Klebsiella*; two strains of *Pseudomonas aeruginosa;* two strains of *Proteus*; and one strain of *Escherichia coli*. These strains were gained from several sources (stool, wound infections, urine, skin lesions) of patients admitted to Al-Fayhaa Hospital in Basrah. All the collected samples were processed upon receipt in the Al-Fayhaa laboratory and cultured in appropriate media [14].

The separated bacteria were classified into Gram-negative and Gram-positive classes, and biochemical tests were carried out by using VITEK2 automated system. A 100 µg/ml bacterial suspension for all isolates was set and balanced to 0.5 McFarland standard. The Mueller–Hinton agar was used to measure the activity of the fungal extract. A nine-millimeter diameter pore was made using a cork borer with triplicate and control plates. All petri dishes were incubated at 37 °C overnight. The inhibition zone was measured in millimeters. The dishes of each extract were examined for the minimum inhibitory concentration (MIC) [14, 15].

### 2.6 Determination of Minimum Inhibitory Concentration(MIC)

The susceptibility of five bacterial isolates *S. aureus* III, *Klebsiella* spp, *E. coli, P. aeruginosa* II, and *Proteus* spp. II was investigated (MIC) against fungal extract using four different concentrations 500, 250, 100, and 50 µg/ml. The results showed different MIC accounts (Table 2; Figure 3) explain MIC values.

### 2.7 Biocompatibility test

*A. terreus* secondary metabolite extract was applied to conduct a biocompatibility test with fresh human blood according to Wang *et al.* [16] four concentrations of *A. terreus* metabolite extract were prepared (10, 50, 100, and 200) µg/ml, and 100 µl was added from the studying concentrations to all tubes that contain blood solution. Test tubes were kept at weather temperature and monitored to notice the development of turbidity.

### 2.8 Isolation, Purification, and Identification of Bioactive Secondary Metabolite

#### *2.8.1. GC–MS Analysis*

GC–MS analysis was carried out on *A. terreus* secondary metabolite extract at Basrah Oil Company, Nahr Bin Omar Laboratory. An Agilent Technologies 7890B GC system was used coupled to an Agilent Technologies 5977A MSD with an EI ion source, using HP-5MS 5 % phenyl methyl siloxane (30 m × 250 um × 0.25 mm). The carrier gas was helium at a 1 ml/min constant flow mode and a purge flow of 3 ml/min. The oven temperature was set at 40 ºC, held for 5 min, then the temperature was held at 280 ºC for the remaining 20 min. The mass spectrometer applied an ion source temperature of 230 ºC with a 1,562 (N2) scan speed. The electron ionization was obtained over the mass range of 35–650 m/z. Data were run through the NIST 2014 library database [17].

#### *2.8.2. Purification*

Preparative thin-layer chromatography, PTLC, (silica gel, aluminum-backed plates, Merck Art 5554) was carried out for the *A. terreus* secondary metabolite extract. The plates were then developed in the selected solvent system ethyl acetate hexane 4:1, and viewed under UV light and marked. The plates were also observed using an anisaldehyde spray reagent (97 % cold methanol; 2 % sulphuric acid; 1 % p-anisaldehyde) following heating. The marked portion of the TLC plate was then scratched off and dissolved in a similar solvent used for the plate [18].

#### *2.8.3. Nuclear Magnetic Resonance Spectroscopy*

For purified compounds of *A. terreus* secondary metabolite extract, 1H, $^{13}C$, and 2D NMR spectra were performed on a Bruker AVANCE III NMR spectrometer operating at 125.76 MHz for $^{13}C$ and 500.13 MHz for 1H at the University of Surrey, Department of Chemistry, UK. The spectra were recorded in deuterated methanol ($CD_3OD$). The $CD_3OD$ was referenced at δC 49.15 in the $^{13}C$ NMR spectrum and at δH 4.87 in the $^{1}H$ NMR spectrum

## 3. RESULTS

### 3.1 Fungal Appearance

Macroscopic features for one isolate of *A. terreus* showed a brown colony with dense growth and sporulation on MEA medium at 25 °C (Figure 1b). Microscopic examination for 100 spores of *A. terreus* showed a founding of a hyaline conidiophore carry vesicle (conidial head) with biseriate of stigmata that held small and globose yellow conidia (Figure 1a).

### 3.2 Biological Activity

The results of the antimicrobial activity of *A. terreus* secondary metabolite extract against 10 different bacterial isolates under study were very encouraging. Both gram-positive and gram-negative bacteria exhibited altitude inhibition rates all results were significantly different except with *Staphylococcus aureus* II and *Klebsiella* spp. I, *Pseudomonas aeruginosa II* and *Escherichia coli*, *Proteus* spp I and *Proteus* spp II (Table 1; Figure 2)

### 3.3 Determination of Minimum Inhibitory Concentration

The susceptibility of five bacterial isolates *Staphylococcus aureus* III, *Klebsiella* spp.,

**Table 1.** Antimicrobial activity of *A. terreus* secondary metabolite extract against bacterial test organism.

| **Bacteria** | **Zone of inhibition in (mm)** |
|---|---|
| *Staphylococcus aureus* I | 12 |
| *Staphylococcus aureus* II | 16 |
| *Staphylococcus aureus* III | 25 |
| *Klebsiella* spp I | 16 |
| *Klebsiella* spp II | 28 |
| *Pseudomonas aeruginosa* I | 7 |
| *Pseudomonas aeruginosa* II | 12.5 |
| *Proteus* spp I | 2 |
| *Proteus* spp II | 2 |
| *Escherichia coli* | 12.5 |

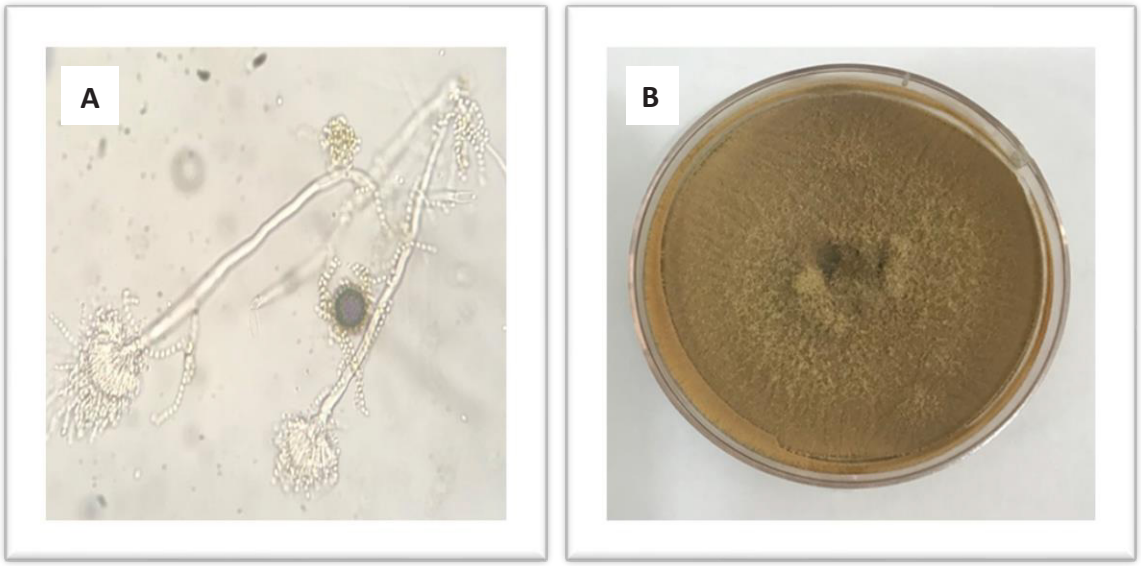



**Fig. 1.** (A) Conidia of *A. terreus,* (B) Colony

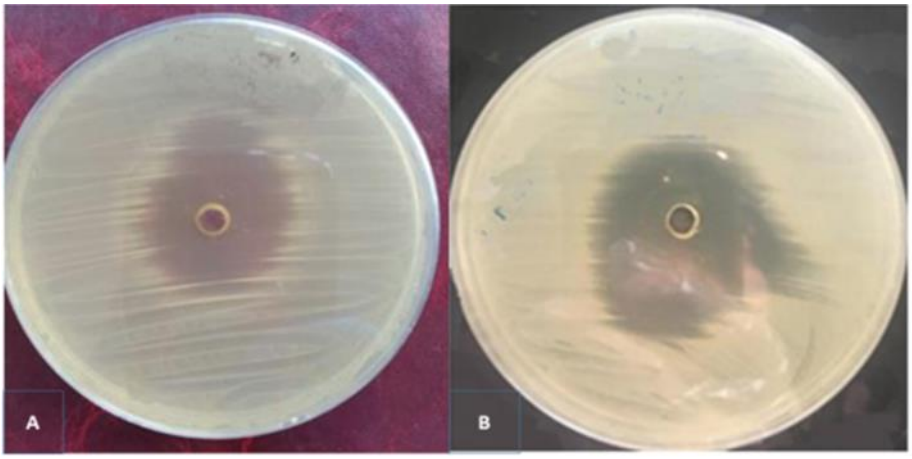



**Fig. 2.** The bacterial inhibitory zone by *A. terreus* extract. (A) *Staphylococcus aureus* (B) *Klebsiella* spp.

*Escherichia coli, Pseudomonas aeruginosa* II, *Proteus* sp. II was investigated against fungal extract using four different concentrations (500, 250, 100, 50). The results show different MIC values (Table 2; Figure 3) explain MIC values.

### 3.3 Biocompatibility test

Biocompatibility test explained there was no turbidity creation in all studying extract concentrations found in blood solution tubes this indicates the fungal extract is not toxic (Figure 4).

### 3.4 GC–MS analysis

GC–MS analysis of *A. terreus* secondary metabolite extract showed 18 peaks (Figure 5; Table 3). The major compounds were bis(2-ethylhexyl) phthalate (47.60 %), n-hexadecanoic acid (16.41 %), dodecamethyl-cyclohexasiloxane (9.79 %), in addition to other fatty acid and amino acid compounds, such as octadec-9-enoic acid (1.14 %) and cysteine (1.62 %).

### 3.5 Purification and NMR Spectra

PTLC of *A. terreus* extract yielded four fractions (A, B, C, D). Only fraction C was identified using NMR to be unreported compounds 2-(4-hydroxyphenyl) tetrahydro-3,4-furandiol was obtained as a yellow oil (Figure 6). The $^{13}C$ NMR and DEPT spectrum together with the analysis of HSQC, COSY, and HMBC experiments showed the presence of one methylene, 7 methines, and 2 quaternary carbons, allowing the molecular formula $C_{10}H_{12}O_4$ to be assigned to compound 2-(4-hydroxyphenyl) tetrahydro-3,4-furandiol (Table 4). The $^{1}H$ NMR spectrum of the compound showed signals attributed to para phenyl moiety at $\delta_H$ 7.64 (2H, d, J = 8.0Hz, H 2", H-6"), $\delta_H$ 8.18 (2H, J= 2.0, 7.0

**Fig. 3.** Different inhibition zones according to different concentrations of *A. terreus extract*. MIC for *Klebsiella* spp

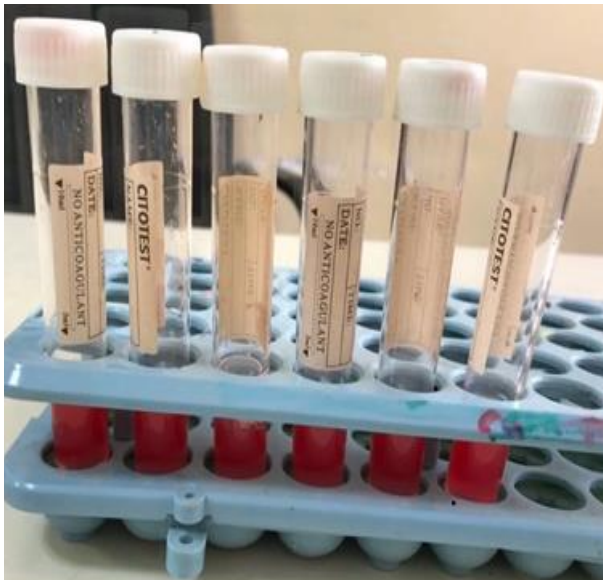



**Fig. 4.** Biocompatibility test display that has no formation of turbidity in blood solution tubes with different concentrations of the used extract.

**Table 2.** Minimum Inhibitory Concentration rate of *A. terreus* secondary metabolite extract against bacterial test organism mg/ml. (Yellow color refers to MIC values)

| Bacteria | 500 µg/ml | 250 µg/ml | 100 µg/ml | 50 µg/ml |
|---|---|---|---|---|
| *Staphylococcus aureus* III | + | + | - | - |
| *Klebsiella* spp I | + | + | + | - |
| *Escherichia coli* | + | + | - | - |
| *Pseudomonas aeruginosa* II | + | - | - | - |
| *Proteus* spp II | - | - | - | - |

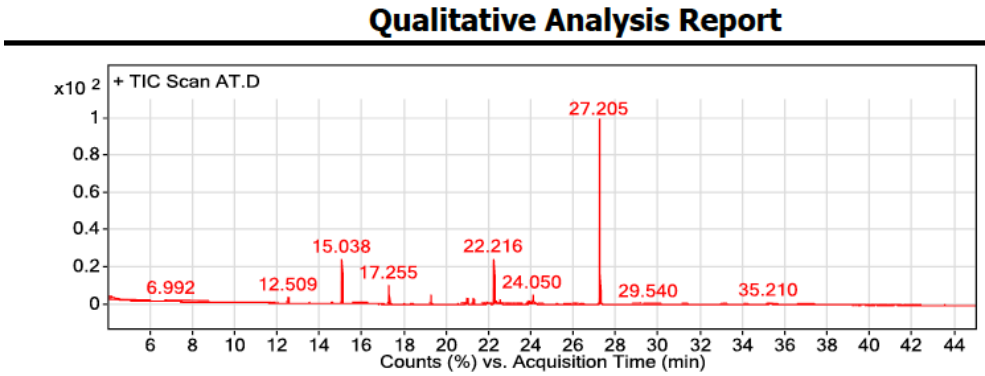
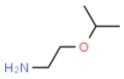
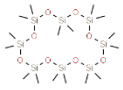
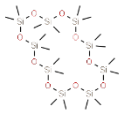
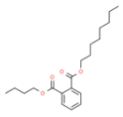
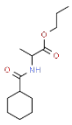
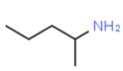
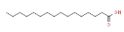
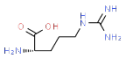
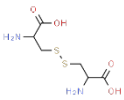
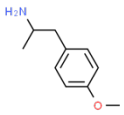
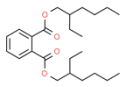
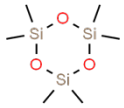
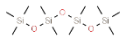
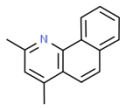



**Fig. 5.** GC–MS chromatogram of A. terreus secondary metabolite extract.

**Table 3.** Chemical Composition of *A. terreus* secondary metabolite extract by GC–MS.

| S. No. | RT | Area% | Library/ID | CAS | Formula | Synonyms | M.W | Chemical structure |
|---|---|---|---|---|---|---|---|---|
| 1. | 12.509 | 1.64 | 2-Propan-2-yloxyetha-namine | 81731-43-3 | $C_5H_{13}NO$ | 2-Isopropoxyethana-mine | 103.16 |  |
| 2. | 15.038 | 9.79 | Dodecamethylcyclo-hexasiloxane | 540-97-6 | $C_{12}H_{36}O_6Si_6$ | Cyclohexasiloxane | 444.92 |  |
| 3. | 17.255 | 4.18 | Tetradecamethylcyclo-heptasiloxane | 107-50-6 | $C_{14}H_{42}O_7Si_7$ | Cyclomethicone 7 | 519.078 |  |
| 4. | 19.235 | 1.98 | Hexadecamethyl cy-clooctasiloxane | 556-68-3 | $C_{16}H_{48}O_8Si_8$ | | 593.24 |  |
| 5. | 20.958 | 1.51 | Octadecamethyl cy-clononasiloxane | 556-71-8 | $C_{18}H_{54}O_9Si_9$ | | 667.4 |  |
| 6. | 21.243 | 1.91 | 1,2-Benzenedicarbox-ylic acid, butyl octyl ester | 84-69-5 | $C_{20}H_{30}O_4$ | Phthalic acid, diiso-butyl ester | 334.44 |  |
| 7. | 21.716 | 1.30 | propyl 2-(cyclohex-anecarbonylamino) propanoate | 1000314-17-4 | $C_{13}H_{23}NO_3$ | Propyl N-(cyclohexylcarbonyl) alaninate | 241.33 |  |
| 8. | 21.896 | 2.10 | 2-Pentanamine | 63493-28-7 | $C_5H_{13}N$ | 1-methyl- butylamine | 87.16 |  |

| S. No. | RT | Area% | Library/ID | CAS | Formula | Synonyms | M.W | Chemical structure |
|---|---|---|---|---|---|---|---|---|
| 9. | 22.216 | 16.41 | n-Hexadecanoic acid | 57-10-3 | $C_{16}H_{32}O_2$ | palmitic acid | 256.429 |  |
| 10. | 22.487 | 1.04 | L-arginine | 74-79-3 | $C_6H_{14}N_4O_2$ | (2S)-2-amino-5-(diaminomethylideneamino)pentanoic acid | 174.20 |  |
| 11. | 23.842 | 1.14 | octadec-9-enoic acid | 112-80-1 | $C_{18}H_{34}O_2$ | Oleic Acid | 282.5 |  |
| 12. | 23.898 | 1.62 | cystine | 56-89-3 | $C_6H_{12}N_2O_4S_2$ | 2R)-2-amino-3-[[(2R)-2-amino-2-carboxyethyl]disulfanyl]propanoic acid | 240.3 |  |
| 13. | 24.05 | 4.48 | Octadecanoic acid | 57-11-4 | $C_{18}H_{36}O_2$ | Stearic acid | 84.483 |  |
| 14. | 26.038 | 1.11 | 1-(4-methoxyphenyl) propan-2-amine | 23239-32-9 | $C_{10}H_{15}NO$ | 4-Methoxyamphetamine | 165.23 |  |
| 15. | 27.205 | 47.60 | Bis(2-ethylhexyl) phthalate | 117-81-7 | $C_{24}H_{38}O_4$ | Dioctylpftalat | 390.56 |  |
| 16. | 33.035 | 0.84 | hexamethylcyclotrisiloxane | 541-05-9 | $C_6H_{18}O_3Si_3$ | | 222.46 |  |
| 17. | 34.119 | 0.49 | Decamethytetrasiloxane | 141-62-8 | $C_{10}H_{30}O_3Si_4$ | | 310.68 |  |
| 18. | 35.196 | 0.85 | 2,4-dimethylbenzo[h] quinoline | 605-67-4 | $C_{15}H_{13}N$ | | 207.27 |  |

Hz, dd H-3", H-5").

An oxymethine proton resonance at δH 5.15 (J=0.01) revealed correlations with the C-2' ($\delta_C$ 128.16) resonance, in the HMBC spectrum, thus it was assigned as H-2. The placement was confirmed by its correlation C-2 ($\delta_C$ 71.16) with one of the oxymethylene proton resonance at $\delta_H$ 3.60 (H-4) and $\delta_H$ 3.56 (H-5) in the HMBC spectrum. Also, the H-3 oxymethine proton resonance at ($\delta_H$ 4.13) displayed a correlation with the C-5 ($\delta_C$ 62.06) resonance in the HMBC spectrum.

The resonance at ($\delta_C$ 150.26) was assigned as C-4' due to correlations seen in the HMBC spectrum with the H-2' ($\delta_H$ 7.64) and H-3' ($\delta_H$ 8.18)

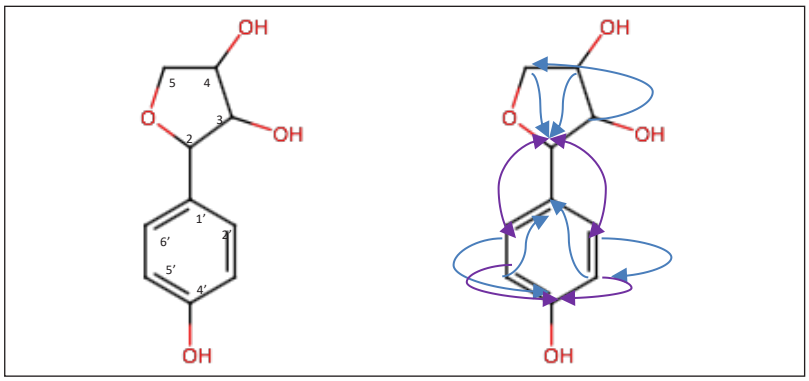



**Fig. 6.** 2-(4-hydroxyphenyl)tetrahydro-3,4-furandiol compound isolated from A.terreus and correlation structure of the compound in the HMBC spectrum

**Table 4.** NMR Data for the 2-(4-hydroxyphenyl)tetrahydro-3,4-furandiol (1) ($CD_3OD$, J in Hz).

| Position | $\partial_C$ type | $\partial_H$ (J in Hz ) | HMBC | COSY |
|---|---|---|---|---|
| 2 | 71.16 CH | 5.15, d (2.6) | 2', 6' | |
| 3 | 58.36 CH | 4.13, dd (2.6, 7.0) | 5 | 4 |
| 4 | 67.26 CH | 3.60, dd (7.0, 10.0) | 2 | 3 |
| 5 | 62.06 $CH^2$ | 3.80, dd (8.4, 10.0) | | |
| | | 3.65, tri (8.6) | 2 | |
| 1' | 151.4628 C | | | |
| 2', 6' | 128.16 CH | 7.64 d (8.0) | 2', 4' | 3', 5' |
| 3', 5' | 124.06 CH | 8.18 dd (2.0, 7.0) | 2', 4' | 2', 6' |
| 4' | 150.26 C | | | |

resonances. The fully substituted aromatic carbon resonance C-1' ($\delta_C$ 151.46) revealed correlations with the H-3' ($\delta_H$ 8.18) resonance in the HMBC. Coupling was seen in the COSY spectrum between the H-2 ($\delta_H$ 7.64) and H-3 ($\delta_H$ 8.18) and between H-4 ($\delta_H$ 3.60) and H-3 ($\delta_H$ 4.13) resonances.

In the NOESY spectrum, The H-3 resonance revealed correlations with the H-4 resonances. Therefore, they were assigned to the same face of the molecule. However, the relative configuration at C-2 could not be set from the NOESY spectrum

## 4. DISCUSSION

Recently, there has been a necessity for the discovery of new antibiotics nearly every day due to the emergence of drug-resistant bacteria, The death of 3.57 million cases associated with resistance of *K. pneumoniae, E.coli, S. pneumoniae, A. baumannii, P. aeruginosa*, and *S. aureus*.[10] Despite the major importance of medicinal plants in producing antibiotics, the soil is still an important source of unique antibiotics [14]. More than 50 % of the annual production of antibiotics comes from microorganisms isolated from soil [15].

*Aspergillus* genus has a strong biosynthetic ability for the production of most of the necessary pharmacological products such as Asperchondols H, butenolide, aspergivones, and Aurasperone [16-17]. The fungus *A. terreus* is one of the important species that is isolated from the soil, especially agricultural soil since it is very rich in organic compounds and inorganic elements that support the fungal community [18]. The literature reported that isolated Terrein a compound from *A. terreus*, this compound showed antimicrobial activity against *P. aerogenes* by reducing virulence factor expression and the formation of biofilm without

effect on cell growth [19]. In response to various growth conditions, *A. terries* produces different secondary metabolites through biosynthesis [20], So, its natural compounds were the target of this study and the phenolic part in particular.

The study identified to be 2-(4-Hydroxyphenyl) tetrahydro-3,4-furandiol based on its $^{1}$H-NMR, $^{13}$C-NMR and 2D-NMR. The findings of the study could be useful in exploring novel bioactive compounds from fungi. The compound 2-(4-Hydroxyphenyl)tetrahydro-3,4-furandiol has not been reported previously. However, a derivative of the compound -2-(3',4'dihydroxyphenyl) tetrahydrofuran-3,4-diol, was isolated from the phytopathogenic fungus *Colletotrichum gloeosporioides* and is known as gloeosporiol [21]. The isolated compound 2-(3',4'Dihydroxyphenyl) tetrahydrofuran-3,4 diol was synthesized previously and displayed a radical scavenging activity at the efficient concentration (EC50) of 0.14 mol/L [ 21-22].

There was considerable antibacterial activity against 10 bacterial isolates. The growth of both gram-positive and gram-negative was inhibited at a substantial rate due to the treatment with the fungal extract [11, 12]. *A. terreus* extract showed strong antibacterial against *Klebsiella* spp II and *Staphylococcus aureus* III, while it displayed exhibited a very limited impact on *Proteus* spp I and II growth. The results come consistent with the conclusions of many studies [23-25]. The antibacterial effect of *A. terreus* extract against bacteria suggests the products of this species as a prospective antibiotic in the future. In terms of toxicity test, all concentrations of the species extracts showed no turbidity in blood aliquots which suggests that it lacks toxicity [19].

The GC–MS analysis of *A. terreus* extract revealed phthalate comprises (47.60%), polysiloxane compounds (18.79 %), and n-hexadecanoic acid (16.41 %) and these were predominant constituents [22, 26-27]. The results agreed with Previous study also detected cyclooctasiloxane, hexadecamethyl in the GC–MS analysis of *A. terreus* and other derivative compounds, such as octadec-9-enoic acid and 1,2-benzene dicarboxylic acid, butyl octyl ester, with different percentages [28]. Phthalic acid esters (PAEs), which were found to be the main constituent, is commonly known as a synthesized plasticizer. Over 50 diverse derivatives of PAEs have been described as a product of different organisms, including plants, animals, fungi, actinomycetes, and bacteria [29].

Detection of Cysteine in the extract of *A. terreus* belongs to that cystine is the element sulphur in filamentous fungi. *A. nidulans* and *Neurospora crassa* employ organic and inorganic S compound pathways for cysteine biosynthesis [30]. Previous studies also reported that cis-9-octadecenoic acid was the predominant compound in crude methanol extract of *Trichoderma* sp. by GC–MS [ 27].

The biological activity of the crude extract of *A. terreus* might belong to n-hexadecanoic acid (palmitic acid), which was found to have a high percentage (16.41 %) in the present study. Palmitic acid showed a strong antifungal effect against scedosporium apiospermum in soap, antibiofilm, and antivirulence potency against *Candida tropicalis* and antibacterial activity [31-33]. In addition, the activity of Amyl-1–18, an antimicrobial peptide, increased by replacing aspartic acid with arginine without a major rise in hemolytic activity [34-35]. Arginine detection in the extract at 1.04 % proposes a potential antibacterial activity since a study by Sepahi *et al*. [32] showed that arginine-rich peptides had antibacterial activity against *E. coli* and *S. aureus*.

## 5. CONCLUSION

It has been concluded from the current study that the fungus *A. terreus* can produce secondary metabolite under laboratory conditions, this metabolite had potent power against clinical bacterial isolates. The extract contained a significant percentage of the antibacterial, antifungal, and anti-virulence n-hexadecanoic. As far as the authors know that the phenolic compound 2-(4-Hydroxyphenyl) tetrahydro-3,4-furandiol is isolated for the first time from *A. terreus* species. The study suggests that the extract of *A. terreus* could be a potentially effective antibiotic against resistant bacteria which is a big challenge these days over all the world.

## 6. CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

## 7. REFERENCES


1. K. Jawaid, M. Shafique, A. Versiani, H. Muhammed, S.A. Naz, and N. Jabeen. Antimicrobial potential of newly isolated *Aspergillus terreus* MK-1: An approach towards new antibiotics. The *Journal of the Pakistan Medical Association* 69(1): 18-23(2019).
2. B. Amina, G. Sana, J. Atef, D. Laid, and K. Noreddin. Antibacterial activity of *Aspergillus* isolated from different Algerian ecosystem. *African Journal of Biotechnology* 16(32): 1699-1704(2017).
3. P. Phainnphoug, C. Rukachaisirikul, C. Srimaroeng, A. Duangja, and J. ayaroj. Analogous from the soil-derived fungus *Aspergillus* sclerotiorum Psu-RSPG178. *Natural Products* 79: 1500-150 (2016).
4. X. Zhang, Z. Li, and J. Gao. Chemistry, and biology of secondary metabolites from *Aspergillus* genus. *The Natural Product Journal* 8(4): 275–304( 2018).
5. D. Chun-Me, L. Shi-Xin, H. Cai-Huan, P. Ji-Yan, and L. Yong-Cheng . Secondary metabolites of Mangrove endophytic fungus *Aspergillus terreus* No.GX7-3B from the South China sea. *Marine Drugs* 11: 2616-2624(2013).
6. Y. Gung, H. Bao, B. Ya.,W. Qiang, Z. Xiao, L. Na, Q. Xue, and X. Li. Secondary metabolites of the fungus *Aspergillus terreus. Chemistry of Natural Compounds* 54(2): 415-418 ( 2018).
7. X. Lan-Lan, C. Fei, T. Sha-Sha, and Z. Hua-Jie. Investigations of Fungal Secondary Metabolites with Potential Anticancer Activity. *Chemistry of Natural Compounds* 53, 1212(2017).
8. M. Cadelis, A. Grey, S. Van de Pas, S. Geese, B. Weir, B. Copp, and S. Wiles. Terrien, a metabolite made by *Aspergillus terreus*, has activity against *Cryptococcus neoformans*. *PeerJ* 10: e14239( 2022).
9. T. Ng, R. Cheung, J. Wong, A. Bekhit, and A.E. Bekhit. Antibacterial products of marine organisms. *Applied Microbiology and Biotechnology* 99(10): 4145-4173(2015).
10. C.J. Murray, K.S. Ikuta, F. Sharara, L. Swetschinski, G.R. Aguilar, A. Gray, C. Han, C. Bisignano, P. Rao, E. Wool, and S.C. Johnson. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis *Lancet* 399(10325): 629–655. (2022).
11. F. Yakop, H. Taha, and P. Shivanand. Isolation of fungi from various habitats and their possible bioremediation. *Current Science* 116(5): 733-740(2019).
12. K.B. Raper, and D. I. Fennell. The genus *Aspergillus*. Williams and Wilkins Co., Baltimore, Md. 686 p. (1965).
13. N. Zainuddin, S.A. Alias, C.W. Lee, R.E bel , N. Othman, M.R. Muktar, and K. Awang. Antimicrobial activites of marine fungi from Malaysia. *Botanica Marina* 53: 507-513(2010).
14. L.A. Al-Timimi. Antibacterial and Anticancer Activities of Fenugreek Seed Extract. *Asian Pacific Journal of Cancer Preventation* 20 (12): 3771-3776(2019).
15. J.M. Miller, J. M. Binnicker, S. Campbell, K.C. Carroll, and K.C. Chapin *et al*. Laboratory Diagnosis of Infectious Disease. *Clinical Infectious Diseases* 67(6): 1-94 (2018).
16. Y.T. Wang, Y.R. Xue, and C.H. Liu. A brief review of bioactive metabolites derived from deep-sea fungi. *Marine Drugs* 13(8): 4594-4616 ( 2015).
17. W.t. Li, D. Luo, J.N. Huang, W. Ll, F.G. Zhang, T. Xi, and J.M. Liao, Y.Y. Lu. Antibacterial constituents from Antarctic fungus, *Aspergillus* sydowii SP-1. *Natural Product Research* 32(6): 662-667(2018).
18. H. Xian-guo, and M. Urasella. Antifungal compound from *Solanum nigrum*. *Journal of Ethnopharmacology* 43: 173-177(1994).
19. B. Kim, J. Park, H. Choi, S.S. Yoon, and W. Kim. Terrein is an inhibitor of quorum sensing and c-di-GMP in *Pseudomonas aeruginosa*: a connection between quorum sensing and c-di-GMP. *Scientific Reports* 8(1): 8617(2018).
20. A. Bracarense, and J.T. Takahashi. Modulation of antimicrobial metabolites production by the fungus *Aspergillus parasiticus. Brazilian Journal of Microbiology* 45(1): 313-321(2014).
21. M. Femenía-Ríos, C.M. García-Pajón, R. Hernández-Galán, A.J. Macías-Sánchez, and I.G. Collado. Synthesis and free radical scavenging activity of a novel metabolite from the fungus *Colletotrichum gloeosporioides*. *Bioorganic & Medicinal Chemistry Letters* 16(22): 5836-5839(2006).
22. G. Mancilla, M. Femenía-Ríos, M. Grande, R. Hernández-Galán, A.J. Macías-Sánchez, and I.G. Collado. Enantioselective, chemoenzymatic synthesis, and absolute configuration of the antioxidant (−)gloeosporiol, *Tetrahedron* 66( 40): 8068-8075(2010).
23. Z.N. Al-Laith, L.A. Naser, and A.D. Al- Hilfi. Determination The Effect Of *Helicobacter Pylori* Toxin On Normal And Cancer Cells Lines. *Turkish Journal of Physiotherapy and Rehabilitation* 32(3) ((2021.

24. F.N. Jafer, and L.A. Naser. The Biological Activity Of Aqueous And Methanolic Extracts Of Juglans Regia On Yeasts And Pathologic Bacteria. *Plant Archives* 20: 2405-2410(2020).
25. L.A. Al-Timimi, and N.A. Al-Tameemi. Role Of Carob Seed In Characterization Of MDR Bacteria In Diabetic Foot Ulcer. *Biochemical and Cellular Archives* 20: 927-934(2020).
26. G. Molinari. Natural products in drug discovery, present status and perspectives. *Pharmaceutical Biotechnology* 655: 13-27(2009).
27. S. Kandasamy, S. Sahu, and K. Kandasamy. In Silico Studies on Fungal Metabolite against Skin Cancer Protein (4,5-Diarylisoxazole HSP90 Chaperone). *International Scholarly Research Notices* 626214-5(2012).
28. S. Rajalakshmi, and N. Mahesh. Production and Characterization of Bioactive Metabolites Isolated from *Aspergillus terreus* in Rhizosphere Soil of Medicinal Plants. *International Journal of Current Microbiology and Applied Sciences* 3: 784-798(2014) .
29. L. Huang, X. Zhu, S. Zhou, Z. Cheng, K. Shi, C. Zhang, and H. Shao. Phthalic Acid Esters: Natural Sources and Biological Activities *Toxins* 13(7): 495(2021).
30. M.J. Sohn, S.J. Yoo, D.B. Oh, O.K. Won, S.Y. Lee, and A.A. Sibirny . Novel Cysteine-Centered Sulfur Metabolic Pathway in the Thermotolerant Methylotrophic Yeast Hansenula polymorpha 9(6): e100725(2014).
31. K. Jung, M. Miyagawa, A. Matsuda, Y. Amagai, K. Oida, Y. Okamoto, M. Takai, S. Nishikawa, H. Jang, S. Ishizaka, G. Ahn, A. Tanaka, and H. Matsuda. Antifungal effects of palmitic acid salt and ultrapure soft water on Scedosporium apiospermum. *Journal of Appllied Microbiology* 115(3): 711-7 (2013).
32. M. Sepahi, R. Jalal, and M. Mashreghi. Antibacterial activity of poly-l-arginine under different conditions. *Iranian journal of microbiology* 9(2): 103-111(2017).
33. K.G. Prasath, H. Tharani, M.S. Kumar, and S.K. Pandian. Palmitic Acid Inhibits the Virulence Factors of *Candida tropicalis*: Biofilms, Cell Surface Hydrophobicity, Ergosterol Biosynthesis, and Enzymatic Activity. *Frontiers in Microbiology* 11: 864(2020).
34. M. Taniguchi, A. Ochiai, K. Takahashi, S. Nakamichi, T. Nomoto, E. Saitoh, T. Kato, and T. Tanaka. Effect of alanine, leucine, and arginine substitution on antimicrobial activity against *Candida albicans* and action mechanism of a cationic octadecapeptide derived from α-amylase of rice, *Peptide Science* 106( 2): 219-229(2016).
35. C.B. Huang, Y. Alimova, T.M. Myers, and J. L. Ebersole. Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. *Archives of Oral Biology* 56(7) : 650-654. (2011).