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Characterization of Bioactive Compounds of Antimicrobial Metabolites Extracted from Soil Fungi

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Abstract

Background: The global spread of bacterial resistance has significantly contributed to the increased mortality and morbidity of patients clinically due to the shortage of suitable and potent antibiotics. The concept of searching for new antibiotics in this era of antibiotic resistance pandemic remains vital therefore fungi-inhabiting uncultivated soil was examined for the antimicrobial activity against multidrug-resistant bacteria.

Methods: Soil samples were collected from uncultivated farmland for fungi isolation. The isolation was done using Potato Dextrose Agar (PDA) and secondary metabolites of fungi were extracted using ethyl acetate as the solvent and the extract was dissolved with DMSO. The occurrence of bioactive compounds was carried out using GC-MS analysis. The antimicrobial susceptibility assay for the metabolites was carried out using the disc diffusion technique and zones of inhibition were measured with a vernier caliper.

Findings: The preliminary antimicrobial screening was carried out on two hundred and fifty-six (256) fungal isolates. Only forty-three (43) of the isolates were able to exhibit antimicrobial capabilities and they were identified to belong to the genera of Aspergillus and Penicillium. The pathogens used as test organisms were multidrug resistance with the maximum mean zone of inhibition to be 25.3 ± 4.619. Crude extract of the fungi was active against all the pathogens with the maximum mean of a zone of inhibition to be 26.0 ± 0.000 however the minimum was 14.0 ± 0.000 zone of inhibition. The activity of the extracted metabolites ranges from 14.0 ± 0.000 to 22.0 ± 0.000 zone of inhibition.

Conclusion: There was the presence of different bioactive compounds like aromatic compounds, terpenes, steroids in the fungal metabolites. Fungi still constitute vital sources of antimicrobial substances and subsequently generate potential antibiotics for the treatment of diseases.

Keywords: Fungi; Metabolites; Bioactive compounds; Novel; Antimicrobials

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Introduction

Soil is an ecological niche for fungi which are mainly producers of several useful bioactive natural products. Fungal secondary metabolites are rich in bioactive compounds which are highly diverse in chemistry and biological activity [1]. These fungal secondary metabolites are inexhaustible sources of new antibacterial substances. Species in the genera of the Penicillium and Aspergillus are globally distributed and are saprophytes [2]. These genera are important sources of bioactive compounds that hold potential for novel drug substances in medicine [3,4]. Secondary metabolites are biologically active organic compounds that are not required for normal growth, development, or reproduction, however provide a competitive advantage to the producing organism in the ecological niche [5].

Endophytic fungi due to the production of secondary metabolites have been reported as growth promoters, stress tolerance, immunity to drought, repellent of insects and herbivores [6]. The first reported antibiotics were from fungi and currently, metabolites of fungi still hold potentials for novel antimicrobials [7]. Microbial metabolites are well known to be rich sources of new potential therapeutic drugs [8,9]. Out of thousands of antibiotics inherent in fungi, only about 100 antibiotics have been adequately documented in the treatment of humans and animal diseases [10].

Bacteria have emerged as essential pathogens associated

with health care for decades. However, there is a continuous emergence of drug and multidrug-resistant pathogens [11], which demands the continuous search for new and highly potent antimicrobial compounds. Resistance of pathogenic microorganisms to antibiotics has become a major threat to the health sector thereby reducing the effectiveness and economic loss of the clinically available drugs. The rapid spread of Vancomycin-Resistant Enterococci (VRE) has been of particular concern around the world [12]. The resistance pressure on the bacterial population due to the constant use of antibiotics without adding novel ones has led to the emergence of resistant bacteria. Resistance to antibiotics belonging to β lactam antibiotics, quinolones, aminoglycosides, nitrofurantoin and others have also been reported [13,14].

Drug resistance in bacteria has become a global problem and there is an urgent and continuing search for new antibacterial agents [15] hence, the primary objective of the current study was to harness the antimicrobial potentials of the extract of fungi that has antibacterial properties along with the bioactive components.

Materials and Methods

Sample collection and fungi isolation

This study was carried out in Oyo town, which is in the south-western part of Nigeria. The town is situated on latitude 8˚00 north of the equator and longitude 4˚00 east. Soil samples were randomly collected aseptically at a depth of 5 cm from 20 different locations. 1 g of the soil was suspended in 9 mL of sterile distilled water and serial dilution was carried out to reduce the load. The fungal isolates were cultured using Potato Dextrose Agar (PDA) and incubated for 5 days at 28˚C. Pure cultures of fungal isolates were identified using both macroscopic (cultural) and microscopic (morphological) features [16].

Extraction of secondary metabolites from pure isolates

The fungal isolates showing great antibacterial activities during the primary screening were selected for secondary screening using the modified method of Sheeba et al. [17]. The fungal isolates were inoculated into 250 mL erlenmeyer flasks containing 100 mL Potato dextrose broth and incubated at room temperature for 21 days under stationary conditions with intermittent shaking. The broth culture was filtered to separate the mycelial and filtrate. After which equal volume of ethyl acetate was added to the filtrate, mixed well for 10 min and kept for 5 min till the two immiscible layers formed. The upper layer of ethyl acetate containing the extracted compounds was separated using a separating funnel. The extract was concentrated by removing the solvents under room temperature thereby allowing the solvent to evaporate and leaving the crude extracts. The extract was dissolved in DMSO and stored at 4°C [18,19].

Gas chromatography and mass spectroscopy (GC-MS) analysis of bioactive compounds

Bioactive compounds in the cell-free extract of the fungi isolates that produced metabolites with higher antifungal activity against the test bacterial isolates were identified by GC-MS analysis. Briefly, 100 µL of each cell-free extract was mixed with ethyl acetate at a ratio of 1:1 and was loaded into GC-MS apparatus for analysis using the protocol of Sengupta et al. with slight modifications. The analysis was conducted using Agilent 7890A gas chromatograph equipped and coupled to Agilent 5975C Mass Spectrometer with a fused HP-5MS 5% Phenyl Methyl Silox (30 m x 0.25 mm ID x 0.25 µm of the capillary column). Helium gas was used at a constant flow rate of 1.0 mL/min-1; and a fixed inlet temperature (285°C); injection volume, 1 µL. The oven temperature program was set to an initial temperature of 90OC, then 3°C min-1 ramp to 180°C and held for 10 min. The ionization voltage used will be 70 eV while a scan of 0.6 s will be applied, covering a mass range from 50 amu to 500 amu and the major constituents identified.

Bacterial identification

Bacterial isolates were obtained from the Laboratory of food microbiology, ajayi Crowther university and sub-cultured onto nutrient agar and incubated at 37°C for 24 h to obtain a pure culture. The isolates were identified as Bacillus cereus and Staphylococcus aureus being Gram-positive as well as Escherichia coli and Klebsiella pneumoniae as Gram-negative bacteria [20].

Preliminary evaluation (agar overlay method) of fungi isolates for antimicrobial activity

Primary screening of the fungal isolates was determined by the agar overlay method. Briefly, the isolated fungi grown on PDA were incubated at 25°C for three (3) days and overlayed by a layer of soft nutrient agar (NA) (0.75%) inoculated with bacterial isolate with 0.5 McFarland. However, control does not have fungal isolates. All plates were incubated at 25°C for 24 hrs.

Antimicrobial susceptibility assay

Antimicrobial susceptibility assay was performed using secondary metabolites of the fungi positive to preliminary evaluation antimicrobial of the fungal antimicrobial properties [17]. Sterile Mueller Hinton Agar was poured aseptically and 1.5 x 108 CFU/mL of bacteria liquid culture in an exponential growth phase was spread onto the surface of the plate. All the culture plates were allowed to dry for about 5 min. Wells were bored on the agar surface using a cork borer and filled with the extracted fungal metabolites respectively. After 24 hrs, the zone of inhibition was measured with a measuring scale and compared with the DMSO as a control.

Determination of Minimum Inhibitory Concentration (MIC) Value

The antibacterial activity of the bioactive compounds of the strain was determined in terms of Minimum Inhibitory Concentration (MIC) against gram-positive and gram-negative bacteria by using the agar well diffusion assay. The bioactive compounds were dissolved in dimethyl sulfoxide at concentrations ranging from 0 to 1000 µg/mL and used to assay against test bacteria pathogen. Determination of MIC value was carried out using the serial dilution method after the antibacterial activity of the fungal crude extracts by the standard method described by Akanksha et al. [21] with minor modification. The final concentration of the extract in each of the test tubes numbered after dilution was dispensed on already solidified Mueller Hinton agar. Wells were bored on the agar surface using a cork borer and filled with extract bacterial metabolites and incubated at 37°C for 24 h, then

examined for a zone of inhibition. The plate in which the zone of inhibition fails to occur was the MIC of the culture.

Results

A total of two hundred and fifty-six (256) fungal isolates were screened for antimicrobial potential. Only forty-three (43) of the isolates were observed to have antimicrobial potential. They have morphological characteristics ranges from grey to brown in Penicillium spp. while cream to yellow was observed in Aspergillus spp. as shown in (Table 1). The fungal isolates had velvety and powdery surfaces (Figures 1 and 2).

Table 1: Morphological characterization of fungi.

Isolates	Texture	Surface	Reverse	Zonation	Probable organism/ microscopy
CUA 210	Velvety thick	Ginger brown	Creamy brown	Concentric zones on the surface	Aspergillus sp.
CUW 149	Powdery	Grey to white	Brown	Concentric zone	Penicillium sp.
CUA 197	Floccose	Creamy yellow	Yellow	Furrowed	Aspergillus sp.
CUA 72	Floccose	Chocolate brown	Orange	Furrowed	Aspergillus sp.
CUA 107	Floccose	Creamy to brown at the centre	Orange	Furrowed	Aspergillus sp.
UCUW 182	Velvety	Green to brown	Creamy dirty white	Concentric zone	Penicillium sp.
CUA 65	Floccose	White to grey	Creamy	None	Penicillium sp.
CUA 71	Velvety thick	White to brown	Orange to cream	Slightly furrowed	Aspergillus sp.

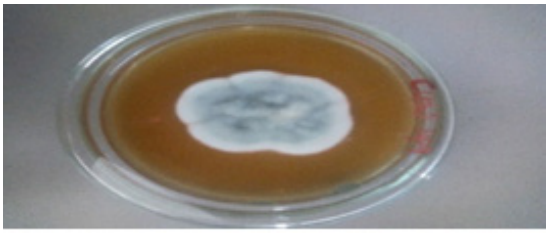

Figure 1 The velvety nature of the fungal Isolates.

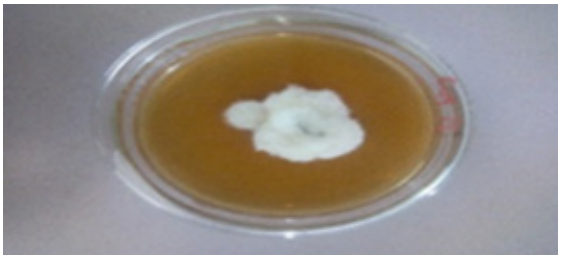

Figure 2 The powdery nature of the isolates.

The bacterial isolates as test organisms were identified as E. coli, Klebsiella pneumonia, Bacillus cereus and Staphylococcus aureus as shown in Table 2. The antibiotics resistance profiling of the test

Table 2: Morphological and biochemical characterization of the bacterial isolates.

Locations	Escherichia coli	Klebsiella pneumonia	Bacillus cereus	Staphylococcus aureus
Gram reaction	-	-	+	+
Endospore	-	-	+	-
Starch hydrolysis	-	-	+	-
Catalase	+	+	+	+
Citrate	-	+	+	+
Motility	+	-	+	-
Mannitol	+	+	-	+
Gas	+	+	-	-
Oxidase	-	-	-	-
VP	-	+	+	+
Indole	+	-	-	-
MR	+	-	-	+
Glucose	+	+	+	+
Lactose	+	+	+	-

organisms used in this study was investigated. Escherichia coli was only susceptible to tetracycline, gentamicin and chloramphenicol out of the sixteen (16) antibiotics used in the profiling. Klebsiella pneumoniae was resistance to all the antibiotics. Staphylococcus aureus was observed to be susceptible to only gentamicin however Bacillus cereus was susceptible to ampicillin and gentamicin as shown in (Table 3; Figure 3) [22].

Table 3: Antibiotic resistance profiling of test bacterial pathogens. S: Sensitive, I: Intermediate and R: Resistant. NZ: No zone, -: Not applicable

Antibiotic	Zones of Inhibition			
	Escherichia coli	Klebsiella pneumonia	Staphylococcus aureus	Bacillus cereus
Tetracycline	16.0 ± 0.000 (S)	NZ	NZ	NZ
Erythromycin	NZ	NZ	16.7 ± 1.155 (R)	17.3 ± 2.309 (R)
Vancomycin	NZ	NZ	NZ	NZ
Meropenem	NZ	17.3 ± 2.309 (R)	NZ	NZ
Ampicillin	NZ	NZ	NZ	17.3 ± 1.155 (S)
Amoxycillin	NZ	NZ	NZ	NZ
Ciprofloxacin	18.7 ± 2.309 (R)	13.3 ± 1.155 (R)	18.7±1.155 (R)	18.0 ± 0.000 (R)
Gentamicin	16.7 ± 2.309 (S)	14.7 ± 1.155 (R)	14.7±1.155 (S)	18.0 ± 0.000 (S)
Cephalexin	NZ	NZ	NZ	NZ
Cefuroxime	NZ	NZ	NZ	NZ
Cotrimoxazole	NZ	NZ	NZ	NZ
Cefoperazone	NZ	15.3 ± 1.155 (R)	NZ	NZ
Chloramphenicol	20.7 ± 1.155 (S)	NZ	NZ	NZ

Ceftriaxone	NZ	15.3 ± 1.155 (R)	-NZ	NZ
Cefotaxime	NZ	16.0 ± 3.464 (R)	NZ	NZ
Amikacin	25.3 ± 4.619 (S)	16.0 ± 0.000 (R)	NZ	NZ

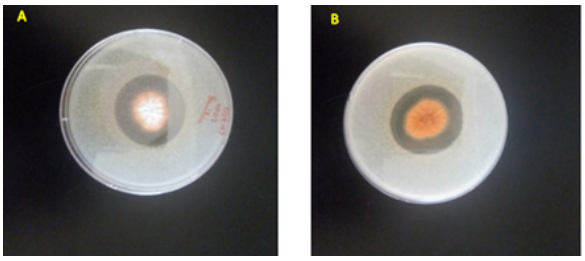

Figure 3 a. The antimicrobial activity of Penicillum sp. against E. coli. b. The antimicrobial activity of Aspergillus sp. against Bacillus cereus.

The main antimicrobial potential of the fungal isolates was observed through the measurement of the zone of inhibition. The metabolites of fungal isolates CUA 210, 197, 72 and 71 were able to inhibit the growth of all the pathogens used as test organisms. Metabolites of fungal isolate CUW 149 possessed activity against all the test organisms except Klebsiella pneumoniae. The antimicrobial potential of CUA 107 was observed only on the Gram-positive bacteria (Staphylococcus aureus and Bacillus cereus). Antimicrobial activity was observed to Staphylococcus aureus in assay with metabolites of fungal isolate CUA 65 while resistance was found with other test organisms (Table 4).

Table 4: Antibacterial activities of fungal crude extract using agar well diffusion method. NZ: no zone

Isolates	Escherichia coli	Klebsiella pneumonia	Bacillus cereus	Staphylococcus aureus
	Inhibition zone (mm) (mean ± SD)			
CUA 210	19.3 ± 2.309	14.0 ± 0.000	15.3 ± 2.309	22.0 ± 0.000
CUW 149	19.3 ± 2.309	NZ	19.3 ± 2.309	14.0 ± 0.000
CUA 197	20.7 ± 4.619	14.0 ± 0.000	16.7 ± 2.309	22.0 ± 0.000
CUA 72	23.3 ± 4.619	16.7 ± 2.309	14.0 ± 0.000	20.7 ± 2.309
CUA 107	NZ	NZ	18.0 ± 0.000	22.0 ± 0.000
CUW 182	NZ	NZ	NZ	NZ
CUA 65	NZ	NZ	NZ	18.0 ± 0.000
CUA 71	24.7 ± 4.619	14.0 ± 0.000	20.7 ± 2.309	26.0 ± 0.000

The minimum inhibitory concentration of the metabolites of the species of Aspergillus was observed to be 421 mg/ml in the isolate CUA 197 against the pathogens (E. coli and Bacillus cereus) however it was 842 mg/mL against Klebsiella pneumonae and Staphylococcus aureus as shown in (Table 5). The level of antimicrobial activity of the metabolites of the isolate of Aspergillus sp CUA 72 through two-fold dilution was 87 mg/mL for E. coli and Bacillus cereus but 174 mg/mL concentration against Klebsiella pneumonae and Staphylococcus aureus (Table 6). It was observed that mg/ml of the extract of Aspergillus sp of CUA 71 was only active against E. coli and Bacillus cereus (Table 7). The antimicrobial activity of the metabolite of Penicillium sp CUW 149 was observed to have a minimum inhibitory concentration

Table 5: Minimum inhibitory concentration of the ethyl extract of Aspergillus sp. CUA 197

Pathogens	Minimum Inhibitory Concentration					
	842 mg/ml	421 mg/ml	210.5 mg/ml	105.25 mg/ml	52.625 mg/ml	MIC value
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	(mg/ml)
E. coli	24.0 ± 2.828	18.0 ± 5.657	-	-	-	421
Klebsiella pneumonae	18.0 ± 0.000	-	-	-	-	842
Staphylococcus aureus	20.0 ± 2.828	-	-	-	-	842
Bacillus cereus	22.0 ± 0.000	16.0 ± 2.828	-	-	-	421

Table 6: Minimum inhibitory concentration of the ethyl extract of Aspergillus sp. CUA 72.

Pathogens	Minimum Inhibitory Concentration					
	174 mg/ml	87 mg/ml	43.5 mg/ml	21.75 mg/ml	10.87 mg/ml	MIC value
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	(mg/ml)
E. coli	20.0 ± 2.828	14.0 ± 0.000	-	-	-	87
Klebsiella pneumonae	18.0 ± 0.000	-	-	-	-	174
Staphylococcus aureus	18.0 ± 5.657	-	-	-	-	174
Bacillus cereus	22.0 ± 0.000	16.0 ± 2.828	-	-	-	87

Table 7: Minimum inhibitory concentration of the ethyl extract of Aspergillus sp. CUA 71

Pathogens	Minimum Inhibitory Concentration					
	38 mg/ml	19 mg/ml	9.5 mg/ml	4.75 mg/ml	2.38 mg/ml	MIC value
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	(mg/ml)
E. coli	22.0 ± 0.000	16.0 ± 2.828	-	-	-	19
Klebsiella pneumonae	18.0 ± 0.000	-	-	-	-	38
Staphylococcus aureus	20.0 ± 2.828	-	-	-	-	38
Bacillus cereus	22.0 ± 0.000	16.0 ± 2.828	-	-	-	19

of 14 mg/mL against E. coli and Bacillus cereus while 28 mg/mL concentration was the MIC against Staphylococcus aureus. There was no inhibitory property against the Klebsiella pneumonae (Table 8).

GC-MS evaluation of the bioactive compounds of the fungal metabolites showed that fatty acid and ester are common bioactive compounds in the metabolites but the only aldehyde is found in the extract of isolate CUW 149 (Table 9). The bioactive

Table 8: Minimum inhibitory concentration of the ethyl extract of Penicillium sp. CUW 149.

Pathogens	Minimum Inhibitory Concentration					
	28 mg/ml	14 mg/ml	7 mg/ml	3.5 mg/ml	1.75 mg/ml	MIC value
	mean ± SD	mean ±SD	mean ± SD	mean ± SD	mean ± SD	(mg/ml)
E. coli	22.0 ± 0.000	18.0 ± 0.000	-	-	-	14
Klebsiella pneumonae	-	-	-	-	-	-
Staphylococcus aureus	18.0 ± 0.000	-	-	-	-	28
Bacillus cereus	22.0 ± 5.657	16.0 ± 2.828	-	-	-	14

compounds amine and ketone were observed in the extract of isolates CUA 71 and 72 (Tables 10 and 11). The bioactive

compounds obtained from isolate CUA 197 were phenol, fatty acid ester and hydrocarbons (Table 12). The GC-MS analysis showed that only metabolite of isolate CUA 210 had steroid as bioactive compound (Table 13) [23].

Discussion

The search for the new potential antimicrobial substance is vital in this era of multi-drug resistant pathogenic bacteria in clinical practice. This study investigates the antimicrobial substances in the metabolites of species of Aspergillus and Penicillium isolated from uncultivated soil. The isolation of these fungi from uncultivated soil has earlier been reported by Kelechi et al. which confirmed the report of our study.

The pathogens used for the antimicrobial study were identified to be E. coli and Klebsiella pneumonia as Gram-negative bacteria. The bacteria used as Gram-positive were Staphylococcus aureus and Bacillus cereus. The Klebsiella pneumoniae was resistance to all the antibiotics used for the susceptibility assay however, E. coli was only susceptible to tetracycline, gentamicin and chloramphenicol. These two Gram-negative bacteria are resistance to more than three antibiotics which showed that

Table 9: Bioactive compound in the metabolite of the fungal isolate CUW 149 extracts

Compound name	Molecular formula	Molecular weight	IUPAC name	Nature of compound
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C18H26O4	306.402g/mol	Bis(2-methylpropyl) 4,5-dimethylbenzene-1,2-dicarboxylate	Fatty acid ester
3-Hydroxy-2-pyridin-3-yl-propenal	C8H7NO2	149.149g/mol	(Z)-3-hydroxy-2-pyridin-3-ylprop-2-enal	Aldehyde
Benzenebutanamine	C10H15N	149.237g/mol	4-phenylbutan-1-amine	Amines
2H-1,4-Benzoxazin-3(4H)-one	C8H7NO2	149.149g/mol	4H-1,4-benzoxazin-3-one	Ketones
Dibutyl phthalate	C16H22O4	278.348g/mol	Dibutyl benzene-1,2-dicarboxylate	Fatty acid ester
1,2-Benzenedicarboxylic acid, butyl octyl ester	C20H30O4	334.456g/mol	1-O-butyl2-O-Octyl benzene-1,2-dicarboxylate	Fatty acid ester
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	C20H30O4	334.456g/mol	1-O-butyl 2-O-(2-ethylhexyl) benzene-1,2-dicarboxylate	Fatty acid ester

Table 10: Bioactive compound in the metabolite of the fungal isolate CUA 71 extracts.

Compound name	Molecular formula	Molecular weight	IUPAC name	Nature of compound
Cyclopentanecarboxaldehyde, 2-methyl-3-methylene-	C8H12O	124.183g/mol	2-methyl-3-methylidenecyclopentane-1-carbaldehyde	Alkanes
Cyclopentane, (2-methyl-1-propenyl)-	C9H16	124.227g/mol	2-methylprop-1-enylcyclopentane	Alkanes
2,4-di-tert-butylphenol	C14H22O	206.329g/mol	2,4-ditert-butylphenol	Phenols
1-hexadecanol	C16H34O	242.447g/mol	Hexadecane-1-ol	Fatty alcohol
5-Octadecene, (E)-	C18H36	252.486	(E)-octadec-5-ene	Alkenes
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C18H26O4	306.402g/mol	Bis(2-methylpropyl) 4,5-dimethylbenzene-1,2-dicarboxylate	Fatty acid ester
Dibutyl phthalate	C16H22O4	278.348g/mol	Dibutyl benzene-1,2-dicarboxylate	Fatty acid ester
Cinnoline, 6-methyl-4-phenyl-	C15H12N2	220.275g/mol	6-methyl-4-phenylcinnoline	Amines
2H-2, 4a-Ethanonaphthalen-8(5H)-one, hexahydro-2,5,5-trimethyl-	C15H24O	220.356g/mol	2,2,8-trimethyltricyclo [6.2.2.ol, 6] dodecan-5-one	Ketones

Dibutyl phthalate	C16H22O4	278.348g/mol	Dibutyl benzene-1,2-dicarboxylate	Fatty acid ester
4,5-dimethoxy-2-ethoxy-1-(2-propenyl) benzene	C13H18O3	222.284g/mol	1-ethoxy-4,5-dimethoxy-2-Prop-1-en-2-ylbenzene	Alkenes

Table 11: Bioactive compound in the metabolite of the fungal isolate CUA 72 extracts.

Compound name	Molecular formula	Molecular weight	IUPAC name	Nature of compound
2,4-Di-tert-butylphenol	C14H220	206.329g/mol	2,4-ditert-butylphenol	Phenols
Cyclododecane	C12H24	168.324g/mol	Cyclododecane	Alkanes
Fluorenone oxime	C13H9NO	195.221g/mol	N-fluoren-9-ylidenehydroxylamine	Amines
Trifluoroacetic acid, n-tridecyl ester	C15H27F3O2	296.374g/mol	Tridecyl 2,2,2-trifluoroacetate	Fatty acid ester
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	C16H22O4	278.348	1-O-butyl 2-O-(2-methylpropyl) benzene-1,2-dicarboxylate	Fatty acid ester
Phthalic acid, butyl oct-3-yl ester	C20H30O4	334.456g/mol	1-O-butyl 2-O-octan-3-yl benzene-1,2-dicarboxylate	Fatty acid ester
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	C16H22O4	278.348	1-O-butyl 2-O-(2-methylpropyl) benzene-1,2-dicarboxylate	Fatty acid ester
L-Proline, N-valeryl-, butyl ester	C14H25NO3	255.358g/mol	Butyl 1-pentanoylpyrrolidine-2-carboxylate	Fatty acid ester
1-Penten-3-one, 4-methyl-1-(2,6,6-trimethyl-2-cycl-ohexen-1-yl)-	C15H24O	220.356g/mol	(E)-4-methyl-1-(2,6,6-trimethylcyclohex-2-en-1-yl) pent-1-en-3-one	Terpenes
2H-1-Benzopyran-2-one, 6,7-dimethoxy-4-methyl	C12H12O4	220.224g/mol	6,7-dimethoxy-4-methylchromen-2-one	Aromatic
Dibutyl phthalate	C16H22O4	278.348g/mol	Dibutyl benzene-1,2-dicarboxylate	Fatty acid ester
Benzene, 1,2-dimethoxy-4-(1-propenyl)-	C11H14O2	178.231g/mol	1,2-dimethoxy-4-[(E)-prop-1-enyl] benzene	Aromatic
2-Butenoic acid, 2-cyano-3-methyl-, ethyl ester	C8H11NO2	153.181g/mol	Ethyl 2-cyano-3-methylbut-2-enoate	Fatty acid ester

Table 12: Bioactive compound in the metabolite of the fungal isolate CUA 197 extracts.

Compound name	Molecular formula	Molecular weight	IUPAC name	Nature of compound
Cyclopentane, (2-methyl-1-propenyl)-	C9H16	124.227g/mol	2-methylprop-1-enylcyclopentane	Alkanes
1,1-dimethyl-4-methylene cyclohexane	C9H16	124.227g/mol	1,1-dimethyl-4-methylidene cyclohexane	Alkanes
Phenol,2,6-bis(1,1-dimethy-lethyl)-	C14H22O	206.329	2,6-ditert-butylphenol	Phenols
Cycloheptane, methyl-	C8H16	112.216	Methylcycloheptane	Alkanes
Phthalic acid, isobutyl non-5-yn-3-yl ester	C21H28O4	344.451	1-O-(2-methylpropyl)2-O-non-5-yn-3-yl benzene-1,2-dicarboxylate	Fatty acid ester
Dibutyl phthalate	C16H22O4	278.348g/mol	Dibutyl benzene-1,2-dicarboxylate	Fatty acid ester

they are multiple antibiotic-resistant bacteria. MDR bacteria have earlier been reported among the family Enterobacteriaceae especially Klebsiella pneumoniae and E. coli which is similar to Peirano et al. [11] that there is an extensive antibiotic resistance pattern in Enterobacteriaceae. In this work, multi-drug resistance was also observed in the Gram-positive bacterial pathogens which are Bacillus cereus and Staphylococcus aureus. There have been a lot of reports on the multi-drug resistance in Staphylococcus aureus which is similar to the findings of this work where Bacillus cereus and Staphylococcus aureus were found multi-drug resistance [20,22].

Table 13: Bioactive compound in the metabolite of the fungal isolate CUA 210 extracts.

Compound name	Molecular formula	Molecular weight	IUPAC name	Nature of compound
2,4-di-tert-butylphenol	C14H22O	206.329g/mol	2,4-ditert-butylphenol	Phenols
1-Octadecene	C18H36	252.486g/mol	Octadec-1-ene	Alkene
Phthalic acid, isobutyl nonyl ester	C21H32O4	348.483g/mol	2-O-(2-methylpropyl) 1-O-nonylbenzene-1,2-dicarboxylate	Fatty acid ester
Dibutyl phthalate	C16H22O4	278.348g/mol	Dibutyl benzene-1,2-dicarboxylate	Fatty acid ester
Dibutyl phthalate	C16H22O4	278.348g/mol	Dibutyl benzene-1,2-dicarboxylate	Fatty acid ester
Cholesterol	C27H46O	386.664g/mol	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,14,15,16,17-dodeca-hydro-1H-cyclopenta[a]phenanthren-3-ol	Steroid
Cholesterol	C27H46O	386.664g/mol	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,14,15,16,17-dodeca-hydro-1H-cyclopenta[a]phenanthren-3-ol	Steroid

The metabolites of the species of Aspergillus and Penicillium were found to have antimicrobial properties against both Gram-negative and Gram-positive bacteria. Four species of Aspergillus were very active in respect to their antimicrobial properties in this work which correspond to Al-Shaibani et al. [18] who reported inhibitory activity of A. niger against P. aeruginosa, S. aureus, and Bacillus sp. They were active against all the pathogens investigated except the metabolite of the Aspergillus isolate CUW 149 that was not active against Klebsiella pneumoniae. This finding is similar to the report of Al-Fakih et al. [2] that metabolites of Aspergillus sp. are majorly active against Staphylococcus sp. Interestingly, the species of Penicillium isolate CUA 65 was only active against Staphylococcus aureus. However, the metabolite of the species of Penicillium isolates CUW 182 did not show any activity against any of the pathogens.

The minimum concentration of the metabolites of the fungal isolates was investigated in this work and it was found that as low as 14 mg/ml concentration was active against even E. coli and Bacillus aureus in the metabolite extracted from Penicillium spp. It was noted in this work that the metabolites whose concentrations are higher were found effective against Klebsiella pneumonae and Staphylococcus aureus across the findings. This finding confirmed the report of a previous study that reported Klebsiella pneumonae and Staphylococcus aureus as notorious is the antibiotic resistance.

The bioactive compounds of the metabolites were mainly fatty acid and ester in all the fungi investigated in this work. Along with these components, amine and ketone were found in some of the fungal metabolites however aldehyde was only in the metabolite of Penicillium isolate CUW 149. The presence of ketones and amine among the bioactive compounds in this work correspond to Jin et al. [23] that reported steroids and alkaloids among other bioactive compounds. The metabolite of the Aspergillus isolates CUA 210 contains a steroid which is an indication of the microbial hormone. Other bioactive compounds found in our work are terpenes, aromatic compounds, phenols, amines, etc which are similar to the report of critical review of bioactive compounds by Zheng et al. [4].

Conclusion

The antimicrobial activity of the fungal metabolites as found in our work provide evidence of the possibility of discovering novel antimicrobials from fungi. Fungi inhabiting virgin or long time uncultivated soil could contain a lot of fungi that would be in natural form with little or no environmental alteration in their biological form.

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Competing interests

All the authors declared that there are no competing interests whatsoever.

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