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Investigation of Antimicrobial Activities of Certain Isolated Fungi and Characterization of Bioactive Compound

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Abstract

This research aimed toidentify, isolate, and screen fungi from 10 soil samples. Using the agar well diffusion technique, the isolated cultures' capacity to generate antimicrobial metabolites was assessed against the virulent strains *E. coli* ATCC 8739*,* *Staphylococcus aureus* ATCC 6538*,* and *Candida albicans* ATCC10231. Ten fungal isolates were crudely extracted, and the extract showed antibacterial action against one or more of the diseases under investigation. Further investigation was directed towards the NS6 isolate, which exhibited the highest level of activity among all the isolated strains against all tested pathogens. They matched them to other pertinent GenBank (NCBI) sequences using a particular internal transcribed spacer primer (ITS1/ITS4). Additionally, a variety of solvents were tested to determine which one produced the highest concentration of antibacterial compounds. PTLC and TLC bioautography have been used to purify antimicrobial substances. Using molecular identification, the most potent isolated fungus was identified as *Aspergillus niger*. Using IR, NMR, and mass spectra, the most promising bioactive molecule extracted from *Aspergillus niger* was discovered as (3-ethoxy-6-ethyle-8-hydroxy-4-p-tolyl-isochroman-1-one). The active compound extract of the *Aspergillus niger* strain was subjectetocolumn chromatography. Among the 12 fractions collected, the fractionnumber4hadthehighestoverall antimicro bial activity with inhibition zone of 28 mm against *Escherichia coli*, 26 mm against *Staphylococcus aureus*and 25 mm against *Candida albicans.*

Keywords: Fungi; Agar well diffusion; antimicrobial activity; optimization; molecular identification; Biooutography; Mass spectra

1. Introduction

Microorganisms produce a wide range of industrially significant metabolites during the growth phase, including dyes, enzymes, pigments, and antibacterial agents. During the stationary phase of microbial growth, microorganisms like fungi, actinomycetes, and bacteria produce chemical compounds known as antimicrobial agents through metabolic processes. Commonly referred to as "antibiotics" these are secondary metabolites that do not contribute to the development or growth of living things [1]. The chemistry of natural products is mostly attributable to a class of substances known as secondary metabolites. Some of the most potent secondary metabolites that have been produced into therapeutic drugs originated from filamentous fungi [2,3]. Many of these fungal metabolites have demonstrated anti-disease efficacy. Secondary metabolites stimulate the host defenses through a variety of signal transduction mechanisms to produce

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their bioactivity [4]. Antibiotics classified as β-lactams were initially identified by Sir Alexander Fleming in 1928 and are the most well-known secondary metabolites generated by fungi. A Penicillium isolate was found to create a chemical that might kill gram-positive bacteria. One of the most significant scientific advances of all time is the finding of antibiotics and their subsequent development. Penicillin is still one of the most effective and safe antibiotics [5]. Since the discovery of penicillin, thousands of antibiotics have been extracted from soil microbes, but there are just 50 of them left because most of them are harmful to humans [6].

Multi-drug resistance to harmful microorganisms has developed since the discovery of antibiotics because of exploitation and misuse [7,8]. To reduce the use of traditional antibiotics, there's an expanding demand for new antimicrobial compounds that are effective against pathogenic resistance microbes.

New methods for preventing and treating bacterial infections are essential due to the evolution, selection, and dissemination of bacterial resistance to a variety of antibiotics [9]. Positive and negative gram bacterial including some eating-related pathogenic bacteria species be resistant to fungi *in vitro* [10]. as well as yeasts and mycelial fungi, including dermatophytes and phytopathogens [11,12,13]. The fact that numerous fungal species have shown effectiveness against bacterial, viral, and fungal infections that are resistant to existing treatment medicines shows that they are a reliable source of natural antimicrobial chemicals [14].Antibacterial and antifungal activities can be used to identify antimicrobial molecules that have been extracted from various stages of the fungal growth process [15].

In the past, research into the antibacterial properties of fungi has primarily concentrated on the isolation of chemicals from the fruiting body, with a negligible amount of attention paid to liquid-cultivated mycelium. Due to the discovery that both the cultured mycelium and culture fluid have antibacterial activity, this has enormous commercial potential [16]. The study aimed to screen isolated fungal isolates which have the capability to produce antimicrobial agents as well as chemical identification of the efficient purified compound.

2. Materials and Methods

2.1. Sample collection

In a sterile plastic bag, the soil samples were taken from Wady El-Natron, Egypt. The acquired material was immediately brought to the lab to air dry. Within 24 hours, the soil samples that were taken were used to isolate soil fungi [17].

2.2. Isolation of fungi

Through the use of the serial dilution agar plate method, the fungi were isolated from a soil sample, under sterile conditions, there was serial dilution (laminar airflow chamber). To isolate fungi, potato dextrose agar (PDA) media was employed. 9ml of sterilized distilled water was used to fill the 5 test tubes. The first test tube, designated 10^{-1}, received 1gm of the soil sample [18]. To prevent bacterial growth, 0.1ml from each test tube was placed onto PDA plates treated with 0.2g/L of streptomycin. The PDA plates with the inoculum were incubated at 30 ^{0}C for 6-7 days. After 5 days of incubation, morphologically distinct fungal colonies emerged on PDA plates; they were sub-cultured separately to produce a pure culture. On PDA slants, pure fungal colonies were injected and stored at 4 ^{0}C for later use [19].

2.3. Screening for antimicrobial activity

The Using the agar well diffusion technique test the antimicrobial activity of fungal isolates, in a 250 ml flask, 100 ml of potato dextrose broth was made. The flasks were autoclaved at 121 ^{0}C for 20 minutes while tightly covered with cotton plugs. After chilling, 2-3 discs from a culture plate of isolated fungi that had been incubating for 6-7 days were added to the media under sterile conditions (Air Laminar Flow Chamber). For 10–14 days, the flasks were incubated at 30 ^{0}C. The broth medium was filtered when the incubation period was over, and Whatman No. 1 filter paper was used to collect the filtrates [20]. Using sterilized cotton buds and sterile conditions, the Mueller Hinton Agar (MHA) plates were swabbed with pathogenic microorganisms, including *S. aureus* ATCC 6538*, C. albicans ATCC 10231* and *E. coli* ATCC 8739. 100µl of the filtrate was added to wells already prepared on plates swabbed with test microorganisms, and the plates were incubated at 37 ^{0}Covernight. Following incubation, the growth inhibition zones around the wells on the plates were detected, and they were measured using a zone scale [21,22]. The maximal dimension of the inhibition zone served as the foundation for the selection of fungal isolate.

2.4. Morphological and molecular identification

For spore staining, lactophenol cotton blue dye was employed for spore staining to identify the fungi under a microscope. The cleaned and dried glass slide was treated with a drop of Lactophenol cotton blue. The drop location was delicately covered by a modest number of mycelia [23, 24]. Glass slides were put under the microscope using the coverslip to observe the conidia structure, spore size, spore morphology, and sporulating structures. The fungal isolate's growth rate, color, and colony form were all noted at this time. Several manuals were used in the identification of the fungus [25]. The genomic DNA extraction of the isolate (NS6) was isolated throughout using 1.5% agarose gel was run through electrophoresis for 20 minutes at 150 V in TBE buffer PH 8.5 [26].

2.5. Extraction of Extracellular Antimicrobial Metabolites

The best organic solvents for producing extracellular antimicrobial metabolites are most effective against all evaluated microbiological infections. Methanol, chloroform, ethyl acetate, n-hexane and n-butanol are among the five different organic solvents, were employed to produce the optimum extraction of antimicrobial metabolites. These solvents are among the best organic solvents that extract the highest number of antimicrobial metabolites [27].

2.6. Purification of active antimicrobial compounds from extracellular crude extract

2.6.1. Thin Layer Chromatography (TLC)

The components in the *Aspergillus niger* NS6 crude ethyl acetate extract were separated using TLC.The extract was loaded onto TLC plates using several solvent solutions and plates made of thin layers of silica gel (Silica Gel F254, Merck, Germany). The bioactive components were then separated by drying the running lane. TheAgar diffusion was employed to evaluate the antibacterial activity of each fraction. Additionally, the chromatogram was viewed in the TLC chamber using a UV laser system at 254 and 366 nm. Spot resolution was used to choose the optimal mobile phase. The distance traveled by the solute divided by the solvent's distance yielded the Rf value for each band. Utilizing PTLC and HPLC, each fraction underwent further purification [28].

2.6.2. Purification by PTLC

Silica gel 60 (GF254Merck for PTLC) and water were combined to create a slurry that was used to cover the TLC plates. Then, this combination was applied as a thick slurry to a clean, 20 20 cm glass plate. The sample was put onto the plate after it had been dried and activated by being heated in an oven at 110 °C for 30 minutes. According to preliminary analytical TLC results, the diluent was discovered to be 1:1 n-hexane-ethyl acetate (v/v). Glass tanks were used to elute the silica gel areas containing with a spatula, and the bands were removed from the plate. Methanol was used to extract each band; silica was removed by centrifugation, and the supernatant was then put into a vial. To verify the purity of each isolated metabolite, the individual metabolites were once again spotted on a TLC plate. Fractions having high antimicrobial activity can be further purified using thin layer chromatography (TLC) using GF254Merck for PTLC) silica gel plates, with a solvent mixture consisting of ethyl acetate: n-hexane 1:1(v/v) [29].

2.7. Detecting the Antimicrobial Metabolites Position by Bioautography

Second purification the TLC plate, paper chromatograms, or fractions are put on the surface of the inoculated agar for a few minutes or hours to facilitate diffusion in the contact bioautography process. The agar layer is then incubated once the plate has been removed. In the regions where the antimicrobial chemicals met the agar layer, growth is inhibited [30].

2.8. Identification of *Aspergillusniger*extracted compounds

2.8.1. Mass Spectra

The extracted mass spectra of the compoundswere recorded on JEUL JMS-AX-500 mass spectrometers.

2.8.2. FT-TR spectra

The isolated compounds' infrared spectra were captured using KBr discs and a Perkin-Elmer infrared spectrometer (681) [31].

2.8.3. ^{1}H-NMR Spectra

The H-NMR spectra were captured in deuterated dimethyl sulfoxide using a JOEL EX-270 MHZ FT-NMR spectrometer (DMSO-d6). The chemical changes' distances from the sol-vent peaks were measured [32].

2.8.4. ^{13}C-NMR spectra

The advantage of 13C-NMR is that it provides clear details on the molecule's carbon skeleton. On a Bruker AV-600 spectrometer, 13C-NMR spectra were captured at 150.917 MHz for the 13C nucleus. In 5 mm NMR tubes, the material was examined in DMSO-d6 solutions at 298 and 373 K. TMS uses the chemical shifts, or ppm, as an internal standard [33].

3. Results and Discussion

3.1. Isolation of fungi

Ten 10 soil samples from Wady El-Natron, Egypt, yielded 28 fungus isolates, which were then tested in submerged culture for the generation of bioactive secondary metabolites.

According to Table 1, about ten isolates have antimicrobial activity against one or more of the examined pathogens. The difference in the morphology of the fungal isolates as well as the constituents of the produced bioactive metabolites may be responsible for the effect of the bioactive metabolites appearing to be related to the tested strains.

The 10 morphologically distinct isolated fungi identified from NS1 to NS18 were tested for antimicrobial activity using the three test microorganisms: *C. albicans* ATCC 10231, *E. coli* ATCC 8739 and*S. aureus* ATCC 6538. Ten different fungal isolates, including NS1, NS4, NS6, NS8, NS13, NS14, NS15, NS16, NS17, and NS18, have shown antimicrobial activity against all the test microorganisms. *S. aureus* and *E. coli* were inhibited by ciprofloxacin, *C. albicans* were inhibited by fluconazole and none of the test microbes were inhibited by uninoculated broth, which was employed as positive and negative, respectively.

3.2. Identification of the most potent fungal isolates (NS6).

According to various guides and colony characteristics (colony color and colony growth) and sporulating structures, the fungal isolates were recognized as *Aspergillus niger* Figure (1) [23, 25, 26].

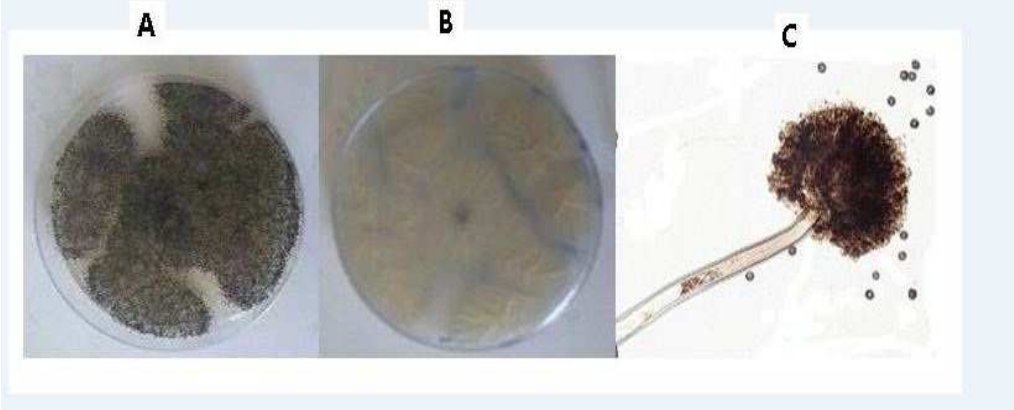


Figure 1: Identification of the most potent fungal isolates (NS6). Colony characteristics of *Aspergillus niger* (A), reversed culture (B) , Micro-morphology by using an image analysis system 400X (C)

Table 1
Antimicrobial activity of fungal isolates

No. of isolated strain	Zone of inhibition in (mm)		
	Staphylococcus aureus (ATCC 6538)	*Escherichia coli* (ATCC 8739)	*Candida albicans* (ATCC 10231)
NS 1	20	16	14
NS 4	18	19	16
NS 6	**27**	**25**	**22**
NS 8	25	19	16

NS 13	22	20	18
NS 14	23	20	19
NS 15	23	21	18
NS 16	19	17	15
NS 17	20	18	14
NS 18	22	19	16
Uninoculated broth (Negativecontrol)	0	0	0
Ciprofloxacin (Positive control)	19	17	0
Fluconazole (Positive control)	0	0	18

The ITS region *gene and sequencing of fungal DNA* were the ITS universal primers of ITS1 were amplified in a polymerase chain reaction (PCR) utilising the genomic DNA as a template (5`-TCCGTAGGTGAACCTGCGG-3`) and ITS4 (5-TCCTCCGCTTATTGATATGC-3`). The sequencing for PCR product occurred in GATC Company by using ABI 3730x1 DNA sequencer (Germany) by using reverse and forward primers. The sequencing product was determined for the isolate D2-1 under study as the following. Based on molecular identification of the most potent fungal isolates NS6, which showed the greatest antimicrobial action, *Aspergillus niger* was discovered (NCBI). According to the results of the molecular identification, the examined fungal isolate NS6 had a 99% sequence similarity to *Aspergillus niger* (Figure2), and submission in gene bank https://www.ncbi.nlm.nih.gov/nuccore/OP764681?fbclid=IwAR1AImNKKY27UXNIxz9yMocjvo9B610qJ1KiAeyhybIiTDmbd7TIL7Naa7E

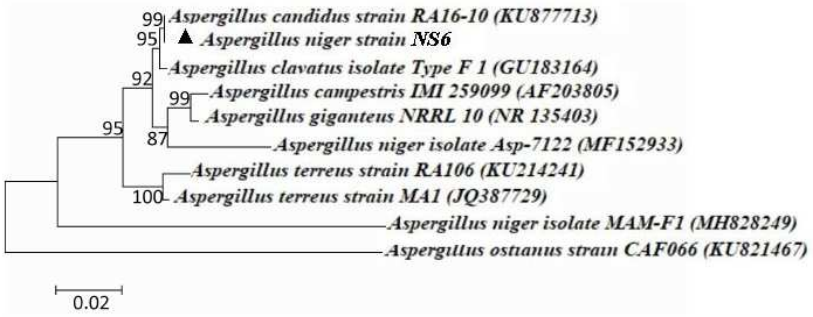


Figure 2: Phylogenetic tree based on the ITS sequences of the *Aspergillus niger* NS6 fungal isolate and NCBI sequences. ITS segments collected from this investigation are indicated by the symbol *. The neighbor-joining approach was used to carry out the study with MEGA 6.

3.3. Extracellular Antimicrobial Metabolites

The results presented in Table (2) represent the antimicrobial activities of *Aspergillus niger* (NS6) extracted compound. For methanol, chloroform, ethyl acetate, n-butanol, and n-hexane extraction, respectively, against investigated microbiological pathogens.

Table (2)
The effect of different organic solvent extracts against all tested microbial pathogens

Different organic solvents	Zone of inhibition in (mm)		
	Staphylococcus aureus (ATCC 6538)	*Escherichia coli* (ATCC 8739)	*Candida albicans* (ATCC 10231)
Methanol	25	28	22
Chloroform	20	22	20
Ethylacetate	**30**	**27**	**24**
n-butanol	24	27	21
n-hexane	20	22	17

Escherichia coli *Candida albicans* *Staphylococcus aureus*

Figure 3: Inhibition zone of the bioactive metabolites produced by NS6 fungal isolate at different PHs

Soil is a significant source of microorganisms that produce bioactive substances. The fungal isolates from Wady El-Natron Egypt were morphologically identified in the current study and determined to belong to the genera *Aspergillus*, *Fusarium*, and *Penicillium*species.Most antibiotics chemically significant substances for industry and other biologically active substances are isolated from fungi that are part of the soil microflora [13].

The soil microflora varies from location to location and is influenced by the environment. All of *Penicillium*, *Fusarium oxysporum*, *Trichoderma viride*, *Aspergillus flavus*, *Aspergillus niger,* and *Alternaria* were isolated by Hussain *et al 2016*. *E. coli* ATCC 8739, *S. aureus* ATCC 6538, and *Candida albicans* ATCC 1023 were used to assess the antibacterial activity of the fungal metabolite [27,28].

4. Purification of the Antimicrobial Compound by Column Chromatography and TLC

According to the results shown in Table (3), a 50:50 mixture of hexane and ethyl acetate was the most effective mobile phase for purifying the ethyl acetate fraction of *Aspergillus niger* NS6 using this solvent system. The ethyl acetate fraction was further separated using the same mobile phase for PTLC which produced pure secondary metabolites.

The crude ethyl acetate extract of the *Aspergillus* strain was subjectedtocolumn chromatography. Among the 12 fractions collected (Figure 4), the fraction number 4had the highest overall antimicrobial activity with inhibition zone of 28 mm against *Escherichia coli*, 26 mm against *Staphylococcus aureus,* and 25 mm against *Candida albicans* (Figure 5). The TLC plate for fraction number 12 only produced one band with a R=0.63 value, suggesting that there was only one TLC-detectable molecule in this fraction (Figure 6).

Table (3)
Solvent system used in column chromatography to separate bioactive compounds from *Aspergillus niger* NS6 crude ethyl acetate extract

Solvent system	Ratio	Volume (mL)	Fraction
Methanol	100%	50	3
Ethyl acetate	100%	50	4
Hexane	100%	50	5
Ethyl acetate: Methanol	1:1	50	7
Ethyl acetate: Methanol	1:1	50	9
Hexane: Ethyl acetate	1:1	50	12
Hexane: Ethyl acetate	1:10	50	8
Hexane: Ethyl acetate	10:1	50	6

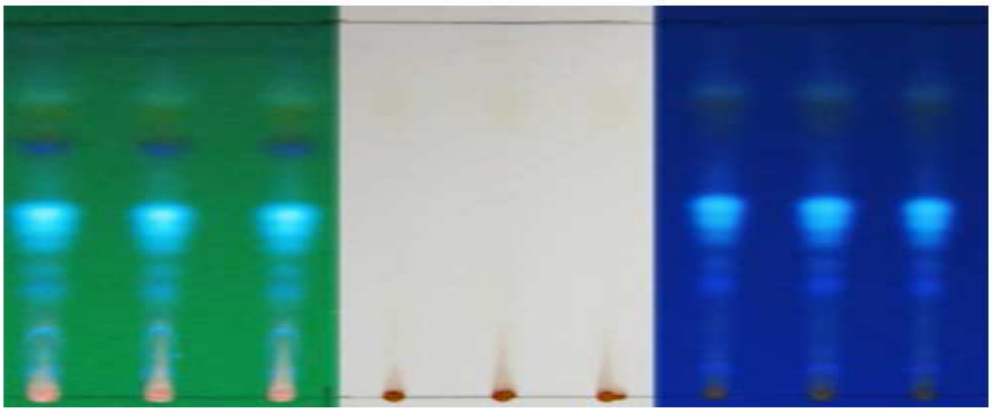


Figure 4: *Aspergillus niger* active compounds under white and UV lamps (365 and 254 nm).

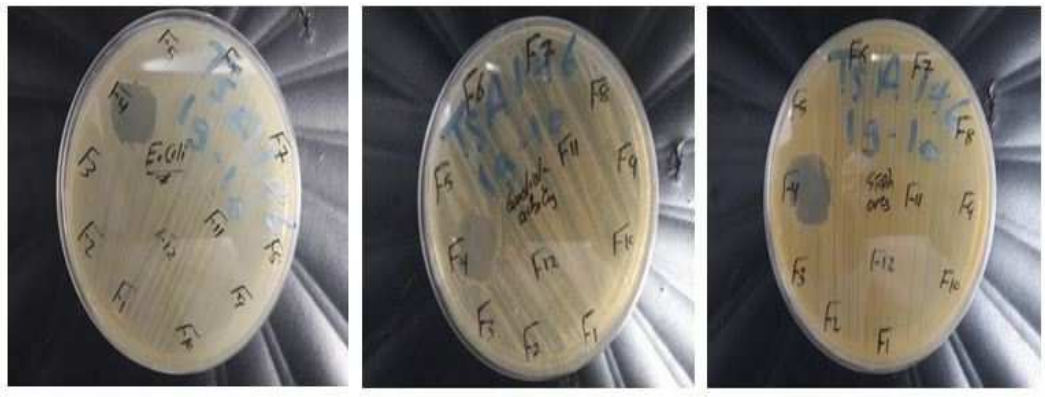


Figure 5: The effect of Aspergillus *niger* active compound against pathogenic microorganisms.

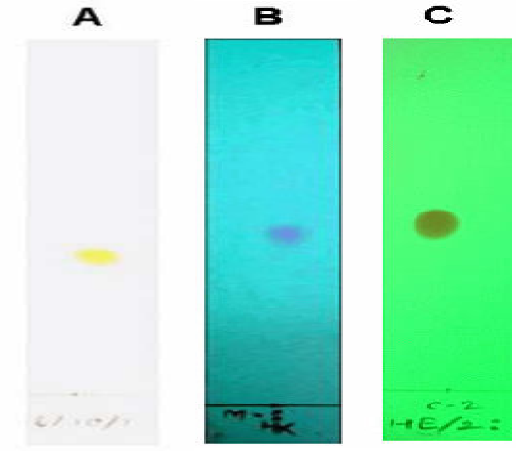


Figure 6: Iodine is used to demonstrate the separation pattern of pure chemicals on a TLC plate (A)., UV light 254 nm (B) and vanillin-sulfuric acid reagent spray (C) for the presence of specific compounds.

4.1. FT-IR Spectra

Data represe**nte**d graphically in Figure (7), refer to the compound's FT-IR spectrum (KBr disc) revealing bands at 1607 and 1576 cm^{-1} that is due to a distinctive stretching vibration of benzene ring. The aromatic ring's C-H bending (out-of-plane) is attributed to the absorption band at 978 cm^{-1}. The absorption band at 3051 cm^{-1} is assigned to the C-H stretching vibration of the aromatic ring [31], while the C-H aliphatic gives a symmetric stretching vibration absorption band at 2920 cm^{-1} and an asymmetric stretching vibration band at 2947 cm^{-1}. A broad absorption band that appears at 3433 cm^{-1} is assigned to O-H stretching vibration owing to intermolecular hydrogen bonding. The C-O stretching vibration for ester appears at 1253 as a medium-intensity band [31]. While the C-O stretching vibration for aliphatic ether appears at 1185 cm^{-1} as a strong band. The C=O stretching absorption band appears as a strong band at 1700 cm^{-1} . Methyl groups (CH_3) have a characteristic absorption at approximately 1348 cm^{-1}, While methylene groups (CH_2) have a characteristic absorption at 1044cm^{-1} [31].

4.2. ^{1}H - NMR Spectra

Figure (8) shows the ^{1}H-NMR spectrum of the substance as it was measured in DMSO-d6 solvent**,** It exhibits signs that are in line with the suggested structure. For the *Aspergillus niger* extracted compound**,** the spectrum displayed a group of peaks that are believed to be the protons of aromatic rings, ranging from 6.3 to 7.08 ppm. [29&32].Resonance appears at 6.2 ppm and is assigned to the proton of methine group O-CH-O-C=O. The proton signal of the OH group was observed at 5.7 ppm. Resonance appears at 3.75 ppm due to the proton of the methine group which attaches to toluene and phenol groups [31]. The peak at 3.34 ppm is due to the proton of methylene group O-CH_2 –C [32]. Also, the spectrum showed a set of peaks in the 2.0 -2.5 ppm ranges that are ascribed to the protons of methylene and methyl groups [32].

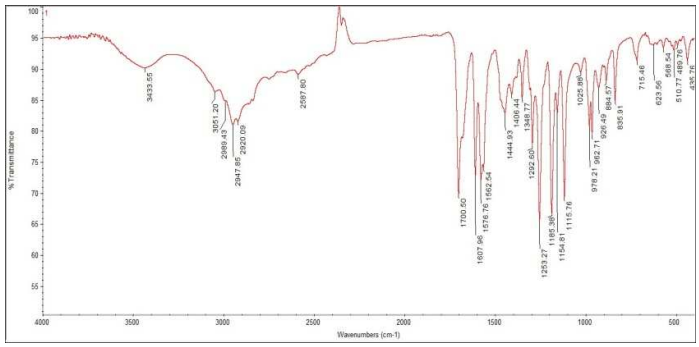


Figure 7: FT-IR of *Aspergillus niger* extracted compound

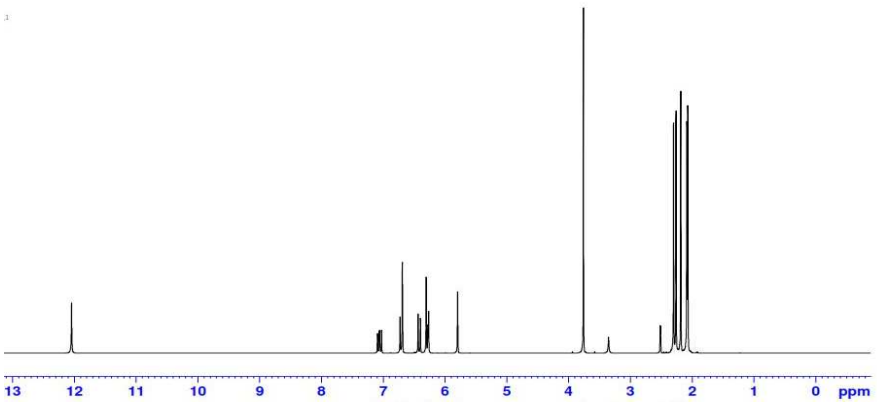


Figure (8): ^{1}H-NMRof*Aspergillus niger* extracted compound

4.3.*^{13}C-NMR spectra*

The simple compound isolated from *Aspergillus niger* (Figure 9), has its ^{13}C-NMR chemical shift data, which gave a total of 20 carbon signals.Five signals corresponding to the five saturated carbons are visible in the ^{13}C- NMR spectra at 12.2, 13.86, 17.6, 21.61, and 40.6 for C24, C21, C18, C20 and C4 respectively. In this case, these signals evidence of the presence of methine, methylene and methyl carbons. The higher chemical shift of C23 at 55.74 ppm is due to this carbon is directly linked to the oxygen atom. The value of the chemical shift C3 in the compound differed significantly in the shift at chemical shift 110.54 ppm due to the influence of the electronegativity of two oxygen atoms.Resonances in the area were produced by the aromatic ring carbons from120–155 ppm. The remaining carbon C1 of the carbonyl signal of ester appeared in the ^{13}C-NMR spectra of the compound at 168.27 ppm [33]. These signals further supported the formation of the compound.

4.4. Mass spectra of the extracted compound

The mass spectra of extracted compounds confirmed their proposed formulations. In (figure 10a, b) the spectrum of the extracted compound reveals the molecular ion peaks (m/z) at 326 amu consistent with the compounds and bases molecular weightspeak at m/z = 235 amu. Furthermore, the fragments observed at m/z = 15, 45, 54, 67,91, 120, 148, 164,162, 178, 206, 235, 259, 272, 281, and 311 correspond to CH_3, C_2H_5O, C_4H_6, C_5H_7, C_7H_7, C_8H_8O, $C_9H_8O_2$, $C_9H_8O_3$, $C_{11}H_{14}O$, $C_{11}H_{14}O_2$, $C_{11}H_{14}O_3$, $C_{13}H_{15}O_4$, $C_{15}H_{15}O_4$, $C_{16}H_{16}O_4$, $C_{18}H_{17}O_3$, and $C_{19}H_{19}O_4$ moieties respectively. The fragments of the compound are represented in Table (4).

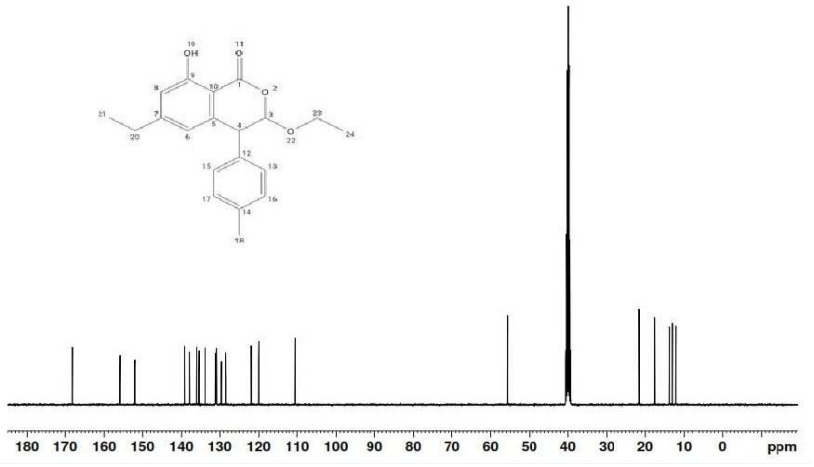


Figure 9: ^{13}C-NMR spectra of *Aspergillus niger* extracted compound

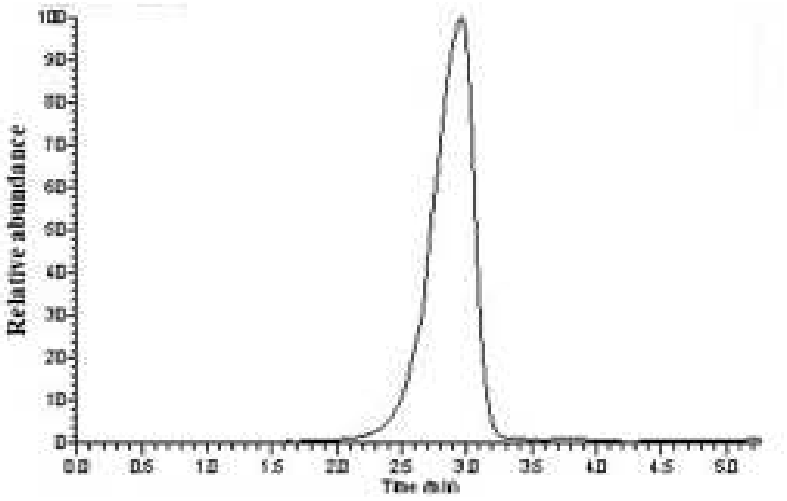


Figure 10a: Mass spectra of *Aspergillus niger* extracted compound

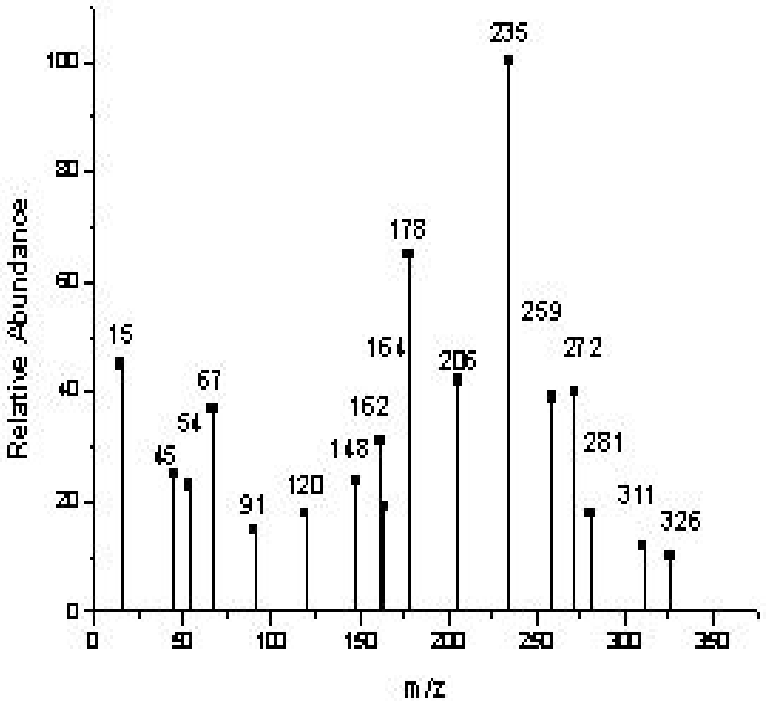
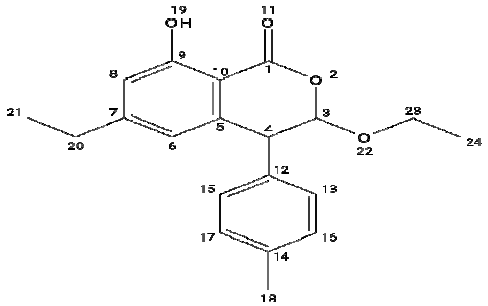


Figure 10b: Mass spectra of *Aspergillus niger* extracted compound

Table 4
Fragments of *Aspergillus niger* extracted compound

m/z	Rel. Int.	Fragment
15	45.6	CH_3
45	25.0	C_2H_5O
54	23.0	C_4H_6
67	37	C_5H_7
91	15	C_7H_7
120	18	C_8H_8O
148	24	$C_9H_8O_2$
164	19	$C_9H_8O_3$
162	31	$C_{11}H_{14}O$
178	65	$C_{11}H_{14}O_2$
206	42	$C_{11}H_{14}O_3$
235	100	$C_{13}H_{15}O_4$
259	39	$C_{15}H_{15}O_4$
272	40	$C_{16}H_{16}O_4$
281	18	$C_{18}H_{17}O_3$
311	12	$C_{19}H_{19}O_4$

The chemical formula of the extracted compound that was produced from Aspergillus niger under certain conditions was identified by some methods of analytical chemistry, all of which indicate that this compound is as is clear in Figure (11).



3-Ethoxy-6-ethyl-8-hydroxy-4-*p*-tolyl-isochroman-1-one
$C_{20}H_{22}O_4$

Figure 11: Chemical formula of active compound from *Aspergillus niger*

Molecular Weight: 326.4 m/e.

Complexity. 586

According to the study's findings, the chemical analysis of fungus from the Aspergillus species that are obtained from soil has significantly increased in recent years, with the discovery of new molecular structures containing a broad of pharmacological properties and served as a good source of antimicrobial agents [34].

In contrast, this study showed that the filamentous fungi have an enormous potential for the production of a wide range of bioactive compounds, with application as among others antibiotic, anticancer or antifungal drugs [35].

A novel metabolite generated by the endophytic fungus Aspergillus fumigatus CY018 is called asperfumin, and it has been reported in similar studies by other researchers to inhibit Candida albicans [36].

These results are consistent with reports indicating that theseveral isochromann compounds have a variety of biological properties, including

antioxidant, antibacterial, plant growth-regulating, antiplatelet, and herbicidal actions. [37].

Similar reports by other researchers also suggest that the Members of the genus *Aspergillus* are well-known producers of secondary metabolites, including polyketides (e.g. aflatoxin), non-ribosomal peptides (e.g. ferricrocin), indole terpenes and terpenesare important resources for new drug exploration [38].

Four different fungal strains, Aspergillus flavus (NCIM No. 524), Aspergillus fumigatus (NCIM No. 902), Penicillium marneffei, and Trichophyton mentagrophytes, have all been reported to be resistant to the antifungal properties of isochrontriazoles and thiadiazole in similar studies by other researchers [39,40]. Isoquinoline derivatives from isochromen, dione discovered to have significant biological characteristics [41-47].

This result is similar to reports observed that the isoquinoline derivatives from isochromen , dione found to exhibit interesting biological properties includingnarcotic, anti-angiogenic, anti-allergic, anti-inflammatory, anti-fungal, and anti-malarial [48], antibacterial [49], antivirus and anti cancer [50].

This results in agreement with studies showed that the several metabolites of isocoumarins have several bioactivities: cytotoxic, antimicrobial, algicidal [51,52], protease inhibitors, acetylcholinesterase, antimalarial, immunostimulatory, allergic- and allergy-free, and plant growth regulatorsare only a few examples of the bioactivities of many isocoumarin metabolites [53,54].Similar studies from other researchers imply that the dihydroisocoumarins, which are primarily natural, were extracted from a variety of natural sources, such as microbes, bacteria and fungi (endophytic, soil, and marine fungi) [55, 56].

5. CONCLUSION

We need to research antimicrobial compounds to fight diseases that are resistant to current treatments. Thus, the native fungi that are the subject of this research on isolation can create bioactive substances that have a significant potential to combat pathogenic microorganisms. *E. coli* ATTCC 7839, *S. aureus* ATTCC 6538, and yeast strain *C. albicans* 10231 were tested for their antibacterial activity using extracts of the secondary metabolites of 10 fungus isolates. Out of all the examined isolates, NS6 exhibits the highest zone of inhibition. TLC bioautography and PTLC have both been used for the purification of antimicrobial substances. The most effective isolated fungus was molecularly identified as *Aspergillus niger*. The highest amounts of extracellular antimicrobial metabolites against all the investigated microbial pathogens were obtained from ethyl acetate extracts, which were the best organic solvents. Using IR, NMR, and mass spectra, the most promising bioactive component obtained from *Aspergillus niger* was identified. The mechanism of action of this new molecule will require more research, but we think it's helpful to use it. We think it is essential to study this novel compound's potential for treating bacterial and fungal infections.

6. Conflicts of interest

"There are no conflicts to declare".

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