# ANALYSIS OF BIOACTIVE CHEMICAL COMPOUNDS OF ASPERGILLUS FUMIGATUS AND EVALUATION OF…

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# ANALYSIS OF BIOACTIVE CHEMICAL COMPOUNDS OF ASPERGILLUS FUMIGATUS AND EVALUATION OF ANTIFUNGAL ACTIVITY USING TWENTY SEVEN MEDICINAL PLANTS

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## ABSTRACT

The objectives of this study were analysis of the secondary metabolite products from *Aspergillus fumigatus* and evaluation antibacterial activity. Fifteen bioactive compounds were identified in the methanolic extract of *Aspergillus fumigatus*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GC-MS analysis of*Aspergillus fumigatus* revealed the existence of the 9-Hexadecenoic acid ,2,4-Dimethyl-5-methylthiopent-4-en-2-ol , E-11-Hexadecenoic acid , ethyl ester , D-Glucose , 6-O-α-D-galactopyranosyl- , α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- , 6-Acetyl –β-d-mannose , 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- , 5-Hydroxymethylfurfural , β-D-Glucopyranoside, methyl , Tetraacetyl-d-xylonic nitrile , n-Hexadecanoic acid , 9-Octadecenoic acid , (2-phenyl-1,3-dioxolan-4-yl)methyl ester , Octadecanoic acid ,9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)- and Pyrimidin-2-ol,4-(3,4-dimethoxyphenyl)-6-phenyl. *Gramineae poaceae* was very highly antifungal activity (8.32±0.09) mm while *Streptococcus faecalis* has a maximum zone formation (6.92±0.24) mm against *Aspergillus fumigatus*.

**KEYWORDS:***Aspergillus fumigatus*, **Antibacterial, Antifungal, GC/MS, Secondary metabolites.**

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# INTRODUCTION

Filamentous fungi can produce a vast array of secondary metabolites, many of which are volatile[1-4]. *Aspergillus* is a spore-forming fungus consisting of ~200 species among which lessthan 20 have been found to cause of IA, contains numerous biosynthetic clusters that enable it to synthesize at least 226 secondary metabolite products[5,6]. *Aspergillus fumigatus*, the most common cause infection in human, known as aspergillosis.The primary route of infection by *Aspergilli* is via inhalation of the airborne conidiafollowed by deposition of these conidia in the bronchioles or alveolar spaces[7-9]. *Aspergillus* volatile organic compounds (VOCs) have been characterized under growth conditions that promote sporulation[10-13], with identification of small alcohols, ketones, and furans, little is known about VOC production in vivo in patients with IA. *Aspergillus* isan opportunistic pathogen and disease development depends on the host's immunefunction. Aspergillosis is frequently found in immunocompromised patient populations,such as those with chronic granulomatous disease, AIDS, acute leukemia, andneutropenia and those treated with immunosuppressants[14]. Although not required for primary growth, these metabolites, which often have antibiotic, cytotoxic, and phytotoxic properties, likely influence interactions between fungi and their ecological niche[15-18]. Human aspergillosis can be clinically divided into three types: saprophytic,allergic, or invasive. Saprophytic aspergillosis is defi ned as a colonization of*Aspergillus* in the respiratory tract, usually does not invade or damage tissues, andmay present as aspergilloma[19-22]. The aims of this study were analysis of the secondary metabolites and evaluation antibacterial and antifungal activity.

# MATERIALS AND METHODS

### *Growth conditions and determination of metabolites*

*Aspergillus fumigatus* was isolated from dried fruit and the pure colonies were selected, isolated and maintained in potato dextrose agar slants[23,24]. Spores were grown in a liquid culture of potato dextrose broth (PDB) and incubated at 25ºC in a shaker for 16 days at 130 rpm.The extraction was performed by adding 25 ml methanol to 100 ml liquid culture in an Erlenmeyer flask after the infiltration of the culture. The mixture was incubated at 4ºC for 10 min and then shook for 10 min at 130 rpm. Metabolites were separated from the liquid culture and evaporated to dryness with a rotary evaporator at 45 $C^0$. The residue was dissolved in 1 ml methanol, filtered through a 0.2 µm syringe filter, and stored at 4ºC for 24 h before being used for GC-MS. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library as well as on comparison of their retention indices either with those of authentic compounds or with literature values**.**

### *Analysis of bioactive compounds by using gas chromatography-mass spectrometry (GC/MS)*

Bioactive compounds were examined for the chemical composition using GC-MS (Agilent 789A) equipped with a DB-5MS column (30 m×0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as for the previous analysis. Helium was used as the carrier gas at the rate of 1.0 mL/min. Effluent of the GC column was introduced directly into the source of the MS via a transfer line (250oC). Ionization voltage was 70 eV and ion source temperature was 230$^o$C. Scan range was 41- 450 amu. The constituents were identified after compared with available data in the GC-MS library in the literatures[25].

### *Determination of antibacterial activity*

The test pathogens (*Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa,* and *E. coli*) were swabbed in Muller Hinton agar plates. 90µl of fungal extracts was loaded on the bored wells. The wells were bored in 0.5 cm in diameter. The plates were incubated at 37$C^0$ for 24 hrs and examined. After the incubation the diameter of inhibition zones around the discs was measured.

### *Determination of antifungal activity*

*Aspergillus fumigatus* isolate was suspended in potato dextrose broth and diluted to approximately 105 colony forming unit (CFU) per ml. They were "flood inoculated onto the surface of Potato dextrose agar and then dried. Standard agar well diffusion method was followed[18,23]. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (*Linum usitatissimum, Anastatica hierochuntica, Gramineae poaceae, Nerium olender, Ricinus communis,* and *Datura stramonium*) were delivered into the wells. The plates were incubated for 48 h at room temperature. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent[26]. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

### *Statistical analysis*

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$ using Duncan's multiple range test (by SPSS software)Version 9.1.

## RESULTS AND DISCUSSION

Based onmorphological characteristics of fungiwas isolated in selective media of potatodextrose agar media.Morphological,Microscopical and microscopicalcharacteristics of fungal strains weredetermined using specific media light andcompound microscope Figure 1. The 400ml of fermentation broth (PDA broth) which contain 200µl of the standardized fugal suspensions were used to inoculate the flasks and incubated at 37°C on a shaker at 90 rpm for 7 days. After fermentation, the secondary metabolites were produced by isolated microorganisms.

***Identify the secondary metabolites from Aspergillus fumigatus***

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *Aspergillus fumigatus*, shown in Table 1. The GC-MS chromatogram of the thirty one peaks of the compounds detected was shown in Figure 2. The First set up peak were determined to be 1,2-cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-htdroxy-1-isopropyl)cy, Figure 3. The second peak indicated to be 2-Furancarboxaldehyde,5-methyl, Figure 4. The next peaks considered to be 2(5H)-Furanone, 6-Hydroxymethyl-5-methyl-bicyclo[3.1.0]hexan-2-one, D-Glucose,6-O-α-D-galactopyranosyl, 2-(3-Hydroxy-propyl)-cyclohexane-1,3-dione, 9-Oxa-bicyclo[3.3.1]nonane-1,4-diol, Benzenemethanol,2-(2-aminopropoxy)-3-methyl, 1,2-Cyclopentanedione,3-methyl, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-ß-D-fruc, 1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol, Desulphosinigrin, Orcinol, Bicyclo[2.2.1]heptane-2-carboxylic acid isobutyl-amide, 2H-Oxecin-2-one.3.4.7.8.9.10-hexahydro-4-hydroxy-10-methyl-.[4, 2H-Pyran,tetrahydro-2-(12-pentadecynyloxy), Maltol, 2-Tridecyl-5-(acetylamino)tetrahydro-γ-pyrone, Cycloundecanone , oxime, D-Glucose,6-O-α-D-galactopyranosyl, 6-Acetyl-ß-d-mannose, 5-Hydroxymethylfurfural, 1-Gala-l-ido-octonic lactone, Pterin-6-carboxylic acid, Uric acid, Acetamide , N-methyl -N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl], l-(+)-Ascorbic acid 2,6-dihexadecanoate, D-fructose , diethyl mercaptal , pentaacetate, 2-Bromotetradecanoic acid, Octadecanal ,2 –bromo, L-Ascorbic acid , 6-octadecanoate, 18,19-Secoyohimban-19- oic acid,16,17,20,21-tetradehydro-16. (Figure 5-17). Many compounds were identified in the presentstudy. Some of them are biologicalcompounds with antimicrobial activities[27-31].

***Antibacterial and antifungal activity***

Clinical pathogens selected for antibacterial activity namely, (Streptococcus pyogenes, Pseudomonas eurogenosa, Streptococcus faecalis, Proteus mirabilis and Staphylococcus aureus).Maximum zone formation against Streptococcus faecalis (6.92±0.24) mm, Table 2. In agar well diffusion method the selected medicinal plants (Linum usitatissimum, Anastatica hierochuntica, Gramineae poaceae, Nerium olender, Ricinus communis, Datura stramonium, Piper nigrum, Zingiber officinale, Linum usitatissimum, Cassia angustifolia, Euphorbia lathyrus, Euphorbia lathyrus, Foeniculum vulgare, Nigella sativa, Ocimum basilicum, Quercus infectoria, Citrullus colocynthis, Althaea rosea, Coriandrum sativum, Melia azedarach, Origanum vulgare, Urtica dioica, Equisetum arvense, Rosmarinus oficinalis, Mentha viridis, Artemisia annua, Punica granatum and Cinnamomum Zeylanicum) were effective against Aspergillus fumigatus, Table 3. Gramineae poaceae was very highly active (8.32±0.09) mm against Aspergillus fumigatus. Aspergillus fumigatus was found to be sensitive to all test medicinal plants and mostly comparable to the standard reference antifungal drug Amphotericin B and fluconazole to some extent.

**Figure 1**
***Morphological characterization of Asp ergillus fumigatus colony.***

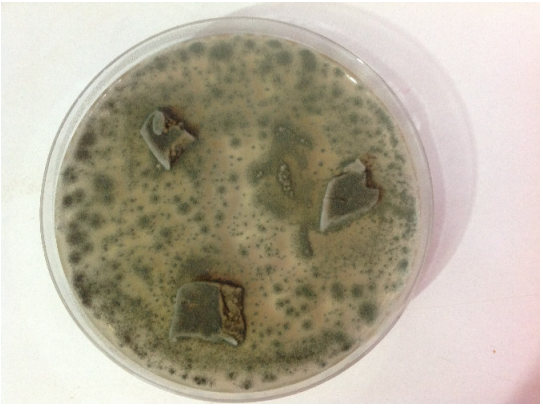


**Figure 2**
***GC-MS chromatogram of methanolic extract of Aspergillus fumigatus***

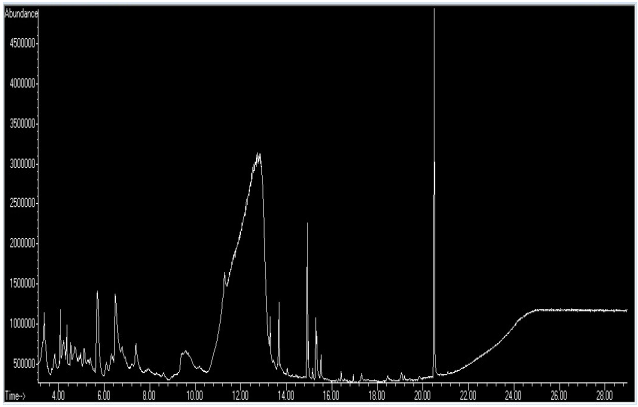



**Figure 3**
***Mass spectrum of 9-Hexadecenoic acid with Retention Time (RT)= 3.196***

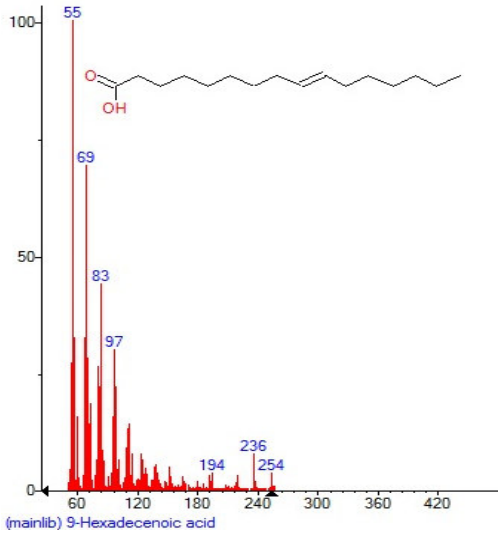



**Figure 4**
***Mass spectrum of 2,4-Dimethyl-5-methylthiopent-4-en-2-ol with Retention Time (RT)= 3.831***

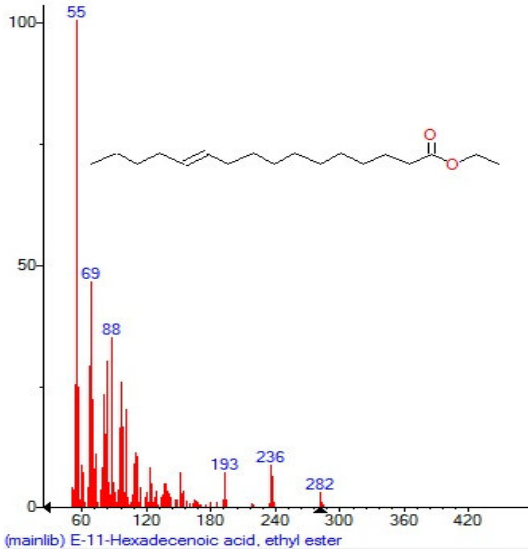


**Figure 5**
***Mass spectrum of E-11-Hexadecenoic acid , ethyl ester with Retention Time (RT)= 4.042***

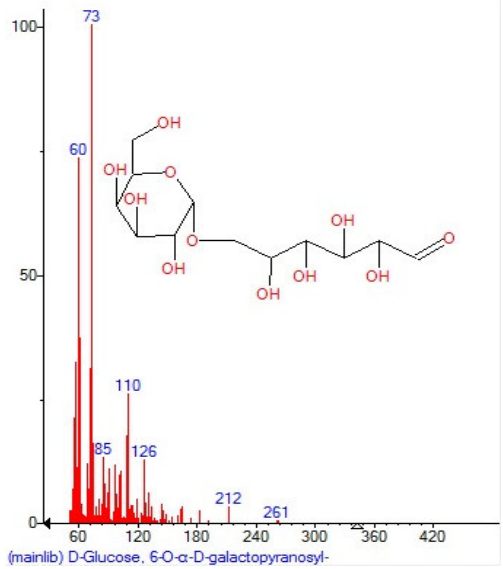



**Figure 6**
***Mass spectrum of D-Glucose , 6-O-α-D-galactopyranosyl- with Retention Time (RT)= 4.203***

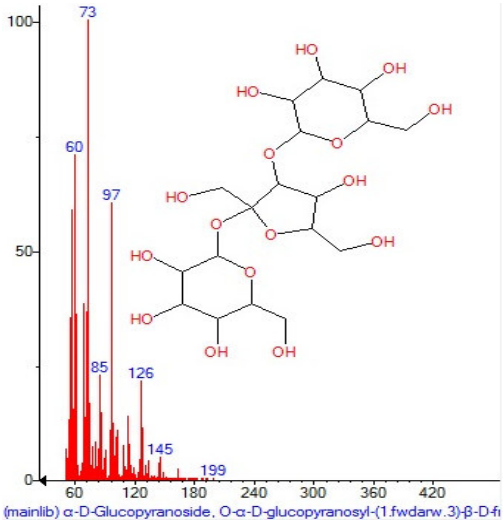


**Figure 7**
***Mass spectrum of α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- with Retention Time (RT)= 4.546***

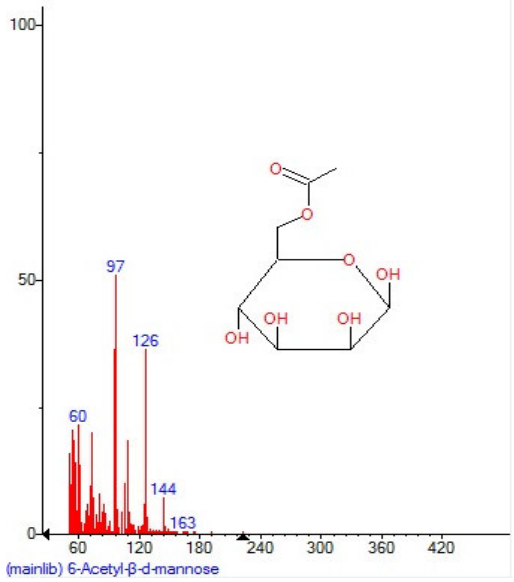



**Figure 8**
***Mass spectrum of 6-Acetyl –β-d-mannose with Retention Time (RT)= 5.124***

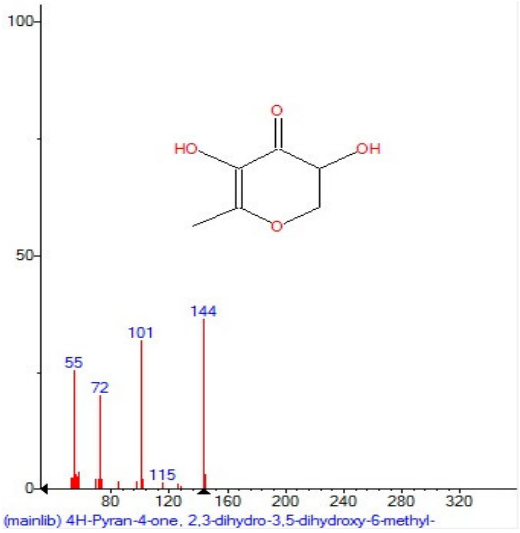



**Figure 9**
***Mass spectrum of 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- with Retention Time (RT)= 5.730***

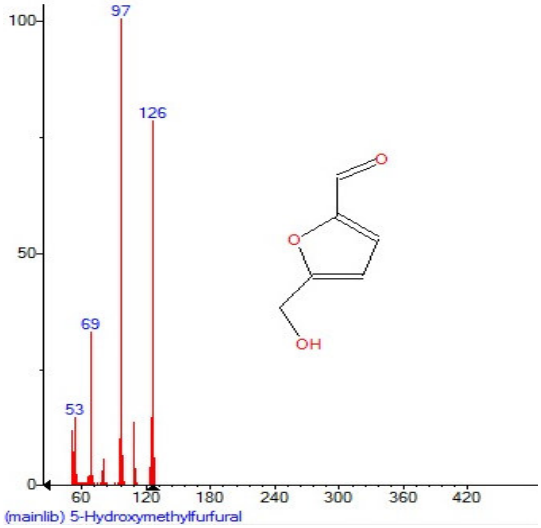


**Figure 10**
***Mass spectrum of 5-Hydroxymethylfurfural with Retention Time (RT)= 6.497***

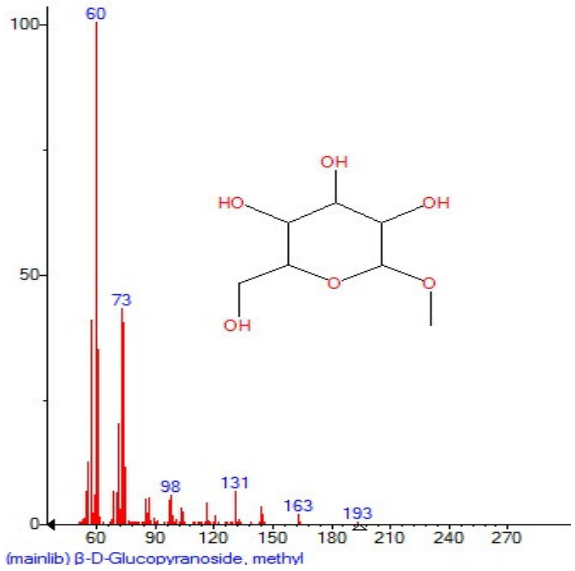



**Figure 11**
***Mass spectrum of β-D-Glucopyranoside, methyl with Retention Time (RT)= 12.625***

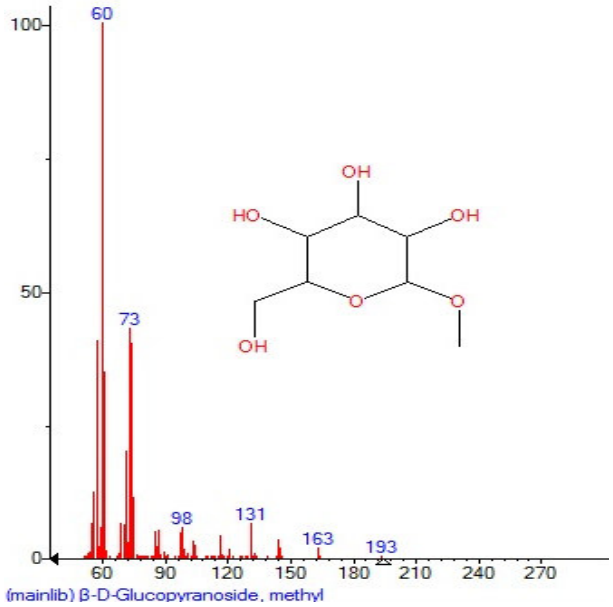


**Figure 12**
***Mass spectrum of Tetraacetyl-d-xylonic nitrile with Retention Time (RT)= 13.495***

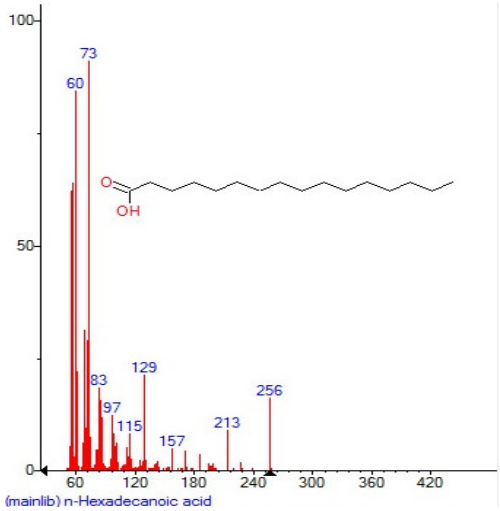



**Figure 13**
***Mass spectrum of n-Hexadecanoic acid with Retention Time (RT)= 13.678***

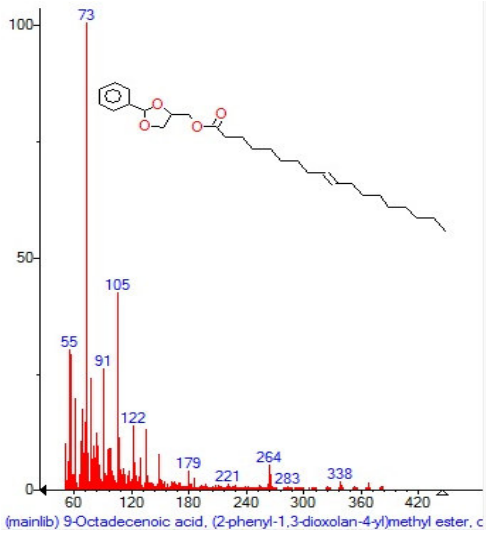



**Figure 14**
***Mass spectrum of 9-Octadecenoic acid , (2-phenyl-1,3-dioxolan-4-yl)methyl ester with Retention Time (RT)= 15.160***

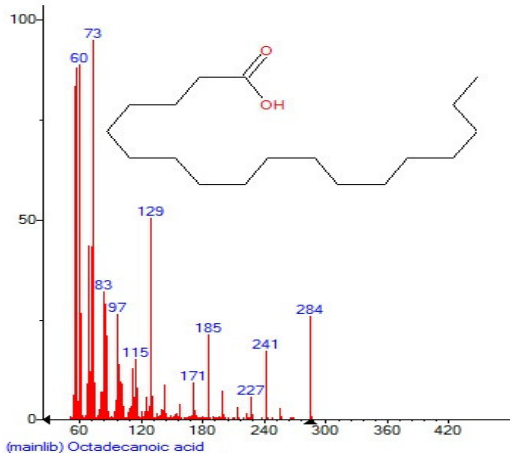


**Figure 15**
***Mass spectrum of Octadecanoic acid with Retention Time (RT)= 15.526***

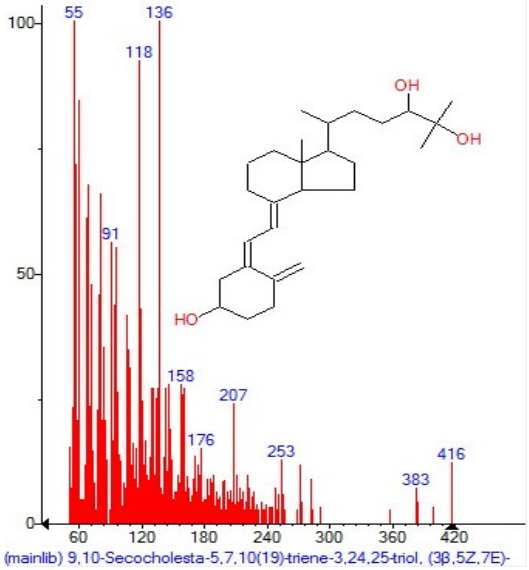



**Figure 16**
***Mass spectrum of 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)- with Retention Time (RT)= 16.425***

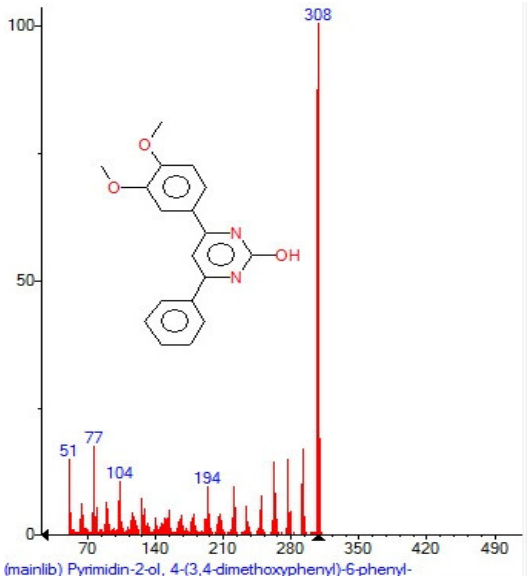
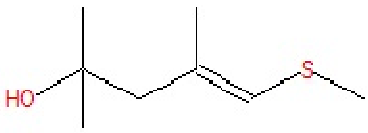
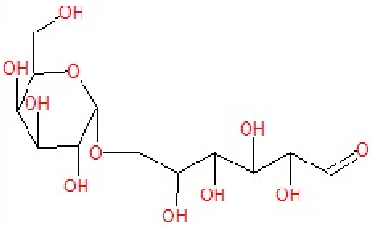
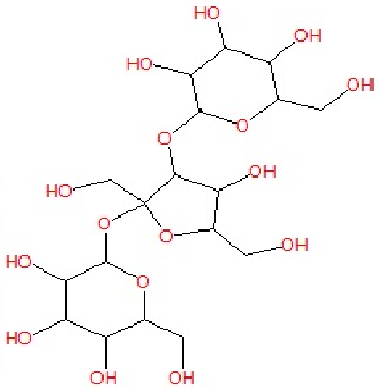
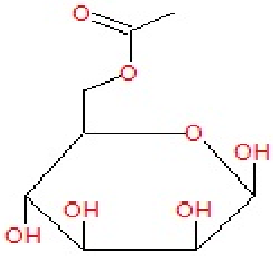
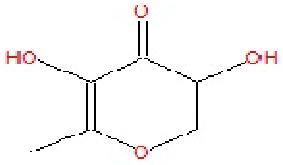
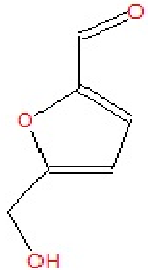
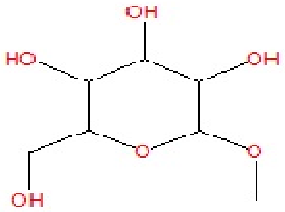



**Table 1**
***Major chemical compounds identified in methanolic extract of Aspergillus fumigatus.***

| Serial No. | Phytochemical compound | RT (min) | Molecular Weight | Exact Mass | Chemical structure | MS Fragment- ions |
|---|---|---|---|---|---|---|
| **1.** | 9-Hexadecenoic acid | 3.196 | 254 | 254.22458 |  | 55,69,83,97,194,236,254 |
| **2.** | 2,4-Dimethyl-5-methylthiopent-4-en-2-ol | 3.831 | 160 | 160.092186 |  | 59,87,102,160 |
| **3.** | E-11-Hexadecenoic acid , ethyl ester | 4.042 | 282 | 282.25588 |  | 55,69,88,193,236,282 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **4.** | D-Glucose , 6-O-α-D-galactopyranosyl- | 4.203 | 342 | 342.11621 |  | 60,73,85,110,126,212,261 |
| **5.** | α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- | 4.546 | 504 | 504.169035 |  | 60,73,85,97,126,145,199 |
| **6.** | 6-Acetyl –β-d-mannose | 5.124 | 222 | 222.073953 |  | 60,97,126,144,163 |
| **7.** | 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- | 5.730 | 144 | 144.042258 |  | 55,72,101,115,144 |
| **8.** | 5-Hydroxymethylfurfural | 6.497 | 126 | 126.031694 |  | 53,69,97,126 |
| **9.** | β-D-Glucopyranoside, methyl | 12.625 | 194 | 194.079039 |  | 60,73,98,131,163,193 |

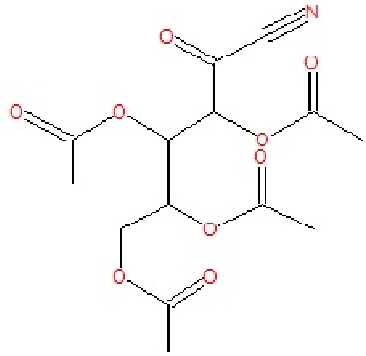
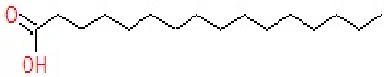
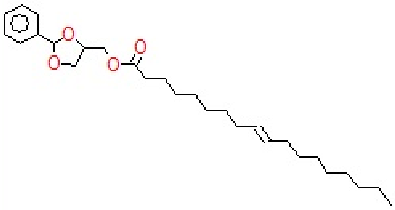
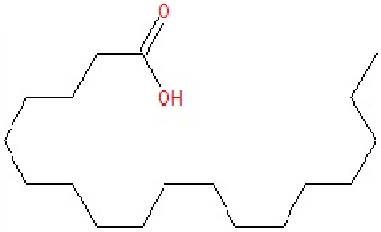
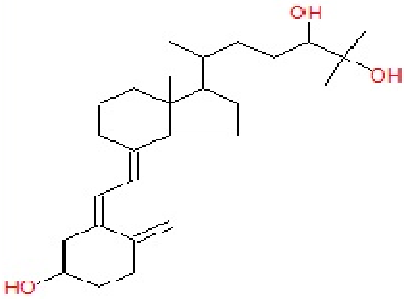
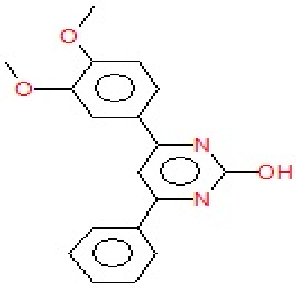
| | | | | | | |
|---|---|---|---|---|---|---|
| **10.** | Tetraacetyl-d-xylonic nitrile | 13.495 | 343 | 343.090332 |  | 60,73,112,133,197,228,270 |
| **11.** | n-Hexadecanoic acid | 13.678 | 256 | 256.24023 |  | 60,73,83,97,115,129,157,213,256 |
| **12.** | 9-Octadecenoic acid , (2-phenyl-1,3-dioxolan-4-yl)methyl ester | 15.160 | 444 | 444.32396 |  | 55,73,91,105,122,179,221,264,283,338 |
| **13.** | Octadecanoic acid | 15.526 | 284 | 284.27153 |  | 60,73,83,97,115,129,171,185,227,241,284 |
| **14.** | 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)- | 16.425 | 416 | 416.329044 |  | 55,91,118,136,158,176,207,253,383,416 |
| **15.** | Pyrimidin-2-ol,4-(3,4-dimethoxyphenyl)-6-phenyl- | 20.464 | 308 | 308.116093 |  | 51,77,104,194,308 |

**Table 2**
***Antibacterial activity of bioactive compounds of Aspergillus fumigatus against bacterial strains***

| Metabolites /Antibiotics | Bacteria | | | | |
|---|---|---|---|---|---|
| | *Streptococcus pyogenes* | *Pseudomonas eurogenosa* | *Streptococcus faecalis* | *Proteus mirabilis* | *Staphylococcus aureus* |
| **Streptomycin** | 2.00±0.30 | 0.99±0.09 | 0.83±0.11 | 2.00±0.10 | 2.01±0.11 |
| **Rifambin** | 1.71±0.10 | 0.98±0.20 | 2.01±0.09 | 1.00±0.21 | 2.91±0.26 |
| **Kanamycin** | 1.09±0.12 | 1.04±0.27 | 1.03±0.22 | 1.03±0.24 | 0.94±0.21 |
| **Cefotoxime** | 2.06±0.30 | 1.09±0.29 | 2.03±0.26 | 0.89±0.27 | 2.07±0.26 |
| **Metabolites** | 5.02±0.27 | 6.07±0.15 | 6.92±0.24 | 5.95±0.10 | 6.05±0.17 |

**Table 3**
***Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of plants to Aspergillus fumigatus.***

| S. No. | Plant | Zone of inhibition (mm) |
|---|---|---|
| **1.** | *Linum usitatissimum*(Crude) | 5.00±0.90 |
| **2.** | *Anastatica hierochuntica*(Crude) | 4.18±0.12 |
| **3.** | *Gramineae poaceae*(Crude) | 8.32±0.09 |
| **4.** | *Nerium olender* (Alkaloids) | 6.21±0.17 |
| **5.** | *Ricinus communis* (Alkaloids) | 3.62±0.19 |
| **6.** | *Datura stramonium*(Alkaloids) | 9.00±0.73 |
| **7.** | *Piper nigrum* (Crude) | 2.99±0.27 |
| **8.** | *Zingiber officinale* (Crude) | 5.00±0.20 |
| **9.** | *Linum usitatissimum* (Crude) | 3.99±0.23 |
| **10.** | *Cassia angustifolia* (Crude) | 5.56±0.11 |
| **11.** | *Euphorbia lathyrus* (Crude) | 5.66±0.21 |
| **12.** | *Foeniculum vulgare* (Crude) | 6.11±0.27 |
| **13.** | *Nigella sativa* (Crude) | 5.09±0.24 |
| **14.** | *Ocimum basilicum* (Crude) | 5.33±0.28 |
| **15.** | *Quercus infectoria* (Crude) | 5.65±0.17 |
| **16.** | *Citrullus colocynthis* (Crude) | 4.65±0.23 |
| **17.** | *Althaea rosea* (Crude) | 5.00±0.33 |
| **18.** | *Coriandrum sativum* (Crude) | 4.07±0.20 |
| **19.** | *Melia azedarach* (Crude) | 4.59±0.11 |
| **20.** | *Origanum vulgare*(Crude) | 7.09±0.24 |
| **21.** | *Urtica dioica* (Crude) | 3.00±0.10 |
| **22.** | *Equisetum arvense*(Crude) | 3.91±0.27 |
| **23.** | *Rosmarinus oficinalis*(Crude) | 2.99±0.20 |
| **24.** | *Mentha viridis* (Crude) | 4.09±0.22 |
| **25.** | *Artemisia annua*(Crude) | 5.00±0.23 |
| **26.** | *Punica granatum* (Crude) | 2.87±0.29 |
| **27.** | *Cinnamomum Zeylanicum* (Crude) | 5.50±0.16 |
| **28.** | Amphotericin B | 7.30±0.26 |
| **29.** | Fluconazol | 7.00±0.13 |
| **30.** | Control | 0.00 |

## CONCLUSION

The results of this study showed that*Aspergillus fumigatus*species produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *Aspergillus fumigatus*species can be useful.

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## CONFLICT OF INTEREST

All authors declare there are no conflict of interest.

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