Antioxidant and cytotoxic activity of ethyl acetate extract from Thermomyces lanuginosus and Aspergillus nidulans isolated from rhizospheric region of peanut (Arachis hypogaea)

Ahmed Raslan (ahmedreslan@agr.aswu.edu.eg)  
Aswan University  
Fatma Abdel-Motaal  
Aswan University  
Mohamed Abou-Ellail  
Aswan University  
Abou El-Hamd Mohamed  
Aswan University

Research Article

Keywords: Ethyl acetate extract, Thermomyces lanuginosus, Aspergillus nidulans, GC-MS, Antioxidant, Cytotoxicity

Posted Date: November 30th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2315764/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Plant rhizospheric region is a good habitat of microbial communities that producing important secondary metabolites and essential bio products such as antioxidants and cytotoxins. Rhizospheric fungi are the best example for these communities. Thermomyces lanuginosus and Aspergillus nidulans were isolated from rhizospheric region of Arachis hypogaea and characterized morphologically and confirmed with Molecular genetic using (ITS) region. Ethyl acetate extract was a common solvent used in extraction and separation. Gas Chromatography Mass Spectrophotometer (GC-MS) was carried out to identify these fungal extracts and estimate their content. DPPH Scavenging was used for Screening of Antioxidant Activity and HeLa (Cervical Cancer), Colo-205 (Colorectal cancer) and MCF-7 (Breast Adenocarcinoma) for determining Cytotoxicity and cell Viability.

1. Introduction

Peanut (Arachis hypogaea) is one of the most important oil crops belongs to Fabaceae family and Arachis genus (Stalker et al., 1997). Rhizosphere is the region around plant roots which is rich in microbial content (Yakov et al., 2015). Biodiversity of microbiota in rhizosphere represents a complex interaction between plant and rhizospheric microorganism especially fungi that utilize the nutrients released by the plant. On the other hand, they provide the plant with a lot of bio products that help plant in its physiological process and be used in a wide range of applications in the field of biomedicine, pharmaceuticals, and industry.

Thermomyces lanuginosus and Aspergillus nidulans are important fungi species that rich in secondary metabolites which showed high effect as a source of antioxidant, anticancer, antimicrobial factors.

Free radicals are highly reactive molecules with an unpaired electron in their valence (Senguttuvan et al., 2014). The production of free radicals is responsible for the cell and tissue damage through oxidation process. So, antioxidants are considered a good free radical scavengers help in repairing the cell damage caused by these free radicals such as 2-Bromo dodecane, Ethanol, 2-(octadecyloxy) and 2-Tetradecene, (E) (Sen et al., 2010). In these scenario fungi are the abundant source of naturally producing antioxidant. Also these bioproducts have an essential role as cytotoxic factors such as 2-Tetradecene, (E), Cycloeicosane, Di 2 ethylhexyl Phthalate and Bis(2-ethylhexyl) phthalate (Tonisi et al., 2020).

2. Materials And Methods

2.1. Collection of Samples:

Three rhizospheric samples of Arachis hypogaea were collected from Aswan university, Egypt. Each sample (about .5 kg) was collected and transferred immediately in sterilized plastic bags to the laboratory for the isolation of fungi.

2.2. Isolation of fungi from the collected samples:
Dilution plate method and Potato dextrose agar (PDA) cultural media were used for isolation of fungi from the collected rhizospheric samples (Avishai et al., 2014). One ml of $10^{-3}, 10^{-2}$ dilution was transferred aseptically into each of 3 sterile Petri-dishes and about 20ml of melted medium were poured. The dishes were rotated clockwise and anti-clockwise by hand for good dispersion of sample suspension and were leaved for solidification then incubated at $45^\circ C$ for 5–7 days. Colony forming units per gram (CFU/g) of sample have been calculated from the following equation:

$$\text{CFU/g} = \frac{\text{Total number of colonies}}{\text{Dilution of soil sample}} \times \frac{\text{Cell/g soil}}{10^3}$$

The purified cultures were transferred to fresh agar slants and stored at $4^\circ C$ in the refrigerator.

2.3. Morphological and Molecular identification:

All the isolated rhizospheric fungi were identified based on their colonial and hyphal characteristics at the genus and species level by using (PDA) according to the detailed study of all microscopic morphological characters (Raper et al., 1965), (Ellis et al., 1971), (Booth et al., 1977), (Christensen et al., 1978). *Thermomyces lanuginosus* and *Aspergillus nidulans* molecular identification was performed by gene sequencing (Gontia et al., 2014) CTAB method was used to extract DNA after cultivation for 7–10 days. Agarose (0.7%) ultra-pure was used for resolving the DNA fragments. Bands were detected on UV-transilluminator. PCR Amplification was performed using Primers ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') to amplify ribosomal internal transcribed spacer (Suarez et al., 2005). Polymerase Chain Reaction (PCR) products were detected by electrophoreses on 1.5% Agarose gel. Bands were photographed by gel documentation system (BIO-RAD Gel DOCTM XR+). These bands were sequenced in Korea Solgent Company. NCBI Blast website was used to analyze sequences. MEGA11 software programme was used for construction of phylogenetic tree (Tamura et al., 2013) and (Kenia et al., 2021).

2.4. Extraction of secondary metabolites from the selected rhizospheric fungi:

*Thermomyces lanuginosus* and *Aspergillus nidulans* were incubated at 6 mm disc in 500 ml flask containing 150 ml PDA media under the condition of shaker incubator (150 rpm) along 10 days. Then ethyl acetate (EtOAc) was mixed with culture and left 24h under continuous shaking at $45^\circ C$, and then the extract of EtOAc was separated by separating funnel and vacuum dried according to (Abdel-Motaal et al., 2010).

2.5. Analysis of secondary metabolites by GC-MS:

The analysis was carried out using gas chromatography-mass spectrometry (GC-MS) instrument stands with the following specifications, Instrument: Agilent Technologies 7890 A GC System, coupled with Agilent Technologies 5977 A MSD Mass Spectrometer. The GC-MS system was equipped with HP-5ms
GC column/Agilent (30 m x0.32mm i.d,0.25µm film thickness). Analysis was carried out using helium as carrier gas at flow rate of 1.0 mL/min and a split ratio of 1:20 using the following temperature program: 80°C for 3 min; rising at 20°C/min to 180°C and held for 1 min, rising at 4°C/min to 220°C and rising at 20°C/min to 250°C and held for 5 min. 1µL of the mixtures were always injected. Result were obtained by electron ionization (EI) at 70 eV using spectral range of m/z 50–550. The identification of the chemical constituents of mixtures was de-convoluted using Agilent software and mass spectrum matching to NIST library database.

2.6. Screening of Antioxidant Activity:

2.6.1. DPPH Scavenging Activity:

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay was carried out according to the method of . Briefly, 100µL of freshly prepared DPPH reagent (0.1% in methanol) was added to 100 µL of the sample in 96 wells plate (n = 6). The reaction was incubated at room temp for 30 min in dark. At the end of incubation time the resulting reduction in DPPH color intensity was measured at 540 nm. Trolox standard preparation by a stock solution of 100 µM concentration of Trolox was prepared in methanol from which 7 concentrations were prepared including 50, 40, 30, 20, 15, 10 and 5 µM.

Data are represented as means ± SD according to the following equation: percentage inhibition= (Average absorbance of blank-average absorbance of the test) / (Average absorbance of blank)) *100.

2.7. Cytotoxicity Assessment:

2.7.1. Cell Culture:

HeLa (Cervical Cancer), Colo-205 (Colorectal cancer) and MCF-7 (Breast Adenocarcinoma) were maintained in RPMI media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO2 atmosphere at 37°C.

2.7.2. Cytotoxicity Assay:

Cytotoxicity assay were assessed by Sulforhodamine B (SRB) assay. Aliquots of 100 µL cell suspension (5x10^3 cells) were in 96-well plates and incubated in complete media for 24 h. Cells were treated with another aliquot of 100 µL media containing drugs at various concentrations. After 72 h of drug exposure, cells were fixed by replacing media with 150 µL of 10% TCA and incubated at 4°C for 1 h. The Tricyclic Antidepressant (TCA) solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70 µL SRB solution (0.4% w/v) were added and incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 µL of Tris (10 mM) was added to dissolve protein-bound SRB stain; the absorbance was measured at 540 nm using a BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany) (Allam et al., 2018).

2.9. Statistical Analysis:
Result was subjected to a one-way analysis of variance (ANOVA). Significant differences between the control and treatments ($P \leq 0.01$) obtained by one-way analysis of variance (ANOVA).

3. Results And Discussion

The results obtained from the research carried out indicated that five 10 fungal species were isolated belonging to 6 fungal genera (Table 1). From the result it was observed that *Aspergillus* was the most predominant fungal genus in all isolated samples (47.34%), followed by *Thermomyces* (27.76) and *Humicola* (12.25). In this study the results observed that fungal species growth rate was variable in each sample as *Thermomyces lanuginosus* was the most common fungal species with the highest percentage, followed by *Aspergillus nodulins* and *Humicola grisea*, while *Fennellia nivea* and *Aspergillus sydowi* had the least percentage. All isolated species were thermophilic which can grow at (45 °C). So *Thermomyces lanuginosus* and *Aspergillus nodulins* were the selected species for this study.

3.1. Morphological characterization of rhizospheric fungi:

*Thermomyces lanuginosus* colonies appear white and are less than 1 mm high, but soon turn grey or greenish grey, commencing from the center of the colony. Subsequently the colony turns purplish brown, and the agar substratum stains a deep pink or wine color, due to diffusible substances secreted by the colony. Mature colonies appear dull dark brown to black.

*Aspergillus nodulins* colonies on potato dextrose agar at 25°C are dark green with orange to yellow in areas of cleistothecial production, reverse is purplish to olive. Exudate is usually present and may be brown to purplish.

3.2. Molecular Characterization of Rhizospheric Fungi:

DNA sequencing of the internally transcribed spacer (ITS) region was used to identify the isolated fungi at molecular level. Segments of the entire (ITS) regions, including partial 5.8S rRNA and internal transcribed spacer 1 were amplified using primers ITS1 and ITS4. It was submitted to the NCBI. The ITS sequence showed 99–100% similarity with each species in the Phylogenetic tree (Fig. 1) and the accession numbers OL891594 and OL907110 were received.

3.3. Extraction of Secondary Metabolites from The Selected Rhizospheric Fungi:

Ethyl acetate used for extraction from each fungal species with 80 mg/L for *Aspergillus nidulans* and 110.66 mg/L for *Thermomyces lanuginosus*.

3.4. Analysis of Secondary Metabolites by GC-MS:

*Thermomyces lanuginosus* extract was analyzed and identified using gas chromatography-mass spectrometer detector (GC-MS) (Fig. 2) (table 2).
3.4.1. Identified Compounds of *Thermomyces lanuginosus* GC-MS Analysis:

Result showed that 7-Hexadecene, (Z)- having peak ratio of 7.645% and retention time 10.028 is the largest compound, followed by 1-Octadecene with peak ratio of %9.957 and retention time 12.523, Cycloeicosane with peak ratio of %8.204 and retention time 15.756, Phenol, 3,5-bis (1,1-dimethylethyl) with peak ratio of %2.947 and retention time 9.268, 1-Nonadecene with peak ratio of %5.935 and retention time 19.400 and 2-Tetradecene, (E)- with peak ratio of %1.592 and retention time 8.229 (Table 4).

7-Hexadecene, (Z)- is reported as an essential compound because of its biological properties as Anticancer and Aggregation-Sex Pheromone (El Fakir et al., 2021) and (Silva et al., 2018). 1-Octadecene and 1-Nonadecene are considered as Antibacterial, Antiinflammatory and a Solvent for ferrofluids for intraocular use (Skanda et al., 2021) and (Tejada P et al., 1997). Cycloeicosane has Cytotoxic and Anticancer activity and Bio-Pesticide’s usage (Abdelkader et al., 2018) and (Saad et al, 2019). Phenol, 3,5-bis (1,1-dimethylethyl) plays an essential role as Antifungal, Antimicrobial and Cytotoxic factors (Sonkar P et al., 2019) and (Dhanya., 2016), while 2-Tetradecene, (E)- have a cytotoxicity activity (Tonisi et al., 2020). All these identified compounds were known to possesses antioxidant activity (table 3).

3.5. Analysis of Secondary Metabolites by GC-MS:

*Aspergillus nidulans* extract was analyzed and identified using gas chromatography-mass spectrometer detector (GC-MS) (Fig. 3) (table 4).

3.5.1. Identified Compounds of *Aspergillus nidulans* GC-MS analysis:

Result showed that Bis(2-ethylhexyl) phthalate having peak ratio of 70.588% and retention time 29.259 is the largest compound, followed by (1R)-2,6,6-Trimethylbicycle [3.1.1] hept-2-ene with peak ratio of 0.701% and retention time 3.276, Octadecane, 1-iodo- with peak ratio of % 4.531 and retention time 8.734, 2-Bromo dodecane with peak ratio of % 3.968 and retention time 12.351, Hentriacontane with peak ratio of % 3.152% and retention time 13.197 (Table 4).

Bis(2-ethylhexyl) phthalate is reported as an essential compound because of its biological properties as Antioxidant, Antiviral, and antitumor Activity (El-Sayed et al., 2015), from the other hand (1R)-2,6,6-Trimethylbicycle [3.1.1] hept-2-ene, Octadecane, 1-iodo-2-Bromo dodecane, Hentriacontane considered as Antioxidant and Antimicrobial factors (Wibawa et al., 2019), (Khammas et al., 2020) (Table 5).

3.5.2. DPPH Scavenging Activity:

Result calculated using Graph pad Prism 6® by converting the concentrations to their logarithmic value and selecting non-linear inhibitor regression equation (log (inhibitor) vs. normalised response – variable
slope equation) by converting the concentrations to their logarithmic value and selecting non-linear inhibitor regression equation (log (inhibitor) vs. normalised response – variable slope equation).

*Thermomyces lanuginosus* extract had the highest significant inhibition percentage of 45.571 percent, whereas *Aspergillus nidulans* extract had the lowest significant inhibition percentage of 21.538 percent, according to the findings (Fig. 4).

### 3.5. In Vitro Cytotoxicity Assay:

The In Vitro Cytotoxicity Assay of extracts of rhizospheric fungal species isolated from *A. hypogaea* was determined against Cervical Cancer (HeLa), breast cancer (MCF-7) and *Colorectal* cancer (Colo-205) human cell lines by using Sulforhodamine B (SRB) assay method. Each fungal extracts showed cytotoxic effects on the three cell lines. *Aspergillus nidulans* extract displayed the highest significant cytotoxicity against Colorectal cancer (Colo-205) cell line with inhibition percent 83.5% followed by *Thermomyces lanuginosus* with inhibition percent 77% while the least cytotoxicity was exhibited by *T. lanuginosus* against Breast Adenocarcinoma (MCF-7) cell line with inhibition percent 69.5% followed by *A. nidulans* with inhibition percent 64.5% and the result showed equal cytotoxic effects of each fungal species against Cervical Cancer (HeLa) with inhibition percent 63.5% (Fig. 5,6,7).

### 4. Conclusion

*Thermomyces lanuginosus* and Aspergillus *nidulans* were the most prevalent fungi species in this investigation. Morphological and Molecular identification for these species were studied. Colonies of *Thermomyces lanuginosus* start out white but they quickly turn grey or greenish grey. On the other hand, *Aspergillus nodulins* colonies on potato dextrose agar at 25°C were dark green with orange to yellow in cleistothecial production zones, Purplish to olive on the reverse. Exudate is common and can range in color from brown to reddish.

DNA sequencing of the internally transcribed spacer (ITS) region of the rRNA gene was used to identify the isolated fungi at a molecular level. It was sent to the National Center for Biotechnology Information (NCBI). The ITS sequence was found to be 99–100% identical to each species in the Phylogenetic tree and accession numbers OL891594 and OL907110 were assigned.

Analysis of secondary metabolites by GC-MS showed that most ethyl acetate extracts have antioxidant and cytotoxic effect. *Thermomyces lanuginosus* extract had the highest DPPH scavenging activity, with an inhibition percentage of 45.571 percent, whereas *Aspergillus nidulans* extract were the lowest, with an inhibition percentage of 21.538 percent. The SRB assay method was used to assess the In Vitro cytotoxicity of extracts of rhizospheric fungus species isolated from *A. hypogaea* against cervical cancer (HeLa), breast cancer (MCF-7) and colorectal cancer (Colo-205) human cell lines. *Thermomyces lanuginosus* had the highest cytotoxicity effect against Colorectal cancer (Colo-205) cell line, with 83.5 percent inhibition, followed by *Aspergillus nidulans* with 77 percent inhibition, while *T. lanuginosus* had the lowest cytotoxicity effect against Breast Adenocarcinoma (MCF-7) cell line, with 69.5 percent
inhibition, followed by *Aspergillus nidulans* with inhibition percent 64.5% and showed equal cytotoxic effects against Cervical Cancer (HeLa) with inhibition percent 63.5%.

**References**


Tables
### Table 1
The percentage of occurrences of the isolated fungi.

<table>
<thead>
<tr>
<th>Fungal isolate</th>
<th>Percentage of occurrences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermomyces lanaginosus</td>
<td>27.76%</td>
</tr>
<tr>
<td>Aspergillus nodulins</td>
<td>26.52%</td>
</tr>
<tr>
<td>Humincola grisea</td>
<td>12.25%</td>
</tr>
<tr>
<td>Aspergillus egyptiacus</td>
<td>7.35%</td>
</tr>
<tr>
<td>Aspergillus subsesilis</td>
<td>6.94%</td>
</tr>
<tr>
<td>Phoma eupyrenal</td>
<td>4.49%</td>
</tr>
<tr>
<td>Myrothecium roridium</td>
<td>4.08%</td>
</tr>
<tr>
<td>Aspergillus versicolor</td>
<td>4.08%</td>
</tr>
<tr>
<td>Fennellia nivea</td>
<td>4.08%</td>
</tr>
<tr>
<td>Aspergillus sydowii</td>
<td>2.45%</td>
</tr>
<tr>
<td><strong>Total Percentage</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

### Table 2
Main compounds of GC-MS Analysis of Thermomyces lanuginosus.

<table>
<thead>
<tr>
<th>RT</th>
<th>Identified compound</th>
<th>Molecular Formula</th>
<th>Area %</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.028</td>
<td>7-Hexadecene, (Z)-</td>
<td>C₁₆H₃₂</td>
<td>7.645%</td>
<td>224</td>
</tr>
<tr>
<td>12.523</td>
<td>1-Octadecene</td>
<td>C₁₈H₃₆</td>
<td>9.957%</td>
<td>252</td>
</tr>
<tr>
<td>15.756</td>
<td>Cycloeicosane</td>
<td>C₂₀H₄₀</td>
<td>8.204%</td>
<td>280</td>
</tr>
<tr>
<td>9.268</td>
<td>Phenol, 3,5-bis (1,1-dimethylethyl)</td>
<td>C₁₄H₂₂O</td>
<td>2.947%</td>
<td>206</td>
</tr>
<tr>
<td>19.400</td>
<td>1-Nonadecene</td>
<td>C₁₉H₃₈</td>
<td>5.935%</td>
<td>266</td>
</tr>
<tr>
<td>8.229</td>
<td>2-Tetradecene, (E)-</td>
<td>C₁₄H₂₈</td>
<td>1.592%</td>
<td>196</td>
</tr>
</tbody>
</table>
Table 3

Biological activity of identified compounds from extract of Thermomyces lanuginosus.

<table>
<thead>
<tr>
<th>N</th>
<th>Identified compound</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7-Hexadecene, (Z)-</td>
<td>Antioxidant Activity, Anticancer and Aggregation-Sex Pheromone</td>
<td>El Fakir et al., 2021</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Silva et al., 2018</td>
</tr>
<tr>
<td>2</td>
<td>1-Octadecene</td>
<td>Antioxidant, antibacterial, Antiinflammatory and a solvent for ferrofluids for intraocular use</td>
<td>Skanda, S. et al., 2021(1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skanda et al., 2021(2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tejada et al., 1997</td>
</tr>
<tr>
<td>3</td>
<td>Cycloeicosane</td>
<td>Cytotoxic Activity, Anticancer and Bio-Pesticides</td>
<td>Abdelkader et al., 2018</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saad et al, 2019</td>
</tr>
<tr>
<td>4</td>
<td>Phenol, 3,5-bis(1,1-dimethylethyl)</td>
<td>Antifungal Activity, Antioxidant Activity, Antimicrobial Activity and Cytotoxic Activity</td>
<td>Sonkar et al., 2019</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rahul Chandra Mishra et al., 2021</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dhanya., 2016</td>
</tr>
<tr>
<td>5</td>
<td>1-Nonadecene</td>
<td>Antioxidant and antimicrobial activities and Anti-inflammatory</td>
<td>Fengping Yi et al., 2019</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skanda, 2021 (3)</td>
</tr>
<tr>
<td>6</td>
<td>2-Tetradecene, (E)-</td>
<td>Antioxidant and cytotoxicity Activity</td>
<td>Tonisi et al., 2020</td>
</tr>
</tbody>
</table>

Table 4

Main Compounds of GC-MS Analysis of Aspergillus nidulans.

<table>
<thead>
<tr>
<th>RT</th>
<th>Identified compound</th>
<th>Molecular Formula</th>
<th>Area %</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.259</td>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>C_{24}H_{38}O_{4}</td>
<td>70.588 %</td>
<td>390</td>
</tr>
<tr>
<td>3.276</td>
<td>(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene</td>
<td>C_{10}H_{16}</td>
<td>0.701 %</td>
<td>136</td>
</tr>
<tr>
<td>8.734</td>
<td>Octadecane, 1-iodo-</td>
<td>C_{18}H_{37}I</td>
<td>% 4.531</td>
<td>380</td>
</tr>
<tr>
<td>12.351</td>
<td>2-Bromo dodecane</td>
<td>C_{12}H_{25}Br</td>
<td>% 3.968</td>
<td>248</td>
</tr>
<tr>
<td>13.197</td>
<td>Hentriacontane</td>
<td>C_{31}H_{64}</td>
<td>% 3.152</td>
<td>436</td>
</tr>
</tbody>
</table>
Table 5
Biological Activity of Identified Compounds from extract of Aspergillus nidulans.

<table>
<thead>
<tr>
<th>No.</th>
<th>Identified compound</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>Antioxidant, Antiviral and antitumor Activity</td>
<td>El-Sayed et al., 2015</td>
</tr>
<tr>
<td>2</td>
<td>(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene</td>
<td>Antifungal and Antimicrobial activity</td>
<td>Wibawa et al., 2019, Ndukwe et al., 2018</td>
</tr>
<tr>
<td>3</td>
<td>Octadecane, 1-iodo-</td>
<td>Antifungal Activity</td>
<td>Khammas et al., 2020</td>
</tr>
<tr>
<td>4</td>
<td>2-Bromo dodecane</td>
<td>Antibacterial and antifungal activities</td>
<td>Sánchez-Calvo et al., 2016</td>
</tr>
<tr>
<td>5</td>
<td>Hentriacontane</td>
<td>Antioxidant and Antimicrobial</td>
<td>Olubunmi et al., 2009</td>
</tr>
</tbody>
</table>

Figures
Figure 1

Phylogenetic tree of two fungal species Thermomyces lanuginosus and Aspergillus nodulinis.
Figure 2

GC_MS chromatogram of the Thermomyces lanuginosus

Figure 3

GC_MS chromatogram of the Aspergillus nidulans.
Figure 4

inhibition percent of two fungal species.

Figure 5

Cell viability percent of *T. lanuginosus* and *A. nidulans* against (Colo-205 Colorectal cancer).
Figure 6

Cell viability percent of *T. lanuginosus* and *A. nidulans* against (MCF-7: Breast Adenocarcinoma).

Figure 7

Cell viability percent of *T. lanuginosus* and *A. nidulans* against (HeLa: Cervical Cancer).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Aspergillusnidulans.gc.mass.docx
- Thermomyceslanuginosus.gc.mass.docx
• cancerdata.docx