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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE ACACIA ALBIDA (DEL.) A. CHEV. (MIMOSOIDEAE) FIXED OIL

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ABSTRACT

This study was designed to identify the constituents of Acacia albida fixed oil, which find many applications in herbal medicine, and to evaluate its antimicrobial activity. The GC-MS analysis showed the presence of 22 components. Major constituents are: Z, Z-9, 12-Octadecadienoic acid methyl ester (38.29%), Z-9-Octadecenoic acid methyl ester (22.31%), Hexadecanoic acid methyl ester (19.55%) and methyl stearate (5.59%). In the diffusion bioassay, the oil showed excellent activity against the bacterial strain Bacillus subtilis in the concentration range: 100-50mg/ml. It also exhibited activity against all test organism at 100mg/ml except for Aspergillus niger which gave a partial activity.

KEYWORDS: Acacia albida oil, GC-MS analysis, Antimicrobial activity.

INTRODUCTION

Over centuries, medicinal plants, which contain bioactive constituents, have been used by humans in primary health care. Now there is a renewed interest in the constituents of medicinal plants which find diverse application in herbal medicine. The multi-drug resistance which became lately a matter of concern, triggered extensive studies in phytochemistry and pharmacology in an attempt to discover new molecules for drug discovery and drug design. Evidently medicinal plants are the best candidates for such leads.

Acacia albida (also known as Faidherbia albida) is a thorny tree with deep penetrating roots and dull grey bark.11 This species which belongs to the Leguminaceae family may reach 20m in height11. Within the fruits are pod-bearing seeds. Seeds are brown with a tough seed coat often eaten by animals2-4. Phytochemical screening revealed the presence of many secondary metabolites including among others: flavonoids, alkaloids, tannins and saponins5-10. Acacia albida is rich in saturated and unsaturated fatty acids including stearic, oleic, linoleic and palmitic acids.11

The plant is used traditionally against a wide spectrum of diseases including: diarrhea12,13, asthma, leprosy and skin diseases14. The plant has also anti-inflammatory5 and antihaemorrhagic12 properties. Furthermore, the antipyretic15 antimalarial16 and antidiabetic properties have also been reported.

Seeds are eaten by humans in time of famine12,17 and the plant is considered as a nitrogen fixer increasing soil fertility18,19.

MATERIALS AND METHODS

Plant material

The seeds of Acacia albida were collected from Khartoum state, Sudan. The plant was authenticated by direct comparison with a herbarium sample.

Test organisms

The following standard bacterial pathogens were used to assess the antimicrobial potency of Acacia albida oil: Bacillus subtilis (Gram +ve), Staphylococcus aureus (Gram +ve), Pseudomonas aeroginosa (Gram –ve), Escherichia coli (Gram –ve) and the fungi Candida albicans and Aspergillus niger.

Methods

Extraction of fixed oil from Acacia albida seeds

Dry-powdered seeds of Acacia albida (400g) were exhaustively macerated with n-hexane at room temperature for 48h. The solvent was removed under reduced pressure leaving the oil. For GC-MS analysis, a methanolic solution of sodium hydroxide and a methanolic sulphuric acid were used to esterify the oil.
GC-MS analysis

*Acacia albida* fixed oil was analyzed by gas chromatography – mass spectrometry. A Shimadzu Ultra instrument was used with RTX-5MS column (30m, length ; 0.25mm diameter ; 0.25 μm, thickness). Analytical grade helium (purity; 99.99 %) was a carrier gas. Oven temperature program and other chromatographic conditions are displayed below:

**Table 1: Oven temperature program.**

<table>
<thead>
<tr>
<th>Rate</th>
<th>Temperature (°C)</th>
<th>Hold time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>60.0</td>
<td>0.00</td>
</tr>
<tr>
<td>10.00</td>
<td>300.0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Table 2: Chromatographic conditions.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column oven temperature</td>
<td>60.0 °C</td>
</tr>
<tr>
<td>Injection temperature</td>
<td>280.0 °C</td>
</tr>
<tr>
<td>Injection mode</td>
<td>Split</td>
</tr>
<tr>
<td>Flow control mode</td>
<td>Linear velocity</td>
</tr>
<tr>
<td>Pressure</td>
<td>93.1 KPa</td>
</tr>
<tr>
<td>Total flow</td>
<td>50.0ml/min</td>
</tr>
<tr>
<td>Column flow</td>
<td>1.50ml/sec</td>
</tr>
<tr>
<td>Linear velocity</td>
<td>44.7 cm/sec</td>
</tr>
<tr>
<td>Purge flow</td>
<td>3.0ml/min</td>
</tr>
<tr>
<td>Split ratio</td>
<td>- 1.0</td>
</tr>
</tbody>
</table>

Antimicrobial assay

Diffusion method was used for screening the oil. Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungi respectively. The media were prepared according to the manufacturer’s instructions.

One ml aliquots of 24 hours broth culture of the test microorganisms were aseptically distributed onto nutrient agar slopes and then incubated at 37°C for 24 hours. The harvested bacterial growth was washed off using sterile normal saline, then it was suspended in (100 ml) of normal saline to give about 108-109 colony forming units per ml. Using the surface viable counting technique, the average number of viable organism per ml of the stock suspension was determined. Serial dilutions of the stock suspension were made in sterile normal saline. (0.02 ml) of the appropriate dilutions were transferred onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature and then incubated at 37°C for 24 hours. Fungal cultures were accomplished on Sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed off with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antibacterial activity

(2ml) of the standardized bacterial stock suspension were mixed with (200 ml) of sterile molten nutrient agar which was maintained at 45°C. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle. Each plate was divided into two halves. In each half two cups (10mm in diameter) were cut using sterile cork borer (No 4). Each half was designed for a test solution.

Agar discs were removed, alternate cups were filled with (0.1 ml) samples of each test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured.

RESULTS AND DISCUSSION

GC-MS analysis

GC-MS analysis of *Acacia albida* oil was conducted and the identification of the constituents was accomplished by comparison with the MS library (NIST). The observed fragmentation pattern was also interpreted. The analysis revealed the presence of 22 components (Table 3). The typical total ion chromatogram (TIC) of hexane extract is shown in Fig.1.

**Table 3: Constituents of Acacia albida oil.**
The following were detected in the chromatograms as major components:

**Z, Z-9, 12-Octadecadienoic acid methyl ester (38.29%)**

Fig. 2 shows the EI mass spectrum of 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294 (R.T. 15.566), corresponds to loss of a methoxyl function.

**Z-9-Octadecenoic acid methyl ester (22.31%)**

Fig. 3 displays the mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296, which appeared at R.T. 15.678, corresponds to loss of a methoxyl group.

**Hexadecanoic acid methyl ester (19.55%)**

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 4. The peak at m/z 270 (R.T. 11.958) corresponds to M+ [C₁₀H₃₂O₂]⁺, while the signal at m/z 239 is attributed to loss of a methoxyl group.

**Methyl stearate (5.59%)**

Fig. 5: Mass spectrum of methyl stearate.

**Antimicrobial activity**

*Acacia albida* fixed oil was screened for antimicrobial activity against six standard human pathogenic bacteria. The results are depicted in Table (4). The results were interpreted in the following conventional terms: (<9mm: inactive; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Tables (5) and (6) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

**Table 4: Antimicrobial activity of *Acacia albida* oil.**

<table>
<thead>
<tr>
<th>Type</th>
<th>Sa</th>
<th>Bs</th>
<th>Ec</th>
<th>Ps</th>
<th>Ca</th>
<th>An</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>100</td>
<td>13</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>10</td>
<td>17</td>
<td>12</td>
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<tr>
<td>25</td>
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<td>14</td>
<td>13</td>
<td>12</td>
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</tr>
<tr>
<td>12.5</td>
<td>--</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>--</td>
</tr>
<tr>
<td>6.25</td>
<td>--</td>
<td>10</td>
<td>7</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 5: Antibacterial activity of standard drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc.(mg/ml)</th>
<th>Bs</th>
<th>Sa</th>
<th>Ec</th>
<th>Ps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>40</td>
<td>15</td>
<td>30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>14</td>
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</tr>
<tr>
<td></td>
<td>10</td>
<td>11</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>40</td>
<td>25</td>
<td>19</td>
<td>22</td>
<td>21</td>
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<tr>
<td></td>
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<td>10</td>
<td>17</td>
<td>14</td>
<td>15</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 6: Antifungal activity of standard drug.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc.(mg/ml)</th>
<th>An</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotrimazole</td>
<td>30</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>17</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>16</td>
<td>29</td>
</tr>
</tbody>
</table>

Sa.: Staphylococcus aureus  
Ec.: Escherichia coli  
Pa.: Pseudomonas aeruginosa  
An.: Aspergillus niger  
Ca.: Candida albicans  
Bs.: Bacillus subtilis

The oil showed excellent activity against the bacterial strain *Bacillus subtilis* in the concentration range: 100-50mg/ml. It also exhibited activity against all test organism at 100mg/ml except for *Aspergillus niger* which gave a partial activity.

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