PHYTOCHEMICAL SCREENING, CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF CARDAMOM

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ABSTRACT
The aim of this study was to examine the phytochemicals of cardamom crude extract and to investigate the chemical composition and antibacterial activity of its essential oil. The results revealed the presence of phenolic compounds, alkaloids, flavonoids, tannins and saponins. The major components of the essential oil from cardamom were: Alpha-ionone (29.295%), Eucalyptol (18.85%), Santolina alcohol (9.23 %), 1,6-octadiene-3-ol,3,7-dimethyl- (8.19 %), 2,6-octadiene-1-ol,3-ol,3,7-dimethyl-,(Z)- (7.71%), Cinnamaldehyde,(E)-(4.20%), Terpinen-4-ol (4.06 %), 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl- (3.82%), Acetic acid, 1-methyl-1-(4-methyl-5-oxy-cy (1.44%). The antibacterial activity was tested against four pathogenic bacteria using the disk diffusion method. Bacillus subtilus NCTTC8236 and Staphylococcus aureusAACC25923 was selected to be gram positive bacteria while E. coli 25922 and Pseudomonas aureus27853 was gram negative. The antibacterial assay showed that the oil had significant inhibitory effects against both Gram-negative and Gram-positive bacteria except E. coli.

KEYWORDS: Phytochemical screening, GC-MS, antibacterial, cardamom, sequential extraction.

INTRODUCTION
Aromatic and medicinal plants offer a wide range of bioactive molecules used to contrary the spread of drug resistance pathogenic bacteria and fungi that cause severe and life-threatening infections.[1,2] The cardamom of commerce is called small green cardamom or the true cardamom (Elettariacardamomum). There are many other plants belonging to the Amomum
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and *Aframomum* genera, both belonging to the cardamom family, producing aromatic seeds. Among them, clubbed together as false cardamoms, the most important and the one that is being grown commercially is *Amomumsubulatum*. Most of these false cardamoms are used as flavoring plants and also as remedies for various ailments.[3]

*Elettaria cardamomum* (L.) Maton is a tall, perennial, reed-like herb growing wild in rainforests of South India, Sri Lanka, and other tropical countries[4,5] *Elettaria cardamomum* (*Zingiberaceae*) or *cardamom* is commonly known as queen of spices for the versatile use in cooking practice. *Cardamom* is a perennial shrub with fleshy, thick and lateral roots and the plant grows to a height of eight feet.[6] It is native to South Asia but it is commercially cultivated in Sri Lanka, Tanzania, Morocco, Guatemala and Southern India.[7] *Cardamom* has antifungal, antibacterial[8-10], antioxidant[10-12], gastro protective effect[13] and anticancer properties.[14]

In Saudi Arabia and the Near East, cardamom is used largely in the preparation of “Gahwa” a strong cardamom coffee concoction.[15] *Cardamom* has been used for carminative and stimulant and stomachic properties and its essential oil show antimicrobial activity in vitro.[16] The medicinal use of *cardamom* is also mentioned in Unani and Ayurveda systems.[17] Besides its use in cooking, *cardamom* also possesses a wide range of therapeutic features such as antifungal, antibacterial, antiviral, diuretic and carminative properties.[16] It is also used to fight against cardiac diseases, renal problems, anorexia, asthma and bronchitis. Furthermore, it demonstrates antioxidant, anti-platelet aggregation, anti-hypersensitive and anti-cancerous attributes.[18] Reports showed that the essential oil has biological activity against the pathogens. Noumi, E.et al[3] were studied the essential oil compositions and also they compared the potential activity of the three different cardamom species *Elettaria cardamomum*, *Aframomum corrorima* and *Amomum subulatum*, oxygenated compounds were the main constituent, 1,8-cineol was the main common compound in the three tested volatile oils. The authors in[19] were revealed that the main compounds of the essential oil of cardamom were α-terpinyl acetate 34.55%, (-)-Terpinen-4-ol (10.63%) and the essential oil showed an antimicrobial activity against all most tested microorganism.

The objective of this study was to screen phytochemicals of the crude extract, chemical compositions and antibacterial activity of the essential oil of cardamom.
MATERIALS AND METHODS

Sample collection and preparation
The sample was collected from a local market in Khartoum, Sudan. The sample was washed, shadow dried and grinded to proper size by using blender. The essential oil of the cardamom was extracted by steam distillation using Clevenger.

Preparation of extract
Fifty gram of the grounded extract was soaked with 80% of ethanol solution and left for 5 days, then filtered and the solvent was evaporated. The extract was sequentially extracted using four solvents of different polarities, including ethyl acetate (fraction 1), acetone (fraction 2), ethanol (fraction 3) and distilled water (fraction 4). All the fractions were filtered then dried and stored for further analysis.

Microorganisms
The microorganisms used included two strains of gram positive bacteria (Bacillus subtilus NCTTC8236 and Staphilla aureus AACC25923) and two other strains of gram negative bacteria (E. coli 25922 and Pseudomonas 27853). The organisms were collected from microbiology laboratory of central lab the ministry of higher education and scientific research, Sudan. The strains were identified the central lab.

Antibacterial activity study
Antibacterial activity of the essential oil of cardamom was tested using disc diffusion method.[20]

GC-MS condition
The essential oil was analyzed by GC.MS-Q-2010 Ultra (Shimadzu, Japan) with column Rtx-5MSLength (30 m), Diameter (0.25 mm), Thickness (0.25 ml). The column oven temperature was programmed from 30 °C to 300 °C. The value of the column flow and linear velocity were 1.55 ml/min, 45.3 cm/sec respectively. The detector (mass spectrometer) and injector (split mode split ratio -1.0 at the pressure (96 kPa) temperatures were programmed.

MS conditions
Detector mass spectrometer was programmed. The temperature of ion source and interface were recorded at 200 °C and 250 °C respectively. Solvent cut time was 2.50 min and detector gain was relative (detector gain 0.85 kV + 0.00 kV). The process of analysis in detector was
started 3.00 min after the injection of sample in full scan mode (Scan Speed: 1666), ratio mass over charge star was from 50 to 500. The time end of process was 31.00 min.

**GC-MS analysis**

GC-MS analysis was carried out by injection (0.1 µL) of the seed oil on a QP-2010 instrument with a mass selective HP 597A detector fitted with Ulbon HR-1 capillary column (30 m x 0.25 mm, film thickness 0.25 µm). GC- MS operation condition split mode: carrier gas helium at a flow rate of 1.5 ml/min; temperature program 60 - 300ºC (100 C/min), injector temperature 300ºC and detector temperature 280ºC. The mass spectrometry conditioned was as follows: ionization voltage, 70 eV; emission current, 40 mA; mass range 0 – 400 Da, ion source temperature, 200ºC.

**RESULTS AND DISCUSSION**

**Phytochemical screening of crude extract**

The phytochemical screening of fractionated cardamom with different solvents revealed the presence of secondary metabolites as shown in Table 1.

Table 1: Phytochemical screening of cardamom.

<table>
<thead>
<tr>
<th>Fraction1</th>
<th>Fraction2</th>
<th>Fraction3</th>
<th>Fraction4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>Distilled water</td>
<td>Ethanol</td>
<td>acetone</td>
</tr>
<tr>
<td>Phenolic</td>
<td>+</td>
<td>Phenolic</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>Saponins</td>
<td>+</td>
</tr>
</tbody>
</table>

The symbol + indicates presence while the symbol – indicates absence.

The results showed that all fractions confirmed the presence of phenolic compounds, alkaloids, flavonoids, tannins and saponins. While only distilled water fraction revealed the absence of tannins.

These results are in disagreements with[21], who performed preliminary phytochemical and antimicrobial screening of Syzygiumaromaticum, Elettariacardamomum and piper nigrum
extracts, his results indicated that only flavonoids were conducted from the fractions of distilled water and acetone while the other metabolites were absent. Similarly another investigation of methanolic extract leaves of elettaria cardamomum\textsuperscript{[22]} reported that tannins where not present.

Another study conducted by Bano S \textit{et al.},\textsuperscript{[23]} methanolic extract of cardamom seeds showed the presence of flavonoids, terpenoids and glycosides while phenolic, alkaloids, tannins and saponins were not deducted.

The diversity in these results may be as a consequence of variations in climate, nutrition, plant chemo type, harvesting time and other factors.\textsuperscript{[18]}

Kumar U \textit{et al.},\textsuperscript{[24]} extracted cardamom (\textit{amomum subulatum}) fruits successively with petroleum ether the phytochemical analysis revealed the presence of alkaloids, glycoside, steroids, protein, carbohydrates, terpenoids, tannins and phenolic compound.

\textbf{Chemical composition of the essential oil}

The major components of the essential oil extracted from cardamom were Alpha.-ionone (29.295%), Eucalyptol (18.85%), Santolina alcohol (9.23%), 1,6-octadiene-3-ol,3,7-dimethyl- (8.19%), 2,6-octadiene-1-ol,3-ol,3,7-dimethyl-, (Z)-(7.71%), Cinnamaldehyde, (E)-(4.20%), Terpinen-4-ol (4.06 %), 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-, (3.82%). Acetic acid, 1-methyl-1-(4-methyl-5-oxy-cy (1.44%). as shown in Table 2.

\textbf{Table 2: Main components of essential oil scanning in GC-MS.}

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Area %</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29.29</td>
<td>Alpha.-ionone</td>
</tr>
<tr>
<td>2</td>
<td>18.85</td>
<td>Eucalyptol</td>
</tr>
<tr>
<td>3</td>
<td>9.23</td>
<td>Santolina alcohol</td>
</tr>
<tr>
<td>4</td>
<td>8.19</td>
<td>1,6-octadiene-3-ol,3,7-dimethyl-</td>
</tr>
<tr>
<td>5</td>
<td>7.71</td>
<td>2,6-octadiene-1-ol, 3-ol,3,7-dimethyl-, (Z)-</td>
</tr>
<tr>
<td>6</td>
<td>4.20</td>
<td>Cinnamaldehyde,(E)-</td>
</tr>
<tr>
<td>7</td>
<td>4.06</td>
<td>Terpinen-4-ol</td>
</tr>
<tr>
<td>8</td>
<td>3.82</td>
<td>1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-</td>
</tr>
<tr>
<td>9</td>
<td>1.44</td>
<td>Acetic acid, 1-methyl-1-(4-methyl-5-oxy-cy)</td>
</tr>
</tbody>
</table>
The obtained results of GC-MS analysis indicated that chemical composition profile obtained for EOs was differed from the previous reported results. Özkan OE et al., [19] stated that α-terpinyl acetate (34.55%), eucalyptol (24.91%), (-)-terpinen-4-ol (10.63%), linalool (7.69%) and neryl butyrate (6.58%) were the major components of the cardamom essential oil. According to the study done by Noumi E., et al[3] on the chemical composition essential oil of three different cardamom species namely; *Elettariacardamomum, Aframomumcorrorima* and *Amomumsubulatum* confirmed that 1,8-cineole was the main common compound between the three tested volatile oil. The tested E. cardamomum showed that the major components were 1,8-cineole (55.4%), α-terpinyl acetat (28.6%) and 4-terpineol (3.3%). Another investigations done by Snoussi M., et al[25] showed the presence of α-terpinyl acetate (45.6%), 1,8-cineole (26%), linalyl acetate (5.6%), linalool (5.2%), α-terpineol and limonene (2.9% both components). Another previous study conducted by Aysegul Mutlu-Ingok et al.[26] reported that major components were α-Terpinyl acetate (43.4 %), 1,8-Cineole (29.2%), linalyl acetate (5.7%), Sabinene (4.3%), Linalool (3.6%), Limonene (2.1%) and α-Pinene (1.3%). Cardamom collected from India contained 1,8–cineole (85.2%) following by cis-ocimene (3.7%) and α-terpinene (2.2%).[18] These differences in chemical composition can be attributed, at least in part, to several factors such as environmental factors, soil characteristics, geographic origin, harvest time and storage conditions.
Antibacterial activity of the essential oil

Antibacterial activity of the essential oil of cardamom was tested using disc diffusion method.[20] The result of the antibacterial activity of essential oil showed in the Table 3.

Table 3: Antibacterial activity of essential oil of cardamom.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Bacillus Subtilis</th>
<th>Pseudomonas aureus</th>
<th>E.coli</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code</td>
<td>NCTTC8236</td>
<td>27853</td>
<td>25922</td>
<td>AACC25923</td>
</tr>
<tr>
<td>Inhibition zone / mm</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

The antibacterial activity of cardamom essential oil was tested in both gram positive and gram negative bacteria. *Bacillus subtilus* NCTTC8236 and *staphilla aureus* AACC25923 was selected to be gram positive bacteria while *E.coli* 25922 and *pseudomonas* 27853 was gram negative. The result revealed that the essential oil was active against gram positive and the gram negative except *E.coli*. The essential oil showed the highest inhibition toward the gram positive bacteria *staphylococcus aureus* AACC25923 10 mm and *Bacillus subtilis* NCTTC8236 5mm, *Pseudomonas aureus* 27853 1mm, while *E.coli* 25922 was not shown any inhibition zone.

Previous reports indicated that the essential oils of cardamom species have been found to have important antimicrobial activities against pathogenic bacterial and fungal strains.[27,28]

**CONCLUSION**

In this study cardamom was successively extracted with methanol and then fractionated by using different solvents to test qualitatively. The phytochemical screening of cardamom showed the presence of phenolic compounds, flavonoids, alkaloids, tannins and saponins. These compounds could be potential control of clinical pathogenic bacteria and fungi. Cardamom essential oil showed diversity in chemical composition due to plant chemo types, climatic conditions, harvesting time and nutritional status. Finally, it can be concluded that the essential oil of cardamom have antibacterial activities against some gram positive and gram negative microorganisms. In view of their wide range of activity, cardamom essential oils may find industrial applications as a natural conservative agent in the food industries, pharmaceutical and medicinal applications.
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REFERENCES


