Chemical Composition and Antimicrobial Activity of the Essential Oil of *Salvia langiera* Poir Herbs Growing Wild in Egypt

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Abstract: *Salvia* has been used since ancient times in folk medicine and many pharmaceutical industries. *Salvia langiera* Poir herbs, growing wild in Egyptian desert, are among nine endemic wild species native to Egypt. The essential oil of Wild Egyptian *Salvia langiera* has not been previously investigated in Egypt. The aim of the present study was to assess the composition and antimicrobial activity of the essential oil of *Salvia langiera* plant growing wild in Egyptian desert. The plant materials were collected from wild population growing in sandy soils near El-Sallum region (41 km eastern of Sallum). The essential oil of the air-dried aerial parts of plant was extracted by hydrodistillation and analyzed by Gas Chromatography-Mass Spectrometry (GC/MS). The essential oil composition of *Salvia langiera* was characterized by a high percentage of oxygenated sesquiterpene (26.44%). The major constituents were Spathulenol (5.90%), Carotol (5.36 %) and cis, trans farnesol (5.34%) followed by caryophyllene oxide (3.96%) and Globulol (2.20%). Oxygenated monoterpene (3.65 %) were Camphor (1.28%) and -Thujone (1.23%) and Linalool (1.14%). -Pinene (2.17%) and Sabinene (1.12%) were the monoterpene hydrocarbons while sesquiterpene hydrocarbons were -Muurolene (1.68%). Other important constituents n-alkane (6.32 %), aliphatic alcohols (9.73%) and fatty acids (21.97%) were present in considerable amounts. On the other hand the essential oil of *Salvia langiera* was more active against *Saccharomyces cerevisiae*, intermediate sensitive with *Bacillus megaterium*, while it was the least against *Bacillus subtilis*.

Key words: Antimicrobial · Essential oil · Lamiaceae · *Salvia langiera*

INTRODUCTION

The genus *Salvia* is one of the most important aromatic and medicinal genera of the Lamiaceae family, includes about 900 species, widespread throughout the world [1]. *Salvia* species richest in the essential oil and have powerful biological activity and pharmacological properties. The oil contain more than 100 different bioactive compounds which mainly include Monoterpene hydrocarbons, Oxygenated monoterpenes, Sesquiterpene hydrocarbons, Diterpenes, not iso-prenoid compounds and Oxygenated sesquiterpenes [2]. *Salvia* species have commonly been widely used as folk medicine as antimicrobial, antioxidant, improvement of cognitive performance and mood, reducing work-related stress, anti-mutagenic, anticancer, anti-inflammatory [3, 4]. Some *Salvia* species have been widely used in coronary heart diseases [5]. *Salvia langiera* belongs to section Salvia. It is a perennial plant growing wild in the Mediterranean area [6]. *Salvia langiera* Poir, growing wild in Egyptian desert and Sinai, is among nine endemic wild species native to Egypt [7, 8]. The leaves of *S. Lanigera* are used as an aromatic tea for a variety of abdominal troubles [9]. Previous reports on the plants have shown the presence of four diterpene-quinones of the royleaneone type [10]. In addition, three diterpenes, namely isocarnosol, 12- hydroxyl isocarnosoal and methyl carnosolate were isolated from the petroleum ether extract of the plant [9].

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The essential oil of wild Egyptian *Salvia langiera* has not been previously investigated in Egypt, the aim of the present study was to assess the composition and antimicrobial activity of the essential oil of *Salvia langiera* plant growing wild in Egyptian desert.

**MATERIALS AND METHODS**

**Plant Materials:** The aerial parts of wildly grown plant of *Salvia langiera* were collected during the flowering period on March, 2010 from wild populations growing in sandy soils near El-Sallum region (41 km eastern Sallum), Matruh Governorate, Egypt. Identification of the species was achieved by Prof. Dr. Loutfy Boulos, National Research Centre (NRC), Dokki, Giza, Egypt. Voucher specimens are kept by Prof. Dr. Loutfy Boulos in the herbarium of NRC, Dokki, Giza, Egypt.

**Isolation of Volatile Oils:** The aerial parts were air-dried 25-28°C for one week. The volatile oil from air dried herb were extracted by hydrodistillation for 3 h using a Clevenger-type apparatus according to the Egyptian Pharmacopoeia [11]. The oil was dehydrated over anhydrous sodium sulfate and stored in refrigerator until analyzed.

**Gas Chromatography:** FID Hewlett-Packard 5890 using DB-5 (methyl-silicone containing 5% phenyl groups) column 25 m x 0.31 mm i.d. Temperature program was; 2 min at 60°C, 60-100°C (2°C/min) and 100-250°C (5°C/min). Carrier gas was helium at flow rate of 1.0 ml/min.

**Gas Chromatography-Mass Spectrometry:** A Hewlett packard 5989A GC-MS system equipped with library software Wiley 138 and NBS75 was used. Capillary GC conditions as above were employed for DB-5 (methyl-silicone containing 5% phenyl groups) column. Injection volume was 1.0µl at 1:50 split. Significant MS operating parameter: ionization voltage 70 eV, scan mass range 40-350u.

**Identification of Components:** Compounds were identified by matching their mass spectra with those recorded in the MS library and further confirmed by injecting the authentic samples of different compounds with the volatile oil and by comparison of the mass spectra with those of reference compounds.

**Biological Activity:**

**Test Organisms:**

**Bacteria:** Strains of bacteria (*Escherichia coli* NRR-B 3704 (Gram negative), *Bacillus subtilis* NRRL B-941, *Bacillus cereus*, *Bacillus megaterium*, *Staphylococcus aureus* (Gram positive) were obtained from the Microbial Chemistry Department, National Research Center, Dokki, Giza, Egypt.

**Yeast:** One pure strain of yeast (*Saccharomyces cervisiae*) was obtained from Agricultural Research Center, Giza, Egypt.

**The Used Media:** Nutrient agar media for bacteria and yeast as described by Harrigan and Margaret [12]. The media consisted of peptone 5g/l, meat extract 3g/l, Agar 15 g/l.

**Method of Antimicrobial Assay:** Disc assay was applied on nutrient agar media with adjusting pH at 7.0. Bacteria were spread on the media and the plates were allowed to dry for 5 minutes. Six millimeters sterilized filter paper discs were dipped into appropriated tested essential oil concentration (10µg/l) in absolute ethanol then the alcohol being evaporated after introducing concentration on the disc. The saturated disc were sterilized under a quartz lamb and put on the plates after inoculation. The control substances used to compare the effectiveness of the investigated preparations were Ampicillin disc (100 µg/disc) antibiotic due to relative high bacteriostatic activity and Canestin (100 µg/disc) for yeast. All these steps were done under aseptic conditions. Bacterial plates were incubated at 30°C for 24 hours while yeast was incubated at 28°C for 48-72 hours. Diameter of inhibition zones was measured to the nearest millimeter for three replicates and the average diameter were calculated. For the estimation of the bacteriostatic and antimicrobial action of the *Salvia langiera* distilled oil preparation, the relative bacteriostatic and yeast activity (Arel) were taken into account being determined from the proportion as follows:

\[
Arel = \frac{\varnothing \text{exam}}{\varnothing \text{A or C}}
\]

\[
\varnothing \text{exam} = \text{Diameter of growth inhibition zone of bacteria and yeast (mm) by the given preparation of determine concentration}
\]

\[
\varnothing \text{A} = \text{Diameter of growth inhibition zone of bacteria by Ampicillin.}
\]

\[
\varnothing \text{C} = \text{Diameter of growth inhibition zone of yeast (mm) by Canestin.}
\]

The numeral values of Arel are the basis for comparing the microbial activity of determined dose of
Salvia langiera distilled oil with the action of Ampicillin and Canestin (control) against the strains of bacteria and yeast, respectively. The following determinations are applied in this comparison:

- For values of Arel > 1: Activity higher than Ampicillin and Canestin activity.
- For values of Arel 0.96 -1: Activity near to Ampicillin and Canestin activity.
- For values of Arel < 0.81: Weak activity less than Ampicillin and Canestin activity.

The degree of sensitivity of the bacteria to Ampicillin and yeast to Canestin determined in the following way as stated by Bauer [13]:

- Zones of growth inhibition above 18 mm sensitive
- Zones of growth inhibition range from 13 to 18 mm intermediate sensitive.
- Zones of growth inhibition below 13 mm resistant.

RESULTS AND DISCUSSION

Essential Oil Composition: Twenty three components were identified, representing approximately 78.47% of the total distilled (essential) oil% (Table 1). The essential oil composition of Salvia langiera was characterized by a high percentage of Oxygenated Sesquiterpene (26.44 %) and the major constituents were Spathulenol (5.90%), Carotol (5.36%) and cis, trans farnesol (5.34%) followed by Caryophyllene oxide (3.96%), than Globulol (2.20%), cis, β-Santalol (1.89%) and β-Ionone (1.79%). Oxygenated monoterpenes (3.65%) were Camphor (1.28%), α-Thujone (1.23%) and Linalool (1.14%). α-Pinene (2.17%) and Sabinene (1.12%) were monoterpenic hydrocarbons, while sesquiterpene hydrocarbon was γ-Muurolene (1.68%). Other important constituents n-alkane (6.32%), aliphatic alcohols (9.73%) and fatty acids (21.97%) were present in considerable amounts. The fatty acids were Hexadecanoic acid (9.70%), Octadecanoic acid (3.06%) and Tetradecanoic acid (1.56%). The essential oil composition of Salvia species showed great variation due to plant origin, genetic and environmental factors. The composition of the distilled oil from S. lanigera growing wild in Egypt resulted different when compared with the composition of the oil from plants grown in different location. In Jordan, Flamini et al. [14] reported that Monoterpenes were the main class of the essential oil of S. lanigera (71.7%), followed by Sesquiterpenes (21.7%) and Phenylpropanoids (3.5%). The high percentage of Monoterpenes was largely due to Thymol (54.9%).

Other important constituents were Cedrol (8.9%), Methyl chavicol (3.5%) and Spathulanol (3.4%). The essential oil was largely composed of Oxygenated derivatives (85.9%), mainly Alcohols, ketones, Adehydes and Phenols. In Cyprus, Tenore et al. [15] also identified Thymol as a major oil constituent of S. lanigera. The terpenoidic fraction of the oil amounted to 40.8% with monoterpenes.
Table 2: Antimicrobial activities of volatile oil isolated from Egyptian wild Salvia langiera aerial parts against certain bacteria and yeast

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Antibiotic 100µg/disc mm</th>
<th>Distilled oil mm</th>
<th>Numeral values</th>
<th>Arel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli NRR-B 3704*</td>
<td>16</td>
<td>16</td>
<td>1</td>
<td>&lt;Amp.</td>
</tr>
<tr>
<td>Bacillus subtilis NRRL B-941</td>
<td>24</td>
<td>8</td>
<td>0.33</td>
<td>&lt;Amp.</td>
</tr>
<tr>
<td>Bacillus cereus*</td>
<td>18</td>
<td>17</td>
<td>0.92</td>
<td>&lt;Amp.</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>22</td>
<td>18</td>
<td>0.795</td>
<td>&lt;Amp.</td>
</tr>
<tr>
<td>Staphylococcus aureus*</td>
<td>26</td>
<td>10</td>
<td>0.385</td>
<td>&lt;Amp.</td>
</tr>
<tr>
<td>Yeast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharomyces cerevisae**</td>
<td>15</td>
<td>18</td>
<td>1.2</td>
<td>&gt;C</td>
</tr>
</tbody>
</table>

* = pathogenic to human
** = pathogenic to plants
Amp.: Ampicillin antibiotic for bacteria

Numerical values: Calculated in comparison with ampicillin in case of bacteria and canestin in case of yeast
C: Canestin antibiotic for yeast
Arel: relative activity of distilled oils against pathogenic bacteria and yeast

accounting to 24.5%, while the Sesquiterpene fraction was lower (16.3%). Phenolic (21.4%) and Carbonylic (10.9%) compounds were the other main fractions of the oil. Thymol was the most abundant compound (12.1%) being hexadecanoic acid (6.0%), Carvacrol and α-thujone (5.7%). In the monoterpenoidic fraction, the oxygen containing monoterpenes predominated (20.8%) with α-thujone (5.7%), β-thujone (3.8%) and Camphor (3.6%) as the main compounds. Among the Sesquiterpenes, Caryophyllene (3.0%) and Caryophyllene oxide (2.4%) were the main components.

Antimicrobial Activities: Sage is one of the most appreciate herbs for its rich essential oil and its plethora of biologically active compounds extensively used in folk medicine. Data in Table 2 showed the antimicrobial characteristics of Egyptian wild Salvia langiera distilled oil. The obtained data showed that the tested oil was active against all the tested Bacillus cereus, Escherichia coli NRR-B 3704 (Gram negative) bacteria and Saccharomyces cerevisae (yeast). On the other hand, S. langiera oil was more active against Saccharomyces cerevisae, intermediate sensitive with Bacillus megaterium, while it was the least against Bacillus subtilis. This killing effect was more pronounced in case of distilled oil However, when these data are compared with Ampicillin in case of bacteria and Canestin in case of yeast, the estimated numeral values showed variable activities. Antimicrobial activity of essential oil is one of the most examined features, important for both food preservation and control of human and animal diseases of microbial origin. These observations may be attributed to the nature of biologically active components. Indeed, various chemical compounds have direct activity against many species of bacteria such as terpenes and a variety of aliphatic hydrocarbon (alcohols, aldehydes and ketones). Therefore, a rank of activity has been proposed as follows phenols > aldehydes >ketones >alcohols >esters >hydrocarbons [16]. However, essential oils consisting of numerous components and other major and/or minor compounds) possibly producing a synergistic effect between other components may affect antibacterial activity [17, 18] Numerous reports suggest strong antimicrobial activities of essential oils, especially those belonging to Salvia species [19-22]. The antimicrobial activity could be mainly due to the presence of phenolic compounds, such as Thymol and Carvacrol [23]. It has been also demonstrated that oxygenated monoterpenes such as camphor, 1, 8-cineole, terpinen-4-ol and borneol, which were detected in S. hydrangea oil as major components, have antibacterial activity [24, 25].

If compared with antimicrobial activity of other Salvia species. The effectiveness of the essential oil of S. lanigera collected in Cyprus resulted significantly higher, showing bacteriostatic and bactericidal properties especially against B. cereus, B. subtilis and S. epidermidis by inhibiting the growth of almost all the human pathogenic and/or food spoilage bacteria, moulds and the yeast tested, S. lanigera essential oil exerted a broad antimicrobial spectrum, it may be considered as a natural preservative against food-borne pathogens for the food production industry. S lanigera essential oil possesses a good antioxidant activity and also shows a broad spectrum of antimicrobial activity against referenced strains, especially the Gram-positive bacteria [16].
REFERENCES


