Essential Oil Secondary Metabolites Variation of *Salvia palaestina* Leaves Growing wild from Different Locations in Palestine

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**ABSTRACT**

Herbal medicine is widely practiced in Palestine. *Salvia palaestina* (Lamiaceae) in particular is heavily used because of its acquired traditional reputation over the years rather than scientific basis. *S. palaestina* essential oils contain secondary metabolites whose production is influenced by many factors that determine their composition and yield. Wild leaves of *S. palaestina* were collected from eight different locations in Palestine. Air dried leaves were subjected to steam distillation (SD) and the composition of essential oils was determined for the first time by GC-MS in the electron impact mode. Twenty volatile and semi-volatile components were identified. The major components in all samples were eucalyptol and the percentages ranged from 51.9% to 63.06%. Other components were found but to a lesser extent mainly β-Thujene, β-Myrcene, (±)-Camphor, α-Terpinol, and β-Caryophyllene.

**INTRODUCTION**

*Salvia* is the largest genus of Lamiaceae family that comprises nearly a thousand species (Hedege, 1992). The name is derived from Latin word (*salvare*) which means ‘to save or to cure’ in reference to its curable medicinal properties (Keller, 1978). Several studies showed that *Salvia* genus is a valuable source of potent essential oils (Packham, 1997). Specific biological activities such as antimicrobial, antimalarial, antioxidant, antitumor, antidiabetic, anxiolytic, sedative and anti-inflammatory activities were reported (Esmaili et al., 2008; Kelen and Tepe, 2008; Loizzo et al., 2008; Loizzo et al., 2007; Jaber et al., 2013). *Salvia palaestina* (*Meramia* in Arabic), is heavily used in almost every Palestinians home in the form of herbal tea by either infusion or decoction. Its reputation plays an integral part of the cultural heritage and the public healthcare practice (Sawalha, 2008). Nonetheless, the usage of this plant is relied on traditional belief rather than scientific consciousness.

*S. palaestina* essential oils secondary metabolites from Palestine has never been analyzed as per our recent intensive literature survey revealed. It was believed that different factors might determine the composition and yield of these essential oils secondary metabolites. These variables include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized, post-harvest drying and storage conditions (Juergens et al., 2003). In the present study, we investigated the secondary metabolites phytochemicals of wild *S. palaestina* leaves by Gas Chromatography Mass Spectrometry (GC-MS) which is a highly efficient and precise tool used for separation and identification of volatile compounds from oil mixtures.

**MATERIALS AND METHODS**

**Plant materials**

Wild *S. palaestina* leaves were collected from eight different locations in Palestine between April and May 2013. Leaves were air dried in the absence of light at room temperature for about one week until constant weight is achieved.
Reagents
GC grade n-hexane solvent and anhydrous sodium sulfate salt were purchased from Sigma-Aldrich Inc. (USA). Kovats retention index (KI) reagent that consist of alkane standard mixture were between C10-C40 (even numbered) were purchased from Fluka, Switzerland. All the reference standards used in the research were kindly supplied by the Central Public Health Laboratory, Ministry of Health, Ramallah-Palestine.

Instrumentation
Essential oils were analyzed using Perkin Elmer, Clarus Gas Chromatography connected to Clarus 600 C mass spectrometer (USA). The GC-MS was operated in the electron impact ionization mode (EI) at 70 eV. Perkin Elmer auto-sampler was used with 2ml vials. The GC is equipped with a fused silica capillary column; DB-5 MS consisted of (5% diphenyl polysiloxane, 95% dimethyl polysiloxane) 28 m × 0.25 mm, coating film thickness is 0.25 µm (Restck, USA). Scanning electron microscope (SEM) was high resolution scanning electron microscope (HR SEM) Sirion (FEI Company) using Shottky-type field emission source and secondary electron (SE) detector. The images were scanned at voltage of 5kV.

Extraction of the essential oils by steam distillation
The essential oils of the S. palaestina leaves were isolated by steam distillation using Clevenger type apparatus for three hours. The water distillate was extracted twice with 100 ml hexane. The hexane fractions were combined and dried over anhydrous sodium sulfate. Then, 300 µL of hexane extract was diluted to 1 mL with hexane and 1 µL of the resulted diluted sample was injected to GC-MS using optimized method. The oil was obtained by evaporation of the hexane by rotary evaporator.

GC-MS chromatographic condition
The flow rate of the carrier gas was 1 ml He/min. Injector temperature was set at 235 °C, the source temperature was at 250 °C and the interface temperature was at 260 °C. Split ratio of 1:20 was adopted during the entire analysis. The column gradient temperature was held at 50 °C for 2 minutes, then raised from 50 °C to 180 °C at a ramp of 5 °C/min and from 180° to 280 °C at a ramp rate of 15 °C/min and held there for extra 5min. Solvent cut time of 4.5 minutes was used to eliminate the solvent gigantic peak. The mass range was from 50 up to 480Da, and of scan interval of 0.2 seconds.

RESULT AND DISCUSSION
Aromatic plants in general exhibit significant morphological and phytochemical variability. Even among the same genus, Salvia, morphology is expected to be very variable since it has about a thousand species (Hedige, 1992).

Usually the secondary metabolites are preserved in the trichomes of leaves in order to avoid direct contact with the vital leaf tissues. Scanning electron microscopy (SEM) of wild S. palaestina fresh leaf was performed. It contains intensive, branched, thin, smooth and dark trichomes which intensity in the lower surface higher than that in the upper surface (Figure 1). This is consistent with the fact that all creatures seek to adapt and cope with its environmental conditions. Thus, S. palaestina leaves is curved to avoid harsh environmental conditions to avoid water and essential oil losses. This also can be observed from the smaller width and from the edges which are curved inward as a cover.

<table>
<thead>
<tr>
<th>Yield of dry S. palaestina leaves oils</th>
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Salvia palaestina leaves were collected from eight different Palestinian governorates between April 2013 and May 2013. Prior the oil extraction, part of fresh leaves was dried and the water loss was calculated to acquire information about water content in the leaves. The essential oils were then isolated by steam distillation (SD). The oil yield was obtained from each location and the ratio between dried and fresh leaves and the water loss % were determined as summarized in Table 1 and Figure 2.

The average oil yield (w/w) % was approximately 0.46 %. In some samples however such as in Anabta, it was undetectable, while it was up to 0.72% in Al-Khader. The water loss of these wild samples was found to range from 36% (Deir Istiya/Salfit) and 69% (Al-Khader/Bethlehem) as shown in Table 1.
and Figure 3. The water loss in the wild leaves apparently depends mostly on the topographical nature, rainfall and relative humidity of their location.

Table 1: *Salvia palaestina* leaves dried/fresh ratio, water loss% and oil yield%.

<table>
<thead>
<tr>
<th>Location</th>
<th>Dried/Fresh Weight Ratio</th>
<th>Average water loss w/w% ± SD(n=3)</th>
<th>Average oil yield w/w% ± SD(n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anabta/Tulkarem</td>
<td>33.2/100</td>
<td>66.8±1.153</td>
<td>ND**</td>
</tr>
<tr>
<td>Beita/Nablus</td>
<td>33.4/100</td>
<td>66.6±1.168</td>
<td>0.074±0.17</td>
</tr>
<tr>
<td>Kafr N'ima/Ramallah</td>
<td>49.6/100</td>
<td>50.4±0.985</td>
<td>0.304±0.096</td>
</tr>
<tr>
<td>Hallul/Hebron</td>
<td>34.6/100</td>
<td>65.4±0.954</td>
<td>0.698±0.085</td>
</tr>
<tr>
<td>Al-Khader/Bethlehem</td>
<td>30.7/100</td>
<td>69.3±1.079</td>
<td>0.72±0.131</td>
</tr>
<tr>
<td>Ya'bad/Enin</td>
<td>46.6/100</td>
<td>53.4±0.874</td>
<td>0.552±0.095</td>
</tr>
<tr>
<td>Deir Istiya/Salfit</td>
<td>63.5/100</td>
<td>36.5±0.493</td>
<td>0.434±0.081</td>
</tr>
<tr>
<td>Abud/Ramallah</td>
<td>45.3/100</td>
<td>54.7±0.862</td>
<td>0.234±0.076</td>
</tr>
</tbody>
</table>

* The oil yield was calculated based on weight of oil to weight of each dried sample. ** ND: Not determined.

Identification of separated components by GC-MS

The essential oils were analyzed by GC-MS in the Electron Impact (EI) mode and identified by comparing with NIST library database and by calculating Kovats Index (KI) values. The total ion chromatograms (TIC) of the GC-MS that comprise the components in wild oil samples collected from Halhul is shown in Figure 4. Twenty major components were identified. The molecular formula, retention time and KI values are summarized in Table 2. The main essential oils contain monoterpenoids, oxygenated monoterpenoids and to a lesser extent sesquiterpenes and diterpenes.

Among all, eucalyptol (oxygenated monoterpenoid) was the predominant component in average concentration exceeding 50%. All the essential oils were analyzed by GC-MS in triplicate. The relative standard deviation percentages (RSD %) were calculated for the essential oil peak areas as shown in Table 3. The precision values were within the acceptable limits.

The GC-MS results were interpreted mainly based on their MS in comparison to typical stored NIST database. The molecular ions and the fragmentation patterns were found to have full match with the NIST library, which indicates excellent conformity of the identified structures.

Wild *S. palaestina* constituents and percentages from different locations

Palestine is unique not only in its geographical location but also in its topographical features which lead to weather and climate changes which in turns leads to biodiversity (Mendelsohn and Yom-Tov, 1999). Since the composition of essential oil is affected by these factors, the differences in oil composition of *S. palaestina* from one location to another was obvious.

Wild *S. palaestina* secondary metabolites constituents and percentages were compared from all locations as in Figure 5. Some of the samples contain the twenty components such as in Ya’bad sample. However, it was obvious that there were certain indigenous volatiles, which distinguished each location. For example, Al-Khader sample was found to be characterized by high levels of eucalyptol, while Beita sample is differentiated with β-myrcene phytochemical. Abud sample however is characterized with high levels of camphene and camphor while Anabta sample is distinguished with the sesquiterpene, caryophyllene.
The predominant components in all the investigated samples were eucalyptol (more than 50%), camphor (up to 8.7%), caryophyllene, terpineol, β-thujene and β-myrcene. These six components represented about 70% of all components. Eucalyptol is mainly used as an active ingredient in mouthwash, lozenges, ointments inhalants, body powder and cough suppressant preparations. It controls airway mucus hyper-secretion and asthma via inhibition of cytokine production in human monocytes (Juergens et al., 2003; Juergens et al., 2004). In addition, it stimulates immune system response by enhancing the phagocytic ability of human monocytes (Juergens et al., 1998). Eucalyptol has noticeable antimicrobial activity with minimal side effects when applied either topically or systemically. Therefore, it is used in many antiseptic preparations and to reduce inflammation and pain. Due to its pleasant smell, it is used as a fragrance to impart a fresh and clean aroma in soaps, lotions, detergents and cosmetics. Recently, several studies revealed that it might have anti-tumour activity since it kills leukaemia cells in vitro (Moteki, et al., 2002). Thus, it is advisable to use wild S. palaestina leaves in the preparation of pharmaceutical dosage forms that contain eucalyptol as active or inactive ingredient.

Due to the aforementioned factors, differences between S. palaestina components from one location to another are reasonable.
CONCLUSION

Twenty volatile and semi-volatile components were detected in wild salvia leaves from different locations in Palestine for the first time and eucalyptol was found to be the predominate component in a percentage exceeding 50%. Other minor components were also found but to a lesser extent namely β-Thujene, β-Myrcene, (±)-Camphor, α-Terpineol, and β-Caryophyllene. Eucalyptolis probably the principle phytochemical responsible for the pharmacological activities of wild S. palaestina.

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