Characterization of Chemical Components in Organs Emissions of Two *Salvia dominica* L. Populations from Jordan

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Abstract: The current investigation reports the chemical composition of volatile organic compounds emitted from the different aerial organs of two populations of *Salvia dominica* L. from Jordan collected from Mediterranean and Irano-Turanian bio-geographical zones. Oxygenated monoterpenes dominated the emission profiles of most organs from the two populations but with variable qualitative and quantitative differences. Mediterranean samples contained higher content of oxygenated monoterpenes (stems: 88.37%, leaves 89.95%, pre-flowering buds 67.14%, fully opened flowers 79.43%, sepal 90.93% and petals 92.25%) as compared to those from Irano-Turanian origin (range 39.85% to 75.06%). trans-Sabinene hydrate dominated the emission profiles of all organs from Irano-Turanian zone (range 38.54% to 73.24%) in addition to the stem, sepal and petal samples from the Mediterranean zone (51.37% to 86.98%). The other organs from the Mediterranean zone were dominated by α-terpenyl acetate (27.66-54.87%). Cluster and Principle Component statistical analysis classified the two populations into two clusters based on their origin. The current study evidenced the different VOCs composition in the two populations, that was mainly related to climatic and environmental conditions and suggested the presence of two ecotypes of *S. dominica* L. in Jordan.

Key words: *Salvia dominica* L., aroma emissions, volatile organic compounds, Principle Component Analysis, ecotype, GC/MS

1 Introduction

Several *Salvia* plants have been the subject of thorough phytochemical and ethnopharmacological investigations due to their importance in cosmetics, foods and pharmaceutical industries¹-². Many *Salvia* species were described in traditional medicine for the treatment of different ailments including bronchitis, tuberculosis, hemorrhage and menstrual disorders³ and many of these plants were reported to possess antimicrobial, anti-inflammatory, anti-tumor, antifungal and anti-diabetic properties⁴-⁶.

There are 25 *Salvia* species growing wild in Jordan⁷-⁸. *Salvia dominica* L. is a perennial herb, shrubby, woody at base that is 30-70 cm long with many basal branches and hairy aromatic 3-9 cm lengthen leaves. The flowers are 1.5-2 cm long characterized by their creamy color and yellow lower lip (Fig. 1). Flowering of this plant occurs during spring season (April-June). The plant is known to grow wild in different locations belonging to the Mediterranean and Irano-Turanian bio-geographical zones of Jordan. The plant is used like *S. triloba* as an anti-colic astringent plant for the treatment of common colds, abdominal pain and indigestion⁹-¹⁰.

Literature survey reveals that the chemical composition of the essential oil obtained from whole aerial parts of *S. dominica* L. was investigated⁹. Phytochemical analysis of *S. dominica* resulted in the isolation and characterization of 18 compounds including oxygenated monoterpenes (88.37-90.93%), sesquiterpenes (7.63-11.67%), alcohols (0.61-0.93%), esters (0.93-1.67%), ketones (0.61-0.93%), ethers (0.61-0.93%), hydrocarbons (0.61-0.93%) and other compounds (0.61-0.93%). The oxygenated monoterpenes were dominated by trans-Sabinene hydrate (51.37-86.98%) and α-terpenyl acetate (27.66-54.87%). The other compounds were dominated by *S. triloba* as an anti-colic astringent plant for the treatment of common colds, abdominal pain and indigestion⁹-¹⁰.

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of many new sesterterpenoids\textsuperscript{11, 12} with interesting tubulin tyrosine ligase inhibition\textsuperscript{11, 13}. Additionally, the extract of the plant showed interesting anti-tumor activity against MCP-7 cancer cell lines and \(\alpha\)-amylase inhibitory activity\textsuperscript{14}.

Lately, there has been an interest in the determination of the chemical composition of the aroma emitted from plants. Plant terrestrial (roots and rhizomes) and aerial organs (stems, leaves, floral organs at pre, full flowering and post flowering stages) emit a large and variable number of volatile organic compounds (VOCs) that are useful for the interaction of the plant with its direct environment and signal communication with neighboring plants\textsuperscript{15}. Factors like soil, light, climate and vegetative state are known to have a direct effect on the composition of VOCs emitted from different plant organs. Moreover, VOCs are emitted by the plants to reduce negative abiotic or biotic stresses\textsuperscript{15}.

In light of our interest in phytochemical investigation of S. dominica \textsuperscript{L.} growing wild in Jordan, we report here Solid Phase Micro Extraction (SPME)-GC/MS characterization of the chemical constituents in the aroma emitted from different organs of two \textit{S. dominica} \textsuperscript{L.} populations collected from two biogeographical zones of Jordan. Moreover, the obtained data were statistically analyzed using Principle Component Analysis (PCA) and Cluster Analysis (CA) to investigate similarities or differences in the chemical composition of aroma VOCs to check the possibility to identify the plant based on chemical composition or origin.

2 Experimental Procedures

2.1 Plant material and SPME extraction of VOCs

The aerial parts of \textit{S. dominica} \textsuperscript{L.} were collected from Mediterranean zone (Al-Salt, BAU/LAM/SDhs-2019) and from the Irano-Turanian zone surrounding Al-Karak governorate (BAU/LAM/SDka-2019) on May-2019. The plant material was identified by Prof. Dr. Hala I. Al-Jaber, Department of Chemistry, Faculty of Science, Al-Balqa Applied University, Al-Salt, Jordan.

SPME was performed according to the procedure mentioned in the literature and using the same SPME-fiber and assemblies for manual sampling (DVB/CAR/PDMS, Stable/DVB; fiber length 2 cm, Supelco, Bellefonte, PA, USA). Sample from each fresh selected organ (0.5 g) was introduced into a 15 mL amber glass vial tightly capped with PTFE-coated septa. Extraction was allowed for 10 min at room temperature using a conditioned fiber assembly. Desorption of the analytes was carried out at 240\degree C for 60 s.

2.2 GC-MS and GC-FID analysis

Both experiments were performed according to the procedure described in the literature using the same instruments, columns and temperature program\textsuperscript{17, 18}. An \(\text{n}\)-alkane hydrocarbon standard mixture (\textit{C}_\text{10}-\text{C}_\text{30}) was analyzed separately under the same chromatographic conditions. The measured relative peak areas of the aroma components were used to calculate the concentration of the detected components. The retention indices (RI) were calculated using a series of \(\text{n}\)-alkanes (\textit{C}_\text{6}-\text{C}_{20}) for the DB-5 column. Each sample was repeated twice.

2.3 Identification of VOC constituents and statistical analysis

Identification of individual components was confirmed by comparing their experimentally obtained retention indices (relative to \textit{C}_\text{6}-\text{C}_{30} \text{n}-alkane hydrocarbon standard mixture) with those listed in the literature\textsuperscript{19} and through computer matching of their MS data with those listed in digital libraries \textit{(NIST and Wiley, USA)}. Moreover, the identity of several compounds including \(\alpha\)-pinene, \(\beta\)-pinene, \(\pi\)-cymene, 1,8-cineol, \(\alpha\)-terpineneol, limonene, ocimen, linalool, sabinene and Caryophyllene (Sigma-Aldrich, Buchs, Switzerland) was further confirmed by the analyzing authentic standards under the same GC/MS conditions. R 3.6.1 (The R Foundation of Statistical Computing) with dev tools, ggbi plot, psych and GPA rotation packages were used for the data processing and chemometric analysis.

3 Results and Discussion

Investigation of the chemical composition of volatile organic compounds emitted spontaneously from the fresh aerial organs of the two \textit{S. dominica} populations collected from Mediterranean and Irano-Turanian zones resulted in the identification of a total of 52 components (Table 1). The relative composition of the main classes of VOCs de-
## Table 1  Chemical composition of VOCs detected in the aroma emitted from the aerial parts of *S. dominica* L. two populations.

| No | Lit RI | Exp RI | Compound             | Mediterranean |          |         |         |         |         |         |         |         |         |         |
|----|-------|--------|----------------------|---------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|    |       |        |                      | % S           | % L²      | % Pre²   | % FI²   | % Pet¹  | % Sep¹  | % S       | % L²      | % Pre²   | % FI¹⁰  | % Pet¹   | % Sep²   |
| 1  | 900   | 895    | α-nomane             |               |          |          |          |          |          |          |          |          |          |          |          |
| 2  | 930   | 925    | α-Thujene            |               |          |          |          |          |          |          |          |          |          |          |          |
| 3  | 939   | 934    | α-Pinene*            |               |          |          |          |          |          |          |          |          |          |          |          |
| 4  | 975   | 972    | Sabine*              | 1.35          | 1.50     | 2.17     |          | 0.30    | 0.37    |          |          |          |          |          |          |
| 5  | 979   | 978    | β-Pinene*            | 1.97          | 1.03     | 7.20     |          | 1.76    | 0.19    |          |          |          |          |          |          |
| 6  | 990   | 989    | Myrcene              |               |          |          |          |          |          |          |          |          |          |          |          |
| 7  | 1002  | 1007   | α-Phellandrene       |               |          |          |          |          |          |          |          |          |          |          |          |
| 8  | 1017  | 1016   | α-Terpinene          |               |          |          |          |          |          |          |          |          |          |          |          |
| 9  | 1026  | 1025   | β-Cymene*            |               |          |          |          |          |          |          |          |          |          |          |          |
| 10 | 1029  | 1028   | Limonene*            | 0.65          | 0.54     | 9.72     | 0.57    | 0.70    | 0.99    |          |          |          |          |          |          |
| 11 | 1037  | 1033   | Z-β-Ocimene*         |               |          |          |          |          |          |          |          |          |          |          |          |
| 12 | 1031  | 1030   | 1,8-Cineol           | 10.0          | 6.59     | 9.31     |          |          |          |          |          |          |          |          |          |
| 13 | 1050  | 1045   | E-β-Ocimene*         |               |          |          |          |          |          |          |          | 1.30     | 2.43     | 2.27     | 1.42     |
| 14 | 1059  | 1058   | γ-Terpinene          |               |          |          |          |          |          |          |          | 0.70     | 0.74     | 0.33     | 0.22     |
| 15 | 1070  | 1072   | cis-Sabinene hydrate |               |          |          |          |          |          |          |          | 0.53     |          |          | 0.50     |
| 16 | 1088  | 1084   | Terpinolene          |               |          |          |          |          |          |          |          |          |          |          |          |
| 17 | 1096  | 1093   | Linalool*            |               |          |          |          |          |          |          |          |          |          |          |          |
| 18 | 1138  | 1128   | iso-3-Thujanol        |               |          |          |          |          |          |          |          |          |          |          |          |
| 19 | 1164  | 1170   | 3-Thujanol           |               |          |          |          |          |          |          |          |          |          |          |          |
| 20 | 1199  | 1198   | γ-Terpinol           |               |          |          |          |          |          |          |          |          |          |          |          |
| 21 | 1221  | 1228   | cis-Sabinene hydrate acetate |   |          |          |          |          |          |          |          |          |          |          |          |
| 22 | 1256  | 1252   | trans-Sabinene hydrate acetate | 51.37       | 24.44    | 26.14    | 24      | 64.49   | 86.98   |          |          |          |          |          |          |
| 23 | 1257  | 1255   | Linalool acetate     | 1.97          | 2.2      | 1.51     | 0.97    | 0.09    | 1.35    |          |          |          |          |          |          |
| 24 | 1259  | 1292   | 3-Thujanol acetate   |               |          |          |          |          |          |          |          |          |          |          |          |
| 25 | 1328  | 1314   | Limonene aldehyde    |               |          |          |          |          |          |          |          |          |          |          |          |
| 26 | 1320  | 1333   | Dihydro citronellol acetate |           |          |          |          |          |          |          |          |          |          |          |          |
| 27 | 1349  | 1346   | α-Terpinyl acetate   | 24.31         | 54.87    | 27.66    | 53.00   | 25.3    | 3.69    |          |          |          |          |          |          |
| 28 | 1351  | 1356   | α-Cubebene           |               | 0.56     |          | 2.93    |          |          |          |          |          |          |          |          |
| 29 | 1361  | 1359   | Neryl acetate        |               | 0.67     | 1.70     |          | 0.36    | 0.27    |          |          |          |          |          |          |
| 30 | 1376  | 1376   | α-Copaene            |               | 1.20     | 0.32     | 0.31    | 0.10    |          |          |          |          |          |          |          |
| 31 | 1375  | 1378   | Linalool isobutanoate| 0.71         | 0.28     | 0.52     | 0.79    | 0.60    | 0.43    | 0.18     |          |          |          |          |          |
| 32 | 1388  | 1382   | β-Bourbonene         |               | 0.67     | 0.24     | 0.36    | 0.20    | 0.17    | 1.59     | 0.53     |          |          |          |          |
| 33 | 1388  | 1389   | β-Cubebene           |               | 0.24     |          |          |          |          |          |          |          |          |          |          |
| 34 | 1407  | 1412   | Longifolene          | 1.48          |          |          |          |          |          |          |          |          |          |          |          |
| 35 | 1409  | 1419   | α-Gurjunene          |               |          |          |          |          |          |          |          |          |          |          |          |
| 36 | 1433  | 1424   | β-Gurjunene          |               |          |          |          |          |          |          |          |          |          |          |          |
| 37 | 1441  | 1439   | Aromadendrene*       |               |          |          |          |          |          |          |          |          |          |          |          |
| 38 | 1451  | 1451   | α-Himachalene        |               |          |          |          |          |          |          |          |          |          |          |          |
| 39 | 1450  | 1455   | cis-Muurola-3,5-diene|               |          |          |          |          |          |          |          |          |          |          |          |
| 40 | 1463  | 1461   | cis-Cadma-1(6),4-diene|           |          |          |          |          |          |          |          |          |          |          |          |
| 41 | 1477  | 1470   | γ-Gurjunene          |               |          |          |          |          |          |          |          |          |          |          |          |
| 42 | 1479  | 1476   | γ-Muurolene          | 0.82          | 0.27     | 0.73     |          | 0.19    | 0.08    | 1.32     | 1.22     | 0.26     |          |          | 0.37     |
| 43 | 1481  | 1486   | Germacrene D         | 2.67          | 1.30     | 0.44     | 4.61    | 0.66    | 0.40    | 4.19     | 2.40     | 0.40     | 0.16     | 2.40     | 0.19     |
| 44 | 1490  | 1492   | β-Selinene           |               |          |          |          |          |          |          |          |          |          |          |          |
| 45 | 1492  | 1493   | δ-Selinene           |               |          |          |          |          |          |          |          |          |          |          |          |

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detected in the scent emitted from the aerial organs of *S. dominica* from Mediterranean (MD, sample 1) and Irano-Turanian zone (IT, sample 2) are shown in Fig. 2 while Fig. 3 shows the structures of the main components detected in the current study.

In the Mediterranean zone, GC/MS analysis revealed that emissions from the different organs contained mainly oxygenated monoterpenes (stems: 88.37%; leaves: 89.95%; pre-flowering buds: 67.14%; opened flowers: 79.43%; petals: 90.93% and sepals: 92.25%) mainly due to high concentration levels of trans-sabinene hydrate acetate and α-terpinyl acetate.

Sesquiterpene hydrocarbons were the second class of compounds detected in the aroma of stems (7.61%) and leaves (5.73%), mainly represented in both organs by germacrene D (2.67% and 1.3%, respectively). Monoterpene hydrocarbons were detected at relatively lower concentration levels (3.97% and 4.29%, respectively). The aroma
lacked the presence of oxygenated sesquiterpenes and non-terpenic compounds.

The emitted aroma of closed pre-flowering buds contained the highest amount of monoterpenic hydrocarbons (29.67%) as compared to the emissions of other organs from this zone and limonene (9.72%) and β-pinene (7.20%) were the main components of this class. The emission profile of fully expanded flowers contained higher amounts of sesquiterpenic hydrocarbons (17.44%) mostly represented by germacrene D (4.61%), α-gurjunene (3.05%) and α-cubebene (2.93%). Petals and sepals emitted comparable concentration levels of oxygenated monoterpenes (90.93% and 92.25%, respectively). However, careful inspection of the results reveals that sepals’ emissions contained the highest amounts of trans-sabinene hydrate acetate that amounted to 86.98% of the total emission.

Inspection of the emission profiles of the S. dominica organs from Irano-Turanian zone revealed qualitative and quantitative differences compared to those observed for Sample 1. Oxygenated monoterpenes and trans-sabinene hydrate acetate dominated the emission profiles of all organs from sample 2, but were detected at lower concentration levels as compared to those belonging to the Mediterranean zone. Moreover, it was noticed that the emission profiles of the different organs belonging to the Irano-Turanian zone were richer in monoterpene and sesquiterpene hydrocarbons.

Oxygenated monoterpenes accounted for 45.71% of the total content of stems emissions, of which, 44.19% were due to trans-sabinene hydrate acetate. Monoterpenic hydrocarbons (31.34%) were mainly represented by Z-β-ocimene (15.76%) which was totally absent from the emissions of the stems collected from Mediterranean zone. Also, stems emissions contained higher amounts of sesquiterpene hydrocarbons (21.19%) and the main components detected in this fraction included germacrene D (4.19%) and δ-cadinene (3.08%).

Fresh leaves emissions contained comparable amounts of monoterpenes (oxygenated: 39.85%; hydrocarbons: 39.47%). Again, trans-sabinene hydrate acetate and Z-β-ocimene were the main components in these two classes (38.54% and 24.39%, respectively).

The emission profiles of all flowering organs (pre-flowering buds, fully expanded flowers, sepals and petals) were characterized by high concentration levels of oxygenated monoterpenes (66.21% - 75.06%), due to trans-sabinene hydrate acetate (range 57.34% - 73.24% of the total content). Fully expanded flowers emitted higher concentration levels of monoterpenic hydrocarbons (30.26%) as compared to other flowering organs and limonene was the main component in this class (14.69%). The emissions of the other flowering organs contained Z- and E-β-ocimene isomers, pinene isomers (α and β) and sabinene.

3.1 SPME-GC/M Statistical analysis

In the current work, Principle Component Analysis (PCA) was applied to the data listed in Table 1 to investigate similarities or differences in the chemical composition of the emissions of the different plant organs from the two different bio-geographical zones and to check the possibility to identify the plant based on chemical composition or origin. Therefore, only the first few PCs were studied, best result was obtained upon using a two dimensional PCA score model that accounted for about 45% of the total variation in the data set using the first two PCs. Figure 4 represents the resulted score PCA plot.

Based on the data listed in Table 1, two crossed cluster were obtained. One cluster referred to the plant samples data collected from Mediterranean zone (Al-Salt) and the other one corresponded to the plant samples data collected from Irano-Turanian zone (Al-Karak governorate). Every point in Fig. 4 in the two clusters represents the
data obtained from a particular organ of the plant collected from each site, and results clearly indicates that there is no significant differences in the chemical composition of the aroma extracted from different organs in each location. As could be deduced from Fig. 4, the common crossed area between the two crossed clusters indicates that there is some similarity in the chemical composition of the emitted aroma from the two populations. In order to identify the chemical constituents responsible for the observed similarities and differences that resulted in the creation of these two crossed clusters, a bi-plot (scores and loadings) PCA was created (Fig. 5).

As could be deduced from this figure, β-pinene and neryl acetate had the greatest impact on the similarity in the first PC, while linalool contributed to a less extent on the similarity of the same PC. On the other hand, mainly linalool isobutanoate and α-cubebene to a less extent had the major weight on the first PC (Al-Salt) and were responsible for the differences observed in this figure. On the other hand, valencene, α-thujene and Z-β-ocimene had the major weight on the second PC (Al-Karak governorate). Cluster Analysis (CA) was applied to the same data matrix used for the PCA to obtain more information. The resulted dendrogram of this test is shown in Fig. 6 using complete linkage distances between the organs. The obtained results were very consistent with those obtained from the PCA application. Concerning the relation between the aromas of aerial organs from the Irano-Turanian zone in Al-Karak governorate, a relation was observed between the chemicals detected in the aroma emitted from sepals and petals, pre-flowering buds and fully expanded flowers and these four organs were related to each other. From Al-Salt area, a good similarity was observed between the aroma emitted from the stems and sepals. The emissions of the leaves and flowers
from this location were also similar to each other.

4 Conclusions
This study reports the first knowledge for characterization the chemical constituents of the VOCs detected in the aroma emitted from the different aerial organs of *S. dominica* L. growing wild in Mediterranean and Irano-Turanian zones. CA and PCA classified the two populations into two different clusters based on their origin and indicated the occurrence of two ecotypes of this species, that could be mainly attributed to climatic and environmental factors. The investigation also revealed *trans*-sabinene hydrate as a stable component emitted by the different organs in appreciable amounts from both populations. Accordingly, this compound could be considered for describing the aroma emission chemotypes of wild growing *S. dominica* L.

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