THE SEPARATION AND IDENTIFICATION OF VOLATILE COMPOUNDS IN OFFICINALIS SPECIES OF SALVIA PLANT BY USING CLEVENER METHOD

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Abstract

Salvia (Officinalis species-mint) due to the large amounts of oil essence is known as one of the most popular medicinal plants and is widely used in traditional medicine. In this thesis, the aerial parts of grown officinalis species of Salvia was collected from the greenhouses around the city of Shiraz and dried in the shade for 15 days. Its essence was extracted by Clevenger method and was analyzed by gas-mass chromatography system. Twenty combination in the essence of salvia was identified as the main component which is containing of cis-Thujone (31/35 percent), Eucalyptol (13/11 percent), Globulol (2/33 percent), trans-Thujone (10/05 percent), camphor (21/05 percent), Isoborneol (1/69 percent), α-pinene (4/54 percent), β-pinene (1/68%) and α-Humulene (2/71 percent). The presence of high concentrations of oxygen-containing compounds such as thujones, Eucalyptol and camphor in the essence of sage can cause bacterial properties, antioxidant, antifungal and anti-inflammatory effects of this plant. So Salvia should be studied for biological treatment. Here, antioxidant activity of the essence of sage should be evaluated through the carotene-Linoleic acid system. This essence showed a good antioxidant activity.

Keywords: Identification, Volatile Composition, Salvia Plant, Clevenger methods

I. Introduction

There are several plants that has long been used in traditional medicine because of having medicinal properties from long time ago. Many of clinical applications have been acquired in an experimental and achievable way. However, due to the toxicity and adverse effects of chemicals and synthetic drugs, special attention has been drawn to the medical plants. Accordingly, a need for a simple and reproducible method for the extraction and isolation of active ingredients in these God-given herbs is strongly felt. Salvia plant is one of those plants that including a variety of herbs that have medicinal properties including antioxidant activity, anti-bacterial, anti-fungal, anti-microbial and more. Many of these properties are strongly dependent on the plant's habitat and climatic conditions. For this reason, in this
study, officinalis species of Salvia plants which are produced in greenhouses of Shiraz city and was selected as a sample and the process of separating and the essence-extracting was conducted on it.

Generally, those plants which have the same gender like salvia are one of the important and consumable plants in many of the countries around the world. In Europe, the local people are used and have used from these kind of plants for curing and the treatment of memory loss. In Iran’s traditional medicine has been used of Salvia for the treatment of Diabetes. Among the other medical properties and useful features of this plant in Iranian traditional medicine can be mentioned of facilitating digestion, diuretic, anticonvulsant, antipyretic, antiseptic and nourishing and tonic for the heart. In traditional medicine of China, has been used of these kind of plants for the treatment of Mellitus Diabetes and Pectoral Angina and in the traditional medicine of Turkey has been used of these kinds for the treatment of Rheumatism. According to the vast use and consumption and different functions of these kind of plants, different researchers have studied the different effects of various species of this gender including of the Antioxidant effects, Anti-Diabetes, Esterase Anti-Colin and etc…. and they have been able to prove the different useful effects of the members of these gender. For this reason, it is required that the volatile compounds presented in the Iranian native species need to be fully recognize and the value and effects of each one should be investigated and evaluated in order to have a better and more effective use of these medical plants and herbs.

Features of Officinalis Species of Salvia Plant

Salvia is an herbaceous and perennial plant, with straight root and many ramification of straight and stem and its height is 50-80 cm. The young branches are like dark green and covered with thickly shaggy gray hair. By extending the life of the plant, stem turns to a wooden form and the color turns to brown. The leaves are long and in a lanciform. Lower leaves have long petioles, while the leaves of upper stems, have short petioles. Upper and lower surfaces of the leaves are covered with fine hairs. The flowers are bluish purple, pink or white and are observed in an integrated way in the upper part of the stem on special cycles. There is 8-5 flowers on each cycle. The fruit is nuts and it is like a bright or dark brown.

In a recent study by using two different methods of Clevenger and Dering have extracted essential oil from the officinalis species of Salvia and the resulting compounds were identified by gas chromatography. There is a significant difference between the percentages of extracted compounds in both ways (Baj et al. ², 2013).

Production and Preparation of Plant Sample

Salvia sample was purchased and washed from the greenhouses around the Shiraz and was dried in the shade for 15 days. 50 g of dried herb was divided into small pieces and was transferred to the 500 ml flask of distilled water contents. First, we should boil the contents at 100 ° C for 2.5 h and then at 290 ° C, we should extract essential oil by Clevenger method.
The Total Composition Percentage
Discussion and Conclusion

Separation of Volatile Compounds in Officinal species of Salvia by using Clevenger method

The Salvia plant firstly was extracted essential oil by Clevenger method then separated by gas chromatography and the results are stated in (Table 1, 4). As can be seen here 20 volatile compounds were isolated from this plant, so that α- Thujone combination with 31/35%, has the highest material and γ-Terpinene combination with 0/32 percent, has the lowest presenting material and ingredients in this plant.

On the other hand, as shown in (Fig. 4: 2) oxygenated monoterpenes, with a total percentage of 79/38, and has the most material and the oxygenated sesquiterpene with a total percentage of 2/57, has the minimal ingredients in this herb.

The introduction of volatile Compounds in Officinal Species of Salvia

1. Camphene

This combination is extracted in a molecular formula of C_{10}H_{16} and molecular weight of 136/23 g / mol with boiling point of 159 C °. Insoluble in water but soluble in organic solvents. Its appearance is like a solid crystalline form and it is available in many of the necessary oils such as Turpentine, Oil Cypress, Oil Camphor, Oil Hyacinth Hindi, Neroli, Oil Ginger, and Hyacinth and Valerian. It is volatile in the temperature of room and makes a strong odor. In the industry they produce Isomerization Catalytic of Pinene Alpha. Camphene is also used in the preparation of the perfumes and as one of the taste additive of foods.

2- Pinene

Pinene is a chemical compound of two-loop monoterpenes with the molecular formula of C_{10}H_{16} and mass molecular of 136/24 g / mol and with the Point boiling of 155 - 156 C ° and it is insoluble in the water. Pinene has two structure of Alpha and Beta Isomer. The alpha of Pinene could be found in conifers and the great number of other plants. Pinene is main part of necessary oil of the Sideritis species and salvia species, resin of the terebinthus pistachios (that usually known as the Luggage tree or the Turpentine tree) are full of the Pinene. Pine nuts which are producted by the pine
trees is also consisting of Pinene. In chemical industry, the selective oxidation of Pinene with some of the catalysts, creates ingredients of many of the perfumes, such as artificial perfume. The most important product of oxidation is *verbenone*.

3. Myrcene

A combination with a molecular formula of C$_{10}$H$_{16}$ and mass molecular of 136.23 g / mol and the boiling point of 166-168 C ° and in herbs such as wild thyme, parsley and sage can be found. Myrcene has an analgesic and anti-inflammatory properties.

4. Para Seaman

An aromatic compound with molecular formula of C$_{10}$H$_{14}$ and molecular weight of 134.21 g / mol and Boiling point of 177 C ° and in plants such as cumin and thyme is seen to the fore.

5. Eucalyptol

A cyclic ether of monoterpens family with molecular formula of C$_{10}$H$_{18}$O and molecular mass of 154.25 g / mol and the boiling point is 176-177 C °. More than 90% of the essential oil of plants is eucalyptus. As well in Odor leaf of camphor, red leaf which is willing to be a bright red, the tea tree, *mugwort*, sweet basil, wormwood, rosemary, salvia, cannabis, and other horn and leaf of the aromatic plants can be found.

One element is in many brand marks of mouth lotion and cough, and as a non-active element in the powder of body.

6. Gamma Trpynn

Chemical compound with the molecular formula of C$_{10}$H$_{16}$ and the molecular weight of 136.23 gmol$.^1$ and the boiling point is 183C °. Trpynn groups are including of alpha, beta and gamma.

7. Tojon

It is a ketone compound from the monoterpenes family with the molecular formula of C$_{10}$H$_{16}$O and molecular mass of 152.23 g/mol$.^1$ and the boiling point is 201 C ° and has both alpha and beta. It will be found in the some of the plants, such as Cypress Pine, some of the *Arar* tree, *mugwort*, Pennyroyal Mountain, common salvia, *tansy*, and Wormwood, especially large mountain Sagebrush (Artemisia Absinthium), and also in the different species of Mentha (mint).

8. Camphor

The chemical composition of the terpenoids family with molecular formula of C$_{10}$H$_{16}$O and molecular mass of 152.23 gmol$.^1$ and the boiling point is 204C °.

9. Borneol
The chemical composition of terpenes family with molecular formula of \( C_{10}H_{18}O \) and molecular mass of 154.25 g mol\(^{-1}\) and the boiling point is 213 °C. Borneol can be found in several species of the Sagebrush, *Dipterocarpaceae, balsamifera Blumea* and *Kaempferia*.

This combination has the both L and D-enantiomer.

10. Trpynn 4-L

The chemical composition of monoterpenes family with molecular formula \( C_{10}H_{18}O \) and molecular mass of154/25 g mol\(^{-1}\). This combination in the oil of tea tree and nutmeg can be found in abundance.

11. Caryophyllene

The chemical composition of the sesquiterpene terpenoides family with molecular formula of \( C_{15}H_{24} \) and molecular weight of 204.36 g / mol and the boiling point is 129-130 °C. This combination can be found in large plants such as *Black Caraway, Oregano, Rosemary*, etc..

12. Humulene

The chemical composition of sesquiterpene Terpenoides family with molecular formula of \( C_{15}H_{24} \) and molecular weight of 204.36 g / mol and the boiling point is 106-107 °C. This compound has anti-inflammatory effects. Can be found in plants such as officinal sage, ginger, oregano, bay leaf and cannabis.

Salvia is used in this study for comparison with other species sage such as *Lavandulifolia, Sclarea, Triloba, Verticillata, Fruticosa* and *Mirzayanii* seven different combinations of these plants were selected and the results of this comparison are presented in (Table 1).

**The table 1: Comparison of Composition Percentage Between Different Salvias**

<table>
<thead>
<tr>
<th>The Composition</th>
<th>Composition Percentage</th>
<th>This Study</th>
<th>(A)</th>
<th>(B)</th>
<th>(C)</th>
<th>(D)</th>
<th>(E)</th>
<th>(F)</th>
<th>(G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Thujone</td>
<td></td>
<td>31/35</td>
<td>18/95</td>
<td>0.45</td>
<td>22.39</td>
<td>0.53</td>
<td>0.31</td>
<td>0.95</td>
<td>0.04</td>
</tr>
<tr>
<td>β-Thujone</td>
<td></td>
<td>10/05</td>
<td>19/96</td>
<td>0.12</td>
<td>3.60</td>
<td>-</td>
<td>-</td>
<td>0.84</td>
<td>-</td>
</tr>
<tr>
<td>Camphor</td>
<td></td>
<td>21.05</td>
<td>18/97</td>
<td>0.99</td>
<td>7.60</td>
<td>-</td>
<td>-</td>
<td>4.48</td>
<td>-</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td></td>
<td>13/11</td>
<td>8.13</td>
<td>0.40</td>
<td>9.60</td>
<td>4.35/</td>
<td>-</td>
<td>58.89</td>
<td>8.03</td>
</tr>
<tr>
<td>α-Pinene</td>
<td></td>
<td>4.54</td>
<td>0.43</td>
<td>-</td>
<td>3.90</td>
<td>5.73</td>
<td>2.32</td>
<td>5.62</td>
<td>0.17</td>
</tr>
<tr>
<td>β-Pinene</td>
<td></td>
<td>1.68</td>
<td>3.96</td>
<td>0.17</td>
<td>9.30</td>
<td>4.78</td>
<td>6.54</td>
<td>5.22</td>
<td>-</td>
</tr>
<tr>
<td>α-Humulene</td>
<td></td>
<td>2.71</td>
<td>3.34</td>
<td>-</td>
<td>5.17</td>
<td>10.16</td>
<td>7.66</td>
<td>1.37</td>
<td>-</td>
</tr>
</tbody>
</table>

(A), (b) and (C) are respectively of Salvia plant and species of *Lavandulifolia, Sclarea* and *Triloba* (Piruzan et al. 3, 2009)
(D) and (e) are respectively of the Salvia plant and wildlife species of *Verticillata* (Naser moadely and colleagues ⁴, 2013).

(F): *Fruticosa* species of Salvia plant (Topcu and colleagues ⁵, 2013).

(G) *Mirzayanii* species of the Salvia plant (Yamini et al ⁶, 2008).

As can be seen the studied officinal species here has the highest percentage of α- Thujone among the plants.

Also, there was a comparison between the percentage composition of the studied officinalis species and other officinalis species in different countries and regions and the results are presented in( Table 5 ² ) and (Fig. 5: 2 ). As you can see there is a great variety among these compounds’ percentages, for example the percentage of α- Thujone composition in the studied species in here, is only less than the officinalis species studied in Brazil. This suggests that weather and climate can strongly affect the percentage of the composition of the plant. These differences can even be observed between varieties grown species in the country (columns (c), (d) and the species studied here).

**The table 2: Comparison of Composition Percentage among Officinalis Species of Salvia in Different Countries**

<table>
<thead>
<tr>
<th>The composition</th>
<th>Composition Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In this Study</td>
</tr>
<tr>
<td>α- Thujone</td>
<td>31/35</td>
</tr>
<tr>
<td>β- Thujone</td>
<td>10.05</td>
</tr>
<tr>
<td>Camphor</td>
<td>21.05</td>
</tr>
<tr>
<td>1,8- Cineole</td>
<td>13.11</td>
</tr>
<tr>
<td>α- Pinene</td>
<td>4.54</td>
</tr>
<tr>
<td>β- Pinene</td>
<td>1.68</td>
</tr>
<tr>
<td>α- Humulene</td>
<td>2.72</td>
</tr>
</tbody>
</table>

(A): Officinalis species of Salvia plant in Brazil (Piruzan and colleagues ², 2009).

(B) Officinal species of the Salvia plant in Netherlands (Ransom et al., 2013).

(C) Officinal species of the Salvia plant in Iran-Abadan (Roshan and Najafian ⁸, 2013).

(D) Officinal species of the Salvia plant in Iran-Isfahan (Badii and colleagues ⁹, 2012).

(E) Officinal species of the Salvia plant in Tunisia (Bouaziz and colleagues ¹⁰, 2009).

(F): Officinal species of the Salvia plant in Algiers (Lakhal and colleagues ¹¹, 2013).

(G) Officinal species of the Salvia plant in Jordan (Abu-Darwish and colleagues ¹², 2013).
Beta Carotene Discoloration Test

In order to assess the anti-radical and the antioxidant effects of studied essence in this research, beta carotene discoloration test was selected. This method is one of the most reliable and useful methods in the measurement of antioxidant activities of herbal essence in vitro environment. This method can be known and used for the simulation of a cellular lipid peroxidation in a laboratory setting. According to the results, it is clear that officinalis essence has a desirable protective effect against the attacks of radicals which are resulted from Linoleic Acid on the composition of beta carotene. In general, the beta carotene discoloration test, is a procedure that is performed in an emulsion system and environment, so the activity of antioxidant compounds is dependent on their polarity. Less polar antioxidants because they usually accumulate in the lipid environment show more antioxidant power in this test than the plasters compounds, and therefore has more effective function in protecting from lipid composition against free radicals and the active species in the model (Denison et al., 2005). However, it should be noted that essential oils are a mixture of different compounds from different chemical groups and therefore, the antioxidant activity of these compounds and their effects resulting from the collection is associated (with various mechanisms that can sometimes intensify, strengthen or weaken each other's effects). Similar results also indicate that the antioxidant potential of this test is desirable officinalis species (Lakhal et al., 2013).

Resources


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1 S. Officinalis
2 Tomasz baj et al., 2013
3 Morgana Pierozan Karen et al., 2009
4 Sepideh Nasermoadeli et al., 2,013th
5 Gulact Topcu et al., 2013
6 Yadollah Yamini et al., 2,008
7 Morgana Pierozan Karen et al., 2009
8 Rowshan and Najafian, 2013
9 Parisa Badiee et al., in 2012
10 Bouaziz et al., 2009
11 Lakhal H et al., two thousand and thirteen
12 MS Abu-Darwish et al., the 2,013th
13 Denison E and Ie, 2,005th