Antioxidant activity, total phenols and variation of chemical composition from essential oil in sage (Salvia officinalis L.) grown under protected soilless condition and open field conditions

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ABSTRACT

Soilless culture considered one of the main components of sustainable protected horticulture. It is desirable to develop techniques of agronomical cultivation to improve essential oil products and their specific compounds. S. officinalis was cultivated as an importation medicinal plant especially nowadays due to different uses such as pharmaceutical, sanitary, cosmetic, and agricultural and food industries over the entire world. The influences of cultivation method (protected soilless vs. open field conditions) on the quality and quantity of nutritional content of essential oil for sage (Salvia officinalis L.) were evaluated in this study. The objective of this study was to compare essential oil content and composition, antioxidant activity by free radical scavenging activity (antiradical) and total phenols as mg Gallic acid equivalents (GAE)/g and flavonoids as mg Catechin Equivalent (mg CE/g of dry weight) in the aerial parts of a sage (Salvia officinalis L.) under protected soilless vs. soil based conditions. Results obtained showed that the highest essential oil percentage (w/w) was obtained under protected soilless conditions (3.297%) which significantly high than that obtained under open field conditions (2.133%). Five major compounds were identified in this study including carvacrol, 1,8 cineole, camphor, α-thujone and β-thujone. The major compound found was camphor and had a concentration of (51.1 μg/g) under protected soilless conditions, which is significantly high than that under open field conditions (16.4 μg/g). 1,8 Cineole was the other major compounds of volatile oils of sage which varied from (37.9 μg/g) under open field conditions, while under protected soilless conditions (20.1 μg/g). Camphor value under open field conditions was (16.4 μg/g) which significantly high than that under protected soilless conditions (5.88 μg/g). The total phenolic content and the antioxidant activity of plant extract were determined, by Folin-Ciocalteau and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays respectively. Phenolic content of plant extract of leaves part S. officinalis was significantly (120 mg GAE/g DW) under open field than that under protected soilless conditions (58 mg GAE/g DW). Antiradical of leaves part under open field conditions was 5mg/g which is highly significant than antiradical of leaves under protected soilless condition which was 3.5 mg/g. The presence of total flavonoids in leaves parts of sage plant were (92 mg CE/g) and (52 mg CE/g) under open field and under protected soilless conditions respectively.

Key words: Salvia officinalis, Essential oil, Phenolic, Antioxidant activity, Flavonoids.

Introduction

Secondary metabolites from medicinal plants having a great attention and desired aromatic with therapeutic qualities, providing source material for the perfume and chemical industries [6]. Among these plants, Sage (Salvia officinalis), is aromatic plant belonging to the Lamiaceae family, which is a perennial plants and native to Mediterranean area. Sage is one of the oldest medicinal plants and has a great economic and industrial importance and most popular aromatic medicinal herbs used for medicinal purposes. In traditional medicine, sage was used for many ailments, including inflammation of the mouth and three. Leaves of sage were used to relieve headache, flatulence, toothache, abdominal pain [4,29,15,1]. Additionally, sage was also used traditionally in food preparation, flavoring agents in perfumery and cosmetics.

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Recently, sage as medical plant with producing high quality essential oil with aromatic properties, used by different industries including pharmaceutical, food and cosmetic, wound treatment, bathing, washing, skin, hair care, spices and for healing of different diseases due natural compounds as essential oil [30,29,9,23,12]. Essential oils are a mixture of volatile and natural substances produced by from different parts of sage plants as secondary metabolites. Essential oils from these plants, used for multiple pharmacological effects including their antibacterial [12] antiviral [25] antioxidant [19] antidiabetic [18] antihyrdrotic, spasmylytic, antiseptic and in the treatment of mental and nervous conditions [8], cardiovascular and anticancer [26], anti-inflammatory [7] antimutagenic [44], antimicrobial [2].

Variation in chemical composition of essential oils, in particular, and extracts of medicinal plants may be observed. Several factors influence the chemical composition of plant essential oils, including genetic structure, climatic factors and the cultural practices such cultivation methods like soilless culture [20, 36, 41], plant organs like leaf, stem and their developmental stages like pre flowering and after flowering [36] the harvesting season[38, 5] developmental stage of collected plant materials [16] and Environmental factors, such as light and moisture content, have strong effects on essential oil production [36,40].

On the other hand, Sage are very rich in phenolic compounds, such as flavonoids, phenolic acids and phenolic diterpenes and possess high antioxidant activities [2, 27, 45]. Phenolic compounds are secondary metabolites, naturally present in all plant materials such as leaf, and shoot [21, 35]. Phenolics are antioxidants with redox properties, which allow them to act as reducing reagents, hydrogen donors, and singlet oxygen quenchers. Phenolic compounds including flavonoids have been reported to accelerate wound healing activity [28, 31]. Antioxidants were usually employed in industry as product additives and in food processing and preservation to prevent undesirable changes due to oxidation an important deterioration process for oil and fats [11]. Moreover, Antioxidants are important because they have the ability of protecting organisms from damage caused by free radical-induced oxidative stress [17]. These compounds can delay or inhibit the oxidative damage caused by free radicals and can protect us against major diseases such as coronary heart disease and cancer in human. Flavonoids and phenolic compounds exert multiple biological effects such as antioxidant, free radical scavenging and anti-inflammatory properties [24, 37]. Oxidative damage in the human body plays an important causative role in disease initiation and progression [22].

At present, the increase on the demand for natural bioactive compounds that can be used as functional compounds for the food industry has led to an exhaustive search of new potential natural sources under protected soilless condition culture conditions. Soilless culture considered one of the main components of sustainable protected horticulture. It is desirable to develop techniques of agronomical cultivation to improve essential oil products and their specific compounds. Soilless culture is a method that permits a good control of plant growth and development, and is currently in practice all over the world [43]. The application of closed systems is important to affect on commercial production of essential oils from medicinal and aromatic plants in the entire world.

In this research, S. officinalis was cultivated as an importation medicinal plant especially nowadays due to different uses such as pharmaceutical, sanitary, cosmetic, and agricultural and food industries over the entire world.

Aims of study was (i) to investigated the yield of volatile oil and identify the chemical compositions of the oil of S. officinalis L. and (ii) to determine its antioxidant, total phenols and falvonoids under protected soilless condition culture conditions and open field conditions in an attempt to contribute to use of these as alternative products and natural antioxidant agent for food and medicinal uses.

Materials and Methods

This study was carried out in Jordan University of Science and Technology (JUST) campus during the growing season 2010-2011 in the open field and protected soilless condition. Seedling of S. officinalis L. was cultivated under open field (Experiment 1) and protected soilless conditions (Experiment 2).

2.1. Experiment 1:

Seeding of sage was transplanted in concrete blocks (95*100*75) in cm (W*L*D) filled with soil mix with peatmoss under soil conditions. Peat moss was applied at 33L/m² at the top layer of soil (20cm) to improve for water holding capacity of soil. Inorganic NPK fertilizer has formula N₂, P₂O₅, and K₂O with (20:20:20) ratio was applied at the rate 30 g/m² at the planting time. After each harvest of sage plants, 25g/m² of NPK fertilizer were added. In addition, 6 g/m² nitrogen fertilizer was added in the form of urea (46%N) after each harvest.

2.2. Experiment 2:

Under protected soilless conditions, seedling of sage was transplanted in woods beds (120*110*25 D) in cm (W*L*D), filled with tuff zeolite (Ø 3-8mm). The irrigation water and nutrients were delivered to the plants via drip irrigation twice a day for 15 minute (early morning and evening). The nutrient solutions were prepared manually once per two week. The concentration of ions was used in...
preparation nutrient solution expressed as (mg/L): N: 360, P: 2, K: 283, Ca: 302, Mg: 48, S: 64, Fe: 2.76, Mn: 0.974, B: 0.536, Zn: 0.3, Cu: 0.076, Mo: 0.155.

2.2. Essential oils Extraction:

Essential oils were obtained from dried aerial parts of *S. officinalis* by steam distillation using Clevenger type apparatus for 3 hours. Extraction of essential oil dried with anhydrous sodium sulfate and stored in sealed amber flasks at 4°C until analysis. All experiments were conducted in triplicates and results were expressed based on dry matter weight.

2.3. Gas Chromatography Analysis (GC):

The isolated oils were dilution with hexane (C₆H₁₂), and sample was injection into the gas Chromatographic analysis. The constituents of essential oils of *S. officinal* were identified. Five components were analyzed from essential oils of sage plants including: α-thujone, campher, β-thujone, carvacrol and 1,8-cineole. Identification of aromatic compounds was based on the calculation of their concentration in (μg/g).

2.4. Preparation of Extract:

The extraction procedure for phenolic compounds was based on [33] with modifications, where about 0.5 g (three replicates) of each plant sample was weighed out, and extracted with 50 ml of methanol(CH₃OH). The sample of sage under protected soilless conditions was weighed from first harvested. While, under open field conditions the sample was weighed from two harvested. Extraction was carried out under shaker at overnight at 30°C. Each extract was filtered into a 50 ml volumetric flask using Whatman filter paper No 42. Volume were completed to mark, and allowed to set in the dark until analysis.

2.5. Total Phenolic Content:

The total phenolic content of the solvent extracts was determined by the method using Folin–Ciocalteu reagent and Gallic acid as standard to produce the calibration curve [39]. Briefly, 2000 μl of the plant extract (triplicate) were transferred into a test tube, and then mixed with 2.5ml of 10% Folin-Ciocalteu reagent. After 3 minutes for allowing the reaction to take place, 2000 μl of a 10 sodium carbonate (Na₂CO₃) was added. The tube was allowed to stand for 1hr at ambient temperature, and the absorption was measured at 760 nm using UV-VIS spectrophotometer (model Spectro Scan 50) against a blank, with contained 50 μl of menthol in place of sample. Different concentrations of Gallic acid in methanol were tested to obtain a standard curve. Total phenolic content was expressed as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g dw).

2.6. Free radical-scavenging activity: DPPH assay:

Scavenging activity of DPPH radicals of sage plant was measured according to the method described by [10]. The antioxidant activity was determined by2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay as described previously with some modifications. Briefly, Fresh DPPH stock solution (25ml) was prepared daily. The solution was prepared by weighing 50mg/100ml, which represent the amounts we need, then 0.0125g of DPPH was dissolved in 25 ml methanol, which resulted in purple color solution. The mixture was mixed thoroughly and allowed to stand in the dark for 60 min. Absorbance at zero time (At o) at 517nm wavelength was determined. Absorbance then was read at 517 nm, against the blank. The percentage inhibition of DPPH free radical was calculated by the formula:

\[
\text{Percentage inhibition (%) = } \left[ \frac{(A \text{ blank} - A \text{ sample})}{A \text{ blank}} \right] \times 100
\]

Where, A blank is the absorbance of control reaction (DPPH alone) and A sample is the absorbance of DPPH solution in the presence of the test compound.

2.7. Determination of Total Flavonoid:

The total flavonoid contents of sage plants under protected soilless and open field conditions were determined by colorimetric method. Each sample (0.5ml) of the plant extract (three replicate) were transferred into with 150 μl of a NaNO₂ solution (15%). After 6 minutes for allowing the reaction take place, 150 μl of an Aluminum chloride (AlCl₃) solution (10%) was added and allowed to stand for 6 minutes, then 2000μl of Sodium hydroxide (NaOH) solution (4%) was added to mixture. 0.2 ml of distilled water was added to bring volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. The tubes were allowed to stand for 1 hr at ambient temperature, and absorption was measured at 510 nm using spectrophotometer against a blank which contain 50 ml of menthol in place sample. Catechin was used as calibration standard with different concentrations 10, 30, 80, 100, 150, 200, 300, 400 and 500 mg/l were tested to obtain standard curve; results were expressed as Catechin equivalents (mg Catechin/g dried extract).

2.8. Statistical analysis:

All Data were statistically analyzed using analysis of variance (ANOVA) according to the statistical package MSTAT-C (Michigan State Univ., East Lansing, MI, USA). Probabilities of significance
among treatments and LSD (P≤ 0.05) were used to compare means among treatments.

Results and Discussion

3.1. Essential Oils content:

Table 1 shows the essential oils content (relative to the amount of dried herbs used) were identified. Results obtained showed that the highest essential oil content (w/w) was obtained under protected soilless condition (3.297%) which were significantly higher than the essential oils percentage obtained under open field conditions (2.133%).

3.2. Identification of aromatic compounds of essential oils:

Table 1: Essential oils content and volatile components (µg/g) identified in sage plants under open field and protected soilless conditions.

<table>
<thead>
<tr>
<th>Cultivation methods</th>
<th>Essential oil (w/w %)</th>
<th>α-Thujone (µg/g)</th>
<th>Camphor (µg/g)</th>
<th>β-Thujone (µg/g)</th>
<th>Carvacrol (µg/g)</th>
<th>1,8 Cineole (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open field</td>
<td>2.13</td>
<td>0.378</td>
<td>16.3</td>
<td>0.378</td>
<td>2.28</td>
<td>37.9</td>
</tr>
<tr>
<td>Protected soilless</td>
<td>3.3</td>
<td>0.300</td>
<td>5.88</td>
<td>0.300</td>
<td>51.1</td>
<td>20.1</td>
</tr>
<tr>
<td>Significance</td>
<td>**</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
</tr>
</tbody>
</table>

*: Significant at P≤0.05, **: Significant at P≤0.01.

The result shows camphor, carvacrol and 1,8 cineole are the major components under two conditions. Carvacrol has a high concentration under protected soilless conditions. Whereas, 1,8 cineole had high concentration under two conditions but it was significantly high under soil conditions. Camphor concentration in (µg/g) of dry matter was (51.1) under protected soilless conditions which is significantly high than that under soil conditions (2.28 µg/g). 1,8 cineole was the other major compounds of volatile oils of sage that had concentrations of (37.9 µg/g) and (20.1 µg/g) under soil conditions and under protected soilless conditions respectively. Camphor value under soil conditions was 16.4 (µg/g) which significantly high than that under protected soilless conditions (5.88 µg/g). α-Thujone and β-thujone were not significant under two conditions. [13, 14] reported the 1,8 cineole, α-thujone, β-thujone, borneol and camphor were the major compounds found in the sage essential oils. Most of these reports showed that borneol and 1,8 cineole are the major and principal components of salvia essential oils [34]. The comparison of the present results of essential oils composition of sage plants with other results reported was under soil conditions. While, to comparison the present results of essential oils composition under protected soilless conditions there was fewer reported available.

Identification of aromatic compounds was based on calculation of their concentration in (µg/g) and listed in Table 1. Five major compounds were identified in this study in this oil, carvacrol, 1, 8 cineole, camphor, α-thujone and β-thujone. It can be conclude from Table1 that α-thujone, β-thujone and 1,8 cineole concentration were not significantly, while variation of camphor and carvacrol were significantly. The analysis of the volatile compounds from sage showed its major compounds of essential oil clearly difference between compounds under soil and protected soilless conditions. The major component of the essential oils under soil was 1,8 cineole, followed by camphor and carvacrol as a major compound in present study. While, under protected soilless condition essential oils had dominated by carvacrol. The next most abundant constituents were 1,8 cineole and camphor, respectively.

According to our results, it seems that chemical composition of *S. officinalis* essential oils varied significantly with cultivation conditions. In this way, cultivation *S. officinalis* under protected soilless conditions was important for major compounds.

3.3. Effect of cultivation conditions on yield of total phenols:

Results of quantitative estimation of phenols in the aerial parts of *S. officinalis* under open field and protected soilless conditions are given in Table 2. Sage as medicinal herbs are rich in total phenols and total flavonoids according to the data shown in Table 2. Total phenolic content of methanol extracts of leaves and shoot for sage plants were evaluated using the Folin-Ciocalteu reagent and expressed as mg Gallic Acid equivalent (mg GAE/g of dry weight) under two conditions. The content of phenols varied significantly with two conditions. The greater amount of phenolic compounds leads to higher radical scavenging and the amount of total phenolic of leaves part of sage plants under open field was highly significantly than under protected soilless conditions. Similarly, total phenolic of shoot part of sage was high significance under open conditions than protected soilless conditions. As shown in Table 3 the phenols yield of leaf (120 mg GAE/g) under open field conditions is significantly high than that total phenols of leaf (58 mg GAE/g) under protected soilless conditions. The yield of total phenols of...
shoot part of sage plans under the open field conditions (99 mg GAE/g) was not significant under yield total phenols for shoot part (52 mg GAE/g) under protected soilless conditions. Variations of phenolic concentration of *S. officinalis* under two conditions affirm the influence of climate factors and cultivation conditions on production and release of these metabolites.

### Table 2: Total phenols and flavonoids of two parts (leaves and shoot) for sage plants under open field and protected soilless conditions.

<table>
<thead>
<tr>
<th>Cultivation methods</th>
<th>Total phenols (mg CAE/g)</th>
<th>Total flavonoids (mg CE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Shoot†</td>
</tr>
<tr>
<td>Open field</td>
<td>120</td>
<td>99</td>
</tr>
<tr>
<td>Protected soilless</td>
<td>58</td>
<td>52</td>
</tr>
<tr>
<td>Significance</td>
<td>**</td>
<td>ns</td>
</tr>
</tbody>
</table>

*: Significant at P≤0.05, **: Significant at P≤ 0.01.
† Shoot: leaves +stem.

3.4. Effect of cultivation conditions on yield of total flavonoids:

The total flavonoid content of the extract was determined by the method described in the literature [46]. Total flavonoids concentration of methanol extracts of leaves and shoot for sage plants were evaluated using the AlCl₃ reagent and expressed as mg catechin equivalent (mg CE/g of dry weight) under open field and protected soilless conditions. As shown in Table 2, there is large variation in the total flavonoids content of the *S. officinalis* investigated, ranging from 52 to 92 mg CE/g for leaf part, and from 41 to 84 mg CE/g for shoot part under two conditions as mg GAE/g dry weight for those of aqueous and methanolic extracts, respectively.

Total flavonoid of leave part for sage plants under open field conditions was higher than under protected soilless conditions but not significance. However, total flavonoids of shoot part for sage plants under open field were higher than that under protected soilless conditions and significance. The presence of total flavonoids in leaves parts of *S. officinalis* under open field conditions was varying from (92 mg CE/g) and (52 mg CE/g) under protected soilless conditions as shown in (Table 3). No significant difference for total flavonoids of leaves part under two cultivation methods. In other part of sage plant, flavonoids of shoot parts were high significant under open field conditions (84 mg CE/g) than the protected soilless conditions (42 mg CE/g).

### Table 3: Antioxidant activity of two parts (leaves and shoot) for sage plants under open field and protected soilless conditions.

<table>
<thead>
<tr>
<th>Cultivation methods</th>
<th>Antiradical(1/antioxidant) mg/g</th>
<th>Antiradical(1/antioxidant) mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Shoot†</td>
</tr>
<tr>
<td>Open field</td>
<td>5.23</td>
<td>7.66</td>
</tr>
<tr>
<td>Protected soilless</td>
<td>3.5</td>
<td>3.10</td>
</tr>
<tr>
<td>Significance</td>
<td>*</td>
<td>**</td>
</tr>
</tbody>
</table>

*: Significant at P≤0.05, **: Significant at P≤ 0.01.
† Shoot: leaves +stem.

Data on antioxidant activity are showed in Table 3. The antiradical (1/antioxidant) values varied highly significant in leaves and highly significance in shoot of soil than that under protected soilless conditions. Antiradical of leaf under open field conditions was 5 mg/g which is highly significant than antiradical of leaves under protected soilless conditions which was 3.5 mg/g, but in shoot the significant was highly under open field conditions and the values of antiradical was 8 mg/g and the values under protected soilless conditions was 3 mg/g. It can be observed from present data in Table 2 and Table 3, that antioxidant activity of *S. officinalis* has been attributed to high phenolic content and agreement with [42] who reported that *S. tomentosa*, which exhibited good antioxidant activity and exhibited to high phenolic content.

4. Conclusions:
In conclusion, Cultural practices factors, in combination with environmental conditions appear to be affected on antioxidant activities, total phenolic content, total flavonoids levels in leaves and shoot of *S. officinalis* plants. Comparison of cultivation condition (protected soilless vs. open filed) giving understanding how cultural practices combination with environmental factors affect the variation in antioxidant content of sage plants could represent a significant factor toward optimizing growth conditions for maximal recovery of phytochemicals and antioxidants. In conclusion, this study provides new knowledge for effect cultivation methods with combination environmental conditions on chemical compositions essential oils for sage plant. To produce the best yield of essential oils with a good concentration, it is necessary to combine suitable cultivation methods that optimized the concentration of volatile compounds. Essential oil composition for open field and ported soilless conditions is very variable, it is important that cultivation methods be optimized for some particular volatile compound than others. The major compound found was camphor under protected soilless conditions, which is significantly high than that under open field conditions. While, 1,8 Cineole was the other major compounds of volatile oils of sage under open field conditions. Selection for cultivation methods should be done according to the target compound and therapeutic value of sage.

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