Essential Oils Yield and Heavy Metals Content of Some Aromatic Medicinal Plants Grown in Ash-Shoubak Region, South of Jordan.

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ABSTRACT

The use of aromatic medicinal herbs to relieve and treat many human diseases is increasing in Jordan and worldwide due to their mild features and low side effects. It is important to have a good quality control for aromatic medicinal herbs in order to protect consumers from contamination. The aim of the present study was to carry out a comparative evaluation of essential oils yield and heavy metals content in some medicinal herbs such as Thymus vulgaris L., Thymus serpyllum L. and Salvia officinalis L. grown in Ash-Shoubak region-south of Jordan. The essential oils were hydro-distilled from studied dried aerial herbs using Clevenger-type system. The heavy metals content in collected herbal samples were analyzed by using atomic absorption flame emission spectrometry by method described by Al-Alawi & Mandiwana. Analytical results have evaluated by statistical analysis system. The mean values of essential oils yields of T.vulgaris, T. serpyllum, and S. officinalis were 4.0, 2.5 and 1.9 %, respectively. The contents of heavy metals in the samples were determined in the ranges of 1.26-32.03, 0.47-23.85, 7.66-13.23, 14.7-44.0, 15.8-114.91, 141.3-756.17ppm for Pb, Ni, Cu, Mn, Zn, Fe, respectively. Cobalt has been detected only in T. serpyllum, while Cd and Cr were not detectable in all studied samples. The highest Pb, Ni and Cu content has been detected in T. vulgaris (32.03ppm, 23.85ppm and 13.23 ppm, respectively. S. officinalis had the highest Mn, Zn and Fe content 44.0ppm, 114.91ppm and 756.17 ppm, respectively. The essential oils and heavy metals contents in studied plants are affected by environmental conditions. Moreover, the obtained results showed that the studied plants grown in Ash-shoubak region (with respect to the lead content in T.vulgaris) can safely be used for pharmaceutical and ethno-pharmacological purposes without any hazardous effect.

Key words: essential oils, heavy metals, sage, thyme,

Introduction

The use of aromatic medicinal herbs to relieve and treat many human diseases has been increased in Jordan and around the world because of their mild features and low side effects. [1,2]. At present, there are many aromatic medicinal herbs widely cultivated and consumed in Jordan. Among these herbs, Thymus vulgaris L. (common thyme), Thymus serpyllum L. (wild thyme), and Salvia officinalis L. (sage) which are the most popular aromatic medicinal herbs used for medical purposes or maintaining good health [1,3,4].

T. vulgaris and T. serpyllum are aromatic producing high quality of essential oil required by the pharmaceutical, food and cosmetic industry [5-8] The pharmacological activities of this medicinal plant are antispasmodic, expectorant, antiseptic, antimicrobial, and antioxidant activity [9-11]. S. officinalis (Sage) is a herb considered mainly for its content of essential
oil, it has been credited with a long list of medicinal uses: e.g. spasmylytic, antiseptic, astringent, anti-oxidant and antimicrobial [12-15]. Also, it is largely used as an infusion where polar compounds play a central role [15]. In Jordan, there are twenty species of Salvia cultivated or grow wildly [4,16] used to improve the taste of tea and included in many phytopreparations, for the treatment of inflammations, catarrhs, mouth and throat gargling [15,17].

Medicinal herbs can be easily contaminated with heavy metals from the environment (soil, water, or air) during growth and the manufacturing processes when the ready-made products are produced [18]. Additional sources of heavy metal contamination are rainfall, atmospheric dust, plant protective agents, and fertilizers [19-21]. The level of essential elements in plants is conditional, the content being affected by the geochemical characteristics of the soil and by the ability of plants to selectively accumulate some of these elements [22-23]. Considering the complexity of these aromatic medicinal herbs and their inherent biological variation, it becomes necessary to evaluate their safety, efficacy and quality [3]. While many investigations of the quality values of aromatic medicinal plants grown in Jordan are being reported in the current literature [15,17,22] less emphasis has been made on the metal content of herbal products. As well as T. vulgaris L., T. serpyllum L. and S. officinalis L. have various applications in ethno-pharmacology. It is important to have a good quality control to protect consumers from contamination [4]. Therefore, the aim of the present study was to carry out a comparative evaluation of essential oils yield and heavy metals content in Thymus vulgaris L., Thymus serpyllum L. and salvia officinalis L. grown in Ash-Shoubak, south environmental region in Jordan.

Materials and methods

Plant Material

Aerial parts of wild T. serpyllum, cultivated T. vulgaris and S. officinalis have been collected at vegetation during May, June and July 2008 respectively, from experimental area of the Ash-Shoubak University Collage, in Ash-shoubak region (30° 32N, 35° 33E) in the southern altitudes of Jordan, 1365 m above the sea level. Ambient temperature is 4.11 – 19.9°C and seasonal means of rainfall is about 294.2 mm/year. The collected plant materials were dried in draught place at about 20°C, all specimens were identified on the basis of macroscopic characteristics by comparison with authentic sample and a voucher specimens were deposited by the Herbarium of Ash-Shoubak University College. The dried material of studied plants were separately crushed and mild in to small pieces and sieved through (0.5mm) mesh sieve.

Determination of Essential Oil

Essential oils contents of particular specimens of studied plants were extracted separately by the hydro-distillation method utilizing Clevenger-type apparatus similar to European Pharmacopoeia (EP) (22), using 50 gr of the dried mild and sieved plant (0.5mm), 500 ml of water in 1000 ml round-bottomed flask. The distillation time was 2 h at a rate of 2-3 min⁻¹. The values reported are the mean of at least 3 distillations [22]. There were three replications for each specimen; all the results obtained were statistically analyzed.

Determination of heavy metals content in medicinal plant samples

Heavy metals content of particular collected samples were analyzed using Atomic Absorption. Flame Emission Spectrophotometer Model (Shimadzu, Japan) AA-6200 [23]. The plant samples were oven dried at 70 °C for 24 hours until the dry weight was constant. The dried samples were then ground and passed through a 0.2 mm plastic sieve. Then, 0.5g of plant sample was wet digested with an Ultra-pure nitric acid (HNO₃, 10-15ml) in a polyethylene test tube using a heating block digestion unit at 120 °C. The final solution was filtered into a 25 ml or 50 ml volumetric flask through a 45-µm filter paper and diluted to the mark with ultra-pure water. Ultra-pure water was used for all dilutions and sample preparation.

Results and discussion

Means of essential oil contents in the studied aerial parts of T.vulgaris L., T.serpyllum L. and S.officinalis L. are presented in table (1). The essential oil contents in T.vulgaris L., T.serpyllum, and S.officinalis were 4.0, 2.5, and 1.90 respectively. The results showed that T. vulgaris L. and T.serpyllum contain higher volumes of essential oil than S.officinalis. However, The yields were found to satisfy the requirements of Pharmacopeias such as European Pharmacopoeia (EP), which requires a yield of oil should be ≥ 1.2, 0.3, and 1.0% v/w for T. vulgaris , T.serpyllum, and S.officinalis respectively [23]. On the other hand, the results of previous studies [7,8] revealed a wide variation in essential oil contents in T.vulgaris cultivated in other neighboring places located in the south and middle regions of Jordan. However, Ash-shoubak region yielded higher than these cultivated in other places located in the south of Jordan such as in Ma’an 2.00±0.00% and in Al-Tafelich 2.30± 0.002%(8),or in Mshaqar (middle
region of Jordan), 1.6% [7], but less than those cultivated in the north region of country, where *T. vulgaris* cultivated in Jeresh and Ajlune yielded 5.4±0.12 and 5.2±0.09%, respectively [8]. However *T. vulgaris* cultivated in Ash-shoubak region yielded higher than those cultivated in other regions of the world, where in Egypt, Iran, Brazil, Iraq, and Turkey yielded 1.07, 1.45, 0.56, 1.67 and 1.6%, respectively) [24-28]. The higher volumes of essential oil in *T. vulgaris* was observed with a lower content in *T. serpyllum*. However, the results showed that, *T. serpyllum* grown in Ash-shoubak region yielded higher volumes of essential oil than these investigated from other regions of the world, where in Estonia, the yield of essential oil was ranged between 0.6-4.4 ml/kg, while in Russia, Latvia and Armenia was ranged between 1.9 and 8.2 ml/kg, [29]. This variation of essential oil yielded from a particular species of thyme plant could be explained by the effect of harvesting season [25,26], geographical environment and other agronomical factors [8,30-32].

The essential oils content in *S. officinalis* was 1.90%. This result is in agreement with others who reported that *S. officinalis* cultivated in other locations from the middle region of Jordan; Hfashiet Al Dbajbe and Al Fesalia, yielded essential oil ranged from 1.18 to 2.13% [15]. However, this variation in essential oil yield of *S. officinalis* cultivated in Ash-shobak region in south and other regions of Jordan could be ascribed according to climate variations among regions [30], growth region [7,8,24] and cultivation practices such as spacing [31], irrigation regimen (25), and harvesting time [26,32].

The results of heavy metal concentrations (ppm) in *T. vulgaris*, *T. serpyllum*, and *S. officinalis* grown in Ash-shobak region are presented in table (2). In all investigated medicinal herbs, Cadmium (Cd) and Chromium (Cr) were not detectable. The undetectable concentration of Cd and Cr in all investigated herbs may be due to a low soil content of Cd in suburbanized areas in Jordan or due to cultivation these herbs in locations free off industrial activities such as steel and glass industries that have been shown to be a source of chromium pollution [33,34]. However, high Cd content was detected in urban areas in Jordan [33] near the road and their level increased with increasing traffic densities [35]. On the other hand, Cd was not detectable in *T. vulgaris* cultivated in other regions of Jordan [8] and had not yet determined in *S. officinalis* cultivated in the middle region of Jordan [15]. Comparing Ash-shoubak region to other locations of the world, it was obtained that the studied species grown in that locations are contaminated with Cd and Cr [36,37-39].

The average concentrations of Pb in *T. vulgaris*, and *T. serpyllum*, were 32.03 and 1.26, respectively. The concentration of Pb in *T. vulgaris* cultivated in Ash-shobak region was higher than that detected in wild *T. serpyllum* at the same region and exceeded the level (< 10 ppm) recommended by WHO [3]. However, it was lower than that detected in the north regions [9]. On the other hand, Pb was not detectable in *S. officinalis* cultivated in Ash-shobak region while, it was characterized by higher level of these trace elements in the middle region [15]. The contamination of studied *T. vulgaris* by Pb, could be clarified according to location of cultivation area of these species, especially it was known that motor vehicles are the leading factor in Pb contamination [15,18,21,33-38] while, in wild *T. serpyllum* grown in natural habitat Pb was low and below the toxic level [3]. On the other hand, the variation in Pb content in studied plants may be due the type of soil, species of plant, the availability of plant to absorb the metal and accumulate it [38-40].

The average content of Ni was 23.85 ppm in *T. vulgaris* and not detectable in wild grown *T. serpyllum*. In *S. officinalis*, it was within normal range (0.47 ppm) since the toxic level ranges from 10 to 100 ppm. Also, the Copper (Cu) contents in all studied plants were within normal level (2-20 ppm). The detected level of Cu was ranged from 10.4 ppm in *T. serpyllum* to 13.23 ppm in *T. vulgaris*, while it decreased to 7.66 ppm in *S. officinalis*. This variation of Cu content in the studied samples, could be due to genetic differences between plant species [41], or different plant selectivity for heavy metals [33]. These results are in agreement with the results of others who found that the contents of Cu in some medicinal plants in the same family such as *salvia officinalis*, Mentha *piperita*, Melissa *officinalis*, Ocimum *basilicum*, and Origanum *vulgaris* were varying from 5.92 to 14.79 mg/kg [42]. On the other hand, the concentration of Cu detected in *S. officinalis* cultivated in Ash-shobak is less than that detected in the middle of Jordan, where it was varied from 61 to 70 mg/kg [15], and less than cultivated in other regions of the world. [37,38]. This indicates the effect of anthropogenic activities and heavy traffic activities by which the Cu metal may accumulate in the soil [33,43].

Table 1: Means±SD of essential oil Yields (%) extracted from *Thymus Vulgaris* L, *Thymus serpyllum* L, and *Salvia officinalis* L. collected from Ash-shoubak region-south of Jordan.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Essential Oil (%)</th>
<th>Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thymus Vulgaris</em> L*</td>
<td>4.0 ±0.007</td>
<td>6.00 ±0.020</td>
</tr>
<tr>
<td><em>Thymus serpyllum</em> L</td>
<td>2.5 ±0.008</td>
<td>6.60 ±0.010</td>
</tr>
<tr>
<td><em>Salvia officinalis</em> L</td>
<td>1.9 ±0.002</td>
<td>9.40 ±0.012</td>
</tr>
</tbody>
</table>

*Results of *T. vulgaris* were delivered from previous paper for the same author [9].
As well as Cu is one of important constituent of many enzymes essential for living organisms, also Zn has the same role and considered an important factor in the biosynthesis of enzymes and some proteins. [18,44,45]. The concentration of Zn in plant may vary between 30-150 ppm, but usually it is 20-50 ppm. In the investigated plant materials the highest concentration was found in *S. officinalis*, while the lowest was detected in *T. serpyllum*. The results indicated that *S. officinalis* cultivated in Ash-Shouback south environment is rich with Zn content compared with the middle growth area in Jordan (114.19 vs. 22-29 ppm) [15].

The concentrations of Manganese (Mn) were found to be closely relative to each other in *T.serpyllum*, and *T.vulgaris*. They were 14.7 and, 15.52 ppm, respectively, where the highest concentration (44.0ppm) was found in *S. officinalis*. However, the concentration of Mn in *S. officinalis* was lower than that detected in *S. officinalis* cultivated in middle of Jordan which was ranged from 92 mg/kg to 108 mg/kg [15]. The high content of Mn could be ascribed as a cultivation of plant in industrial and residential sites rich of Mn and Ni due to their using as fuel additives just like Pb [45] since the critical threshold for Mn deficiency in plants is <10 ppm, it is obvious that studied plants were sufficiently provided with Mn.

Ferrous (Fe) concentration in dry plant material typically reaches 1000 ppm or more [38]. In the studied herbs, Fe content was in the following order *T.vulgaris* < *T.serpyllum* < *S. officinalis*. The highest content of Fe was been detected in *S. officinalis* (756.17 ppm), while the lowest was detected in *T. vulgaris* (142.3 ppm). The variation in Fe content in Salvia species and thymus species may be due to plant availability of the essential micronutrients and plant uptake of micronutrients which vary and depend on genetic differences between plant species [20,41]. On the contrary, a low Fe content ranged from 122 mg/kg to 184 mg/kg was found in *S. officinalis* grown in the middle region of Jordan (near highway between Amman and Sahab) [15]. These differences in Fe contents could be ascribed to a well-supplied soil in Ash-shoubak south region, and according to the neighborhood location the cement factory in the south of Jordan [46].

Cobalt (Co) concentration in *T.serpyllum* was within the optimum range 0.02-1.0 mg/kg in the plant [47]. It was 0.40 ppm and not detectable in *T.vulgaris* and *S. officinalis*. In 303 samples, which represent 20 different types of spices and medicinal plants that were collected from areas of exportation in Egypt, Co was not detectable [48]. Also in infusion of *S. officinalis* consumed in Turkey these metal was not detected [37]. The presence of Co in *T.serpyllum* and its absence in other studied herbs could be ascribed that cobalt distribution in plants is entirely species-dependent. The uptake is controlled by different mechanisms in different species. Physical conditions like salinity, temperature, pH of the medium, and presence of other metals influence the process of uptake and accumulation of Co in medicinal plant [49].

**Conclusion**

The essential oils yields in *Thymus vulgaris L.*, *Thymus serpyllum L.* and *Salvia officinalis L.* grown in Ash-shoubak region were found to satisfy the requirement of European pharmacopoeia.

The contents of heavy metals in Ash-Shouback south environment are variable and mainly affected by plant species. *Thymus vulgaris* is rich in Pb content, *Thymus serpyllum* and *Salvia officinalis* are more safely and rich sources of Fe. Further studies of heavy metals concentrations in medicinal plants grown in Jordan environment are recommended.

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**References**


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**Table 2: Concentration of heavy metals (ppm) in Thymus Vulgaris L., Thymus serpyllum L. and Salvia officinalis L. collected from Ash-shouback south region, Jordan.**

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Cd</th>
<th>Co</th>
<th>Pb</th>
<th>Ni</th>
<th>Cu</th>
<th>Mn</th>
<th>Zn</th>
<th>Fe</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thymus vulgaris</em></td>
<td>ND</td>
<td>ND</td>
<td>3.02±0.04</td>
<td>23.85±0.03</td>
<td>13.23±0.13</td>
<td>13.52±0.16</td>
<td>16.10±0.24</td>
<td>141.3±0.67</td>
<td>ND</td>
</tr>
<tr>
<td><em>Thymus serpyllum</em></td>
<td>ND</td>
<td>ND</td>
<td>1.26±0.01</td>
<td>ND</td>
<td>10.40±0.150</td>
<td>14.7±0.06</td>
<td>15.80±0.02</td>
<td>142.00±0.879</td>
<td>0.40±0.003</td>
</tr>
<tr>
<td><em>Salvia officinalis</em></td>
<td>ND</td>
<td>ND</td>
<td>0.47±0.23</td>
<td>7.60±0.77</td>
<td>44.00±0.46</td>
<td>114.91±0.6h</td>
<td>756±5.82</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

*Results of Cd, Cr, Pb, Ni, Cu, Mn, Zn, contents in *T. vulgaris* were delivered for Comparison from a previous paper for the same author [9].