Investigation of the change in essential oil components with the composition of fatty acids from Hypericum heterophyllum Vent., an endemic species in Turkey

Belgin COSGE ŞENKAL[1] and Tansu Uskutoglu[1]

[1]Yozgat Bozok University

June 22, 2020

Abstract

Turkey is an important center in terms of Hypericum species and 49 of the 119 taxa are endemic. Hypericum heterophyllum is one of these endemics. This study was carried out in order to determine some chemical properties of H. heterophyllum. In dry capsules, 14% protein and 23% oil were detected. This oil has been found to be rich in unsaturated fatty acids (oleic and linoleic). The highest essential oil rate (0.09%) in flowering dry herb was recorded in the period before flowering. Also, germacrene-D, bicyclogermacrene, δ-cadinene, spathulenol, α-guaiene, and valencene in herbage essential oil and limonene, cis-ocimene, β-myrcene, and α, β-pinene in dry capsule were determined as main components. It has been noted that both the essential oil content and the components show variation depending on the developmental stages of the plant and the part used.

1. INTRODUCTION

There are 482 *Hypericum* species distributed in different geographies of the world from the equatorial zone to the Nordic countries in the north (Crockett & Robson, 2011; Cırak & Kurt, 2014). Species of the genus *Hypericum* have been used in many parts of the world for many years because of their healing, bactericidal, diuretic, anti-inflammatory and sedative effects (Cırak & Kurt, 2014). Especially, several extracts from *H. perforatum* are used as a drug in Europe (Brutovska et al., 2001). Turkey is an important center in terms of *Hypericum* species and 49 of the 119 taxa are endemic (Guner et al., 2012). One of these endemics is *H. heterophyllum*which is a perennial, shrub form, and blooming in August. Its habitat is reported as *Pinus* woodlands (1200-1600 m altitude) (TUBIVES, 2019). This species is known locally as "Yaraotu" in Turkey (Guner et al., 2012).

Secondary metabolites such as essential oils, alkaloids, glycosides, steroids, saponins, resins etc. are invaluable effects phytochemicals (Baydar, 2013). *Hypericum* species contain a large number of secondary metabolites of at least 11 different classes, including naphthydiantrons, flurogonol derivatives, flavonoids, organic acids, essential oils, amino acids, xanthones, tannins, proxyanidins and other water-soluble components (Greeson et al., 2001; Tanaka & Takaishi, 2006). However, Patocka (2003) refers to the pharmacological effects of*Hypericum* extracts to hyperfine, flavonoids, and essential oils, which are hyperfine and sedohipericin, and flurugonol derivatives, with naphthydiantron pigments. Essential oils secreted by aromatic plants are stored in droplets in some specific metabolic cells and tissues such as secretion hairs, secretion channels and resin channels (Baydar, 2013). They are obtained from different organs of the plant such as leaves, flowers, and stalks. It is known that essential oils have various biological activities. The essential oil isolated from *H. heterophyllum* exhibited antifungal activity (Cakir et al., 2004). The aqueous extracts prepared from aerial parts of this species shown clastogenic and genotoxic effects in human lymphocytes cultures (Ocal & Eroglu, 2012). Also, it was observed that *H. heterophyllum* had significant impact on several bacteria (*Bacillus* sp.,*Esherichia coli* , *Klebsiella* sp., *Pseudomonas* sp.,*Staphylococcus* sp., and *Salmonella* sp.) (Tanker et al.,

1980; Akgoz, 2015). The amount of bioactive substances composed in secondary metabolites (essential oils, saponins, and tannins etc.) varies significantly according to the plant's organs (morphogenetic variability), life cycles of the plant (ontogenetic variability) and the time of collection of the plant (ontogenetic variability) (Baydar, 2013; Ramakrishna & Ravishankar, 2011).

Although there is a great deal of research on the chemical composition of *Hypericum* species, there are limited studies on the distribution of fatty acids. In studies on fatty acid composition, it has been observed that the oil obtained from different parts of some species in this genus are rich in palmitic, linoleic and linoleic fatty acids (Ozen & Bashan, 2003; Ozen et al., 2004).

This study was carried out in order to determine some chemical properties of *H. heterophyllum* , which is an endemic species.

2.EXPERIMENTAL PROCEDURES

2.1. Location information

The aerial parts and dry capsules of *H. heterophyllum* were collected from natural area (Study Area: Inside the Yozgat Bozok University Campus Area; Altitude: 1340 m; Locality: 9°46´48,04´´N-34°48´02,34´´ E) in Yozgat/Turkey. According to the climate data of the area where plant samples were collected for many years, total precipitation was 562.5 mm, average temperature was 9.1 °C, average highest temperature was 14.6 °C, average lowest temperature was 4 °C, average sunshine time was 82.0 hours and average number of rainy days was 113.5 (TSMS, 2020). Identification of plants collected was performed in Biology Laboratory at Yozgat Bozok University/Turkey.

2.2. Plant material

The aerial parts were collected in four different stages as before flowering (BF1, in May), beginning flowering (BF2, in June), 50% of flowering (50%F, in July), and full flowering (FF, in July). Dry capsules were collected in October. In the laboratory, seeds were removed from dry capsules.

2.3. Determination of Chemical Properties

Within the scope of the study, the following chemical properties were examined:

1- Capsule oil content and fatty acids composition

2- Capsule protein content

3- Capsule and herbage essential oil contents and chemical compositions

2.3.1 Capsule oil content and fatty acids composition

For the oil content of the capsule, dry capsules were ground and 2 grams of sample were extracted in a Soxhlet apparatus containing 80 mL of petroleum ether as a solvent. The solvent was then evaporated and the oil content of the sample was determined gravimetrically (AOAC, 2005). 0.1 g oil sample was dissolved in 5 ml hexane. 0.5 ml 0.2N KOH (prepared in methanol) was added. After shaking well, it was kept in the dark for a while. Centrifugation was applied. 1 ml was taken from the upper phase and placed in the vials. Finally, the device was injected with a syringe. The fatty acid compositions of the oil were detected with GC/MS-QP 2010 (Schimadzu) in the Science and Technology Application and Research Center of Yozgat Bozok University (Turkey). The chromatographic method is given in Table 1.

2.3.2 Capsule protein content

Total nitrogen was determined in 0.5 grams ground dry capsule according to Kjeldahl method and the percentage (%) nitrogen ratio was multiplied by 6.25 coefficient and the crude protein content (%) was determined according to Kacar & Inal (2008) principles. Essential oil, oil and protein analyses were performed in triplicate.

2.3.3 Capsule and herbage essential oil contents and chemical compositions

The amount of essential oil in drug herbage and dry capsule were determined by hydro Clevenger distillation device. 100 grams of sample for drug herbage and 50 grams of sample for dry capsule were used. The samples were ground in the blender and 10 times pure water was added, and distilled for 3h. Essential oil values (%, v/w) were calculated by volume over dry mater. The obtained essential oils were taken into dark colored flasks and stocked at 4°C in a refrigerator until they were analyzed (Damyanova et al., 2016).

The chemical components of the essential oil samples from four collection times were defined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) analyses. The analyses were performed using a Shimadzu GC-2010 Plus and Shimadzu GC/MS-QP2010 Ultra instruments. The 0. 1mI oil sample was dissolved in 10ml hexane and shaken vigorously. It was kept in the dark for 1-2 hours. The sample was taken into vials and given to the device. The information about the chromatographic method is given below:

Column: RXI-5MS (0.25um X 30m X 0.25mm)

Scan range: 35-600 m/z

Split ratio: 30

Oven temperature: 60°C for 1 min followed by a temperature rise at a 4 °C/min rate to 250°C (held for 4 min).

Flow rate: 1.50 ml/min

The essential oil components were identified by comparing their mass spectra, retention times, retention indices, and relative to C_5-C_{40} n-alkanes, the FFNSC 1.2 and W9N11.1 mass spectral library, and the literature (Babushok et al., 2011).

2.4. Data analysis

The numerical data were stated as means ± standard error of the mean. The amount of essential oil was made with three replications. Analysis of variance was performed using TARIST package program, and the means were compared using LSD test (p [?]0.05) (Acikgoz et al., 2004).

3. Results and Discussion

The investigated chemical properties of *H. heterophyllum* are summarized in Figure 1 and Figure 2. In dry capsules, 14% protein and 23% oil were detected.

3.1. Fatty acid compositions

The data related to fatty acid compositions of oil obtained from capsule of *H. heterophyllum* are presented in Table 2. This oil consists of 12% saturated and 87% unsaturated fatty acids. In the oil, 13 saturated fatty acids were determined, of which the values of the others, except $C_{16:0}$ and $C_{18:0}$ acids, were recorded below 1%. On the other hand, monounsaturated fatty acids constituted 48% of the oil and $C_{18:1}$ acid had a share of 48% in the six identified fatty acids. As the polyunsaturated fatty acid, only $C_{18:2}$ acid was determined (Figure 3).

The limited research is available on the fatty acid composition of*Hypericum* taxa. Some research results related to the subject are summarized in Table 3 and Table 4. It is seen that the fatty acid composition of the oil obtained from *Hypericum* taxa varies according to the plant and the part of the plant used. For example, $C_{10:0}$ acid was 20.74% in the aerial part of the*H. ericoides* ssp. *roberti* taxon, 1.34% in the aerial part of the *H.tomentosa* species (Hosni et al.,2017), and 3.62% in the flowers of the *H. uniglandulosum* species (Turkoglu et al., 2015). $C_{8:0}$, $C_{10:0}$, $C_{11:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$, and $C_{24:0}$ acids were determined as saturated fatty acids in oils obtained from *Hypericum* taxa. However, it was observed that the ratio of $C_{16:0}$ was higher in these (Table 3). The oil obtained from different parts of *Hypericum* taxon has been found to contain both mono ($C_{16:1}$, $C_{18:1}$, $C_{22:1}$, and $C_{24:1}$) and polyunsaturated ($C_{18:2}$, $C_{18:3}$, and $C_{20:3}$) fatty acids, and the amount of $C_{18:\ 1}$, $C_{18:\ 2}$, and $C_{13:\ 3}$ acids was higher (Table 4).

Oil was obtained from aerial parts, leaves, seeds and flowers of*Hypericum* taxa, but no studies on the fatty acid composition of the fruit capsule were found among the studied literature. The fatty acid composition of oils is the most important factor that determines the commercial uses. Fatty acids and their chemical derivatives play an important role in almost every area of modern life. The main fatty acids found in oils and fats are grouped into saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids (Mariod et al., 2011). The prevailing fatty acids in vegetable oils are unsaturated fatty acids, and also saturated fatty acid ratio is less than 20%. Vegetable oils are divided into six groups as "$C_{12:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, and $C_{22:1}$" considering the dominant fatty acid. Oleic-linoleic acid group is the largest and most important group among the oils (Nas et al., 2001). According to our results, *H. heterophyllum* capsule oil is in the oleic-linoleic acid group. Oleic and linoleic acids have important uses in our daily lives (Table 5). Generally, $C_{16:0}$ acid is the most common saturated fatty acid found in all vegetable oils and animal fats. It is relatively common in $C_{18:0}$ acid. Similar results were taken from *H. heterophyllum* capsule oil (Table 2).

3.2. Essential oils

The aerial parts of *H. heterophyllum* were recorded to contain 0.09+-0.01%, 0.087+-0.006%, 0.05+-0.00% and 0.077+-0.02% essential oil for BF1, BF2, 50%F, and FF stages, respectively (Figure 4). Essential oil contents recorded in the BF1, BF2 and FF stages did not show statistically (p [?]0.05) significant differences. However, the highest and lowest essential oil content was obtained from BF1 and 50%F stage, respectively. In the previous study, it was stated that hydrodistillation of the dried aerial parts of *H. heterophyllum*yielded 0.09% of the essential oil (Cakir et al., 2004). The amount of the essential oil from aerial parts of *H. aucheri* , *H. montbretii* , *H. kazdaghensis* , and *H. perforatum* was 0.28%, 0.22%, 0.17% and 0.23% in the before flowering, 0.27%, 0.20%, 0.24% and 0.33% in the beginning of flowering, 0.33%, 0.23%,0.26% and 0.37% in the full flowering, 0.02%, 0.03%, 0.02%, and 0.05% in the capsule during, respectively (Pasa, 2013). In the four*Hypericum* species, the highest essential oil ratio was obtained from the plants harvested in full flowering period. In our study, the highest rate of essential oil was recorded in BF2, followed by FF.

The chemical components of *H. heterophyllum* essential oils from four different development stages was given in Table 6 The total of 15, 15, 13, and 16 components representing 92.19%, 80.08%, 90.13%, and 84.24% of the total essential oils were detected in the BF1, BF2, 50%F, and FF stages, respectively.

In this study, germacrene-D, bicyclogermacrene, δ-cadinene, spathulenol, α-guaiene, and valencene having significant biological activities were found to be main components of essential oils obtained from different ontogenetic stages of *H. heterophyllum* (Table 6 and Table 8). In the study carried out by Cakir et al (2004), in the essential oil of*H. heterophyllum* , 35 compounds, representing 99.4% of the total essential oil, were determined, and isocaryophyllene (17.1%), α-pinene (11.6%), δ-cadinene (9.5%), γ-muurolene (8.2%), γ-cadinene (5.5%), n-decane (5.8%), and β-Caryophyllene (4.5%) were recorded as major compounds in this essential oil. Although there is similarity between these findings and present study, there are some differences. Essential oil components have been reported to be affected by many intrinsic (genetic, plant origin, type of plant part, stage of development or seasonal sampling period etc.) and extrinsic factors (environmental factors such as climate and habitat conditions, sowing date, cultivation conditions, and postharvest techniques such as drying methods and extractions, distillation time, and conditions of analysis) (Moghaddam & Mehdizadeh, 2017).

The variation of the main components has been given in Figure 5. The highest concentrations of germacrene-D were recorded in the BF1, 50%F, and BF2 stages, respectively. This component showed a significant decrease by average 3 times in the FF stage. Bicyclogermacrene reached the maximum concentration in the BF1 stage. The amount of this compound reduced approximately to half in the 50%F and BF2 stages, and it was not detected in the FF stage. Although the highest amount of δ-cadinene was recorded in the FF period, similar rates were obtained in BF1 and 50%F stages. But a decrease of about 7% was observed in the BF2 stage. The highest ratio of spathulenol was recorded in the 50%F stage, followed by the BF2 stage. The lowest ratio was obtained from BF1 stage. The amount of valencene being among the minor components in the BF1, BF2 and 50%F stages was found to be 9.76 % in the FF stage. α-Guaiene was detected only in the essential oil in the FF stage (Table 6). Significant differences in the concentrations of the main components of*H.*

heterophyllum essential oil were determined according to the developmental stages. A similar situation was observed in the minor components of the essential oil such as β-caryophyllene, α-humulene, aromadendrene, viridiflorol, globulol, salvial-4(14)-en-1-one, isospathulenol, tau-muurolol, α-amorphene, and α-cadinol (Table 6).

In terms of the effect of ontogenetic variability on essential oil components, the full flowering stage was more effective in *H. perforatum* and *H. aucheri* species (Pasa, 2013). The amount of essential oil and the changes in its chemical composition during ontogenesis are specific to each taxon (Németh, 2005). The findings from previous studies showed that there may be similarities and differences in the its chemical composition and amount of essential oil of various species at different phonological stages or harvesting time in*Menhta aquatic* L. (Andro et al., 2013), *Origanum vulgare*L. (Chauhan et al., 2013), *Ocimum basilicum* L. (Lemberkovics et al., 1998), *Cuminum cyminum* L. (Moghaddam et al., 2015), and*Thymus capitatus* L. (Casiglia et al., 2015).

The timing of the harvest or collection of the herbal crops is one of the most important factors affecting the quality of the essential oils obtained from them. The therapeutic properties of herbal drugs are related to the bioactive substances they contain. The amount of bioactive substances composed of secondary metabolites shows significant changes according to the development stage of plant. For this reason, the drug producer must first of all know the bioactive substance exchange of the medicinal and aromatic plant very well and gather the drug which is the richest of the active substances (Baydar, 2013). Essential oils are the most important of other volatile secondary metabolites derived from medicinal and aromatic plants. Therefore, obtaining high essential oil yields with the most desirable chemical compounds is very important for industrial purposes. The selection of appropriate phonological stage can be help researchers to fulfill this requirement (Afshari & Rahimmalek, 2018).

In the dry capsules, 0.087±0.015% essential oil was detected. In the obtained essential oil, 23 components were determined, which make up 99.50% of the essential oil. α-Pinene had the highest value with 33.28% among these components. This component was followed by β-pinene (17.80%), β-myrcene (9.30%), limonene (5.95%), and cis-ocimene (5.45%), respectively. In addition, trans-caryophyllene, α-terpineol, carvacrol, copaene, verbenone and trans- β -ocimene have been recorded as other important components (Table 7). No studies on the capsule essential oil content and composition of *H. heterophyllum* have been found in the literature review. The essential oils examined were found to be rich in the major components that exhibit various biological activities (Table 8).

4. Conclusion

The oil obtained from dry capsules has been found to be rich in unsaturated fatty acids (oleic and linoleic). Considering the usage areas of these two fatty acids, the oil obtained from Hypericum capsules has the potential to be evaluated in different areas. Significant effects of different development periods on herbage essential oil rate and composition were determined. The chemical composition of essential oil in medicinal and aromatic plants is an important factor that determines quality. The amount and composition of essential oil contained in plants is an indicator of the economic value of that product. Considering the change of bioactive substances in plants, it should be well known in which development period the plant will be collected/harvested. According to result of the literature searches, germacrene-D, bicyclogermacrene,δ -cadinene, spathulenol, α -guaiene, and valencene in herbage essential oil and limonene, *cis* -ocimene,β -myrcene, and α, β -pinene in dry capsule were determined that they exhibited important biological activities. A lot of information obtained from this study, in which some plant and chemical properties of *H. heterophyllum* , which is an endemic plant, is examined, is the first. Therefore, the findings obtained will form an important basis for future studies.

Conflict of Interest The authors declare that they have no conflict of interest.

References

Abdalla, A.E.M., Darwish, S.M., Ayad, E.H.E., & El-Hamahmy, R.M. (2007). Egyptian mango by-product 2: Antioxidant and antimicrobial activities of extract and oil from mango seed kernel. Food Chemistry,**103**

:1141-1152.

Acikgoz, N., Ilker,E., & Gokcol, A. 2004. Biyolojik Araştırmaların Bilgisayarda Değerlendirilmeleri. Ege University, Izmir, Turkey.

Afshari, M., & Rahimmalek, M. (2018). Variation in essential oil composition, bioactive compounds, anatomical and antioxidant activity of*Achillea aucheri* , an endemic species of Iran, at different phonological stages. *Chemistry & Biodiversity* , **15:**e1800319. DOI:10.1002/cbdv.201800319.

Akgoz, Y. (2015). The effects of *Hypericum* (Hypericacea) species on microorganisms: A review. *International Research Journal of Pharmacy* , **6:** 390-399.

Ando, H., Ryu, A., Hashimoto, A., Oka, M., & Ichihashi, M. (1998). Linoleic acid and α-linolenic acid lightens ultraviolet-induced hyperpigmentation of the skin. *Archives of Dermatological Research* , **290** :375–381.

Andro, A-R., Boz, I., Zamfirache, M-M., & Burzo, I. (2013). Chemical composition of essential oils from *Mentha aquatic* L. at different moments of the ontogenetic cycle. *Journal of Medicinal Plants Research* , **7:** 470-473.

AOAC. (2005). Official Method of Analysis. Association of Officiating Analytical Chemists, Method 935.14 and 992.24, 18th Edition.

Babushok, V.I., Linstrom, P.J., & Zenkevic I.G. (2011). Retention indices for frequently reported compounds of plant essential oils.*Journal of Physical and Chemical Reference Data* ,**40:** 1-47.

Baydar, H. (2013). Tıbbi ve Aromatik Bitkiler Bilimi ve Teknolojisi. Süleyman Demirel University, Isparta, Turkey.

Boga, M., Ertas, A., Eroglu-Ozkan, E., Kizil, M., Ceken, B., & Topcu, G. (2016). Phytochemical analysis, antioxidant, antimicrobial, anticholinesterase and DNA protective effects of *Hypericumcapitatum* var. *capitatum* extracts. *South African Journal of Botany,* **104** :249-257.

Brutovska, R., Celarova, E., & Schubert, I. (2001). Cytogenetic characterization of three *Hypericum* species by in situ hybridization. *Theoretical and Applied Genetics* ,**101:** 46-50.

Cakir, A., Kordali, S., Zengin, H., Izumi, S., & Hirata, T. (2004). Composition and antifungal activity of essential oils isolated from*Hypericum hyssopifolium* and *Hypericum heterophyllum* .*Flavour and Fragrance Journal* ,**19:** 62-68.

Carrasco, F. (2009). Ingredientes Cosméticos. Diccionario de Ingredientes (4^{th} ed.).

Casiglia, S., Bruno, M., Scandolera, E., Senatore, F., & Senatore, F. (2015). Influence of harvesting time on composition of the essential oil of *Thymus capitatus* (L.) Hoffmans.&Link. growing wild in northern Sicily and its activity on microorganisms affecting historical art crafts. *Arabian Journal of Chemistry* , https://doi.org/10.1016/j.arabjc.2015.05.017.

Chauhan, N.K., Sing, S., Haider, S.Z., & Lohani, H. (2013). Influence of phonological stages on yield and quality of oregano (*Origanum vulgare* L.) under the agroclimatic condition of Doon Valley (Uttarakhand). *Indian Journal of Pharmaceutical Sciences* ,**75:** 489-493.

Cirak, C., & Kurt, D. (2014). *Hypericum* species as important medicinal plants and their usage. *Journal of Aegean Agricultural Research Institute* , **24:** 38-52.

Crockett, S.L., & Robson, N.K.B. (2011). Taxonomy and Chemotaxonomy of the Genus *Hypericum* . *Medicinal and Aromatic Plant Science and Biotechnology* , **5:** 1-13.

Damyanova, S., Mollova, S., Stoyanova, A., & Gubenia, O. (2016). Chemical composition of Salvia officinalis L. essential oil from Bulgaria. *Ukrainian Food Journal* ,**5:** 695-700.

De-Oliveira, A.C.A.X., Ribeiro-Pinto, L.F., & Paumgartten, F.J.R. (1997). In vitro inhibition of CYP2B1 monooxygenase by β myrcene and other monoterpenoid compounds. *Toxicology Letters,* **92** : 39-46.

Eldeen, I.M.S., Mohamed, H., Tan, W-N., Siong, J.Y.F., Andriani, Y., & Tengku-Muhammad, T.S. (2016). Cyclooxygenase, 5-Lipoxygenase and acetylcholinesterase İnhibitory effects of fractions containing, α-guaiene and oil isolated from the root of *Xylocarpus moluccensis. Research Journal of Medicinal Plants* , **10** : 286-294.

Greeson, J., Sanford, B., & Monti, D.A. (2001). St. John's worth (*Hypericum perforatum*): a review of the current pharmacological, toxicological and clinical literature. *Psychopharmacology* ,**153:** 402-414.

Guner, A., Aslan, S., Ekim, T., Vural, M., & Babac, M.T. (2012). Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, Turkey.

Hosni, K., Msaâda, K., Taârit, M.B., & Marzouk, B. (2017). Fatty acid composition and tocopherol content in four Tunisian *Hypericum*species: *Hypericum perforatum* , *Hypericum tomentosum* ,*Hypericum perfoliatum* and *Hypericum ericoides* ssp.*roberti* . *Arabian Journal of Chemistry* ,**10:** 2736-2741.

Jovanovic, T., Kitic, D., Palic, R., Stojanović, G., & Ristic, M. (2005). Chemical composition and antimicrobial activity of the essential oil of *Acinos arvensis* (Lam.) Dandy from Serbia. *Flavour and Fragrance Journal* , **20** :288-290.

Kacar, B., & Inal, A. (2008). Bitki Analizleri. Nobel Publication, Turkey.

Lemberkovics, É., Kéry, Á., Marczal, G., Simándi, B., & Szőke, É. (1998). Phytochemical evaluation of essential oils, medicinal plants and their preparations. *Acta Pharmaceutica Hungarica* ,**68:** 141-149.

Mariod, A., Matthäus, B., & Hussein, I.H. (2011). Fatty acids, tocopherols, and sterols of cephalocroton cordofanus in comparison with sesame, cotton, and groundnut oils. *Journal of the American Oil Chemists' Society* ,**88:** 1297-1303.

Mishra, D., Joshi, S., Sah, S.P., Dev, A., & Bisht, G. (2011). Chemical composition and antimicrobial activity of the essential oils of*Senecio rufinervis* DC. (Asteraceae). *Indian Journal of Natural Products and Resources,* **2** :44-47.

Moghaddam, M., & Mehdizadeh, L. (2017). Soft Chemistry and Food Fermentation. Ed. Grumezescu, A., & Holban, A-H., Chemistry of essential oils and factors influencing their constituents (1st ed.). Academic Press.

Moghaddam, M., Miran, S.N.K., Pirbalouti, A.G., Mehdizadeh, L., & Ghaderi, Y. (2015). Variation in essential oil composition and antioxidant activity of cumin (*Cuminum cyminum* L.) fruits during stages of maturity. *Industrial Crops and Products* ,**70:** 163-169.

Montanari, R.M., Barbosa, L.C.A., Silva, C.J., Carvalho, L.S., & Andrade, N.J. (2011). Chemical composition and antibacterial activity of essential oils from Verbenaceae species: Alternative sources (E)-caryophyllene and gemacrene-D. *Quimica Nova* ,**34** :1550-1555.

Nas, S., Gokalp, H.Y., & Unsal, M. (2001). Bitkisel Yağ Teknolojisi, Pamukkale Üniversitesi Mühendislik Fakültesi, Yayın No:005, Mühendislik Fakültesi Matbaası, Turkey.

Németh, É. (2005). Changes in essential oil quantity and quality influenced by ontogenetic factors. *Acta Horticultarae,***675:** 159-165.

Ocal, A., & Eroglu, H.E. (2012). Genotoxic effects of *Hypericum heterophyllum* Vent. in human lymphocytes cultures. *Advancements in Life Sciences* , **2:** 65-67.

Ozen, H.C., & Bashan, M. (2003). The composition of fatty acids in*Hypericum scabrum* , *H. scabroides* and *H. amblysepalum* . *Turkish Journal of Chemistry* , **27:** 723-725.

Ozen, H.C., Bashan, M., Toker, Z., & Keskin, C. (2004). 3-Hydroxy fatty acids from the flowers of *Hypericum lysimachioides* var.*lysimachioides* . 2004. *Turkish Journal of Chemistry* ,**28:** 223-226.

Pasa, C. (2013). A Research on determination of diurnal, ontogenetic and morphogenetic variations of essential oil content and composition in some *Hypericum* species growing wild in Ida. Ph.D. Thesis, Namık Kemal University, Tekirdağ, Turkey.

Patocka, J. (2003). The chemistry, pharmacology, and toxicology of the biologically active constituents of the herb *Hypericum perforatum*L. *Journal of Appliede Biomedicine,* **1:** 61-73.

Pérez-López, A., Cirio, A.T., Rivas-Galindo, M., Aranda, R.S., & Waksman de Torres, N. (2011). Activity against *Streptococcus pneumonia* of the essential oil and δ-cadinene isolated from*Schinus molle* fruit. *Journal of Essent Oil Research* ,**23** :25-28.

Ramakrishna, A., & Ravishankar, G.A. (2011). Influence of abiotic stress signaling on secondary metabolites in plants. *Plant Signaling Behavior* , **6:** 1720-1731.

Schmidt, J.M., Noletto, J.A., Vogler, B., & Setzer, W.N. (2007). Abaco Bush Medicine: Chemical composition of the essential oils of four aromatic medicinal plants from Abaco Islands, Bahamas. *Journal of Herbs, Spices & Medicinal Plants* , **3** :43-65.

Shafaghat, A. (2012). Omega-3 content, antimicrobial and antioxidant activities of hexanic axtract from seed and leaf of *Hypericum scabrum* from Northwestern Iran. *African Journal of Microbiology Research* , **6** :904-908.

Sieniawska, E., Swatko-Ossor, M., Sawicki, R., Skalicka-Woźniak, K., & Ginalska, G. (2017). Natural terpenes influence the activity of antibiotics against isolated *Mycobacterium tuberculosis .Medical Principles and Practice* , **26** :108-112. DOI: 10.1159/000454680

Smolinske, S.C. (1992). Handbook of Food, Drug, and Cosmetic Excipients (1st ed.). CRC Press.

Tanaka, N., & Takaishi, Y. (2006). Xanthones from *Hypericum chinense* . *Phytochemistry* , **67:** 2146-2151.

Tanker, N., Gurturk, S., & Kol, U. (1980). Antibiyotik aktivite gösteren bazı tohumlu bitkiler üzerinde araştırmalar. *Ankara Ecz Fak Mec.* **10:** 17-29.

Tognolini, M., Ballabeni, V., Bertoni, S., Bruni, R., Impicciatore, M., & Barocelli, E. (2007). Protective effect of *Foeniculum vulgare*essential oil and anethole in an experimental model of thrombosis.*Pharmacological Research* , **56** : 254-260.

TSMS. (2020, March 6). Turkish State Meteorological Service. Retrieved from*www.mgm.gov.tr/forecast-cities.aspx*.

TUBIVES. (2019, February 04). Turkish Plants Data Service. Retrieved from http://www.tubives.com.

Turkoglu, S., Turkoglu, I., Celik, S., Bahsi, M., Gur, S., & Parlak, A. E. (2015). Some biological compounds, antioxidant and antimicrobial activities of endemic *Hypericum uniglandulosum* . *Chemistry of Natural Compounds* , **51:** 615-619.

Ucciani, E., Mallet, G., Gamisans, J., & Gruber, M. (1994). Composici_on en _acidos grasos de los aceites de semillas de especies mediterraneas. *Grasas Aceites* , **45** :107-112.

Yang, I.J., Lee, D-U., & Shin H.M. (2016). Inhibitory effect of valencene on the development of atopic dermatitis-like skin lesions in NC/Nga mice. *Evidence-Based Complementary and Alternative Medicine* , **11** . DOI:10.1155/2016/9370893.

Hosted file

`Table 1.docx` available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Table 2.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Table 3.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Table 4.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Table 5.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Table 6.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Table 7.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Table 8.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Figure 1.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Figure 2.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Figure 3.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Figure 4.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey

Hosted file

Figure 5.docx available at https://authorea.com/users/335846/articles/461632-investigation-of-the-change-in-essential-oil-components-with-the-composition-of-fatty-acids-from-hypericum-heterophyllum-vent-an-endemic-species-in-turkey