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Full Length Article

Chemical Variation on the Essential Oil of *Thymus praecox* ssp. *scorpilii* var. *laniger*

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ABSTRACT

The rate and main components of essential oil from aerial parts of *Thymus praecox* ssp. *scorpilii* (Velen) Jalas var. *laniger* (Borbas) Jalas were investigated. For the purpose, samples of the species were collected from three altidutinal populations at Southern part of Turkey at flowering stage of the species. The essential oil obtained by hydrodistillation were analysed by GC-MS to determine its components. The rate of essential oil changed in the populations, with an average of 2.26%. Thymol and carvacrol were characterized as main components of the essential oil according to GC-MS analysis, while their rates were changed according to the populations. The averages of thymol and carvacrol were 48.10% and 22.20%, respectively. Thymol, thymol-carvacrol and carvacrol-thymol were determined as chemotypes. Altitude was significantly ($p \leq 0.05$) effective on essential oil, carvacrol and thymol rates according to results of correlation analysis. Variations in essential oil rates and compositions may be due to on genetic, ecological or individual variability. © 2011 Friends Science Publishers

Key Words: Chemotype; Component; Essential oil; Altitude

INTRODUCTION

Turkish flora contains just over 11000 infrageneric taxa, of which more than 34% are endemic (Başer, 2002). The family of Labiatae in the flora is represented by 45 genera, 546 species and 730 taxa in Turkey and the rate of endemism is 44.2% (Akgul & Ozcan, 1999). *Thymus* is one of the largest genera in Labiatae family, which is polymorphic with 60 taxa belonging to 39 species in Turkey and the rate of endemism is 45% (Başer, 2002). *Thymus praecox* is one of the most widespread species in Turkey and generally prevalent in European-Siberia region (Stahl-Biskup & Sáez, 2002). There are many varieties and subspecies because of widespread area of the species (Yıldız *et al*., 2004).

Thymus species are called as "Kekik" in Turkey and dried parts of plants are mostly used for different purposes such as tea mixture, flavor and medicine. Beside, essential oil *of Thymus* obtained by tradational method could be also used as folk medicine by local people, because of its valuable components (Başer, 2001). Azaz *et al*. (2004) reported that essential oil of *Thymus* species was categorized by concentration of isomeric phenolic monoterpens like thymol and/or carvacrol. The species contain different level of phenolic compounds and named as follows; phenol-rich, phenol-poor and phenol-less. Phenol-rich species are used in stomach and intestinal diseases, diabetes and for cough; phenol-poor and phenol-less species are used as herbal tea (Tümen *et al*., 1995). Moreover, while it is known that essential oil of *Thymus* species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities (Imelouane *et al.,* 2009), the oil of the species is not been used in Turkish essential oil industry, whereas *Origanum*, *Thymbra*, *Coridothymus* and *Satureja* are commonly used in trade (Tümen *et al.,* 1995).

The aims of the study were to determine rate and components of essential oil of endemic *T. praecox* ssp. *scorpilii* (Velen) Jalas var. *laniger* (Borbas) Jalas sampled from Southern part of Turkey, to compare the populations for the rate and content, and also to contribute for future studies in the species.

MATERIALS AND METHODS

Plant material: Plant samples were collected from three altidutinal locations (also called populations in the paper) from Southern part of Turkey during the flowering period of 2008 and 2009. The altitudes of the locations (L) were 1250 (L1), 1230 (L2) and 1030 m (L3), respectively. Voucher specimens have been deposited in the Faculty of Art and Sciences Herbarium of Suleyman Demirel University.

Essential oil isolation: The essential oils were extracted by hydrodistillation for 3 h using Clevenger type apparatus using 10 g of the air-dried aerial parts of the plant samples. The volatile oils were stored in dark glass bottles at 4^{o}C until analysis (British Pharmacopoeia, 1980).

GS-MS analysis of essential oil: The essential oils were analyzed by gas chromatography-mass spectrometry (GS-MS) system. The GC-MS analyses were performed using a Shimadzu GC-MS-QP 5050 A GC/MS system operating on

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electro spray ionization (EI) mode (equipped with a CP Wax 52 CB (50 m x 0.25 mm *i.d*., film thickness 1.2 µm), using He (1 mL/min) as the carrier gas. Oven temperature was programmed from 60°C to 220°C at 2°C/min, then isothermal at 220°C for 20 min. The temperature of injector and detector was 240°C. Mass spectra were taken on 70 eV. After compounds in gas chromatography column were separated, each individual ion-mass spectrum was taken. Evaluation process was made using the library "Wiley, NIST and Tutor".

Statistical analysis: The populations were compared for rate and components of essential oil by one-way analysis of variance (ANOVA) at SPSS statistical package program. The populations were grouped for the characters by LSD test according to results of ANOVA. Beside, Cluster analysis was applied for classifications of the locations for the main components of essential oil. The analysis based on components was calculated by the unweighted pair-group method with arithmetic mean (UPGMA) of the Euclidean distance measure method at Multi Variate Statistical Package (MVSP, Version 3.11 c) package software (Kovach, 1999) for grouping the chemotypes. Correlation analysis was also applied to determine the relations among the altitudes, rate and components.

RESULTS

The averages of components of the essential oils are given in Table I. The essential oil rates of the locations were ordered according to the altitudes (Fig. 1). Averages of rates were 1.98% in L1, 2.07 in L2 and 2.73 in L3. Thymol and carvacrol were characterized as two main components of the essential oil (Table I). Thymol was the highest component in L1 (69.09%) and L2 (45.10), while carvacrol was the highest in L3 (46.02) (Fig. 1). There were also large differences among the locations for the essential oil composition. For instance, L1 and L2 had Myrcene and α-Terpinene, the components were not found in L3 (Table I). Besides, there were fifteen times differences for rate of carvacrol between L3 (46.02%) and L1 (3.08%) (Table I).

Fourteen constituents representing 98.97% of the total oil were identified in the L1. Thymol (69.09%), borneol (5.54) and caryophyllene (5.33) were the main components in the locations. γ-terpinene (4.15%), carvacrol (3.08), cymene (2.56), 1,8-cineole (2.29) were also identified. Eighteen components were characterized, representing 99.1% of the essential oil in L2, with thymol (45.10%), carvacrol (17.5), borneol (8.71), caryophyllene (6.46), 1,8-cineole (4.27) and γ-terpinene (4.17) as the main components. Other noticeable constituents included sabinene hdrate (2.58%), cymene (2.22) and 1-octen-3-ol (2.00). However, eight constituents (99.83%) were identified in the sample collected from the L3. It was characterized by carvacrol (46.02%), thymol (30.13), sabinene hydrate (7.15) and borneol (5.08). Caryophyllene (4.86%), bisabolene (3.04) and 1,8-cineole (2.95) were also identified in the volatile oil (Table I). The results were also well accordance with results of cluster analysis and three chemotypes were also determined according to analysis as follows; thymol, thymol-carvacrol and carvacrol-thymol (Fig. 2).

There were significant ($p \leq 0.05$) differences among populations for rate of essential oil and components according to analysis of variance. The populations were in two groups for essential oil and carvacrol rates, while it was three groups for thymol (Table II & Fig. 2).

Altitude was significantly ($p \leq 0.05$) effective on essential oil, carvacrol and thymol rates according to results of correlation analysis (Table III & Fig. 1). It had positive effect on thymol, while it had negative effect on essential oil and carvacrol. There was a negative relation between carvacrol and thymol (Table III).

Table I: Essential oil composition of *Thymus praecox* ssp. *scorpilii* (Velen) Jalas var. *laniger* (Borbas) Jalas for the locations (%)

Components	Rt*	L1**	L2	L3	Averages***
Myrcene	15.8	0.27	0.35	-	0.31
α-Terpinene	17.1	0.39	0.42	-	0.41
1,8-cineole	18.8	2.29	4.27	2.95	3.17
γ-terpinene	21.0	4.15	4.17	-	4.16
3-octanone	21.4	-	0.53	-	0.53
Cymene	22.6	2.56	2.22	-	2.39
1-octen-3-ol	33.6	1.49	2.00	0.60	1.36
Sabinene Hydrate	35.0	1.94	2.58	7.15	3.89
Camphor	39.4	-	0.75	-	0.75
Linalool	40.0	0.25	-	-	0.25
Terpineole-4	44.3	0.47	0.46	-	0.47
Caryophyllene	44.5	5.33	6.46	4.86	5.55
α-Terpineole/fenchyl alcahol	50.0	-	0.75	-	0.75
Borneol	50.5	5.54	8.71	5.08	6.44
Germacrane-D	51.5	-	1.18	-	1.18
Bisabolene	52.0	2.12	1.65	3.04	2.27
Thymol	76.8	69.09	45.10	30.13	48.11
Carvacrol	78.4	3.08	17.5	46.02	22.20
Total	-	98.97	99.1	99.83	-
Averages of essential oil rate		1.98	2.07	2.73	2.26

*;Retention time; **; Locations; ***; Averages of essential oil components

Table II: Averages of essential oil, thymol and carvacrol rates of *Thymus praecox* ssp. *scorpilii* (Velen) Jalas var. *laniger* (Borbas) Jalas

Locations	Essential oil (%)	Carvacrol (%)	Thymol (%)
L1 (1255 m)	1.98 b*	3.08 b	69.08 b
L2 (1230 m)	2.07 b	17.50 c	45.10 a
L3 (1030 m)	2.73 a	46.02 a	30.13 c
Averages	2.26	22.20	48.10

*; The columns with different letters mean statistically different according to LSD ($\rho \leq 0.05$) test

Table III: Relations among the studied characters

Characteristics	Altitude	Essential oil	Carvacrol
Essential oil	-0.850**	-	
Carvacrol	-0.973**	0.790*	-
Thymol	0.850**	-0.639^{ns}	-0.949**

^{ns}; non significant; *; $p \leq 0.05$; **; $p \leq 0.01$

Fig. 1: Averages of essential oil, thymol and carvacrol rates of *Thymus praecox* ssp. *scorpilii* (Velen) Jalas var. *laniger* (Borbas) Jalas (%)

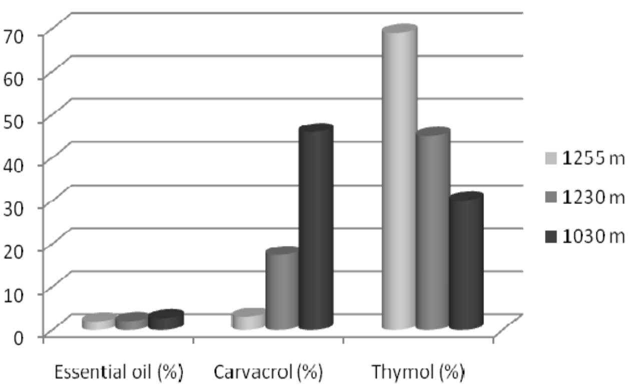
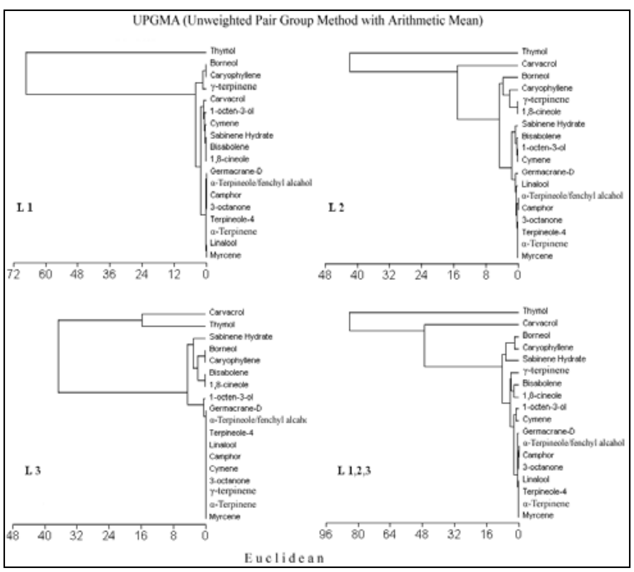


Fig. 2: Dendrogram of cluster analyses of *Thymus praecox* ssp. *scorpilii* (Velen) Jalas var. *laniger* (Borbas) Jalas



DISCUSSION

Baser *et al*. (1996) and Stahl-Biskup and Sáez (2002), reported 17.8-41.4% thymol, 10.5-7.6% carvacrol in the essential oil for *T. praecox*, while they were 48.10% and 22.20%, respectively in the present study (Table I). There were some other studies on different subspecies of the species in Turkey. *T. praecox* ssp. *grossheimii* (Ronn.) Jalas var. *grossheimii* had thymol 26.6%, ρ-cymene 24.9% and ssp. *skorpilii* (Velen.) Jalas var. *skorpilii* had geraniol 24.2% and α-terpinyl acetate 22.7% as major components (Baser *et al*., 1996). Ozen *et al.* (2011) emphasized that ssp. *skorpilii* (Velen.) Jalas var. *skorpilii* contained thymol 40.31% and *o*-cymene 13.66%. Şekeroğlu *et al.* (2007) revealed that ssp. *caucasicus* var. *caucasicus* included thymol 47.45%.

The highest essential oil (2.73%) was extracted in the lowest altitude (L3), while it was opposite (1.98%) in the highest altitude (L1). The highest thymol rate (69.08%) was ensured in the highest altitude (L1) (Table I). There were significant ($p \leq 0.05$) differences among populations for essential oil, thymol and carvacrol rates (Table II). However, there could be many effects on rate of essential oil and components. Edaphic, climatic and genetic factors were reported on chemical and compositional variations of essential oil in different plant species (Tkachev *et al.,* 2006; Mirjalili *et al.*, 2007; Lyra *et al*., 2008; Echeverrigaray *et al*., 2009; Hazzit & Baaliouamer, 2009; Toncer *et al*., 2010). Farah *et al*. (2006) reported that differences could be for rate of essential oil among populations in *Myrtus communis.* Avcı and Bayram (2008) emphasized importance of harvesting period and time on essential oil rate in *M. communis*. Large individual differences were reported for essential oil rate in *Eucalyptus camaldulensis* by Avcı and Bilir (2009). In results of present study, altitude was significantly ($p \leq 0.05$) effective on the carvacrol and thymol contents (Table III & Fig. 1). Altitudinal variations for essential oil rate were reported in *Origanum onites* by Gönüz and Özörgücü (1999). Besides, Baydar (2002) reported that oregano species growing in higher altitudes contain lower essential oil than grown in lower altitudes.

In conclusion, it could be said that altitude could be used as a criterion for selection of gene conservation and harvesting areas in the species. Essential oil rates and compositions showed variations for the location/populations; however the study was carried out in restricted populations. These differences emphasized the large variations among population and within population of the species. New studies should be carried out with large popualtions and individuals for such as determination industrial potential of essential oil of the species.

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REFERENCES

Akgul, A. and M. Ozcan, 1999. Essential Oils of Four Turkish Wild-Growing Labiatae Herbs: *Salvia cryptantha* Montbr. Et. Auch., *Satureja cuneifolia* Ten., *Thymbra spicata* L. and *Thymus cilicicus* Boiss. et Bal. *J. Essen. Oil Res.,* 11: 209–214

Avcı, A.B. and E. Bayram, 2008. Mersin Bitkisi (*Myrtus communis* L.) 'nde farklı hasat zamanlarının uçucu yağ oranlarına etkisi. *SDU. J. Nat. Appl. Sci.*, 12: 178–181

Avcı, A.B. and N. Bilir, 2009. *Denizli Yöresi Eucalyptus Camaldulensis Genotiplerinde Uçucu Yağ Oranı ve Tekrarlanma Derecesi*. 1.Uluslararası 5.Ulusal Meslek Yüksekokulları Sempozyumu, s. 46-467, SÜ, 27-29 Mayıs, 2009, Konya

Azaz, A.D., H.A. Irtem, M. Kurkcuoğlu and K.H.C. Baser, 2004. Composition and the *in vitro* Antimicrobial Activities of the Essential Oils of some Thymus Species. *Z. Naturforsch*, 59c: 75–80

Baser, K.H.C., N. Kirimer, N. Ermin, T. Ozek and G. Tumen, 1996. Composition of essential oils from three varieties of Thymus praecox Opiz growing in Turkey. *J. Essen. Oil Res.*, 8: 319–321

Başer, K.H.C., 2001. Her derde deva bir bitki Kekik. *Bilim Ve Teknik*, *Mayı*s: 74–77

Başer, K.H.C., 2002. Aromatic biodiversity among the flowering plant taxa of Turkey. *Pure Appl. Chem.,* 74: 527–545

Baydar, H., 2002. Isparta koşullarında İzmir kekiğinin (O*riganum onites* L.) verimi ve uçucu yağ kalitesi üzerine araştırmalar. *S.D.Ü. Fen Bilimleri Enstitüsü Dergisi*, 6: 17–24

British Pharmacopoeia, 1980. Vol II.H. M. Stationary Office, Londan. PA 109

Echeverrigaray, S., M. Albuquerque, J. Zacaria, A.C. Atti Dos Santos and L. Atti-Serafini, 2009. Chemical Variations on the Essential Oils of *Cunila spicata* Benth. (Lamiaceae), an Aromatic and Medicinal Plant From South Brazil. *J. Essent. Oil Res.*, 21: 241–245

Farah, A., A. Afifi, M. Fechtal, A. Chhen, B. Satrani, M. Talbi and A. Chaouch, 2006. Fractional distillation effect on the chemical composition of Moroccan Myrtle (*Myrtus communis* L.) essential oils. *Flavour and Fragr. J.*, 21: 351–354

Gönüz, A. and B. Özörgücü, 1999. An investigation on the morphology, anatomy and ecology of *Origanum onites* L. *Turkish J. Bot.*, 23: 19–32

Hazzit, M. and A. Baaliouamer, 2009. Variation of Essential Oil Yield and Composition of *Thymus pallescens* de Noé from Algeria*. J. Essent. Oil Res*., 21: 162–165

Imelouane, B., H. Amhamdi, J.P. Wathelet, M. Ankit, K. Khedid and A. El Bachiri, 2009. Chemical composition of the essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco. *Int. J. Agric. Biol.*, 11: 205–208

Kovach, W.L., 1999. *A Multi Variate Statistical Package*. United Kingdom: Kovach Computing Services.

Lyra, C.C.G.V., R.F. Vieira, De C.B.A. Oliveira, S.C. Santos, J.C. Seraphind and P.H. Ferri, 2008. Infraspecific Variability in the Essential Oil Composition of *Lychnophora ericoides***.** *J. Brazilian Chem. Soc*., 19: 842–848

Mirjalili, M.H., S.M.F. Tabatabaei, J. Hadian, S.N. Ebrahimi and A. Sonboli, 2007. Phenological Variation of the Essential Oil of *Artemisia scoparia* Waldst. et Kit from Iran. *J. Essent. Oil Res.*, 19: 326–329

Ozen, T., I. Demirtas and H. Aksit, 2011. Determination of antioxidant activities of various extracts and essential oil compositions of Thymus *praecox* subsp. *skorpilii* var. *skorpilii*. *Food Chem.*, 124: 58–64

Stahl-Biskup, E. and F. Sáez, 2002. *Thyme The Genus Thymus, Medicinal and Aromatic Plants-Industrial Profile*. Taylor and Francis, New York

Şekeroğlu, N., M. Deveci, C.K. Buruk, B. Gürbüz and A. İpek, 2007. Chemical composition and antimicrobial activity of Anzer tea essential oil. *J. Sci. Food Agric.,* 87: 1424–1426

Tkachev, A.V., E.A. Korolyuk, W. König, Y.V. Kuleshova and W. Letchamo, 2006. Chemical screening of volatile oil-bearing flora of Siberia VIII.: Variations in Chemical Composition of the Essential Oil of Wild Growing *Seseli buchtormense* (Fisch. ex Sprengel) W. Koch from Different Altitudes of Altai Region. *J. Essent. Oil Res*., 18: 100–103

Toncer, O., S. Basbag, S. Karaman, E. Dıraz and M. Basbag, 2010. Chemical Composition of the Essential Oils of some *Achillea* Species Growing Wild in Turkey. *Int. J. Agric. Biol.*, 12: 527–530

Tümen, G., N. Kirimer and K.H.C. Başer, 1995. Composition of the Essential Oils of *Thymus* Species Growing in Turkey. *Chem. Nat. Comp.*, 31: 42

Yıldız, B., G. Tümen, N. Demirkuş, N. Adıgüzel, H. Akyalçın and Z. Bahçecioğlu, 2004. *Türkiye'de Yetişen Thymus L. (Lamiaceae) Trülerinin Revizyonu ve Türler Üzerinde Palinolojik ve Kimyasal Araştırmalar*. TÜBİTAK-TBAG-1715 (198T003) No'lu Proje Sonuç Raporu, 201

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