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Antioxidant Capacity and Essential Oil Composition of *Hypericum thymopsis Boiss.* (Hypericaceae) from Turkey

Türkiye'den *Hypericum thymopsis Boiss.* (Hypericaceae) türünün Antioksidan Kapasitesi ve Uçucu Yağ Bileşimi

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ABSTRACT

Antioxidant capacity and essential oil composition of Hypericum *thymopsis* Boiss. (Hypericaceae), an endemic species, distributed in Turkey was determined. The samples of three different populations were used for the analysis. Antioxidant capacity was determined by DPPH method from the leaves and flowers. Essential oil analysis was performed from areal parts of plant by gas chromatography (GC) and GC/mass spectrometry (MS). The major components of the essential oil were determined as α-pinene (31.86%), spathulenol (11.16%) and limonene (4.3%) in the specimen TA3004, α-pinene (28.07%), spathulenol (12.37%) and limonene (6.07%) in the specimen TA3014 and α-pinene (26.03%), limonene (14.83%) and spathulenol (9.74%) in the specimen TA3017. According to the 50% inhibition (IC50) values (µg/mL) the highest antioxidant values were measured in the methanolic extract of flowers.

Key Words

DPPH, endemic,GC/MS, guttiferae.

ÖZ

Türkiye'de yayılış gösteren ve endemik bir tür olan *Hypericum thymopsis* Boiss. (Hypericaceae) türünün antioksidan kapasitesi ve uçucu yağ bileşimi belirlendi. Analizler için üç farklı popülasyonun örnekleri kullanıldı. Antioksidan kapasiteler yaprak ve çiçeklerden DPPH yöntemi ile belirlendi. Uçucu yağ analizi bitkinin toprak üstü kısımlarından gaz kromatografisi (GC) ve GC/kütle spektrometresi (MS) ile yapıldı. Uçucu yağın ana bileşenleri TA3004 numaralı örnekte α-pinen (%31.86), spathulenol (%11.16) ve limonen (%4.3), TA3014 numaralı örnekte α-pinen (%28.07), spathulenol (%12.37) ve limonen (%6.07), TA3017 numaralı örnekte α-pinen (%26.03), limonen (%14.83) ve spathulenol (%9.74) olarak belirlendi. %50 inhibisyon (IC50) değerlerine (µg/mL) göre, en yüksek antioksidan değerler çiçeklerin metanolik ekstraktında ölçüldü.

Anahtar Kelimeler

DPPH, endemik, GC/MS, guttiferae.

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INTRODUCTION

Hypericum L. is a monotypic representatives of family Hypericaceae in Turkey with the distribution of 96 species of which 46 are endemic [1]. The genus is characterized by the glands which are important in classification. The translucent glands containing essential oils. The red or black glands sometimes containing hypericin [2].

The members of the genus are utilized in traditional medicine such as eczemas, burns, gastric ulcers, hemorrhoids, incontinence, stomach-ache, wound healing, antiseptic, antispasmodic, constipation, sedative [3-5]. The genus has a wide range of biologically active secondary metabolites. Naphtodianthrones (hypericin, pseudohypericin, protohypericin, protopseudohypericin), phloroglucinols (hyperforin, adhyperforin), flavonoids (quercetin, quercitrin, isoquercitrin, hyperoside, astilbin, miquelianin, I3,II8-biapigenin) and phenolic acids (chlorogenic acid, 3-O-coumaroylquinic acid) were identified from *Hypericum perforatum* L. [6]. The oil yield of *Hypericum* species are generally poor with the ratio less than 1%, (w/w). The constituents of the essential oils are reported as aliphatic hydrocarbons (n-nonane and n-undecane), the monoterpenes (α- and β-pinene), and the sesquiterpenes (β-caryophyllene and caryophyllene oxide) in *Hypericum* species [7]. Significant antioxidant activities was determined on *Hypericum* species by several methods such as; DPPH radical scavenging assay, NO scavenging, superoxide scavenging, lipid peroxidation, hydrogen peroxide scavenging activity, metal chelating ability etc. The antioxidant activity related to flavonoids and phenolic acids [8-11]. The phenolic compounds, such as hypericin, pseudohypericin, hyperoside, and quercetin in the extract of the flowers of *Hypericum venustum* Fenzl has shown powerful reducing activity, in term of free radicals (superoxide anion, DPPH), hydrogen peroxide scavenging capacity and metal chelating activity [11]. The essential oil of *Hypericum gaitii* Haines showed a moderate antioxidant capacity when compared with butylated hydroxytoluene (BHT) and ascorbic acid [12].

Hypericum thymopsis Boiss. (in Turkish: Darende Kantaronu) is an endemic species with a narrow distribution in Turkey. The species belongs to Section *Drosanthe* (Spach) Endl. In this study, essential oil composition and antioxidant capacity of *Hypericum thymopsis* was determined. DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay was used in antioxidant capacity study.

MATERIALS and METHODS

Plant material and isolation of the oils

The plant materials of *Hypericum thymopsis* were collected from field studies conducted in the year 2016 from three localities. In addition, *Hypericum perforatum* specimens were obtained from the Medicinal and Aromatic Plants garden of İnönü University Faculty of Pharmacy, for the comparison in antioxidant capacity study. The localities including habitats and collector numbers of *H. thymopsis* and *H. perforatum* are given (Table 1). Voucher specimens are deposited in the herbarium of the Faculty of Pharmacy, İnönü University, Malatya, Turkey.

Essential oil analysis

Dried plant samples obtained from aerial parts were hydrodistillated for 3 h by Clevenger-type apparatus. The essential oil obtained from distillation were analysis with Agilent Technologies 6890N Network system gas chromatograph equipped with a FID and an Innowax column (60 m x 0.25 mm i.d., 0.25 µm film thickness) and GC/MS analyses were carried with the GC system of Agilent Technologies 6890N Network system gas chromatograph equipped with an Agilent Technologies 5973 inert Mass Selective Detector (Agilent G3180B Two-Ways Splitters with Makeup gas) in the electron impact mode (70eV) by the method given by Arabacı et al. (2020) [13]. The library of FLAVOR2, NIST05a, NIST08 and WILEY8 were used. Relative indices calculated by reference of linear alkanes series of C_8-C_{20}. The essential oil compounds, the relative retention indices (RRI) and relative percentages (%) of the essential oils are given (Table 2).

Preparation of the extracts

The methanolic extracts are prepared from the leaves and flowers by the method described by Arabacı et al. (2020) with some modification [13]. The air-dried and grinded leaves and flowers macerating for 24 h with solvent (sample/solvent, 1:10, w/v). The maceration was repeated 2 times for each samples. The extracts were filtered and the solvent methanol was removed by rotary evaporator. The extracts were stored at 4 °C until use.

DPPH radical scavenging assay

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay was performed according to the method described by Brand-Williams et al. (1995) with some modification [14]. The extracts diluted serially between 1000 and 31.25 µg/mL concentrations. 150 µL of diluted extracts and 50 µL freshly prepared solution of DPPH were added in a 96-well

Table 1. The localities including habitats and collector numbers of *Hypericum* thymopsis and *H. perforatum.*

Taxa	Specimen number	Localities
Hypericum thymopsis	TA3004	Malatya: Between Malatya and Hekimhan, Çebiş pass, deep soiled areas, Quercus L. shrub openings, 1050 m, 15.06.2016.
H. thymopsis	TA3014	Sivas: 3-5 km from Gürün to Gökpınar, rocky steppes, 1620 m, 15.06.2016.
H. thymopsis	TA3017	Malatya: 11 km from Darende to Gürün, rocky cliff edges, 1500 m, 15.06.2016.
H. perforatum	EK1001	Malatya: Medical and Aromatic Plants Garden of İnönü University, Faculty of Pharmacy, 15.06.2017, (cultivar).

Table 2. Essential oil composition of *Hypericum* thymopsis collected from three different localities (TA3004, TA3014 and TA3017).

RRI	Compound	Composition%		
		(TA3004)	(TA3014)	(TA3017)
1019	α-Pinene	31.86	28.07	26.03
1041	3-Carene	0.74	1.29	0.53
1049	Camphene	1.99	2.55	1.34
1093	β-Pinene	0.89	2.14	0.93
1175	Mrycene	0.36	0.5	0.52
1234	Limonene	4.3	6.07	14.83
1333	p-Cymene	0.94	0.64	0.4
1352	Terpinolene	0.9	1.14	0.73
1391	cis-3-Hexenyl acetate	0.22	-	-
1539	p,α-Dimetilstiren	0.29	0.24	-
1616	α-Cubebene	-	1.37	-
1618	α-Copaene	-	-	0.52
1594	α- Campholenic Aldehyde	1.42	-	-
1620	3-Pinanone	0.83	-	-
1640	Linalol	0.87	0.72	0.39
1666	Pinocarvone	0.22	-	-
1674	Fenchyl alcohol	0.58	0.69	0.28
1697	4-Terpinenol	0.33	0.32	-
1698	β-Elemene	-	-	0.16
1722	Aromadendrene	-	0.59	-
1722	Myrtenal	1.01	-	-
1736	trans-Pinocarveol	0.76	0.27	0.11
1741	Pulegone	-	0.3	-
1756	trans-carveol	0.88	0.44	-
1775	Bornyl formate	3.72	4.04	-
1776	Bornyl acetate	-	-	2.14
1786	γ-Muurolene	2.22	3.05	1.93
1793	Verbenone	0.66	-	-
1797	α-Phellandren-8-ol	0.58	-	-
1808	Germacrene D	0.85	3.08	1.08
1819	α-Muurolen	0.39	0.57	-
1819	β-Caryophyllene	-	-	0.34

Table 2. Essential oil composition of *Hypericum* thymopsis collected from three different localities (TA3004, TA3014 and TA3017). (Continued)

1828	Bicyclogermacrene	0.79	1.79	-
1850	δ-Cadinene	-	3.52	1.67
1860	Myrtenol	0.6	-	-
1909	cis-Carveol	0.35	-	-
1919	Geraniol	0.3	0.33	0.16
1930	Calamenene	0.39	2.28	1.66
2056	α-Calacorene	-	0.67	-
2282	Oktanoik asit	-	0.31	-
2491	Spathulenol	11.16	12.37	9.74
2560	Nonanoic acid	0.25	0.34	0.24
2592	Cyclosativene	0.31	-	-
2592	α-Elemene	-	0.82	-
2610	α-Cadinol	0.53	1.52	0.37
2657	Cadalene	0.26	0.38	-
2717	Decanoic acid	0.1	0.16	0.17
2740	β-Ionone	-	0.87	1.03
2770	Ledene	0.34		
2917	Dodecanoic acid	0.44	0.54	0.67
2966	Diisobutyl phthalate	-	-	0.31
3023	Benzoic acid	0.36	0.27	0.21
3062	Myristic acid	-	0.32	-
3107	Eugenone	0.59	-	0.48
3189	Hexadecanoic acid	0.59	1	1.07
	Total identified	75.17	85.57	70.04
	Monoterpene hydrocarbons	42.27	44.01	45.83
	Oxygenated monoterpenes	9.49	3.23	1.11
	Sesquiterpene hydrocarbons	5.55	16.75	6.84
	Oxygenated Sesquiterpenes	11.69	13.89	10.11
	Others	6.17	7.69	6.15

RRI: Relative Retation Index.

Table 3. DPPH radical scavenging capacities of the Hypericum thymopsis (TA3004, TA3014 and TA3017) and H. perforatum (Hp) extracts. Results are mean ± standard deviation of three separate analyses.

Extract	(TA3004/L)	(TA3004/F)	(TA3014/L)	(TA3014/F)	(TA3017/L)	(TA3017/F)	(Hp/L)	(Hp/F)
DPPH (IC50 µg/mL)	120.84	67.53	148.20	77.52	84.69	76.24	142.84	50.41
	±8,66	±1.18	±6,07	±3.82	±3.23	±2.53	±7,02	±2.70
References	BHT	GA	Trolox	α-Toc				
DPPH (IC50 µg/mL)	40.48	8.48	68.74	39.59				
	±0.81	0.33±	0.47±	±3.22				

L: Leaves, F: Flowers, BTH (Butylated hydroxytoluene), GA (Gallic acid), α-Toc (α-Tocopherol).

microplate with 3 repetitions and incubated in a dark place for 30 minutes. The absorbance was calculated at 517 nm. The IC_{50} values were calculated by the graphical plot of the percent inhibition versus extract concentrations. Butylated hydroxytoluene (BHT), Gallic acid (GA), Trolox and α-Tocopherol were used as references.

RESULTS and DISCUSSION

Three specimens, collected from different localities were analyzed for determined the essential oil composition of *Hypericum thymopsis*. The essential oil yield was calculated as 0.20% (v/w) in the specimen TA3004 and 0.11 % (v/w) in the specimens TA3014 and TA3017. The major components of the essential oil were determined as α-pinene (31.86%), spathulenol (11.16%) and limonene (4.3%) in TA3004, α-pinene (28.07%), spathulenol (12.37%) and limonene (6.07%) in TA3014 and α-pinene (26.03%), limonene (14.83%) and spathulenol (9.74%) in TA3017. There are two previous studies about the essential oil composition of *H. thymopsis.* In the study given by Özkan et al. (2009) the major components of the essential oil were determined as spathulenol (10.8%), δ-cadinene (7.1%), germacrene D (6.1%), γ-muurolene (5.9%), 2,3,6-trimethylbenzaldehyde (5%) and γ-cadinene (4.4%) while the major components were determined as α-pinene (44.0%), baeckeol (32.9%), spathulenol (8.0%), limonene (7.6%) and camphene (5.2%) in *Özkan* et al. (2013) [15-16]. The major compounds of essential oil of *H perforatum* from six localities in southeastern France were found as monoterpenoids, especially the α-pinene [17]. The main essential oil components were reported as α-pinene (58%, 50%, 26% and 24%) in the species *H. cerastoides* (Spach) Robson, *H. perforatum, H. montbretii* Spach and *H. calycinum* L., respectively, from Turkey [18].

Antioxidant capacities of the methanolic extracts of *Hypericum thymopsis* leaves and flowers were determined with DPPH radical scavenging assay (Table 3). In addition, *H. perforatum* specimens were used for comparison. The specimen which have lower value of inhibitory concentration (IC_{50}) show the higher antioxidant activity [19]. The methanolic extracts of flowers are more active than the leaves in the DPPH assay. The highest inhibitory activity was measured as 50.41 µg/mL (IC_{50}) in the flowers of *H. perforatum.* Among the specimens of *H. thymopsis,* the highest inhibitory activity was observed as 67.53 µg/mL (IC_{50}) in the flowers of specimen labeled TA3004. Various positive control such as BHT, Gallic acid, Trolox and α-Tocopherol were used as references for compare the activity in the assay and the IC_{50} values were determined as 40.48, 8.48, 68.74 and 39.59 µg/mL respectively. When we compared the results we have seen that *H. perforatum* and *H. thymopsis* specimens showed remarkable antioxidant activity. *Hypericum* species have antioxidant activity due to their phenolic content. The correlation between antioxidant activity and total phenol content in the extracts of *H. origanifolium* Willd. and *H. montbretii* Spach. was observed by Öztürk et al. (2009) [19]. The antioxidative potential of ethanol extracts of *H. triquetrifolium* Turra and *H. scabroides* Robson & Poulter were found to be highly active in the DPPH radical scavenging assay and the IC_{50} values in these species were determined as 39.0 and 33.8µg/mL, respectively [10]. Antioxidant activity of the methanolic extracts of *H. thymopsis* was determined as 28.49 % inhibition with DPPH radical scavenging assay in a previous study [20].

In conclusion, essential oil composition of *Hypericum thymopsis* was determined and discussed with the chemotypes and antioxidant capacity of species was observed with the DPPH radical scavenging assay.

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References

1. S. Aslan, *Hypericum* L. In: A. Güner, S. Aslan, T. Ekim, M. Vural, M.T. Babaç (Ed), A Checklist of the Flora of Turkey (Vascular Plants), Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, 2012, 523-530.
2. P.H. Davis (Ed.), Flora of Turkey and the East Aegean Islands, Vol.2, Edinburgh University Press, Edinburgh, 1967, 355-401.
3. M. Koçyiğit, N. Özhatay, Wild Plants Used as Medicinal Purpose in Yalova (Northwest Turkey), Turkish J. Pharm. Sci., 3 (2006) 91-103.
4. G. Bulut, E. Tuzlaci, An ethnobotanical study of medicinal plants in Turgutlu (Manisa-Turkey), J. Ethnopharmacol., 149 (2013) 633-647.
5. F. Tetik, S. Civelek, U. Cakilcioglu, Traditional uses of some medicinal plants in Malatya (Turkey), Journal of Ethnopharmacol., 146 (2013) 331-346.
6. E.C. Tatsis, S. Boeren, V. Exarchou, A.N. Troganis, J. Vervoort, I.P. Gerothanassis, Identification of the major constituents of *Hypericum perforatum* by LC/SPE/NMR and/or LC/MS, Phytochemistry, 68 (2007) 383-393.
7. S.L. Crockett, Essential Oil and Volatile Components of the Genus *Hypericum* (Hypericaceae), Nat. Prod. Commun., 5(9) (2010) 1493-1506.
8. E.E. Özkan, A. Mat, An overview on *Hypericum* species of Turkey, J. Pharmacogn. Phytotherap., 5 (2013) 38-46.

9. D.Z. Orčić, N.M. Mimica-Dukić, M.M. Francišković, S.S. Petrović, E.D. Jovin, Antioxidant activity relationship of phenolic compounds in *Hypericum perforatum* L., Chem. Centr. J., 5 (2011) 1-8.
10. G. Kızıl, M. Kızıl, M. Yavuz, S. Emen, F. Hakimoğlu, Antioxidant Activities of Ethanol Extracts of *Hypericum triquetrifolium* and *Hypericum scabroides,* Pharmaceut.Biol., 46 (2008) 231-242.
11. M. Spiteller, T. Ozen, A. Smelcerovic, S. Zuehlke, N. Mimica-Dukic, Phenolic constituents and the in vitro antioxidant activity of the flowers of *H. venustum*, Fitoterapia, 79(3) (2008) 191-193.
12. P.K. Kamila, A. Ray, S. Jena, P.K. Mohapatra, P.C. Panda, Chemical composition and antioxidant activities of the essential oil of *Hypericum gaitii* Haines–an endemic species of Eastern India, Nat. Prod. Res., 32 (2018) 739-742.
13. T. Arabacı, M.S. İçen, T. Dirmenci, F. Göğer, K.H.C. Başer, Evaluation of Antioxidant Activities, Phenolic Constituents and Essential Oil Composition of *Marrubium heterodon* (Benth.) Boiss. & Balansa from Turkey, Lat. Am. J. Pharm., 39 (2020) 109-115.
14. W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of free radical method to evaluate antioxidant activity, Lebensm.-Wiss.-u-Technol., 28 (1995) 25-30.
15. A.M.G. Özkan, B. Demirci, K.H.C. Başer, Essential Oil Composition of *Hypericum thymopsis* Boiss., J. Essent. Oil Res., 21 (2009) 149-153.
16. E.E. Özkan, B. Demirci, Ç.Ü. Gürer, Ş. Kültür, A. Mat, K.H.C. Başer, Essential oil composition of five endemic *Hypericum* species from Turkey, Med. Aromat. Plants, 2 (2013) 1000121.
17. I. Schwoba, J-M Bessièreb, J. Vianoa, Composition of the essential oils of *Hypericum perforatum* L. from southeastern France, C. R. Biologies, 325 (2002) 781-785.
18. S. Erken, H. Malyer, F. Demirci, B. Demirci, K.H.C. Baser, Chemical investigations on some *Hypericum* species growing In Turkey-I, Chem. Nat. Comp., 37 (2001) 434-438.
19. N. Öztürk, M. Tunçel, İ. Potoğlu-Erkara, Phenolic compounds and antioxidant activities of some *Hypericum* species: A comparative study with *H. perforatum*, Pharmaceut. Biol., 47 (2009) 120-127.
20. B. Okyay, *Hypericum thymopsis* Boiss. (Hypericaceae) Üzerinde Fitokimyasal Ve Biyoaktivite Çalişmaları, Anadolu Üniversitesi, Sağlık Bilimleri Enstitüsü, Farmakognozi Anabilim Dalı (Yüksek Lisans Tezi), Eskişehir, 2020.