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Research Article

Composition and Acetylcholinesterase inhibition properties of *Tripleurospermum inodorum* (L.) Sch. Bip. Essential Oil from Istanbul

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Abstract: Essential oil composition and Acetylcholinesterase (AChE) inhibition properties of *Tripleurospermum inodorum* (L.) Sch. Bip. were investigated. Essential oils of flowers, leaves and stems were obtained through hydro-distillation using a Clevenger type apparatus with 0.03, 0.02 and 0.01% (v/w) yields, respectively. Essential oil composition of the oils was determined by GC-MS analyses. Total of ninety compounds were identified in flower oil comprising of 80.7% of the essential oil. The main components of the flower oil were artemisia ketone 14.4%, terpinen-4-ol 5.5%, 1,8-cineole 5.1%, sabinene 4.7% and tricosane 4.6%. Leaf oil was characterized by 35 compounds representing 62.7% of the oil. Leaf oil contained caryophyllene oxide 16.0%, phytol 12.1%, spathulenol 5.9%, hexahydrofarnesyl acetone 3.8% and salvia-4(14)-en-1-one 3.5% compounds with high relative percentages. Stem essential oil comprised of twenty-eight compounds representing 87.2% of the oil. The main components of the stem essential oil were were neryl acetate 12.8%, (*E*)-β-farnesene 12.5%, phytol 12.1%, guaia-6,10(14)-dien-4β-ol 10.8%, γ-cadinol 7.8%, nonacosane 7.3%, decanoic acid 6.3% and caryophyllene oxide 4.6%. *In vitro* AchE inhition of the oils were evaluated by the Ellman spectrophotometric method. AChE inhibitory property was investigated for the flower oil with at four different concentrations. Highest AChE inhibitory property was observed for 20 mg/mL oil concentration (53.35 ± 1.37 %; *n* =3). This AChE inhibitory concentration was equivalent to the activity of 1.26 µg galanthamine hydrobromide in the assay. Activity of the essential oil was concentration dependent.

Key Words: *Tripleurospermum inodorum,* Essential oils, AChE inhibition, Artemisia ketone, Caryophyllene oxide, Neryl acetate.

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Introduction

Tripleurospermum inodorum (L.) Sch. Bip. is Syn = *Matricaria inodora* L., *Matricaria perforata* Mérat, *Tripleurospermum perforatum* (Mérat) M. Lainz is a member of Asteraceae family. *Tripleurospermum* species are widely distributed in Europe, Asia and North Africa. The genus is represented by 31 taxa where 15 are endemic for Turkey (Davis, 1975). Ethnobotanical reports indicate that *Tripleurospermum* species were reported to be used as an edible plant as well as for medicinal purposes (Şimşek et al., 2004). Ethnomedicinal uses of this genus include uses for the treatment of asthma, cardiac disorders, cold, diabetes, gastric pain, gynecological inflammations, high cholesterol, kidney stones, sour throat, wounds and for easing respiration, haircare, suppressing of cough, as an antiseptic, antipyretic, febrifuge (Akaydın et al., 2013; Amiri et al., 2012; Han & Bulut, 2015; Isil et al., 2004; Mohammadi et al., 2016; Naghibi et al., 2014; Özüdoğru et al., 2011; Sarper et al., 2009; Tetik et al., 2013). In regard to the extensive folk medicinal uses of this genus considerable work has been conducted in order to provide proof for the mentioned medicinal uses. So far, acetylcholinesterase inhibitory, analgesic, antioxidant, antibacterial, antimicrobial, antimycobacterial, anti-inflammatory, antiproliferative, anti-ulcer, cytotoxic activities of *Tripleurospermum* species were reported (Bakhtiarian et al., 2007; Ćavar Zeljković et al., 2015; Chehregani et al., 2010; Erdoğan et al., 2013; 2015; Mandegary et al., 2014; Minaiyan et al., 2007; Parvini et al., 2007; Réthy et al., 2007; Tofighi et al., 2015; Tosun et al., 2005). According to these reports, 70% ethanol extract of aerial parts of *Tripleurospermum conoclinium* (Boiss. et Ball.) Hayek produced considerable antimycobacterial effect (Tosun et al., 2005). Additionally, water extract of *T. disciforme* (C.A. Mey) Shultz Bip was reported to have considerable anti-inflammatory activity in carregeenan induced rat paw edema model *in vivo* and analgesic activity in formalin test model *in vivo* (Bakhtiarian et al., 2007; Parvini et al., 2007). *T. disciforme* 70% ethanolic extract was also reported to have protective effect on ulcer formation in pylorus-ligated rats (Minaiyan et al., 2007). *T. parviflorum* Willd. Pobed. and *T. tenuifolium* Kit. extracts (*n*-hexane, ethyl acetate, methanol and water) were also investigated for their anti-inflammatory properties according to carregeenan induced rat paw edema model and serotonin-induced hind paw edema model, extracts of both plants produced noticeable activity (Erdoğan et al., 2015). Methanolic extract of *T. disciforme* was also reported to have a high inhibition effect on AChE (5 µg/mL extract concentration: 71.18 ± 4.9 % AChE inhibition) (Mandegary et al., 2014).

Previous literature revealed the phytochemistry of non-volatile fraction of *Tripleurospermum* species. So far, flavonoids: luteolin, quercetin-7-*O*-glucoside, kaempferol, kaempferol-7-*O*-glucoside, apigenin, apigenin-7-*O*-glucoside, apigenin 7-methyl ether, (Tofighi et al., 2015; Williams et al., 2001); acetylenic substances: 7-[octa-2,4-diyn-6-enylidene]-4-[3-methyl but-2-enoyloxyl]-1,6-dioxaspiro [4,4] nona-2,8-diene, *cis-cis*-matricaria ester, (Souri et al., 2005, Sørensen, 1963); aromatics: Chlorogenic acid, 3,5-dicaffeoylquinic acid, 1,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid were reported from this genus (Fraisse et al., 2011).

Previously, essential oil composition of *T. disciforme* from Iran was evaluated and essential oil of aerial parts was characterized by 18.8% *p*-methoxy-β-cyclopropylstyrene, 15.6% (*E*)-β-farnesene and 15.4% β-sesquiphellandrene (Javidnia, et al., 2008). Essential oil composition of *T. disciforme* obtained by different distillation methods was investigated and according to the method essential oil composition

changed considerably. First method provided an essential oil composed of 41.2% viridiflorene, 31.9% *trans–trans*–matricaria ester and second method produced an essential oil composed of 51% *trans-trans*-matricaria ester and 13% viridiflorene (Jaimand & Rezaee, 2003). Variation in the essential oil composition of *T. disciforme* according to different development stages was also investigated, β-farnesene and β-sesquiphellandrene composition was reported to change considerably prior to flowering, after flowering and during the flowering stages (Chehregani et al., 2010). Essential oil composition of *Tripleurospermum corymbosum* E. Hossain from Turkey was reported to contain 18.2% (*Z*)-β-farnesene, 16.1% 1-*epi*-cubenol, 8.5% β-patchoulene, 7.2% α-cadinene, 6.4% β-sesquiphellandrene, 4.6% (*E*)-γ-bisabolene and 4.5% dodecanoic acid (Öztürk et al., 2010). Another report indicates that essential oil of aerial parts of *T. insularum* Inceer & Hayırlıoglu-Ayaz from Turkey contained high amounts of 13.5% globulol and 9.3% β-sesquiphellandrene where as its headspace analysis showed high amounts of *n*-octacosane, linoleic acid, *n*-hexacosane and β-sitosterol in its volatiles (Ćavar Zeljković et al., 2015). Chloroform extracts of different organs of *Tripleurospermum callosum* (Boiss. & Heldr) E. Hossain from Turkey was reported to contain 11.7% moretenol in flower, 16.2% linoleic acid, 17.9% hexadecanoic acid, 13.4% 1-tricosene in stem and 6.2% hexadecanoic acid in root extracts (Yaşar et al., 2005). Essential oil composition of *T. decipiens* (Fisch. & Mey.) Bornm. from two different locations in Turkey was investigated and both samples of the plant showed high similarity in their compositions which could be clearly seen from the obtained results. According to this study essential oil from the Adana and Eskişehir sample afforded 57.9%; 70.0% (2*Z*,8*Z*)-matricaria ester, 10.4%, 2.7% β-sesquiphellandrene, 8.1%, 1.7% (2*E*,8*Z*)-matricaria ester, 7.5%, 0.3% (*Z*)-β-farnesene, 2.3%, 0.4% (2*E*,8*E*)-matricaria ester and 1.4%, 0.5% (2*Z*,8*E*)-matricaria ester respectively (Kürkçüoğlu et al., 2016). Finally, *M. perforata* (Syn = *T. inodorum*) flower from Germany essential oil was reported to contain very high amount of polyacetylene (2*Z*,8*Z*)-matricaria ester (Bär & Schultze, 1996).

Previous studies point out that extracts, essential oils of *Tripleurospermum* species finds various ethnobotanical uses and possess various beneficial biological activities including action on the AChE. So far only handful of reports exist in the literature, there is no report on most of the taxa that is found in Turkey. Aim of the current study is to identify the chemical composition and AChE inhibitory property of *Tripleurospermum inodorum* essential oil from Turkey.

Materials & Methods

Plant Materials

Tripleurospermum inodorum (L.) Sch. Bip. was collected in İkitelli-Başakşehir, Istanbul, Turkey in May 2015 by Hüseyin Servi Ph.D. The plant was identified by Ahmet Doğan Ph.D. A voucher specimen was deposited in the Herbarium of Department of Pharmaceutical Botany, Faculty of Pharmacy, Marmara University with the voucher specimen number MARE 17943.

Isolation of the Essential Oil

Air-dried plant parts, flower, leaves and stem were subjected separately to hydro-distillation using a Clevenger type apparatus for 3 h, to produce essential oils. During the distillation condenser of the Clevenger apparatus was attached to a micro-chiller that was set to 4°C. *T. inodorum* flowers, leaves and stems produced 0.03, 0.02 and 0.01% (v/w) essential oil yields, respectively. All of the oils were trapped with 1 ml *n*-hexane and preserved in amber vials under -20°C until the day they were analyzed.

Gas chromatography – mass spectrometry analysis

The GC-MS analysis was performed with an Agilent 5975C Inert XL EI/CI MSD system operating in EI mode. Essential oil of flower, leaves and stem which were trapped in *n*-hexane was injected (1 µL) in splitless mode. Injector and MS transfer line temperatures were set at 250˚C. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) and helium as carrier gas (1 mL/min) were used in GC/MS analyses. Oven temperature was programmed to 60˚C for 10 min. and raised to 220˚C at a rate of 4˚C/min. Temperature kept constant at 220˚C for 10 min. and then raised to 240˚C at a rate of 1˚C/min. Mass spectra were recorded at 70 eV with the mass range m/z 35 to 425. Relative percentage amounts of the separated compounds were calculated from integration of the peaks in MS chromatograms.

Identification of Essential Oil Components

Identification of essential oil components were carried out by comparison of their relative retention indices (RRI) obtained by series of *n*-alkanes (C5 to C30) to the literature (Başer et al., 2000a; 2000b; 2001; 2002; 2003; 2006; Demirci et al., 2003; 2006; Gören et al., 2001; Karamenderes et al., 2008; Kırımer et al., 2000; Kıvçak et al., 2004; Kıyan et al., 2014; Kürkçüoğlu et al., 2006; Maggio et al.,2012; Polatoğlu et al., 2010; 2011; 2013; 2014; 2015; 2017; Suleimenov et al., 2001; Tabanca et al., 2001; 2006a; 2006b; 2007; Tunalıer et al., 2002; Tunalıer et al., 2003; Viljoen et al., 2006) and with mass spectra comparison to the in-house libraries (Wiley W9N11, NIST11).

Acetylcholinesterase Inhibition Assay

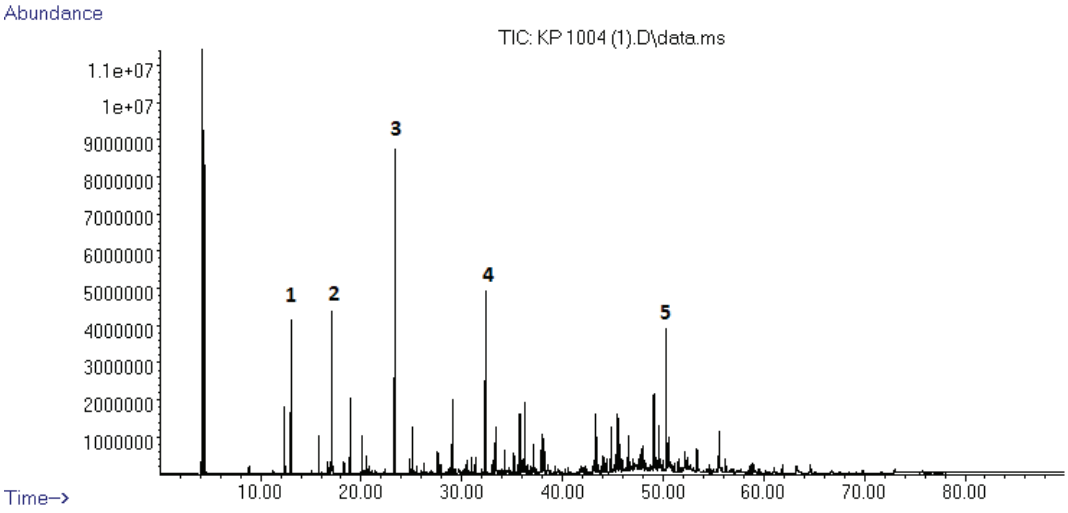
The inhibitory effect of the *Tripleurospermum inodorum* flower essential oil on AChE was determined with the previously described protocol (Ellman et al., 1961). The assay solution contained 240 µL, 1.25 mM 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), 192 µL acetylthiocholine iodide (AChI), 1200 µL, 100 mM Tris–HCl buffer pH 8.0 and 20 µL essential oil. The blank solution contained 20 µL of buffer solution instead of the essential oil. Galanthamine hydrobromide (from *Lycoris* sp.), was used as a positive control in the assay. The calibration curve obtained by testing AChE inhibition of different concentrations of galanthamine was constructed the data points were fitted with logarithmic function to obtain the calibration curve $y = 24.968\ln(x) + 32.003$, that have $R^2 = 0.9934$. Reactions were started by adding 0.0325 U/mL of AChE (electric eel) into the reaction mixture. The reaction was monitored for 2 min at 412 nm wavelength using a spectrophotometer (Carry 60 single beam spectrophotometer, Agilent Technologies, USA). The enzymatic activity was calculated as the percentage of the reaction rate in

accordance with the activity obtained from the blank. The data obtained from the linear section of the initial 60 s were used in the calculation of the activities. The AChE inhibition was calculated by the subtraction of the ratio of the sample activity versus blank activity from 100. The results of the experiments were given as mean ± standard deviation of three parallel experiments.

Results & Discussion

Tripleurospermum inodorum flowers, leaves and stems afforded trace amount of essential oils with yields 0.03, 0.02 and 0.01% (v/w) respectively. Ninety compounds were identified in the flower essential comprising of 80.7% of the oil. Flower oil was dominated by oxygenated monoterpenes. The main components of the essential oil were artemisia ketone 14.4%, terpinen-4-ol 5.5%, 1,8-cineole 5.1%, sabinene 4.7% and tricosane 4.6%.

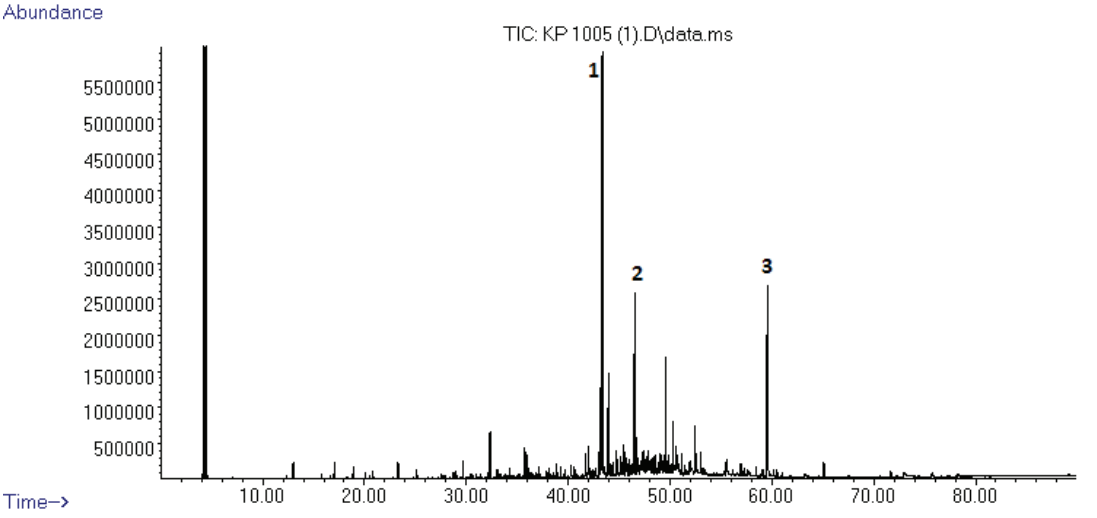
Figure 1. GC-MS Chromatogram of *Tripleurospermum inodorum* flower essential oil.



1: Sabinene; **2:** 1,8-Cineole; **3:** Artemisia ketone; **4:** Terpinen-4-ol; **5:** Tricosane.

Sixty-four compounds were identified in the leaves essential oil which corresponds to 62.7% of the oil. Unlike the flower essential oil, leaf oil contained higher amounts of oxygenated sesquiterpenes and diterpene "phytol". Main components of the leaf essential oil were caryophyllene oxide 16.0%, phytol 12.1% and spathulenol 5.9%. Twenty-eight compounds were identified in the stem essential oil that sums up to 87.2% of the oil.

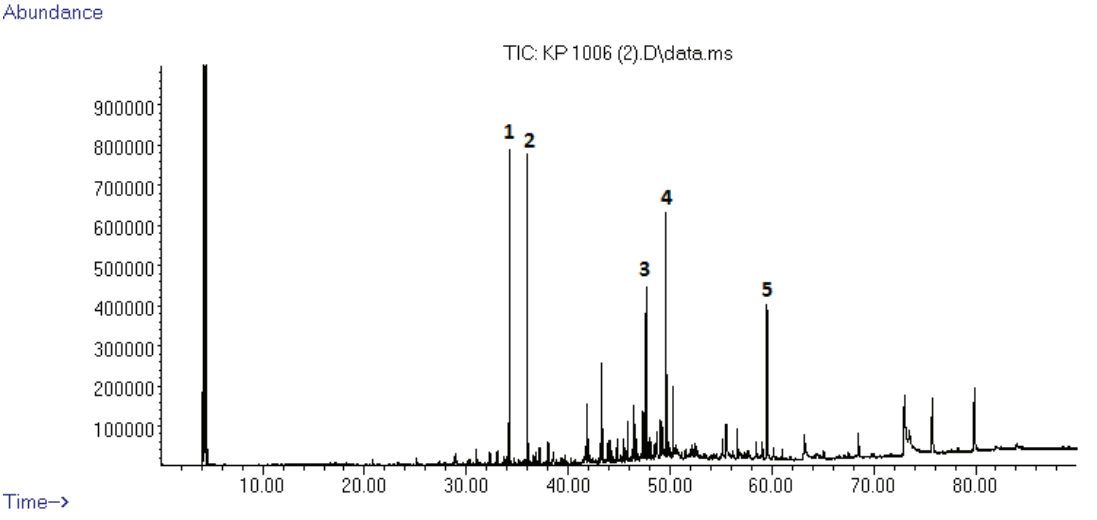
Figure 2. GC-MS Chromatogram of *Tripleurospermum inodorum* leaf essential oil.



1: Caryophyllene oxide, **2:** Spathulenol; **3:** Phytol.

Stem essential oil was dominated by oxygenated sesquiterpenes and saturated *n*-alkane derivatives. The main components of the stem essential oil were neryl acetate 12.8%, (*E*)-β-farnesene 12.5%, phytol 12.1%, guaia-6,10(14)-dien-4β-ol 10.8%, γ-cadinol 7.8%, nonacosane 7.3%, decanoic acid 6.3% and caryophyllene oxide 4.6%.

Figure 3. GC-MS Chromatogram of *Tripleurospermum inodorum* stem essential oil.



1: (*E*)-β-Farnesene; **2:** Neryl acetate; **3:** γ-Cadinol; **4:** Guaia-6,10(14)-dien-4β-ol; **5:** Phytol.

Previously, essential oils of *Tripleurospermum* species with high amounts of (*E*)-β-farnesene was reported like the stem essential oil of *T. inodorum* (Javidnia, et al., 2008). Other reports indicate high amounts of (*Z*)-β-farnesene from *Tripleurospermum corymbosum* and *T. decipiens* (Kürkçüoğlu et al., 2016; Öztürk et al., 2010). Previous reports on the essential oils of *Tripleurospermum* species mostly indicate an essential oil profile either rich in sesquiterpenes, oxygenated sesquiterpenes, acetylenes or *n*-alkane derivatives (Bär & Schultze, 1996; Javidnia, et al., 2008; Kürkçüoğlu et al., 2016; Öztürk et al., 2010; Yaşar et al., 2005). Flower essential oil composition of *T. inodorum* from İstanbul showed a different chemical profile from the previous reports. The flower essential oil contained irregular monoterpenes such as artemisia ketone and artemisia alcohol. Additionally, leaf and stem oils contained high amounts of the diterpene "phytol" and a different profile of sesquiterpenes in comparison to the previous reports. Furthermore, none of the investigated essential oils contained any acetylene derivative.

Table 1. Essential oil composition of *Tripleurospermum inodorum* flowers, leaves and stems.

RRI[1]	RRI Lit.[2]	Compound	Flower (%)	Leaves (%)	Stem (%)	Identification method[3]
1023	1032	α-Pinene	0.2	Tr[4]	-	RI, MS
1028	1035	α-Thujene	0.2	Tr	-	RI, MS
1087	1093	Hexanal	Tr	Tr	-	RI, MS
1114	1118	β-Pinene	2.0	Tr	-	RI, MS
1126	**1132**	**Sabinene**	**4.7**	**0.6**	**-**	RI, MS
1168	1174	β-Myrcene	Tr	-	-	RI, MS
1183	1188	α-Terpinene	1.0	Tr	-	RI, MS
1202	1203	Limonene	0.3	Tr	-	RI, MS
1212	**1213**	**1,8-Cineol**	**5.1**	**0.6**	**-**	RI, MS
1212	1218	β-Phellandrene	0.2	-	-	RI, MS
1239	1244	2-Pentyl furan	0.3	Tr	-	RI, MS
1251	1255	γ-Terpinene	2.1	0.4	-	RI, MS
1277	1280	*p*-Cymene	1.0	Tr	-	RI, MS
1282	1285	Isoamylisovalerate	Tr	-	-	RI, MS
1284	1286	2-Methylbutyl-2-methylbutyrate	0.1	-	-	RI, MS
1287	1290	Terpinolene	0.5	Tr	-	RI, MS
1294	1296	Octanal	0.2	0.3	-	RI, MS
1299	1303	Amyl isovalerate	Tr	-	-	RI, MS
1356	**1358**	**Artemisia ketone**	**14.4**	**0.5**	**-**	RI, MS
1394	1393	3-Octanol	0.1	-	-	RI, MS
1401	1403	Yomogi alcohol	1.3	-	-	RI, MS
1399	1400	Nonanal	-	0.3	-	RI, MS

1400	1400	Tetradecane	Tr	Tr	-	RI, MS, Ac
1430	1430	α-Thujone	0.3	-	-	RI, MS
1451	1451	β-Thujone	Tr	-	-	RI, MS
1452	1452	1-Octen-3-ol	-	-	-	RI, MS
1469	1474	*Trans*-sabinene hydrate	0.6	Tr	-	RI, MS
1476	1482	Longipinene	0.3	Tr	-	RI, MS
1497	1504	α-Copaene	0.2	Tr	-	RI, MS
1510	1510	Artemisia alcohol	1.8	Tr	-	RI, MS
1528	1532	Camphor	Tr	-	-	RI, MS
1530	1535	Dihydroedulan I	Tr	-	-	RI, MS
1528	1535	β-Bourbonene	-	0.6	-	RI, MS
1554	1556	*Cis*-sabinene hydrate	0.3	Tr	-	RI, MS
1570	1571	*Trans*-*p*-menth-2-en-1-ol	0.4	-	-	RI, MS
1582	1586	Pinocarvone	0.6	Tr	-	RI, MS
1599	1600	β-Elemene	0.1	Tr	-	RI, MS
1611	**1611**	**Terpinen-4-ol**	**5.5**	**2.0**	**Tr**	RI, MS
1634	1638	β-Cyclocitral	-	0.5	-	RI, MS
1634	1641	*Cis*-β-terpineol	0.4	-	-	RI, MS
1641	1641	α-Thujenal	0.8	-	-	RI, MS
1645	1648	Myrtenal	1.3	Tr	-	RI, MS
1648	1651	Sabinaketone	0.2	-	-	RI, MS
1655	1658	Umbellulone	Tr	-	-	RI, MS
1667	1664	*Trans*-pinocarveol	0.1	-	-	RI, MS
1673	**1671**	**(*E*)-β-Farnesene**	**0.6**	**0.3**	**12.5**	RI, MS
1685	1687	α-Humulene (= α-Caryophyllene)	Tr	Tr	-	RI, MS
1688	1689	*Trans*-piperitol	0.2	-	-	RI, MS
1702	1704	γ-Muurolene	Tr	-	-	RI, MS
1704	1706	α-Terpineol (=*p*-Menth-1-en-8-ol)	0.6	-	-	RI, MS
1724	1726	Germacrene D	1.6	1.0	Tr	RI, MS
1734	**1733**	**Neryl acetate**	**0.4**	**0.8**	**12.8**	RI, MS
1739	1740	α-Muurolene	0.5	0.4	Tr	RI, MS
1747	1748	Piperitone	1.9	Tr	-	RI, MS
1748	1755	Bicyclogermacrene	0.2	Tr	-	RI, MS
1756	1758	*Cis*-piperitol	0.2	Tr	Tr	RI, MS
1758	1758	(*E*,*E*)-α-Farnesene	0.1	Tr	Tr	RI, MS
1771	1773	δ-Cadinene	0.7	0.4	Tr	RI, MS

1800	1802	Cuminic aldehyde	0.7	-	-	RI, MS
1804	1804	Myrtenol	1.0	0.4	-	RI, MS
1824	1827	(*E*,*E*)-2,4-Decadienal	0.4	Tr	Tr	RI, MS
1837	1838	(*E*)-β-Damascenone	-	0.5	Tr	RI, MS
1864	1868	(*E*)-Geranyl acetone	-	0.3	Tr	RI, MS
1940	1945	1,5-Epoxy-salvial-4(14)-ene	Tr	1.3	-	RI, MS
1946	1953	Palustrol	0.2	Tr	-	RI, MS
1947	1946	Dendrolasine	-	-	2.8	RI, MS
1955	1958	*(E)*-β-ionone	0.2	1.4	Tr	RI, MS
1997	2001	Isocaryophyllene oxide	-	1.2	-	RI, MS
2007	**2008**	**Caryophyllene oxide**	**1.6**	**16.0**	**4.6**	RI, MS
2031	2037	Salvial-4,14-en-1-one	0.4	3.5	Tr	RI, MS
2039	2041	Pentadecanal	0.4	**-**	-	RI, MS
2049	2057	Ledol	0.3	0.4	-	RI, MS
2069	2073	*p*-Mentha-1,4-dien-7-ol	1.6	-	-	RI, MS
2092	2092	β-Oplopenone	2.1	-	-	RI, MS
2117	2113	*p*-Cymene-7-ol	0.4		-	RI, MS
2134	2131	Hexahydrofarnesyl acetone	0.4	3.8	2.2	RI, MS
2144	**2142**	**Spathulenol**	**1.0**	**5.9**	**Tr**	RI, MS
2150	2179	Nor-copanone	-	0.8	-	RI, MS
2174	**2170**	**3,4-Dimethyl-5-pentylidene-2(5H)-Furanone**	-	-	**4.0**	RI, MS
2187	**2187**	**γ-Cadinol**	0.7	-	**7.8**	RI, MS
2200	2200	Bisabolene oxide A	0.4	-	-	RI, MS
2214	2219	δ-Cadinol	1.1	0.4	-	RI, MS
2225	2226	Hexadecanoic acid methyl ester	0.2	-	-	RI, MS
2236	2241	*p*-Isopropylphenol	0.2	-	-	RI, MS
2242	2242	Isospathulenol	-	0.3	-	RI, MS
2250	2255	α-Cadinol	2.2	0.9	Tr	RI, MS
2272	**2269**	**Guaia-6,10(14)-dien-4β-ol**	1.3	-	**10.8**	RI, MS
2282	**2282**	**Decanoic acid**	0.6	-	**6.5**	RI, MS
2300	**2300**	**Tricosane**	**4.6**	**2.1**	**3.9**	RI, MS, Ac
2317	2316	Caryophylla-2(12),6(13)-dien-5β-ol (= caryophylladienol-I)	1.2	-	-	RI, MS
2353	2324	Caryophylla-2(12),6(13)-dien-5-α-ol	-	0.6	-	RI, MS
2362	2359	Cedr-8-en-13-ol	0.2	-	-	RI, MS
2373	2399	Aromadendrene oxide	-	0.4	-	RI, MS

2400	2400	Tetracosane	0.1	-	-	RI, MS, Ac
2500	2500	Pentacosane	1.7	1.2	Tr	RI, MS, Ac
2523	2533	γ-Costol	0.5	-	-	RI, MS
2589	2594	9-Hexacosene	Tr	-	-	RI, MS
2589	2589	1-Octadecanol	-	0.5	-	RI, MS
2596	2604	α-Costol	0.4			
2602	2606	β-Costol	0.4	Tr	-	RI, MS
2617	**2617**	Phytol	**Tr**	**12.1**	**12.1**	RI, MS
2652	2655	Benzyl benzoate	0.3	Tr	Tr	RI, MS
2700	2700	Heptacosane	0.6	Tr	Tr	RI, MS, Ac
2900	**2900**	**Nonacosane**	**Tr**	**Tr**	**7.3**	RI, MS, Ac
		Total	**80.7**	**62.7**	**87.2**	

[1]RRI: Relative retention time indices calculated against *n*-alkanes (C5-C30); [2]RRI Lit.: Relative retention time given in the literature for the compound in similar columns and analysis conditions; [3]Identification method: RI: identification based on the relative retention times (RRI) of genuine compounds on the HP Innowax column and the literature data; MS: identification based on MS comparison with the database or the literature data, Ac: Identification is done according to RRI and MS values of the authentic compounds; [4]Tr: Trace amount 0.1> .

Acetylcholinesterase inhibitory property of the flower oil was also investigated at four different concentrations of the oil. Highest AChE inhibitory property was observed for 20 mg/mL oil concentration (53.35 ± 1.37 %; *n* =3). This AChE inhibitory concentration was equivalent to the activity of 1.26 µg galanthamine hydrobromide in the assay. Activity of the essential oil was concentration dependent. Previously, AChE inhibitory property of artemisia ketone was reported to be less than 20% (Seo et al., 2014). Furthermore, AChE inhibitory properties of terpinen-4-ol and 1,8-cineole were reported. Both compounds reported to show competitive inhibition of AChE with IC_{50} = 0.04 ± 0.00 mM and 10.30 ± 0.61 mM for 1,8-cineole and terpinen-4-ol respectively (Mills et al., 2004). As reported earlier the main components of the *T. inodorum* flower essential oil were reported to have AChE inhibitory properties, therefore it is evident that the observed AChE inhibitory activity is caused by the collective effects of these compounds and other substances present in the essential oil.

Table 2. Acetylcholinesterase inhibitory property of *Tripleurospermum inodorum* flower essential oil.

Essential oil concentration (mg/mL)[1]	AChE Inhibition %	Standard deviation	Number of replications (*n*)
20	53.35	1.37	3
10	36.56	0.93	3
5	32.64	0.83	3
1	23.72	1.50	3

[1] Concentration of the oil sample in methanol.

Conclusion

The essential oil composition of *T. inodorum* from Turkey was investigated for the first time. The essential oil composition was completely different from the previously reported *M. perforata* (*Syn = T. inodorum*) flower oil from Germany (Bär & Schultze, 1996). Especially, alkyne derivatives reported from some of *Tripleurospermum* species do not exist in all species. According to the previous reports and the current study, it is clear that *Tripleurospermum* species have a high amount of chemical diversity which should be investigated further. It is evident from the previous literature that the essential oil composition of *Tripleurospermum* could show variation due to the methodology used in obtaining the essential oils as well as the collection period of the plant species. Further studies on the chemical variation of this species is required in order to verify this variation as well as to provide information on whether certain classes of compounds might be used as chemo-taxonomical markers for the genus. The flower essential oil of *T. inodorum* also afforded a moderate AChE inhibitory activity which could provide potential beneficial uses of this essential oil.

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Conflict of Interests

Authors declare no conflict of interests

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