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Determination of volatile components of *Viscum album* L. distributed naturally in Eğirdir (Isparta) province, Turkey

Abstract. This study aims to determining the volatile oil components of naturally distributed *Viscum album* L. belonging to the Santalaceae family. The study was carried out in villages of Eğirdir province of Isparta in Turkey. The isolation of volatile components was performed from of the leaves of the mistletoe collected from five different tree species. Qualitative analysis of volatile components were carried out by the Head Void-Solid Phase Micro Extraction (HS-SPME) technique combined with gas chromatography/mass spectrometry (GC-MS). The identification of the constituents was carried out by comparing the retention index (RI) and mass spectral data (MS) to those reported in the published literature. As a result of the Head Void-Solid Phase Micro Extraction (HS-SPME) technique combined with gas chromatography/mass spectrometry (GC-MS). 54 different components were determined and (E)-2-Hexenal, Benzaldehyde and Nonanal were found as the main components. It is known that there are limited number of studies on the determination of essential oil and volatile components of the *Viscum* genus. According to this study, the use of mistletoe as a raw material source in different industrial areas have importance.

Keywords: *Viscum*, volatile components, GC-MS, Isparta, Turkey.

Introduction

Turkey is one of the richest countries in the world in terms of plant diversity due to the fact that it is under the influence of various climatic types it has and is at the junction of three different phytogeographic regions, its geographical situation, geological structure, different topographic structures and soil groups [1].

The need for natural resources is increasing day by day and prompts people to explore the possibilities of benefiting from these resources by the increasing population and industrialization. Today, the importance of natural foods and nutrition with these foods is increasing rapidly; medicinal plants and treatment with these plants, the use and importance of medicinal plants are increasing at the same speed [2]. Medicinal and aromatic plants are used in traditional and modern medicine as medicine for disease prevention and health. It is also used in nutrition as nutritional supplements, herbal tea, flavor and spice. In addition to being used in perfumery and cosmetics as perfume and body care products, it is used in different areas of industry as polishes and even insecticides [3]. Many medicinal and aromatic plants are used in various fields due to their different mechanisms of action with their seeds, flowers, fruits, leaves or roots [4]. Plants have important biological effects on human metabolism through their components. In particular, components such as flavonoid, alkaloid, terpenoid, tannin, essential oil, berberine, quinine and emetine come to the fore [5-6].

Among medicinal and aromatic plants, plants rich in essential oils are becoming increasingly important, and the demand for these resources has increased and continues to increase in recent years [7].

Mistletoe, which has 36 genera and 1400 species in the world, is one of the plants that live as parasitic and semi-parasitic in many tree species. *Viscum* genus members are mainly distributed in Europe, Africa, Asia, America, and Australia. Its extracts are used as complementary and alternative medicines in the treatment of various ailments [8-10].

There are 3 subspecies of *Viscum album* L., which is naturally distributed in our country, belonging to the Santalaceae family. These are *Viscum album* subsp. *abietis* spreading on fir, *Viscum album* subsp. *austriacum* on pine trees and also *Viscum album* subsp. *album* on broad-leaved plants. Although mistletoe causes damage to trees with increase and loss of quality, it is used as a fodder in the livestock sector and as a medicine in pharmacy against human health [11].

Mistletoe is a plant with many medicinal properties. Fruit and leafy branches are metabolism drug useful for regulating blood pressure, cleaning the blood, strengthening the eyebrows, increasing urine, strengthening and beneficial effects on the digestive system, diuretic, secretory glands and also manage inflammation, hypertension, ulcers, and other diseases due to the presence of different bioactive compounds, among them mistletoe lectins and viscotoxins [12-16]. Some activities of *Viscum album* extracts are varied relating to the host trees, such as antioxidant, apoptosis-inducing, anticancer activities of the plant [17].

Its fruits are crushed with burning gum and used for rheumatism in Gaziantep, Urfa and Van regions in Turkey. In addition, crushed fruits are placed on the boil and cause inflammation [13]. It is used as an ointment mixed with tallow and mistletoe, and its consumption as tea is used to treat chronic cramps and pancreatic diseases, to regulate diabetes, liver bleeding, to stop intestinal bleeding after typhoid fever and dysentery, and to stop blood from nose bleeding when cold tea is inhaled [18].

In this study, determination of the essential oil content and components of *Viscum* species that were obtained from Isparta Eğirdir Beydere village, Eğirdir Center, Barla village, Akdoğan village, were aimed.

Materials and methods

The samples of *Viscum album* subsp. *album* were collected from plum, pear, almond and hawthorn species and also samples of *Viscum album* subsp. *austriacum* were obtained on Black pine trees. The coordinates and altitude information of the areas where the samples were collected are given in Table 1 and Figure 1. The plant was identified by us using the discrimination key in “Flora of Turkey” [19].

Table 1 – Location of sampling sites

	Province	Host Tree	Location	Altitude
Viscum album subsp. *album*	Eğirdir	Hawthorn	37°52’32.03”N 30°49’25.73”E	952
	Eğirdir Barla Village	Pear	38°01’09.50”N 30°45’36.43”E	1281
	Eğirdir Beydere Village	Almond	37°56’16.84”N 30°44’56.05”E	1020
	Eğirdir	Plum	37°52’32.03”N 30°49’25.73”E	952
Viscum album subsp. *austriacum*	Eğirdir Akdoğan Village	Black pine	37°46’32.10”N 30°58’31.20”E	1206

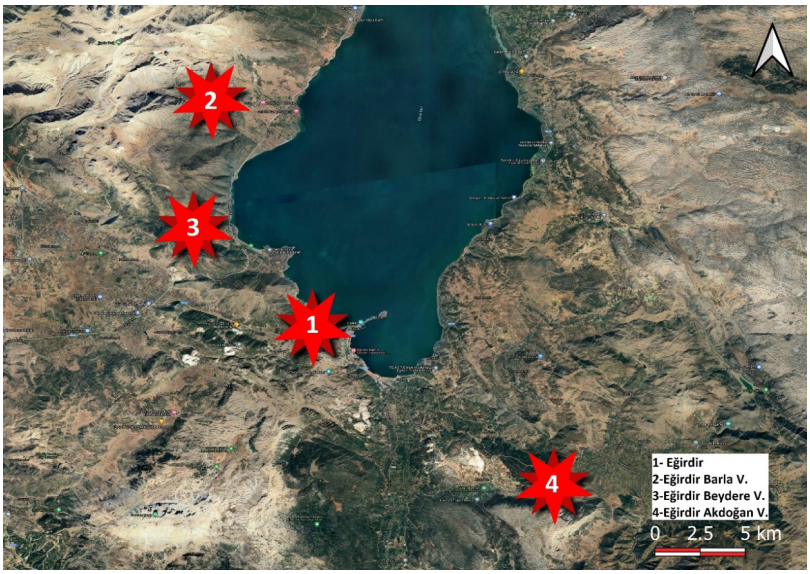

Figure 1 – Sampling sites in Eğirdir (Isparta) province

After the leaf samples of *Viscum album* were harvested, they were placed in paper bags and transferred to the laboratory within the same day, without waiting and protected from sunlight. Collected thyme samples were dried at room temperature (25°C). The volatile components of the leaves were determined by the Head Void-Solid Phase Micro-Extraction (HS-SPME) technique combined with gas chromatography/mass spectrometry (GC/MS). Based on the solid phase micro-extraction technique, 2 g of leaf flower samples from each plant were placed in a 10 mL vial and kept at 60°C for 30 minutes after being covered with a silicone cap (Figure 2).

To adsorb the volatile substances, the SPME apparatus head-passed with 75 µm thick Carboxene/ Polydimethylsiloxane (CAR/PDMS) coated fused silica fiber, then inserted into the capillary column of the Shimadzu 2010 Plus GC-MS device (Restek Rx-5 Sil MS 30 mx 0.25 mm, 0.25 µm) injected directly. This process was repeated three times, the accuracy of the results was compared, and the averages were taken and the results were given. The device operated in EI mode (70 eV) was connected to the same brand mass selective detector. Helium with a flow rate of 1.61 mL per minute was used as the carrier gas. Injection block and detector temperatures were set at 250°C and Retention Indices (RI) of volatile components were calculated according to the standard for C7-C30 alkane mixtures under the above-mentioned chromatographic conditions. Wiley, NIST Tutor and FFNSC libraries were used to identify volatile components (Figure 2).

Figure 2 – The volatile components of the leaves were determined by HS-SPME technique combined with GC/MS

Results and discussion

The volatile components of the collected plant samples were determined by SPME (solid phase microextraction method) analysis method, 21 mistletoe samples collected from hawthorn trees, 23 mistletoe samples collected from pear trees, 25 mistletoe samples collected from almond trees, 29 mistletoe samples collected from plum trees and 17 samples from black pine trees. A total of 54 different components were determined. In all of the mistletoe samples collected from five different tree species, (E)-2-Hexenal (respectively hawthorn: 14.11%; pear: 16.88%; almond: 20.59%; plum: 29.56%; larch: 21%, 49), Benzaldehyde (respectively hawthorn: 27.97%; pear: 41.44%; almond: 28.92%; plum: 44.60%; larch: 23.75%) and Nonanal (hawthorn: 24%, respectively, 97; pear: 15.88%; almond: 19.23%; plum: 10.80%; larch: 17.05%) were determined as the main components. Among the volatile component classes, it was found that aromatic aldehydes were high (Table 2).

Table 2 – Volatile components and classes of mistletoe collected from different tree species

	R.Time	Components	Hawthorn	Pear	Almond	Plum	Black pine	Formula	Class
	4.693	n-Hexenal	*	*	*	*	9,62	$C_6H_{12}O$	AA
	6.187	**(E)-2-Hexenal**	14.11	16.88	20.59	29.56	21.49	$C_6H_{10}O$	AA
	6.320	cis-3-Hexenyl formate	*	*	*	0.29	*	$C_7H_{12}O_2$	FA
	6.350	3-Hexen-1-ol, (Z)	*	*	1.10	*	*	$C_6H_{12}O$	AA
	6.361	4-Hexen-1-ol, (Z)-	*	*	*	*	2.01	$C_6H_{12}O$	AA
	7.791	Heptanal	0.59	0.27	*	0.17	0.66	$C_7H_{14}O$	AA
	8.113	Sorbaldehyde	*	*	*	0.71	*	C_6H_8O	AAI
	8.420	Ocimene	*	*	0.07	*	*	$C_{10}H_{16}$	MH
	8.607	I-Phellandrene	*	*	1.41	*	*	$C_{10}H_{16}$	MH
	8.845	Z-Methyl 3-hexenoate	*	*	*	0.43	*	$C_7H_{12}O_2$	FA
	8.850	α-Pinene	*	*	1.96	*	9.11	$C_{10}H_{16}$	MH
	9.434	Camphene	*	*	2.13	*	*	$C_{10}H_{16}$	MH
	9.797	(E)- 2-Heptenal	0.33	*	*	0.18	*	$C_7H_{12}O$	AA
	9.906	**Benzaldehyde**	27.97	41.44	28.92	44.60	23.75	C_7H_6O	AAI
	10.125	2-Hexenoic acid	*	*	*	0.09	*	$C_6H_{12}O_2$	FA
	10.886	6-Methyl-5-Hepten-2-One B	*	0.70	*	0.43	1.39	$C_8H_{14}O$	AA
	10.900	3-Octanone	*	*	1.20	*	*	$C_8H_{16}O$	AAI
	11.019	β-Myrcene	*	*	1.13	*	*	$C_{10}H_{16}$	MH
	11.330	(E,E)- 2,4-Heptadienal,	*	*	*	1.53	*	$C_7H_{10}O$	AA
	11.564	Octanal	2.00	0.78	2.78	0.34	1.46	$C_8H_{16}O$	AA
	12.027	α-Terpinene	*	*	2.02	*	*	$C_{10}H_{16}$	MH
	12.310	Cymol	*	0.73	2.96	*	0.69	$C_{10}H_{14}$	MH
	12.323	m-Cymene	1.87	*	*	0.18	*	$C_{10}H_{14}$	MH
	12.653	1,8-Cineole	*	*	0.36	*	*	$C_{10}H_{18}O$	OM
	13.050	Benzeneacetaldehyde	5.93	1.77	0.95	2.75	*	C_8H_8O	OC
	13.230	β. Ocimene	*	*	*	0.37	1.13	$C_{10}H_{16}$	MH
	13.645	γ-Terpinene	*	*	7.67	*	*	$C_{10}H_{16}$	MH
	13.676	Isopinocarveol	0.86	*	*	*	*	$C_{10}H_{16}O$	OM
	13.690	(E)- 2-Heptenal,	*	*	*	0.04	*	$C_7H_{12}O$	AA
	14.160	3,5-Octadien-2-one	*	*	*	0.58	0.35	$C_8H_{12}O$	AAI
	15.330	d-Nerolidol	*	*	0.26	*	*	$C_{15}H_{26}O$	OSH
	15.339	α-Terpinolene	0.62	*	*	*	*	$C_{10}H_{16}$	MH
	15.345	Linalool	*	0.34	*	0.31	*	$C_{10}H_{18}O$	OM
	15.499	**Nonanal**	24.97	15.88	19.23	10.80	17.05	$C_9H_{18}O$	AAI
	16.875	Thujyl alcohol	*	0.40	*	*	*	$C_{10}H_{18}O$	OM
	19.190	Dodecane	*	*	*	0,06	*	$C_{12}H_{26}$	AH
	19.388	Decanal	1.41	1.47	1.04	0.22	*	$C_{10}H_{20}O$	OM
	20.297	Cis-3-Hexenyl-2-methylbutyrate	*	*	*	*	1.47	$C_{11}H_{20}O_2$	FA
	21.452	Dec-2(E)-enal	*	*	*	0.09	*	$C_{10}H_{18}O$	OM
	22.530	Thymol	*	2.29	*	*	*	$C_{10}H_{14}O$	OM
	22.848	Carvacrol	8.71	2.56	0.72	0.60	0.42	$C_{10}H_{14}O$	OM

Table continuation

	R.Time	Components	Hawthorn	Pear	Almond	Plum	Black pine	Formula	Class
	23.083	Undecanal	3.87	0.73	0.33	*	*	$C_{11}H_{22}O$	AE
	25.719	β-Bourbonene	1.09	2.23	0.53	*	*	$C_{15}H_{24}$	SH
	26.315	Tetradecane	1.13	1.15	1.19	2.06	*	$C_{14}H_{30}$	AH
	26.591	Lauric aldehyde	0.93		*	0.22	*	$C_{11}H_{24}O$	AAI
	26.926	trans-Caryophyllene	0.61	1.95	0.33	0.45	*	$C_{15}H_{24}$	SH
	27.265	Germacrene-D	*	2.80	*	*	*	$C_{15}H_{24}$	SH
	27.379	α-trans-Bergamotene	*	0.91	*	0.21	0.84	$C_{15}H_{24}$	SH
	28.026	(E)-, β Farnesene	0.71	1.49	0.33	0.96	5.67	$C_{15}H_{24}$	SH
	28.252	Alloaromadendrene		2.28	0.79	0.99	*	$C_{15}H_{24}$	SH
	28.256	Aromadendrene	0.79	*	*	*	*	$C_{15}H_{24}$	SH
	28.736	Cyclosativene	*	0.41	*	*	*	$C_{15}H_{24}$	SH
	29.694	Farnesene	0.98	0.54	*	0.78	2.89	$C_{15}H_{24}$	SH
	29.811	β-Bisobolene	0.52	*	*	*	*	$C_{15}H_{24}$	SH
Total:			**100**	**100**	**100**	**100**	**100**		
Component Number			21	23	25	29	17		
AA: Aromatic alcohol			17.03	18.63	24.47	32.25	36.63		
AAI: Aromatic aldehyde			53.87	57.32	49.35	56.91	41.15		
AE: Acetylenic compound			3.87	0.73	0.33	0	0		
AH: Aromatic hydrocarbon			1.13	1.15	1.19	2.12	0		
FA: Fatty acids methyl ester			0	0	0	0.81	1.47		
MH: Monoterpene hydrocarbon			2.49	0.73	19.35	0.55	10.93		
OC: Other compound			5.93	1.77	0.95	2.75	0		
OM: Oxygenated monoterpene			10.98	7.06	2.12	1.22	0.42		
OSH: Oxygenated sesquiterpene hydrocarbon			0	0	0.26	0	0		
SH: Sesquiterpene hydrocarbon			4.70	12.61	1.98	3.39	9.40		

***** Components in bold are the main components.

Studies on the determination of essential oil and volatile components of *Viscum* genus are limited. Tabe et al. [20] determined the essential oil components of the flowers of mistletoe. They stated that Eicosyl vinyl ester carbonic acid (19.94%), Heptadecyle isobutyl ester carbonic acid (17.81%), Dimethyl-sitanediol (16.46%), Heptadecyle isobutyl ester carbonic acid (16.02%) and 9-methyl-Nonadecane (15.58%) as main components. Wang et al. (2019) determined that benzaldehyde (9.64%), geranylacetone (7.92%), epoxy-β-ionone (7.71%), β-linalool (7.35%), methyl salicylate (6.96%) and hotrienol (6.14%) as main components of *Viscum articulatum* Burm.f.

The main components determined in the studies performed differ from the components determined in our study. Benzaldehyde component, which is among the main components in our study, Wang et al. [21] was also found among the main components in their study. In other studies, on *Viscum album*, the presence of various active substances such as lectin [22], viscotoxin, phenylpropane, lignan, flavonoid [23], alkaloid and polyholoside [24] were determined. Mistletoe has antioxidant properties that inhibit bacterial growth and cell damage. Because of this feature, it is used to cure cancer and cardiovascular diseases [25-26].

Conclusion

Medicinal and aromatic plants are used in traditional and modern medicine as medicine for disease prevention and health. Also, fruit and leafy branches of *Viscum album* are metabolism drug useful for regulating blood pressure, cleaning the blood, strength-

ening the eyebrows, increasing urine, strengthening and beneficial effects on the digestive system, diuretic, secretory glands and also manage inflammation, hypertension, ulcers. It is known that the mistletoe plant, which lives as a parasite and semi-parasite, is used as a feed plant in the livestock sector and as a raw material for drugs against many diseases in pharmacy. As a result, in our study to determine the volatile components of mistletoe samples collected from five different tree species, 54 different components were determined, and (E)-2-Hexenal, Benzaldehyde and Nonanal were determined as the main components. It is thought that our study will shed light on and be a basis for scientific studies to be carried out to determine the volatile components of mistletoe and to investigate the usability of the plant in various fields.

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