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RESEARCH PAPER

Determination of the leaf and flower volatile components of *Thymus jankae* Celak

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Abstract

Nowadays, medicinal and aromatic plants are widely used both among the public and in various sectors, so researching the essential oil contents of medicinal and aromatic plants is of great scientific and economic importance. It was aimed to determine the volatile components of *Thymus jankae* (Syn: *Thymus praecox* subsp. *skorpilii*), which has medicinal and aromatic value and is naturally distributed in Bursa province (Türkiye) and used as a spice and tea plant in the region. The volatile components of leaf and flower of *Thymus jankae* were detected by using the solid phase microextraction technique (SPME) with the help of GC-MS. 42 volatile components were determined. Of them, thymol (17.65%), *p*-cymene (15.34%), carvacrol (13.27%) and γ-terpinene (10.73%) were found to be the main components. This study is important in terms of ensuring the conscious use of plants consumed as natural herbal tea and spices in the region and revealing the economic values of such plants.

INTRODUCTION

Today, medicinal and aromatic plants are widely used both among the public and in various sectors as food, spice, medicine, cosmetics and treatment purposes. For this reason, researching the essential oil contents of medicinal and aromatic plants is of great scientific and economic importance. Medicinal and aromatic plants, known for their rich essential oil content, are widely utilized in the fragrance and taste industries. These plants play a crucial role in the preparation of perfumes, food additives, cleaning products, cosmetics, and medicines. They serve as sources of aroma chemicals and as starting materials for synthesizing nature-identical and semi-synthetic aroma chemicals. Cellulose, pectin, sugar, and other substances are commonly found in many drugs. In addition to these, active ingredients such as essences derived from essential oils are also present in medications. These essential oil essences are known to have significant pharmacological effects (Weiss, 1997; Kılıç, 2005).

Essential oils are strong-smelling and oily mixtures that are generally liquid at room temperature and sometimes freeze. Essential oils are very effective against bacteria, fungi and protists, thanks to the strong components they contain. Essential oils are mostly found in the flower, fruit, rhizome, resin and wood parts of plants (Cowan, 1999; Çelik, 2007;

Hanamanthagouda et al., 2010).

The Lamiaceae family, which is dense in volatile components, has 236 genera and 7172 species in the world (Harley et al., 2004). In Türkiye, plants belonging to the genera *Origanum* L., *Thymus* L., *Satureja* L., *Coridothymus* Rchb.f. and *Thymbra* L. are known as thyme species (Başer 2001; Sarı and Oğuz 2002; Temel 2000; Turgut et al. 2008). *Thymus*, a genus of the Lamiaceae family, has 43 species and 48 taxa, 20 of which are endemic to the Turkish flora (Davis, 1982; Celep and Dirmenci, 2017; Öztürk et al., 2022). Thymus are used as flavorings (spices and condiments), aromatic medicinal plants and herbal teas due to their pleasant smell. It is used in the treatment of diseases such as tonsillitis, eczema, urinary system diseases and hemorrhoids (Tümen et al., 1995; Polat et al., 2007; Topal et al., 2008; Özgen et al., 2011; Nouasri et al., 2015; Yaşar et al., 2016).

When reviewing the literature, Başer et al. (1996) identified geraniol (24.21%), α-terpinyl acetate (22.67%), and geranyl acetate (9.31%) as the primary components in *Thymus praecox* subsp. *skorpilii* var. *skorpilii*. Additionally, Vidic et al. (2010) reported linalyl acetate (28.7%) and linalool (14.4%) as significant constituents, while Özen et al. (2011) determined thymol (40.31%) and o-cymene (13.66%) to be predominant. In our study, it was aimed to determine the volatile components of *Thymus jankae* (Syn: *Thymus praecox* subsp. *skorpilii*), which have medicinal and aromatic value and are naturally distributed in Bursa and used as a spice and tea in the region. Headspace Solid Phase Micro Extraction (HS-SPME) technique combined with gas chromatography/mass spectrometry (GC-MS) was chosen, as it is a solvent-free analysis technique for the determination of volatile components, which significantly reduces processing time and costs and is environmentally friendly (Vas and Vekey, 2004; Malik et al., 2006; Araujo et al., 2007; Başer and Buchbauer, 2010; Dönmez and Salman, 2017).

MATERIALS and METHODS

Plant Material

The leaves and flowers of *Thymus jankae* (Syn: *Thymus praecox* subsp. *skorpilii*) collected during the flowering period from different points of Bursa, which has a significant plant diversity, constitute the material of the study (Figure 1). The study material was collected from rocky and stony areas and the vicinity of dwarf *Juniper* communities at altitudes ranging from 1800 to 2200 meters. The above-ground of the plant samples collected from the research areas were placed in paper packages and transported to the laboratory on the same day, without any waiting or exposure to sunlight. Plant samples were identified by us in the Forest Botany Laboratory of Bursa Technical University, Faculty of Forestry, Department of Forest Engineering. The source "Volume 7 of Flora of Türkiye and the Eastern Aegean Islands" was used to identify dried plant samples. Furthermore, comparisons were made with samples belonging to the species with the specimen number BULU 29257 A, which are available in the Uludağ University Herbarium (BULU). The identified plant materials were dried at room temperature.

Figure 1. An image of the leaves and flowers of *Thymus jankae*.

Determination of leaf and flower volatile components by HS-SPME/GC-MS analysis

Based on the solid phase microextraction technique, 2 g of the leaf and flower samples taken from the sample dried at room temperature were placed in a 10 mL vial and kept at 60 °C for 30 minutes after its mouth was closed with a silicone cap. The SPME apparatus was passed through the headspace with 75 µm thin Carboxene/Polydimethylsiloxane (CAR/

PDMS) coated fused silica fiber to adsorb volatile substances and then transferred to the capillary column of the Shimadzu 2010 Plus GC-MS device (Restek Rx-5 Sil MS 30 m x 0.25 mm). , 0.25 µm) was injected directly. The oven temperature is programmed to reach 250ºC with an increase of 4ºC per minute after waiting at 40ºC for 2 minutes. Injector and detector temperatures were set at 250ºC. EI (70 eV) was used as the ionization type and Helium (1.61 mL/min) as the carrier gas. This process was repeated three times for each collection time, the accuracy of the results was compared, and the results were given as an average. Retention Indices (RI) of volatile components were calculated according to the standard for C7-C30 alkane mixtures under the above-mentioned chromatographic conditions. Identification of the compounds was carried out by comparing them to the compounds found in the mass spectra and spectral library (Wiley, Nist, Tutor, FFNSC).

RESULTS and DISCUSSION

The volatile components of *Thymus jankae* leaves and flowers were detected using SPME (Solid Phase Microextraction) coupled with GC-MS (Gas Chromatography-Mass Spectrometry). 42 different volatile compounds were detected in the leaves and flowers of *T. jankae* such as thymol (17.65%), *p*-cymene (15.34%), carvacrol (13.27) and γ-terpinene (10.73%) being the main compounds. When the component classes were examined, it was determined that monoterpene hydrocarbons and aromatic hydrocarbons were abundant (Table 1; Figure 2).

Table 1. Volatile component composition of *Thymus jankae*.

	R. Time	Components	%	Class	Formula
1.	8.728	*α*-Pinene	2,40	MH	$C_{10}H_{16}$
2.	9.279	Camphene	2,17	MH	$C_{10}H_{16}$
3.	10.173	β- Phellandrene	0,66	MH	$C_{10}H_{16}$
4.	10.322	*β*-Pinene	1,09	MH	$C_{10}H_{16}$
5.	10.561	1-Octen-3-ol	0,56	AAl	$C_8H_{16}O$
6.	10.731	3-Octanone	0,34	AAl	$C_8H_{16}O$
7.	10.929	β-Myrcene	6,62	MH	$C_{10}H_{16}$
8.	11.448	*α*-Phellandrene	0,61	MH	$C_{10}H_{16}$
9.	11.871	*α*-Terpinolene	0,36	MH	$C_{10}H_{16}$
10.	12.166	**p-Cymene**	15,34	MH	$C_{10}H_{14}$
11.	12.379	Limonene	3,66	MH	$C_{10}H_{16}$
12.	12.470	1,8-Cineole	6,11	OM	$C_{10}H_{18}O$
13.	12.671	cis-Ocimene	0,64	MH	$C_{10}H_{16}$
14.	13.108	β-Ocimene	2,05	MH	$C_{10}H_{16}$
15.	13.495	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	2,42	OM	$C_{10}H_{16}O$
16.	13.645	**γ-Terpinene**	10,73	SH	$C_{10}H_{16}$

17.	13.966	trans-Sabinene hydrate	0,16	OM	$C_{10}H_{18}O$
18.	14.641	L-Fenchone	0,08	OM	$C_{10}H_{16}O$
19.	15.165	Linalool	2,13	OM	$C_{10}H_{18}O$
20.	16.280	p-Mentha-1,5,8-triene	0,17	MH	$C_{10}H_{14}$
21.	16.700	2,4,6-Octatriene, 3,4-dimethyl-	0,19	MH	$C_{10}H_{16}$
22.	16.754	Trans-3-Caren-2-Ol	0,69	OM	$C_{10}H_{16}O$
23.	16.883	1,7,7-trimethyl-heptan-2-ol,	1,56	OM	$C_{10}H_{18}O$
24.	17.890	Borneol	1,56	OM	$C_{10}H_{18}O$
25.	18.216	4-Terpineol	0,15	OM	$C_{10}H_{18}O$
26.	18.782	.β. Fenchyl Alcohol	0,15	OM	$C_{10}H_{18}O$
27.	19.229	Verbenone	0,08	AH	$C_{10}H_{14}O$
28.	22.495	**Thymol**	17,65	AH	$C_{10}H_{14}O$
29.	22.852	**Carvacrol**	13,27	AH	$C_{10}H_{14}O$
30.	25.554	β-Bourbonene	0,11	SH	$C_{15}H_{24}$
31.	25.760	β-Elemene	0,12	SH	$C_{15}H_{24}$
32.	26.789	Caryophyllene	1,96	SH	$C_{15}H_{24}$
33.	27.627	Cadina-1(6),4-diene <10betaH->	0,07	SH	$C_{15}H_{24}$
34.	27.873	β-Farnesene	0,47	SH	$C_{15}H_{24}$
35.	27.943	α-Humulene	0,79	SH	$C_{15}H_{24}$
36.	28.166	Epi-Bicyclosesquiphellandrene	0,09	SH	$C_{15}H_{24}$
37.	28.787	Germakren-D	0,68	SH	$C_{15}H_{24}$
38.	29.265	.δ.-Gurjunene	0,23	SH	$C_{15}H_{24}$
39.	29.516	Valencene	0,15	SH	$C_{15}H_{24}$
40.	29.660	β-Bisabolene	0,82	SH	$C_{15}H_{24}$
41.	29.821	γ-Cadinene	0,75	SH	$C_{15}H_{24}$
42.	29.992	δ-Cadinene	0,16	SH	$C_{15}H_{24}$
Total			**100**		
Number of Components			42		
AAI: Aromatic aldehyde			0,90		
AH: Aromatic hydrocarbon			31,00		
MH: Monoterpene hydrocarbon			35,96		
OM: Oxygenated monoterpene			15,01		
SH: Sesquiterpene hydrocarbon			17,13		

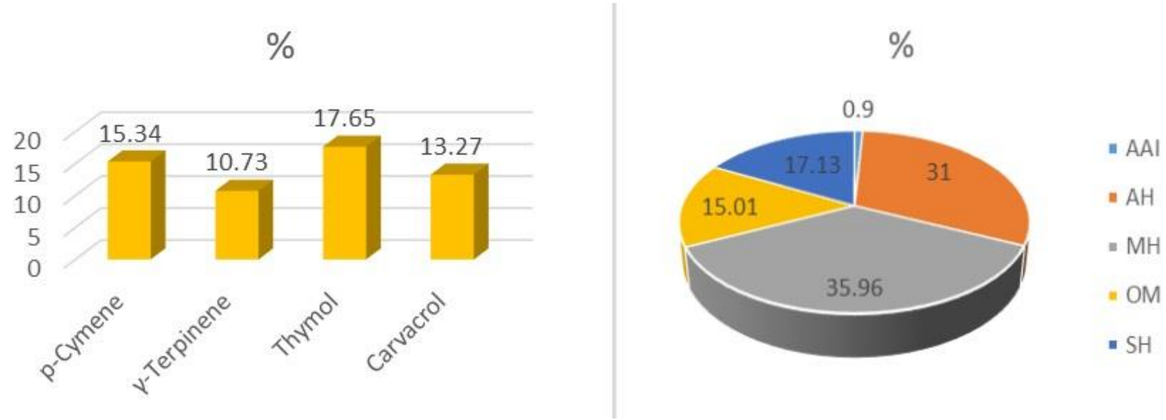

Figure 2. Main volatile components and classes of *Thymus jankae*.

CONCLUSION

Using SPME technique coupled with GC-MS, 42 different volatile components were identified in the leaves and flowers of *T. jankae* (Syn: *Thymus praecox* subsp. *skorpilii*) and thymol (17.65%), *p*-cymene (15.34%), carvacrol (13.27%), and γ-terpinene (10.73%) were found as the main components. Thymol is important due to its diverse pharmacological properties like antioxidant, anti-inflammatory, antibacterial, and cardioprotective effects, making it valuable in various industries and traditional medicine (Agarval et al., 2020). *p*-cymene, a monoterpene, exhibits antimicrobial properties along with antioxidant, anti-inflammatory, and anticancer effects (Marchese et al., 2017). Carvacrol, exhibits diverse therapeutic properties such as antioxidant, anti-inflammatory, antimicrobial and anticancer activities, making it a potent compound with a significant pharmacological potential (Gandova et al., 2023). γ-Terpinene is known for antibacterial and antiviral properties (Mueller-Uri et al., 2015). When *Thymus praecox* subsp. *skorpilii* var. *skorpilii* water-distilled essential oil was analyzed using GC and GC/MS, the main components identified were geraniol (24.21%), α-terpinyl acetate (22.67%), and geranyl acetate (9.31%). Vidic et al. (2010) found linalyl acetate (28.7%) and linalool (14.4%), while Özen et al. (2011) identified thymol (40.31%) and o-cymene (13.66%). Thymol was identified as one of the main components in research, including the study by Özen et al. (2011).
It can be seen that the main components detected in our study differ from the literature and the extraction method used, the regions where the plants are collected and the differences in environmental factors are effective on the main components.

In conclusion; in addition to being used as a spice, the *T. jankae* is also used in the food, cosmetics and pharmaceutical industries. The volatile oil obtained from its leaves and flowers is also used in different areas. As a result of the increase in the use of the species, pressure on its natural distribution areas is increasing due to its collection only from nature. It has been determined that the quality of the plant decreases, the distribution areas are destroyed and the plant yield decreases due to problems such as harvesting by unconscious collectors and lack of transportation. In-situ and ex-situ protection measures should be taken to ensure the sustainability of the plant. In addition, since it is a source of raw materials in many industrial areas, its transformation into value-added products is important for the country's economy. It is recommended that studies in this context should be increased.

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