

Chemical composition of *Vitex agnus-castus* L. flowers collected from populations distributed in Aydın, Türkiye

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Abstract

Essential oils (EOs) are compounds that are biosynthesized autonomously by aromatic plants as their secondary metabolites. *Vitex agnus-castus* plant leaves, flowers and fruits are of pharmacological importance. In this study, the chemical composition determination of the essential oils of the *Vitex agnus-castus* flowers with different colors (white, pink, purple) growing in Aydın ecological conditions was carried out with the extraction using Clevenger hydro-distillation apparatus and then the essential oil composition was determined by Gas Chromatography-Mass Spectrometer (GC-MS). As a result of the study, 'Çakmar' pink-flowered population had the main component (-)-allospathulenol (14.59%), 'Çakmar' purple-flowered and 'Koçarlı' purple-flowered populations had the main component pyrrolo (3,2,1-jk) carbazole (14.42% and 14.68%, respectively), while 'Çakmar' white flowered, 'Koçarlı' white flowered and 'Koçarlı' pink flowered populations had the main component caryophyllene (15.54%, 12.64%, and 19.05 respectively).

Keywords: GC-MS; essential oils; *Vitex agnus-castus*; Türkiye

Introduction

All over the world, various plants have been used for various reasons. Especially, medicinal and aromatic plants are grown for use in preventing diseases, as flavoring agents in incense, cosmetics, preserving food and improving the taste of many types of food (Toplan *et al.*, 2022; Yılmaz *et al.*, 2022). In addition, many studies reporting medicinal plants with important biological activities such as antioxidant, antimicrobial, antiviral, and antiproliferative activities have been revealed (Zhang *et al.*, 2014; Suleman *et al.*, 2021; Gecer, 2022). *Vitex* is one of the largest genera of the Lamiaceae family (formerly belonging to the Verbenaceae family), and consists of 217 species. Some of the known and studied *Vitex* species are *Vitex negundo*, *Vitex agnus-castus*, *Vitex trifolia*, *Vitex rotundifolia*, *Vitex cymosa* and *Vitex peduncularis* (Souto *et al.*, 2020; Kamal *et al.*, 2022). The *Vitex* genus is represented by two species (*V. agnus-castus* and *V. pseudo-negundo* (Hausskn. Ex Bornm.) Hand.-Mazz) in

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the flora of Türkiye (Eryiğit *et al.*, 2015). *Vitex agnus-castus* is defined as vitex, agnos and lygos by ancient literature (Kolancı, 2017). In Turkish, is known as 'hayıt, ayd, hayed, beşparmak grass, and bitter ayit' (Kavaz *et al.*, 2022). It spreads especially in the Eastern Black Sea, Marmara, Aegean, Mediterranean, and partially in the Southeastern Anatolia region of Türkiye (Fakir *et al.*, 2014). When this plant is looked at from an aesthetic point of view; the length of its spike-shaped flowers, its plump flower structure, its rich colour range from white-light pink to purple, its sustained blooming, and the striking appearance of its large seeds on the spike formed after its flowers, increase the variety of usage areas in herbal landscaping (Girmen and Karagüzel, 2005). *Vitex agnus-castus* is rich in numerous bioactive substances it is a well-known herbal plant (Zhelev *et al.*, 2022). *V. agnus castus* fruits, flowers and leaves contain phenolic acids and derivatives, flavonoids, tannins, iridoids and diterpenoids (Sarikurkcü *et al.*, 2009; Sävulescu *et al.*, 2019; Bakr *et al.*, 2020). Since ancient times, local flowers and fruits have been applied in various cultures (Özderin, 2021). Traditionally, chasteberry extract has been used to treat many gynecological conditions, including menstrual disorders, premenstrual syndrome (PMS), corpus luteum insufficiency, infertility, acne, menopause, impaired lactation, and various gynecological problems (Stojković *et al.*, 2011; Ogaly *et al.*, 2021). In this study, essential oil content of different colored (white, pink, purple) *V. agnus-castus* flowers grown in Aydın ecological conditions was determined.

Materials and Methods

Collection of plant samples and extraction of essential oils

For the essential oil extraction process, approximately 500 grams of *V. agnus-castus* flower samples were collected from different regions of Aydın, Türkiye, six populations with white, purple, and pink flowers in three different colors. The collected flower samples were kept with their moisture evaporated in an oven at 35 °C for 24 hours, then distilled for about 4 hours by hydro-distillation method in the Clevenger apparatus to extract the essential oil, and kept at +4 °C for chromatographic analysis.

Chromatographic analysis by GC-MS

The qualitative analysis of the essential oil obtained from the plant sample was detected by Gas Chromatography-Mass Spectrometry (GC-MS) analysis system. Helium was used as the carrier gas and separation was performed with an HP-5MS capillary column coated with 5% phenyl-methylpolysiloxane (30.0 m x 250µm x 0.25 µm). GC-MS temperature program was applied from 50 °C to 80 °C ramped with 5 °C/min waiting for 2 minutes, 80 °C to 100 °C ramped with 2 °C/min waiting for 1 minute, 100 °C to 150 °C ramped with 2 °C/min waiting for 1 minute, 150 °C to 240 °C ramped with 2 °C/min waiting for 1 minute and finally from 240 °C to 270 °C ramped with 2 °C/min waiting for 7 minutes (Rambla *et al.*, 2015; Apaydın, 2018). The volatile compounds which detected were identified with Wiley and NIST mass spectrometry libraries.

Results and Discussion

Essential oils (EOs) which are biosynthesized by aromatic plants as their secondary metabolites and represented by a strong odor are natural, volatile and complex mixtures of lipophilic compounds, often including terpenes, phenol-derived aromatic compounds and aliphatic compounds (Bakkali *et al.*, 2008; Santos *et al.*, 2016; Marić *et al.*, 2021). There have been numerous studies to determine the chemical composition of essential oils obtained from chasteberry (Moudachirou *et al.*, 1998; Senatore *et al.*, 2003; Sarikurkcü *et al.*, 2009; Stojković *et al.*, 2011; Duymuş *et al.*, 2014; Fakir *et al.*, 2014; Gulsoy Toplan *et al.*, 2015; Ulukanlı *et al.*, 2015; Eryiğit *et al.*, 2015; Tin *et al.*, 2017; Zhelev *et al.*, 2022 etc.). In our study, essential oil composition of *V.*

agnus-castus flowers analyzed (components with a total ratio of $\geq 2\%$) is shown in Table 1, while GC-MS chromatogram images are shown in Figures 1, 2, 3, 4, 5 and 6. As a result of the study, 'Çakmar' pink-flowered population had the main component (-)-allospathulenol (14.59%), 'Çakmar' purple-flowered and 'Koçarlı' purple-flowered populations had pyrrolo (3,2,1-jk)carbazole (14.42% and 14.68% respectively), while 'Çakmar' white flowered, 'Koçarlı' white flowered and 'Koçarlı' pink flowered populations had the main component of caryophyllene (15.54%, 12.64%, and 19.05 respectively). PCA (Principal Component Analysis) (simca 14.1) was used to determine the effect of flower varieties on volatile compounds.

Table 1. Composition of the essential oil from *Vitex agnus-castus* flowers of different populations

Çakmar pink flowers			Çakmar purple flowers			Çakmar white flowers		
RT	Component	%	RT	Component	%	RT	Component	%
7.423	α -pinene	2.45	21.516	cyclohexene	3.24	10.530	1.8-cineole	2.22
8.539	sabinene	2.99	24.446	trans β -farnesene	4.63	21.499	α -terpinyl propionate	4.90
15.108	cyclopentasiloxane	2.05	25.556	bicyclogermacrene	6.08	23.490	caryophyllene	15.54
21.591	1-p-menthen-8-yl acetate	6.19	27.839	spathulenol	6.11	24.423	trans- β -farnesene	4.66
23.554	caryophyllene	5.53	27.965	caryophyllene oxide	4.28	27.782	spathulenol	6.36
24.480	trans β -farnesene	4.35	28.480	ledol	2.37	27.925	caryophyllene oxide	5.85
28.051	(-)-allospathulenol	14.59	29.418	delta-cadinene	4.08	29.390	delta-cadinene	5.57
28.120	caryophyllene oxide	3.71	34.866	guaia-3,9-diene	2.07	36.187	n-(m-fluorophenyl)-maleimide	4.68
28.675	ledol	2.56	36.250	n-(m-fluorophenyl)-maleimide	13.70	36.977	pyrrolo(3,2,1-jk)carbazole	11.46
29.447	α -cadinol	2.57	36.685	1-(3-hexyl)-2,5-dimethylbenzene	3.57	38.688	α -selinene	4.29
36.279	4(1h)-pteridinone	6.54	37.040	pyrrolo(3,2,1-jk)carbazole	14.42	39.346	sigmosceptrellin-b	2.34
37.103	pyrrolo(3,2,1-jk)carbazole	10.77	38.728	bakkenolide-a-diol	6.28			
38.763	cycloheptane	4.67						
39.386	kolavelool	2.29						
Koçarlı purple flowers			Koçarlı white flowers			Koçarlı pink flowers		
RT	Component	%	RT	Component	%	RT	component	%
8.493	sabinene	3.49	9.987	β -phellandrene	2.12	6.811	α -pinene	3.00
10.513	1.8-cineole	3.79	25.179	(+)-2-carene	6.10	8.058	sabinene	4.24
21.505	4-terpinenyl ester of isobutanoic acid	5.63	28.915	caryophyllene	19.05	9.998	β -phellandrene	2.19
23.485	caryophyllene	7.47	32.468	bicyclogermacrene	6.19	15.360	cyclopentasiloxane	5.32
24.423	trans- β -farnesene	4.08	39.129	(+)-epi-bicyclosesquiphellandrene	2.54	25.110	(+)-2-carene	3.34
25.522	bicyclogermacrene	5.23	45.486	2-methyl-6-propylpyridine-3-carboxylic acid	3.02	28.829	caryophyllene	12.64
27.811	spathulenol	7.25	48.810	(z)- β -caryophyllene	3.32	30.563	trans- β -farnesene	5.38
27.942	caryophyllene oxide	4.23	50.407	1,2,4-triazolo(3,4-c)(1,2,4)-benzotriazin-1(5h)-one	2.98	32.463	bicyclogermacrene	4.50
29.396	α -cadinol	3.53	51.437	β -isomethylionone	4.96	45.520	2h-1,4-benzothiazine	3.04
36.222	n-(m-fluorophenyl)-maleimide	13.15	53.685	levopimaradiene	2.38	48.822	(z)- β -caryophyllene	3.08
36.674	1-(3-hexyl)-2,5-dimethylbenzene	3.54	55.682	cycloheptane	2.60	50.464	1,2,4-triazolo(3,4-c)(1,2,4)-benzotriazin-1(5h)-one	3.49
37.006	pyrrolo(3,2,1-jk)carbazole	14.68				51.431	β -isomethylionone	3.74
38.711	bakkenolide-a-diol	6.72				52.879	3-fluoro-5,7-dimethylquinol-2(1h)one	3.67

39.358	sigmosceptrellin-b	2.62				55.654	methylpropyleyne	7.73
						56.604	kolavellool	3.57
						68.706	4,4'-dimethyl-2,2',5,5'-tetramethoxy-1,1'-biphenyl	2.02

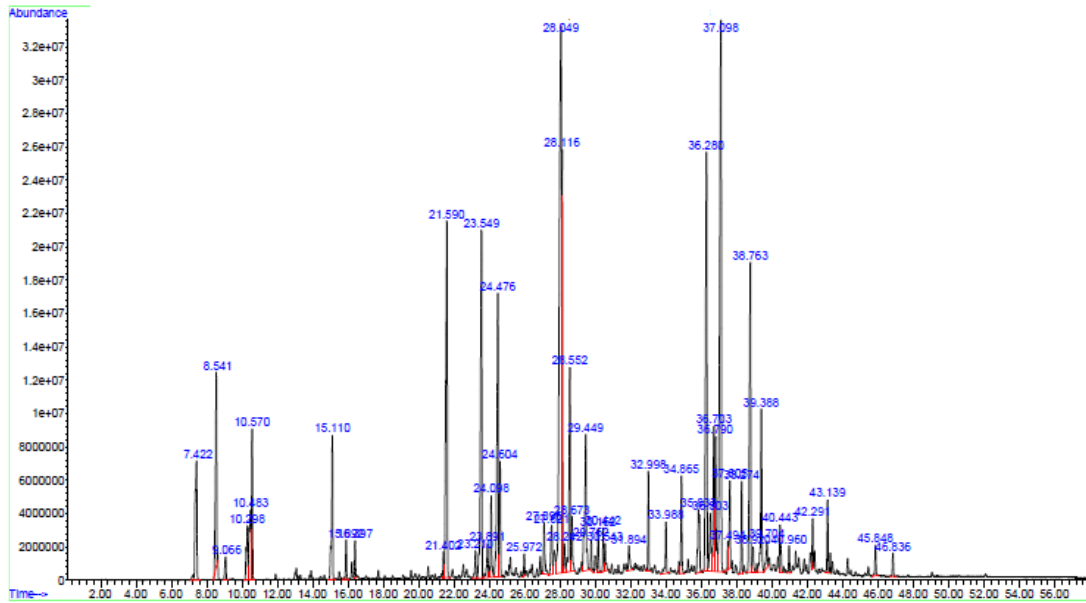


Figure 1. Gas chromatogram of the essential oil of 'Çakmar' pink flowers

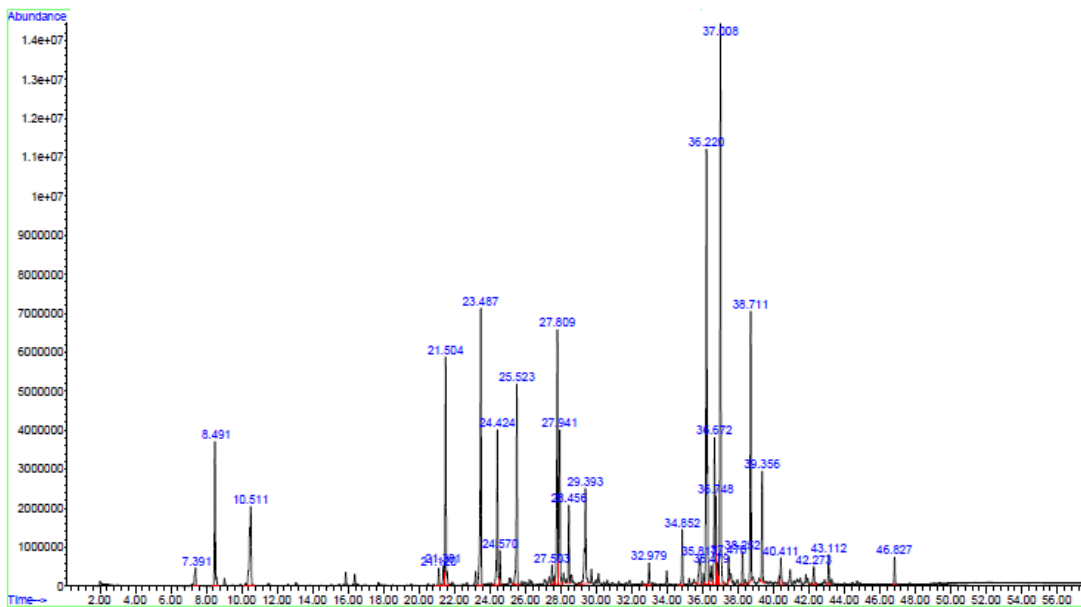


Figure 2. Gas chromatogram of the essential oil of 'Koçarlı' purple flowers

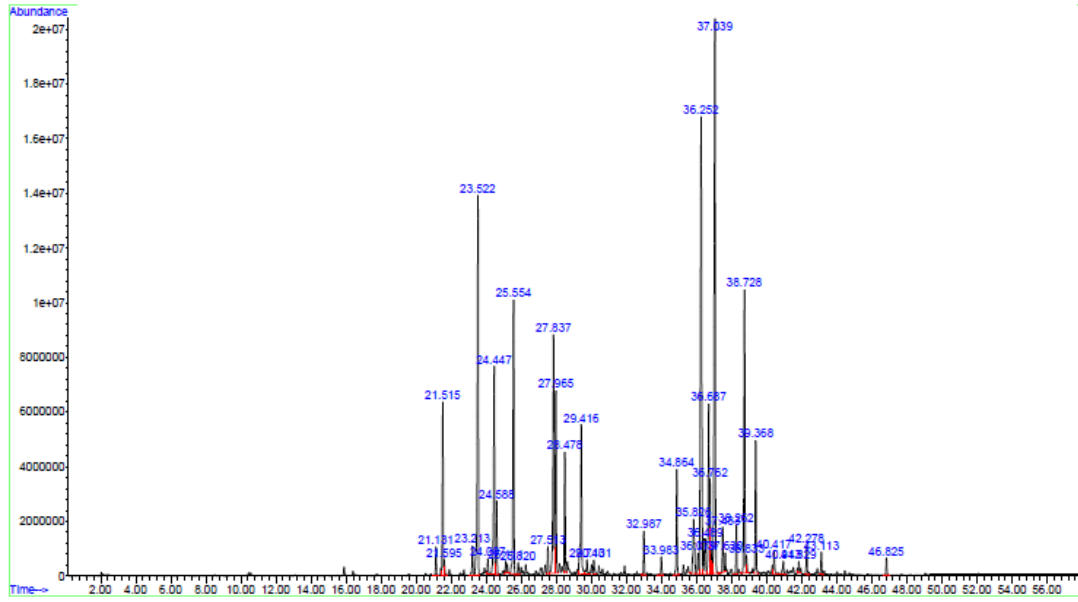


Figure 3. Gas chromatogram of the essential oil of 'Çakmar' purple flowers

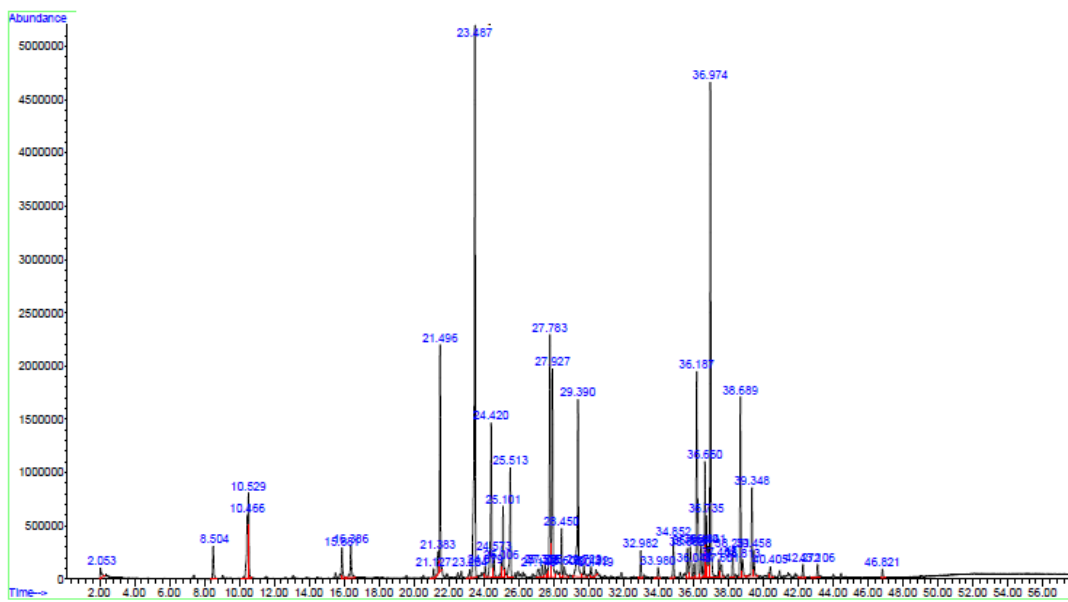


Figure 4. Gas chromatogram of the essential oil of 'Çakmar' white flowers

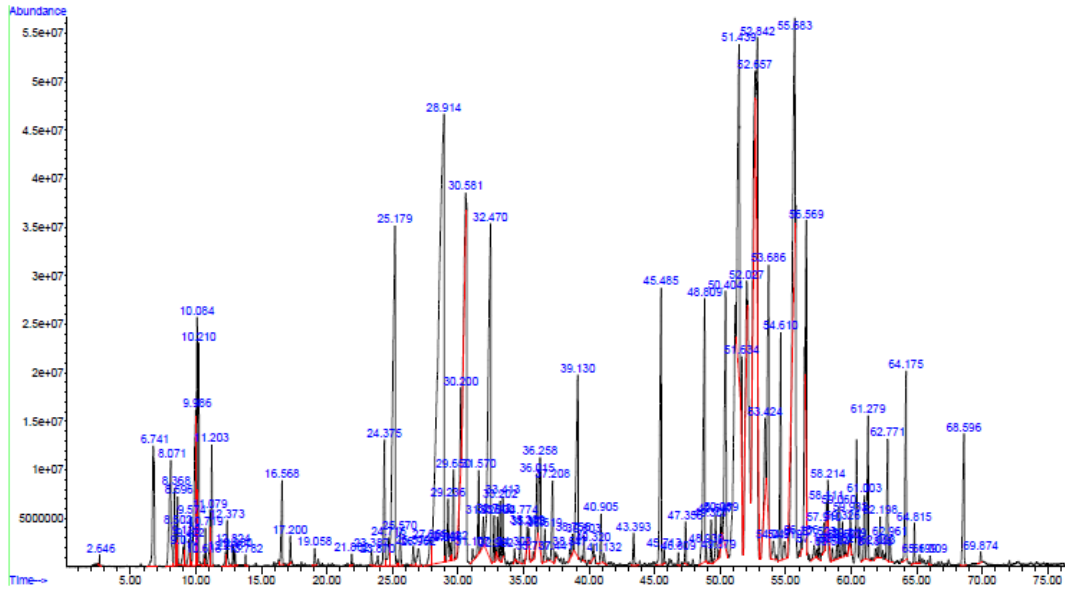


Figure 5. Gas chromatogram of the essential oil of 'Koçarlı' white flowers

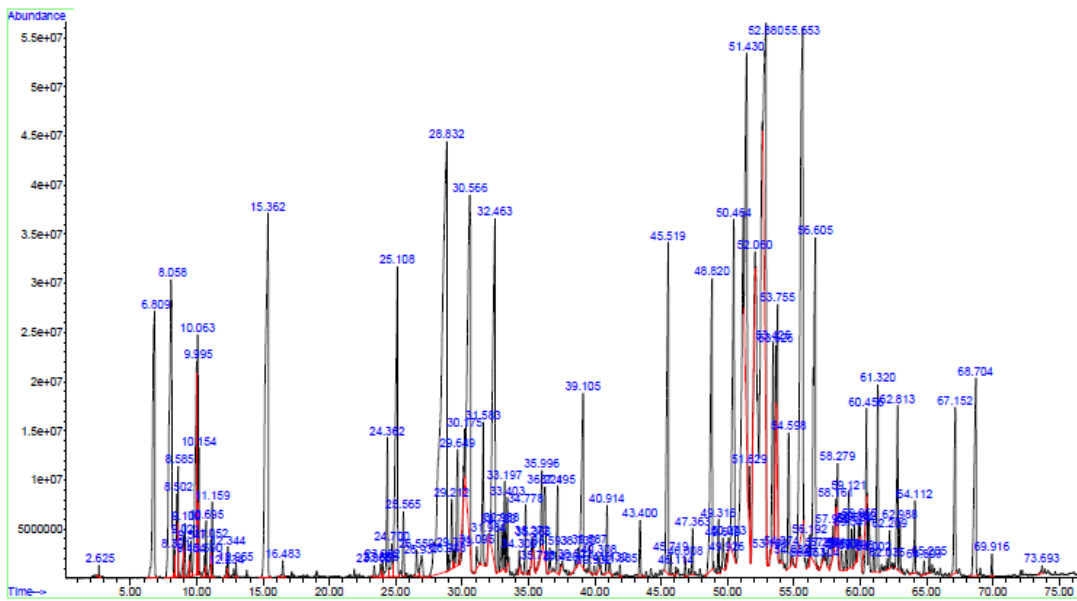


Figure 6. Gas chromatogram of the essential oil of 'Koçarlı' pink flowers

The biplot diagram for the first and second components shows the correlation of the studied flowers based on the volatile compounds detected. According to the data obtained, the first and second components (PC1 and PC2) explained 59.7% of the total variance. Except for 'Çakmar' purple flower, 'Çakmar' and 'Koçarlı' flower populations were collected in two different groups according to PC1 (Figure 7).

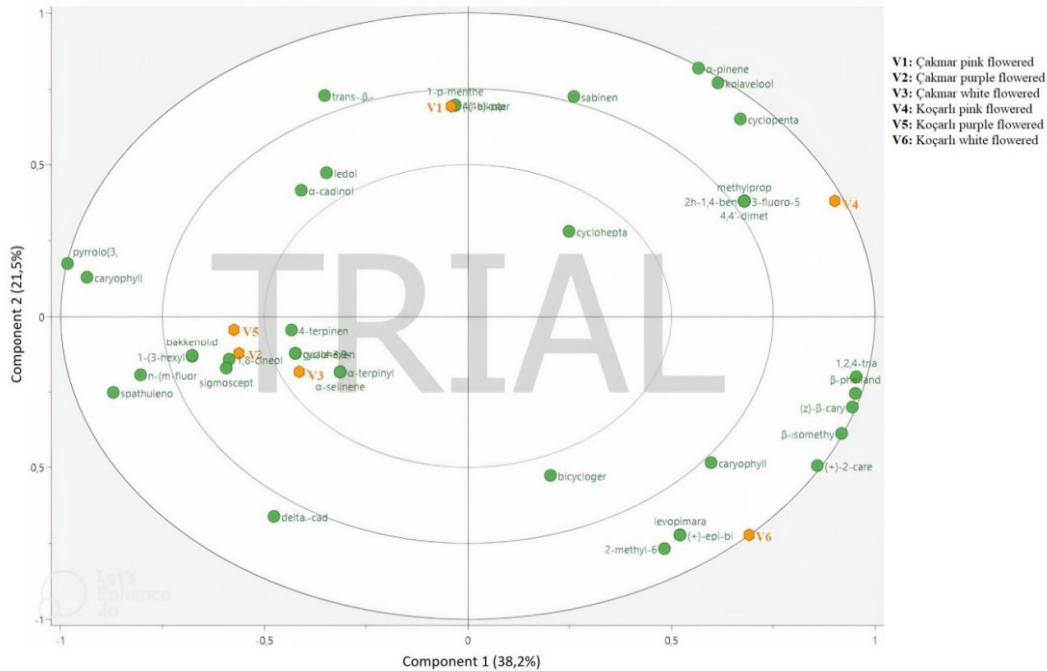


Figure 7. Biplot of the first two principal components

In chasteberry fruits collected from Aydın, Türkiye, Tin *et al.* (2017) determined 1.8 cineole (8.24%) as the main component. Gulsoy Toplan *et al.* (2015) examined the essential oils of *V. agnus-castus* fruits collected from Zonguldak, Edirne, Balıkesir, Muğla, Antalya and the main components were determined as β -caryophyllene (11.7%), bicyclogermacrene (22.1%), 1.8-cineole (17.3%), 1.8-cineole (13.2%), sabinene and bicyclogermacrene (12.1%), respectively. In the essential oils of the fruits of the chasteberry plant collected from Izmir/Turkey, Eryiğit *et al.* (2015) determined trans-caryophyllene (19.17%) as the main component. Duymuş *et al.* (2014) determined sabinene (22.7%) and 1.8-cineole (22.4%) as the main components of fruits and the fruitless herbs, respectively, of *V. agnus-castus* collected from İzmir, Türkiye. Fakir *et al.* (2014) determined essential oil content of *V. agnus castus* collected from Isparta, Türkiye during the flowering period and the main component was α -pinene (26.99), while the main component was 1.8 cineole (28.34%) during the fruit ripening period. Ulukan *et al.* (2015) determined the main component as 1.8-cineole (24.38%) from essential oils of chasteberry leaves collected from Osmaniye, Türkiye. Sarikurkcü *et al.* (2009) identified 1.8-cineole (24.98%) as the main component of chasteberry fruits from Manisa, Türkiye. Senatore *et al.* (2003) determined 1.8-cineol (21.6%) as the most abundant in the essential oil obtained from the white flowered fruit of *Vitex agnus-castus* collected from İçel, Türkiye, and caryophyllene (30.9%) as the most in essential oil obtained from the fruits of the purple flowered ones. In Montenegro, Stojkovic *et al.* (2011) found the main component as sabinene (17.8%) in immature chasteberry fruits, 1.8-cineole (16.3%) in mature fruits, and 1.8-cineol (22.0%) in leaves. Bakr *et al.* (2020) have determined 1.8 cineole (44.98 %) as the most abundant component in leaves of *V. agnus-castus* collected from the city of Giza, Egypt. Zhelev *et al.* (2022) identified 1.8-cineole (16.9–18.8%) as the main component in chasteberry fruits collected from South-Central Bulgaria and North-East Bulgaria. Moudachirou *et al.* (1998) determined 1.8-cineole (22.6%) as the main component in the essential oil content of the leaves of *Vitex agnus-castus* collected from Benin. In the past, in some studies of essential oils on the fruits, leaves and flowers of the *Vitex agnus-castus*, 1.8 cineole, α -pinene, caryophyllene, trans-caryophyllene, β -caryophyllene, sabinene and bicyclogermacrene were determined as main components.

When we compare our study with previous ones, the results are not consistent. The reason for this could be that the geographical and environmental conditions in which the samples distributed were different, confirming that the essential oil composition and content of the plants could be different (Sevindik *et al.*, 2016). In our study, for 'Çakmar' white flower, 'Koçar' white flower and 'Koçarlı' pink flower populations, the main component was determined as caryophyllene. Sesquiterpenoids are an extremely large group of secondary metabolites found in plants. Among them, the caryophyllene or β -caryophyllene group is the most common in nature. In the last few years, β -caryophyllene has represented an important topic of study (Francomano *et al.*, 2019; Gyrdymova and Rubtsova, 2021). In the past, the antimicrobial effect of β -caryophyllene has been tested against some human pathogens (Dahham *et al.*, 2015).

Conclusions

In this study, we evaluated the chemical composition of essential oils obtained from white, pink and purple chasteberry flowers. As a result, we found (-)-allospathulenol, pyrrolo(3,2,1-jk)carbazole and caryophyllene as main components. These findings may be a source for further antimicrobial, anti-inflammatory, anti-cancer, and antioxidant studies.

Authors' Contributions

E.S, D.K İ.M.G and F.B collected flowers samples. The experiments were performed and analyzed by E.S. E.A and E.K. E.S, E.A and E.K wrote the paper.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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