

## Evaluation of Chemical Composition of *Vitex agnus-castus* (Verbenaceae) Fruits Essential Oils Grown in Aydın/Turkey

### Aydın/Türkiye’de Yetiştirilen *Vitex agnus-castus* (Verbenaceae) Meyve Uçucu Yağlarının Kimyasal Bileşiminin Değerlendirilmesi

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#### Abstract

In this study was aimed to investigate the chemical composition determination of essential oils of *Vitex agnus-castus* fruits growing under the ecological conditions of Aydın’s. The essential oil was carried out with Clevenger apparatus and composition was determined by Gas Chromatography-Mass Spectrometry (GC-MS). As a result, a total of 157 components were detected in *V. agnus-castus* fruits. Among them, 1,8 cineole (8.24%), propenamide (6.07%), caryophyllene (5.56%), bicyclogermacrene (5.51%), sabinene (5.37%), maleimide (5.28%), *trans*- $\beta$ -farnesene (4.45%), and  $\alpha$ -pinene (3.98%) were found as the major components.

**Key Words:** GC-MS, essential oils, *Vitex agnus-castus*, Turkey.

#### Öz

Bu çalışmada Aydın ekolojik koşulları altında yetiştirilen *Vitex agnus-castus* meyvelerinin uçucu yağlarının kimyasal bileşim tayini Ekstraksiyon Clevenger cihazı ile gerçekleştirildi ve uçucu yağ bileşimi, Gaz Kromatografisi-Kütle Spektrometresi (GC-MS) ile belirlendi. Sonuç olarak, *Vitex agnus-castus* meyvelerinde toplam 157 bileşen tespit edildi. Bunların arasında 1,8 sineol (% 8.24), propenamid (% 6.07), karyofillen (5.56%), bisiklogermakren (% 5.51), sabinen (% 5.37), maleimid (% 5.28), *trans*- $\beta$ -farnesen (% 4.45) ve  $\alpha$ -pinen (%3.98) ana bileşenler olarak bulundu.

**Anahtar Kelimeler:** GC-MS, uçucu yağ, *Vitex agnus-castus*, Türkiye.

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## 1. Introduction

Recently, in order to prevent the growth of food-borne pathogens or to delay the onset of food spoilage there has been a considerable interest in essential oils, medicinal and edible plant extracts, herbs, and spices as for the development of alternative food additives (Çetin et al. 2011). Primers and secondary metabolites produced by plants include a wide variety of functions. Secondary metabolites were later exploited by humans for their beneficial role. At the same time, volatile oils and their components are also being developed for potential multipurpose functional use (Sawamura 2000; Ormancy et al. 2001; Gianni et al. 2005; Chishti et al. 2013). *V. agnus-castus* L. is a medicinal plant belonging to the *Vitex* genus of *Verbenaceae* family (Rice-Evans et al. 1997) which distributed in West Asia and West Africa although its origin is Mediterranean countries (Brickell and Zuk 1996; Blamey and Grey-Wilson 1998). The *Vitex* genus is represented by two species (*V. agnus-castus* and *V. pseudo-negundo* (Hauskn. Ex Bornm.) Hand.-Mazz) in the flora of Turkey (Eryiğit et al. 2015). *V. agnus-castus* is also known as Chastebery or Monk’s

pepper which widely used for the treatment of several health problems such as acne, insufficient lactation, gynecological disorder, certain menopausal conditions and spasmodic dysmenorrhea in traditional folk medicine. On the other hand, clinical research has proven the efficacy of fruits extract in pre menstrual syndrome (Halaška et al. 1999; Schellenberg 2001; Lucks et al. 2002; Wuttke et al. 2003; Maltaş et al. 2010). In a study, vitexine and vitexinine known as glycoside were found in the leaves of *V. agnus-castus* plant. Moreover, a composition of 0.47% essential oil, a bitter substance castin, glycoside agnoside and some hormone-like substances were also found in the same study (Garnier and Debraux 1961). In another study, researchers stated that the hormone-like substance has a progesterone-like effect on the female mices (Belic et al. 1958). Furthermore, a different study showed that the pharmacological activity of *V. agnus-castus* extract was found similar to Corpus luteum preparations (Bhattacharya et al. 1980). In addition, it is also known that fruit and leaf powder of the *V. agnus-castus* plant

protects wooly fabrics against moths (Baytop 1984; Tümen and Sekendiz 1989), besides that luteoline is used in natural painting and colors such as orange-yellow, olive green and light yellow with diverse mordanting methods (Fakir et al. 2014). The aim of the present study was to determine the essential oil contents of fruits of *V. agnus-castus* plants grown under West Anatolian ecological conditions.

## 2. Materials and Methods

### 2.1 Plant Materials

The fruit samples of *V. agnus-castus* were collected during September 2015 from Aydın/Koçarlı roadside (approximately 290 m altitude). The collected samples were placed in fabric bags and kept in a room without sunlight.

### 2.2 Isolation of Essential Oils

Approximately, 150 g fruit samples were used for the essential oil extraction process. Extraction was performed using Clevenger apparatus with water distillation.

### 2.3 GC-MS Analysis

The qualitative and quantitative of essential oil analysis were conducted at Eskişehir Anadolu University Medicinal Plants, Drugs and Scientific Research Center (AUBİBAM) by Hewlett Packard 5973 Mass Selective Detector System and GC-MS 6890 instrument equipped with an Agilent HP-Innowax colon (60m X 0.25 mm film, 0.25  $\mu$ m thickness). Helium was used as a carrier gas. Conditions were as follows; from 50°C to 240°C by an increase of 4°C / minutes. At 240°C, 40 minutes of waiting time were implemented. Injection port and detector temperatures were 250°C and 280°C respectively. Characterization of essential oil components was based on the library (Wiley and NIST) comparison with the mass spectra of the injected essential oil samples.

## 3. Results and Discussion

### 3.1 Chemical Composition of the Essential Oils

Essential oils extracted from different parts of woody and grassy plants are almost a complex mixture of hydrocarbons, alcohols, esters, aldehydes, carboxylic compounds and phenylpropanoids. Most hydrocarbons are monoterpene compounds, but sesquiterpenes can also be found in plants. Essential oils have well recognized antimicrobial properties, due to their phenolic components (Burt 2004; Holley and Patel 2005; Cassiano 2007; Pirigharnaei et al. 2012). In our study, totally 157 components were detected as *V. agnus-castus* fruit essential oil composition. 81.42% of the total essential oils in 42 components (components which are  $\geq 0.4\%$  in total ratio) were given in Table 1. The essential oils obtained from the fruit of the *V. agnus-*

*castus* plant were detected to contain 1,8 cineole (8.24%), propenamide (6.07%), caryophyllene (5.56%), bicyclogermacrene (5.51%), sabinene (5.37%), *N*-(4-fluorophenyl)-maleimide (5.28%), *trans*- $\beta$ -farnesene (4.45%),  $\alpha$ -pinene (3.98%) at most (Table 1). There were many previous studies relating to the chemical composition of the essential oils obtained from leaf and fruits of the *V. agnus-castus* plant. Eryiğit et al. (2015) detected *trans*-caryophyllene (19.17%), sabinene (18.05%) and 1,8-cineole (16.13%) at most in the essential oils of fruits of *V. agnus-castus* plant collected from Izmir district of Turkey. Fakir et al. (2014) collected *V. agnus-castus* samples distributed in Isparta region of Turkey in both bloom and fruit maturity periods and examined their essential oils contents. Main components of the essential oils obtained in bloom period were defined as  $\alpha$ -pinene (26.99%), 1,8-cineole (14.20%), *trans*-caryophyllene (9.13%), sabinene (8.29%), germaseren-B (8.20%), limonene (6.53%), 1,6,10-dodecatriene (6.37%), while main components of the essential oils obtained in fruit maturity period were detected as 1,8-cineole (28.34%),  $\alpha$ -pinene (26.96%), sabinene (16.36%) and limonene (9.08%). According to the study of Moudachirou et al. (1998) examining essential oil contents of the leaves of *V. agnus-castus* samples collected from Benin, major components were 1,8-cineole (22.6%), sabinene (19.4%) and (*E*)- $\beta$ -farnesene (7.7%). Hamid et al. (2010) found, in a study conducted in Nigeria, that the most abundantly found essential oils in leaves of *V. agnus-castus* in were detected as  $\beta$ -pinene (20.0%), viridifloral (9.8%),  $\alpha$ -pinene (9.1%), *cis*-o-cymene (8.4%), 1,8 cineole (6.7%) and  $\beta$ -farnesene (5.4%). Galletti et al. (1996) extracted essential oils of leaves and fruits of the *V. agnus-castus* in South Italia and the highest components were defined as 1,8 cineol (35.2%), sabinene (23.6%),  $\alpha$ -pinene (7.6%) in leaves; and  $\beta$ -farnesene (17.2%),  $\alpha$ -terpinyl acetate (17.1%) and 1,8 cineole (15.1%) in fruits. Senatore et al. (2003) examined essential oils contents of fruits of white-flowered and purple-flowered *V. agnus-castus* collected from İçel/Turkey. The essential oils obtained from the fruit of the white-flowered plant contained 1,8-cineole (21.6%), caryophyllene (17.1%), sabinene (14.7%) and terpinen-4-ol (8.7%) at most, and the essential oil obtained from the fruit of the purple-flowered plant contained caryophyllene (30.9%), sabinene (15.1%), 1,8-cineole (13.1%) and (*E*)- $\beta$ -farnesene (12.4%) at most. Toplan et al. (2015) examined the essential oils of *V. agnus-castus* fruits collected from Zonguldak, Edirne-Enez, Balıkesir-Antınoluk, Muğla-Bodrum, Antalya-Manavgat districts of Turkey. The essential oils obtained from the fruit of Zonguldak population contained  $\beta$ -caryophyllene (11.7%),  $\alpha$ -pinene (10.0%), bicyclogermacrene (8.9%) at most, the essential oils obtained from the fruit of Edirne-Enez population contained bicyclogermacrene (22.1%), sabinene (7.8%), (*Z*)- $\beta$ -farnesene (5.9%), 1,8-cineole (4.9%) at most, the essential oil obtained from the fruit of Balıkesir-Altınoluk population contained 1,8-cineole (17.3%), sabinene (15.4%), (*Z*)- $\beta$ -farnesene (13.5%), bicyclogermacrene (12.1%) at most, the essential oils

obtained from the fruit of Muğla-Bodrum population contained 1,8-cineole (13.2%), sabinene (12.8%),  $\beta$ -caryophyllene (12.7%), bicyclogermacrene (11.0%) at most, and the essential oils obtained from the fruit of Muğla-Bodrum population contained sabinene (12.1%), bicyclogermacrene (12.1%), 1,8-cineole (11.8%),  $\beta$ -caryophyllene (11.4%), (*Z*)- $\beta$ -farnesene (9.4%) at most. Ulukanlı et al. (2015) detected 1,8 cineole (24.38%), sabinene (22.77%), *trans*- $\beta$ -farnesene (8.50%) and  $\beta$ -caryophyllene (6.49%) at most in essential oils obtained from leaves of *V. agnus-castus* plant collected from Osmaniye district of Turkey. Ibrahim et al. (2009) examined essential oils contents of leaves *V. agnus-castus* collected from El-Sadat city, Menofya Governorate, Egypt. The essential oils obtained from leaves *V. agnus-castus* major components trans caryophyllene (18.76%), 1,8-cineole (17.38%), sabinene (15.83%), germacrene B (13.72%). Katirae et al. (2015) examined essential oils contents of leaves *V. agnus-castus* collected from Maraghe, East Azerbaijan

province, Iran. The essential oils obtained from leaves *V. agnus-castus* major components  $\alpha$ -pinene (19.48%), cyclohexene, 1-methyl-4-(1-methylethenyl) (13.37%), caryophyllene (8.55%), sabinene (6.89%),  $\beta$ -sesquiphellandrene (6.00%), phenol, bis(1,1-dimethylethyl) (4.09%) and camphene (3.59%). As a result of the study, most common obtained components were 1,8-cineole, propenamide, bicyclogermacrene, caryophyllene, sabinene, *N*-(4-fluorophenyl)-maleimide, *trans*- $\beta$ -farnesene and  $\alpha$ -pinene. Components obtained from a previous studies (Moudachirou et al. 1998; Senatore et al. 2003; Ibrahim et al. 2009; Hamid et al. 2010; Fakir et al. 2014; Eryiğit et al. 2015; Katirae et al. 2015; Ulukanlı et al. 2015), were similar but their ratios were different than our study. This is could be explained by the fact that essential oil composition may have different quality and quantity under different geographical and environmental conditions, and also during the different periods of the plant growth (Mazandarani et al. 2013).

**Table 1.** Essential oil composition of *V. agnus-castus* fruits.

RT (min)	Component	Quantity (%)	RT (min)	Component	Quantity (%)
11.72	$\alpha$ -pinene	3.98	42.05	bicyclogermacrene	5.51
15.99	$\beta$ -pinene	0.65	49.43	caryophyllene oxide	0.58
16.94	sabinene	5.37	50.49	ledol	1.05
19.14	$\beta$ -myrcene	1.90	51.06	2-amino-5-hydroxy-acetophenone	0.86
19.83	$\alpha$ -terpinene	0.43	51.60	(-)-globulol	0.46
20.94	dl-limonene	2.59	52.82	(+) spathulenol	1.27
21.44	1,8-cineole	8.24	53.42	3,6-dihydropyrrolo[3,2-e]indazole-7,8-dione	0.68
21.49	$\beta$ -phellandrene	0.99	53.86	$\alpha$ -farnesene	0.98
23.12	$\gamma$ -terpinene	0.64	54.00	$\alpha$ -cadinol	1.03
23.44	1,3,6-octatriene	0.74	54.94	o-cymen-5-ol	1.97
24.35	benzene	0.83	55.45	isospathulenol	0.45
34.91	$\alpha$ -gurjenene	0.75	56.49	1-(3-methylbutyl)-2,3,5-trimethyl	1.79
37.58	caryophyllene	5.56	56.73	tricyclo(3,2,1,0 <sub>2,4</sub> ) octane	0.41
38.74	$\beta$ -sesquiphellandrene	0.68	60.01	<i>trans</i> -7,7-dimethyl-6-methylidene tricyclo[6,2,1,0]undecane	0.48
39.05	nealloocimene	1.37	61.60	<i>N</i> -(4-fluorophenyl)-maleimide	5.28
39.41	<i>trans</i> - $\beta$ -farnesene	4.45	63.02	3,7-dimethoxy-1-methyl xanthen-9-one	0.52
39.62	3-cyclohexene-1-methanol	0.46	63.89	propenamide	6.07
39.85	$\alpha$ -humulene	0.68	64.17	longiborn-9-ene	1.09
40.64	3-cyclohexene-1-methanol	1.72	70.11	9,9'-biphenanthrene	2.60
40.58	camphene	3.22	72.76	1,3-dimethyl-3-(3'-methylbut-2'-enyl)-1 <i>H</i> -quinoline-2,4-dione	0.91
41.19	germacrene-D	1.69	91.69	germacrene D- 4- ol	0.49

RT: Retention time

#### 4. Conclusion

Considering the current study with the essential oils from the fruits of *V. agnus castus* plants, the major components were detected as, 1,8 cineole (8.24%), propenamide (6.07%), caryophyllene (5.56%),

bicyclogermacrene (5.51%), sabinene (5.37%), *N*-(4-fluorophenyl)-maleimide (5.28%), *trans*- $\beta$ -farnesene (4.45%),  $\alpha$ -pinene (3.98%). According to the results obtained in this study, these findings may be a valuable

resource for further biotechnological, biodiversity, pharmaceutical and medicinal studies. It will also help to understand the importance of the biological diversity and conservation biology efforts.

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