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Analyses of Volatile Components of Albanian *Vitex Agnus Castus* L. Fruits

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Abstract

The aim of the present study is the determination of the chemical composition of Vitex agnus-castus fruits essential oils growing wild in two different zones of Albania. Vitex agnus-castus L. (Verbenaceae) is a small tree or deciduous shrub, native to the Mediterranean region and Asia. It is known in Albania as " konopicë " and grows wild in the western lowland. The GC/FID and GC/MS analyses of the fruit soils revealed that the major constituents identified were sabinene from 8.98% (Tepelena) to 16.41% (Divjaka); 1,8 cineole from 9.38% (Tepelena) to 12.63% (Divjaka); ß-caryophyllene from 7.88% (Divjaka) to 10.97% (Tepelena); ß-farnesene from 9.23% (Tepelena) to 10.84% (Divjaka); α-terpinyl acetate from 3.64% (Divjaka) to 6.56% (Tepelena) ect.
The major group of components in both areas are the total sesquiterpenes, 34.47% and 33.34%, respectively inDivjaka and Tepelena, with the main rapresentatives β-caryophyllene and β-farnesene. Aromadendrene was found in Divjaka oils with 6.24%, but was not dedected in Tepelena zone. The quantity of the monoterpene hydrocarbons was higher in Divjaka fruits oils, due to the high quantity of sabinene (16.41%). Meanwhile the quantity of total diterpenes was higher in Tepelena oils because of the higher percentage of manoyl oxide. It was also found verticiol in the Tepelena fruits oils which was not detected in Divjaka oils. The examination of the data reveals differences in the chemical composition of Agnus Castus L. fruits essential oils, from two different areas of Albania.

Key words: *Vitex agnus-castus*, verbenaceae, sabinene, 1,8-cineole, β-pinene, β-farnesene and ß-caryophyllene.

1. Introduction

The aim of the following study was the determination of the chemical composition of *Vitex agnus-castus* fruits essential oils growing wild in two different zones of Albania. *Vitex agnus-castus L.* (Verbenaceae) is a small tree or deciduous shrub, approximately 1–6 m in height, native to the Mediterranean region and Asia [1,2]. In Albania, it is called "konopica" or "mrina e bardhë"[3] and grows wild in the western lowland [4].

The plant is used for the symptomatic treatment of gynaecological disorders including corpus luteum insufficiency and hyperprolactinaemia, premenstrual syndrome, menstrual irregularities, and also to treat hormonally-induced acne and also as a digestive aid, sedative, anti-infective and for the treatment of hot flushes. Traditionally it has beenused as an anaphrodisiac, calefacient, contraceptive, emmenagogue, sedative and as a tonic [1,2,5].

Earlier studies on the plant essential oil had revealed the presence of bicyclogermacrene, β-farnesene, 1,8-cineole, β- farnesene, sabinene, α-terpinyl acetate, β-caryophyllene and manool as the main constituents of the fruits and leaves of Italian *V. agnus-castus*[6]. Sabinene, 1,8-cineole, α- pinene, trans β- farnesene , trans β- caryophyllene were the most abundant constituents of the leaf and fruit essential oils of *V. agnus-castus* grown in Montenegro [7]. Sabinene, 1,8-cineole, α- pinene, β- farnesene, β-caryophyllene were identified as the principal constituents of the Creatan *V. agnus- castus* fruit essential oils [8].

2. Materials and methods

2.1. Plant Materials

Aerial parts of the plants were collected, in the

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zone of Tepelena and Divjaka during 2013. Voucher specimens of the plant were deposited at the herbarium of Faculty of Pharmacy, University of Tirana. The air-dried plant material, were subjected to hydrodistillation using a Clevenger-type apparatus according to standard procedures [9]. The essential oils obtained were stored under refrigeration until further tests.

2.2. Essential oils analysis(GC and GC/MS)

Agilent Triple Quadrupole GC/MS 7890 series System was used for the identification of essential oils components, fitted with HP-5MS capillary column (30m x 0.25 mm x 0.25 μm thick). Ion source 70 eV. Oven program: from 50°, then 4°C/min, till 290°C (5 min), was using Helium as the carrier gas with a flow rate of 2.25 mL/min. The identification of individual components was made by comparison of their retention times and mass spectra with those of the literature data and by computer searching, matching mass spectral data with those held in Nist Library of Mass spectra 2. For quantification purposes were used area percent reports, obtained by GC/FID techniques.

The quantitative and qualitative analysis essential oils were performed using a Varian GC-450 apparatus equipped with FID detector. PTV injector was set at 280 °C and split 1:50 for introduce the essential oil samples. Helium was used as carrier gas with flow rate of 0.8 ml/min. VF- 1ms (30 m x 0.33 mm x 0.25μm) capillary column was used for the separation of the compounds. Oven program: 2 min at 60°C, then 5°C/min to 200°C and again 10°C/min till 280°C (held 5 min). Temperature of FID detector was set at 280 °C and the gases of detector were: H2 (30 ml/min); O2 (300ml/min) and N2-make up gas (25ml/min).

3. Results and Discussion

The quantitative yield of essential oils, expressed in terms of percentage of dry matter, were 0.83% and 0.86% respectively for Divjaka and Tepelena samples.

The results of GC/MS and GC/FID analyses of essential oils are reported in Table 1. A total of 51 compounds weredetected in the oils, fromwhich 42 wereidentified, representing the 84.91% and 78.57% respectively for Divjaka and Tepelena. The qualitative and quantitative data examinated reveals that the main constituents identified were:sabinene from 8.98% (Tepelena) to 16.41% (Divjaka); 1,8 cineole from 9.38% (Tepelena) to 12.63% (Divjaka); ß-caryophyllene from 7.88% (Divjaka) to 10.97% (Tepelena); ß-farnesene from 9.23% (Tepelena) to 10.84% (Divjaka); α-terpinyl acetate from 3.64% (Divjaka) to 6.56% (Tepelena); aromadendrene from 0% (Tepelena) to 6.24% (Divjaka); manoyl oxide from 3.94% (Divjaka) to 5.35% (Tepelena), caryophyllene oxide 2.25% (Divjaka) to 3.35% (Tepelena), tau-Cadinol etc.

The monoterpene hydrocarbons ranged from 13.2% (Tepelena) to 22.36% (Divjaka) with the dominant compound sabinene 8.98% (Tepelena) – 16.41% (Divjaka), followed by α-pinene 1.21% (Tepelena) - 2.34% (Divjaka).

The oxygenated monoterpenes costituted 18.93% (Tepelena) and 19.89% (Divjaka) of total oils. The major component of this group was 1,8- cineolein higher quantity in Divjaka zone (12.63%) then in Tepelena (9.38%) and α-terpinyl acetate with 3.64% in Divjaka and 6.56% in Tepelena.

The major group of components are the total sesquiterpenes with 34.47% and 33.34%, respectively in Divjaka and Tepelena. β-caryophyllenewas the major component with 7.88% in Divjaka fruits oils and 10.97% in Tepelenaoils, followed by β-farnesene ranged 9.23% (Tepelene) - 10.84% (Divjaka). Aromadendrene with 6.24% was found in Divjaka oils and but was not dedected in Tepelena zone. Caryophyllene oxide ranged from 2.25% (Divjaka) – 3.35% (Tepelena).

The quantity of the total diterpenes ranged from 8.19% (Divjaka) to 13.1% (Tepelena). The main component of this group was manoyloxide in the quantity of 3.94% in Divjaka fruits essential oils and 5.35% in Tepelena, followed by sclareol with 1.78% (Divjaka) – 2.75% (Tepelena) and β- iraldeine 1.72% (Divjaka) – 1.93% (Tepelena). It was also found in the Tepelena fruits oils verticiol 1,74%, not detected in Divjaka oils.

According to the literature the composition of Albanian fruits essential oils, comparing with those of Italy, showed some differences [6], due to the content of the main component sabinene, which is higher in Albanian oils compared with the Italian. Byciclogermacrene and spathulenol which are main components in the Italian oils were not detected in the Albanian *V. agnus-castus* fruits oils. On the other hand, the Albanian *Agnus Castus L*. fruits essential oils was found to be more similar to the Montenegro unripe fruits oils [7], because of similar main

components and respective quantity such as sabinene, 1,8- cineole, β-caryophyllene, β-farnesene.

Table 1: Chemical composition (%) of *Vitex agnus-castus* fruits essential oil.

	RT	COMPONENTS	Divjake fruits (%)	Tepelene fruits (%)
1	8.253	α-Thujene	0.29 ± 0.11	0.18 ± 0.02
2	8.479	α-Pinene	2.34 ± 0.59	1.21 ± 0.53
3	9.668	Sabinene	16.41 ± 2.12	8.98 ± 2.38
4	9.806	β-Pinene	1.09 ± 0.72	0.69 ± 0.07
5	10.173	β-Myrcen	1.12 ± 0.33	0.71 ± 0.02
6	10.667	α-Phellandrene	0	0.15 ± 0.01
7	11.059	α-Terpinen	0	0.23 ± 0.01
8	11.461	Limonen	0.49 ± 0.01	0.32 ± 0.04
9	11.549	1,8 Cineole	12.63 ± 3.07	9.38 ± 3.21
10	12.03	δ 3-carene	0	0.14 ± 0.06
11	12.464	γ-Terpinen	0.62 ± 0.02	0.59 ± 0.05
12	13.473	α- Terpinolen	0.16 ± 0.01	0.29 ± 0.06
13	13.816	Linalool	0.24 ± 0.08	0.22 ± 0.03
14	16.186	p-Menth-1-en-8-ol, (S)-(-)-	0.43 ± 0.03	0.24 ± 0.01
15	16.563	Terpinen-4-ol	1.67 ± 0.67	1.23 ± 0.22
16	17.013	α-Terpineol	1.12 ± 0.55	0.54 ± 0.08
17	18.174	β-Citronellol	0	0.34 ± 0.09
18	21.964	exo-2-Hydroxycineole acetate	0	0.13 ± 0.04
19	22.194	α-Terpinyl acetate	3.64 ± 0.82	6.56 ± 1.09
20	24.192	α-Gurjunene	0.11 ± 0.06	0.91 ± 0.04
21	24.51	β-Caryophyllen	7.88 ± 2.76	10.97 ± 3.27
22	24.97	α-Bergamotene	0.29 ± 0.09	1.21 ± 0.21
23	25.499	β-Farnesene	10.84 ± 4.11	9.23 ± 4.22
24	25.641	α-Humulene	0.93 ± 0.18	0
25	25.773	Aromadendrene	6.24 ± 3.04	0
26	25.822	Allo-Aromadendrene	0	0.53 ± 0.08
27	26.371	β-Cubebene	0.28 ± 0.09	0
28	26.831	γ-Elemene	0.27 ± 0.04	0.49 ± 0.12
29	27.34	γ-Cadinene	0	Tr
30	28.932	Palustrol	0.31 ± 0.05	0
31	29.504	Caryophyllene oxide	2.250.79	3.35 ± 0.96
32	29.7	Viridiflorol	0	0.34 ± 0.05
33	29.95	Ledol	1.42 ± 0.54	2.14 ± 0.44
34	30.929	tau-Cadinol	2.54 ± 0.87	2.64 ± 0.92
35	32.114	α-Bisabolol	0	0.32 ± 0.07
36	33.8	Sclareoloxide	1.11 ± 0.03	1.21 ± 0.76
37	35.15	Pimara-7,15-dien-3-one	0	1.33 ± 0.05
38	36.786	β- Iraldeine	1.72 ± 0.56	1.93 ± 0.04
39	37.212	Sclareol	1.78 ± 0.83	2.75 ± 0.73
40	38.891	Cembrene	0.75 ± 0.03	0
41	39.107	Verticiol	0	1.74 ± 0.21
42	39.983	Manoyl oxide	3.94 ± 0.65	5.35 ± 1.22
		TOTAL	**84.91**	**78.57**
		Monoterpenes	**22.36** ± 3.22	**13.2** ± 2.27
		OxygenatedMonoterpenes	**19.89** ± 1.82	**18.93** ± 3.82
		Total Sesquiterpenes	**34.47** ± 4.25	**33.34** ± 5.41
		Total Diterpenes	**8.19** ± 2.38	**13.1** ± 3.08

*All results are calculated as average value of 3 samples.

4. Conclusion

Close examination of the GC/FID and GC/MS data reveals differences in the chemical composition of *Vitex Agnus Castus L*. fruits essential oils, growing wild in two different areas of Albania.The major group of components in both areas are the total sesquiterpenes, with the main rapresentatives β-caryophyllene and β-farnesene. Aromadendrene was found in Divjaka oils with 6.24%, but was not

dedected in Tepelena zone. The percentage of monoterpene hydrocarbons was higher in Divjaka oils, due to the high quantity of sabinene in this area, compared with that of Tepelena oils. Meanwhile the quantity of total diterpenes was higher in Tepelena oils because of the higher percentage of manoyl oxide, the main component of this group. It was also found verticiol in the Tepelena fruits oils which was not detected in Divjaka oils.

In addition, due to the similarity of the main components, the Albanian *Agnus Castus L*. fruits essential oils was found to be more similar to the Montenegro fruits oils compared with those of Italian origin.

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