J. Essent. Oil Res., **11**, 599-605 (Sep/Oct 1999)

Variation in Essential Oil Yield and Composition of Cretan *Vitex agnus castus* L. Fruits

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Abstract

The hydrodistilled essential oils of mature fruits from five populations of *Vitex agnus castus* L. growing wild in Crete, Greece, were analyzed by GC and GC/MS. More than 70 components were detected, of which 47, comprising 87% of the total oil were identified. The oil yield and quantitative composition differed significantly between populations. The main constituents of all the oils were sabinene (16.6-31.2%), 1,8-cineole (4.4-14.5%), (E)-β-farnesene (4.8-9.9%), α-pinene (0.4-10.2%) and β-caryophyllene (1.4-6.0%). Yields varied from 0.15% to 1.0%. The effect of the comminution of the fruits prior to distillation was studied, and the results showed a slightly inferior yield and an elevated concentration of monoterpenoids at the expense of sesquiterpenoids in the oil obtained from entire fruits. An oil obtained by a pilot scale distillation for commercial production was also investigated.

Key Word Index

Vitex agnus castus, Verbenaceae, essential oil composition, sabinene, 1,8-cineole, (E)-β-farnesene, α-pinene.

Introduction

Vitex agnus castus L. is a medicinal plant with a long tradition in folk medicine (1). The drug consists of the ripe fruits. Dioscorides described the use of the drug to stimulate lactation in nursing mothers and to be beneficial in the treatment of several female disorders (2). Commonly called chastetree or monks pepper, *V. agnus castatus* is widely distributed in the Mediterranean area, up to Central Asia, the tropics and the south of Europe. In Greece it is especially widespread on the islands of Crete and Karpathos. In Crete it is abundant, especially in the Western part of the island, where it can be found growing spontaneously in marshes, damp places, lake shores and riverbanks, gorge beds and maritime sands from 0-600 m altitude (3).

Today, the plant is considered one of the most important drugs of natural origin for the treatment of female hormonal problems (4,5). The drug balances the female sex hormones and counteracts estrogen overproduction (6-8). An estrogen domination has been related to several serious female diseases, such as osteoporosis (10) and even breast and endometrial cancer (10,11). The mode of action

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Received: February 1998
Revised: November 1998
Accepted: December 1998
1041-2905/99/0005-0599$04.00/0

has recently been elucidated by several studies (7,12), whereas the compounds responsible for this action could not yet be ascertained.

Among other active compounds, the ripe fruits contain an oil, which, until the middle of the 1990's, had been little studied. In the earlier studies, mainly monoterpene hydrocarbons had been identified (13,14). The composition of a variety of Yugoslavian oils was reported by Kustrak et al. (15), and an oil of African origin investigated by Elgengaihi et al. (16). In 1996, two studies investigated in more detail the chemical composition of oils of Italian origin (17,18) and one study focused on the oil obtained from commercially available fruits of undefined origin (19). Finally in 1998, Valentini et al. (20) reported comparative observations on the oils obtained from several locations in Italy and Cyprus. These studies reported a considerable sesquiterpene fraction. The possible existence of several chemotypes of *V. agnus castus* related to geographical origin was discussed (16-18). All studies were carried out with comminuted fruits or under undefined conditions.

The aim of the present study is the investigation of the oil yield and composition of Cretan *V. agnus castus* populations, contributing also to the knowledge of geographical differences in oil yield and composition of this species. Additionally, the influence of the comminution of the fruits prior to distillation, and the applicability of the obtained results for commercial production by pilot still distillation was studied.

Experimental

Sample Collection: For this study, mature fruits of five *V. agnus castus* populations were collected in the Western part of Crete, Greece. Tavronitis (T) at the Northern West coast of Crete at 35°31' longitude, 23°46' latitude. Cournas (C) a large population located around the only Cretan lake, at 50 m altitude, 35°20' longitude, 24°16' latitude. Agia Marina (A) is a beach situated near the village of Argoules, Sfakia on the southern coast of Crete, 35°11' longitude, 24°17' latitude. Frangokastello (F) Sfakia at 35°11' longitude, 24°15' latitude, 100 m from the sea at its level and Nomikiana (N) on the southern coast of Crete in the district of Sfakia at 35°12' longitude, 24°11' latitude. At each sampling site mature fruits were collected randomly from a large number of plants, representing the population. Voucher specimens are kept in the Herbarium of the Mediterranean Agronomic Institute of Chania, Crete, Greece.

Laboratory Distillations: One lot of fruits from each population was comminuted in a closed type Tecator mill (Cemotec, Sample Mill) for approximately 5 min, until all particles passed through 0.8 mm laboratory test sieves. Distillation was commenced immediately after comminution of each batch.

Hydrodistillations of entire and comminuted fruits were carried out with a Clevenger-type apparatus of the British Pharmacopoeia. Each distillation of the fruits was carried out with 30 g of plant material and 510 mL of distilled, deionized water in all cases (21). Entire fruits were distilled for 5 h, comminuted fruits for 3 h in threefold replication. Upon termination of the process, the oil yield was measured, the oil collected, dried over anhydrous sodium sulfate and stored at –20°C in 2 mL vials for further analysis.

Pilot Still Distillation: Thirty kg of entire fruits comprising six kg from each population were distilled in a pilot still. This pilot still of 700 L volume capacity is constructed entirely of stainless steel and allows water- or water-and-steam distillation, the latter with the help of stainless steel perforated grids which can be fitted into the retort. The retort is 165 cm high, with an internal diameter of 60 cm, fitted with a rounded goose-neck lid (45 cm high), a 160 cm condensation pipe and a 6 m coiled cooling pipe located in a cooling water barrel.

The fruits were soaked overnight in the still in 500 L of water, and subsequently distilled the next morning for 5 h from the initiation of boiling. Oil and distillation water were separated in a stainless steel separator, the oil yield was recorded and samples taken for further analysis.

Quantitative Analyses: The comparative quantitative analyses of the oils was performed by GC (Hewlett Packard 5890 Series 2 model controlled by the HP 3365 series 2 Chemstation software and equipped with an HP 7673 autosampler). Capillary column: FFAP (Free Fatty Acid Phase) of 50 m x 0.2 mm, coated with 0.33 µm cross-linked polyethylene glycol-TPA phase. Injection was performed by autosampler.

Program: 1 min at 60°C, then 3°C/ min to 210°C and additional 20 min at 210°C. Carrier gas: Helium at a flow rate of 0.8 mL/min. All samples were diluted with pentane to a 1 x 15 concentration and the amount injected was 0.5 µL. Split ratio 1:25. Injector temperature: 250°C, detector (FID) 280°C.

Qualitative Analyses: Qualitative analyses were carried out with a GC identical to the above, coupled to a VG Trio 2000 Mass spectrometer. The column and program was identical to the one mentioned above. Mass spectra were taken at 70 eV at a scanning speed of 1 scan/sec from 40 to 240 m/z. Components were identified by comparison with the computerized MASS LYNX software from the Wiley Library, by their relative retention times and by comparison with authentic samples and literature.

Additional analysis was carried out with a GC/MS as above, fitted with an HP 5 column (30 x 0.25 mm fused silica capillary column, coating thickness 25 µm), programmed under the following conditions: linear from 60°-240°C at 3°C/min, Injector: 250°C, detector (FID) 280°C. Carrier gas helium at a flow rate of 1 mL/min and scans were taken at 70 eV at a scanning speed of 1 scan/sec from 40 to 240 m/z, as recorded by Adams (22). The oils obtained the following season from comminuted fruits, distilled for 3 h (Cournas population) and from the pilot still distillation (mature entire fruits, distilled for 5 h) were subjected to this additional analysis.

Results and Discussion

The *V. agnus castus* fruits yielded a mobile, fragrant pale-yellow to yellow oil after hydrodistillation. The oil had an agreeable, interesting somewhat peculiar top-note, reminding one of cannabis and eucalyptus. After a few minutes, it revealed an aromatic floral, warm but fresh, somewhat peppery, sweet, spicy fragrance with lemon-like, woody undertones.

The mean oil yield of all populations obtained in the laboratory experiments was 0.70% for comminuted fruits distilled for 3 h and 0.65%. for entire fruits, distilled for 5 h. More than 70 compounds were detected in the oils, of which 47 (Table I), comprising 70-87% of the total oil, were identified. However, a substantial amount of high boiling constituents present in considerable percentages in the oils could not be identified or verified by GC/MS. The mean composition of Cretan *V. agnus castus* oils were characterized by the main components sabinene, 1,8-cineole and (E)-β-farnesene.

Considering all the Cretan samples as one population or 'geographical origin,' the qualitative composition is more similar to oils of the Mediterranean region (15,18-20), differs, however, substantially from the oils of Egyptian origin (16). The most interesting difference is probably the identification of α-cadinene (8.6%) and β-cadinene (10.7%) in the Egyptian oil, compounds which were neither detected in our oils nor in the other studies. Also the content in α-terpineol, which was a major component in this oil (16.1%), differentiates it from the Cretan oils. The oil of Russian origin (14) differed especially in α- and β-pinene contents, which were very high, in the high limonene content, and in the amount of camphor and bornyl acetate from the Cretan oils. In the above mentioned study, only 10 monoterpene compounds have been reported, comprising 81% of the oil, the factor which differentiates this Russian *V. agnus castus* oil most from all the others, which showed to contain a broad spectra of sesquiterpenoids (15-19).

The quantitative composition of the Cretan oils was found similar to the Yugoslavian oils analyzed by Kustrak et al. (15), but differed from the Italian fruit oils (17,18) and the oil analyzed by Zwaving and Bos (19) by much higher percentages of the main component sabinene. However, Valentini et al. (20) reported their Italian fruit oils contained sabinene (11-17%). The 1,8-cineole content in the presently investigated oils was lower than reported for the Russian (14) and Italian oils. In the Italian oils, 1,8-cineole was reported as the major component (18,20). In the oils of Egyptian origin (16) 1,8-cineole was not detected at all, while Zwaving and Bos (19) found 1,8-cineole to be present only at 0.15%, whereas β-caryophyllene and germacrene B were the main components.

The sesquiterpenoid fraction of *V. agnus castus* oils has only recently been more thoroughly elucidated with the studies carried out by Galletti et al. (17), Senatore et al. (18), Zwaving and Bos (19), and finally Valentini et al. (20), with the exception of the cadinene isomers identified by Elgengaihi (16),

Table I. The composition of Cretan *Vitex agnus castus* fruit oil (column HP5)

Compound	Pilot still	Cournas population	Compound	Pilot still	Cournas population
α-thujene	0.8	0.3	α-terpinyl acetate	4.2	3.3
α-pinene	6.6	1.3	citronellyl acetate	0.4	0.3
sabinene	24.2	26.7	α-gurjunene	0.4	0.2
β-pinene	1.3	0.8	β-caryophyllene	3.8	4.2
3-octanone	t	0.3	α-bergamotene*	0.2	0.1
myrcene	2.1	1.4	(Z)-β-farnesene	0.2	0.1
α-phellandrene	0.3	0.1	α-humulene	0.2	0.2
δ-3-carene	t	0.1	(E)-β-farnesene	6.2	10.7
α-terpinene	1.3	0.3	allo-aromadendrene	0.1	0.1
p-cymene	0.9	0.2	γ-muurolene	0.2	t
limonene	0.6	1.1	germacrene D	0.8	1.3
1,8- cineole	19.6	9.6	β-bisabolene	0.1	-
(E)-β-ocimene	0.2	0.2	δ-cadinene	0.1	0.1
γ-terpinene	2.4	0.5	nerolidol*	0.1	0.1
cis-sabinene hydrate	0.3	0.4	ledol	0.2	0.4
terpinolene	0.7	0.2	spathulenol	0.5	0.3
trans-sabinene hydrate	0.3	0.4	caryophyllene oxide	0.7	0.4
linalool	t	t	viridiflorol	0.1	-
cis-p-menth-2-en-1-ol	0.1	0.1	τ-cadinol	0.9	0.4
octyl acetate	0.1	0.1	α-muurolol	0.9	0.6
trans-p-menth-2-en-1-ol	0.1	t	eudesmol*	0.1	-
terpinen-4-ol	2.4	1.1	β-bisabolol	0.1	t
α-terpineol	0.8	0.6	abietatriene	1.1	2.0
citronellol	0.2	0.3			

* correct isomer not determined; t = trace

β-caryophyllene, which was detected by all authors except Mishurova et al. (14), and ledol, spathulenol and τ-cadinol identified by Kustrak et al. (15).

A substantial amount of (E)-β-farnesene as found in the Cretan oils has been reported as β-farnesene + allo-aromadendrene (17.2%) by Galletti and Russo (17) in an oil from Calabria. The Italian oil investigated by Senatore et al. (18), was found to contain (E)-β-farnesene (0.4%) and (Z)-β-farnesene (6.9%). Valentini et al. (20) reported (E)-β-farnesene (1.0-1.4%) from three Italian locations, however, this component was absent in the fruit oil from Cyprus. Zwaving and Bos (19) found only (Z)-β-farnesene (1.8%) in their oil. Neither isomer was detected in the other reported studies. It could be suggested, that in agreement with Elgengaihi (16) and Senatore et al. (18), different chemotypes exist in this species and our results showed that Cretan *V. agnus castus* is more similar to other populations growing in the Mediterranean area than to plants of other origins and might belong to a distinct chemotype differentiating from other geographical regions such as Russia or Egypt.

The unidentified part of the oils does represent a bulk of components in the highest boiling range (Table I). Except for abietatriene, no other compound could be positively identified. Sclareol and epi-13-manool, manoyl oxide, phyllocladene and kaurene found by Senatore et al. (18) could probably be present in the oil, but could not be verified by MS in the present study. These authors (18) identified a number of longer chain hydrocarbon compounds presumably in the range of the unknown components in the Cretan oils as dodecane, 1-dodecene, 1-tetradecene, 1-hexadecene and 1-octadecene.

The Pilot Still Distillation: The yield obtained by the pilot still distillation of entire fruits (0.60%) was slightly inferior to the laboratory scale distillation yield of entire fruits (0.65%) This fact might be

Table II. Variations in the oil composition among Cretan *Vitex agnus castus* populations

	N[1]		A		F		C		T	
Compound	**Com.**	**Entire**	**Com.**	**Entire**	**Com.**	**Entire**	**Com.**	**Entire**	**Com.**	**Entire**
Monoterpene hydrocarbons	*34.6*	*39.0*	*33.0*	*38.3*	*33.6*	*37.1*	*35.8*	*39.4*	*23.4*	*25.6*
α-pinene	8.5	10.2	4.9	5.2	8.9	10.2	1.4	1.6	0.4	0.5
α-thujene	t	t	t	0.5	t	t	0.4	0.5	0.3	0.4
β-pinene	0.9	1.0	0.9	1.1	1.0	1.1	0.9	1.0	0.6	0.6
sabinene	17.5	18.6	21.4	24.3	16.6	17.4	29.0	31.2	19.2	19.8
δ-3-carene	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.3	t	t
myrcene	2.0	2.2	1.6	1.8	1.8	2.0	1.5	1.7	0.9	0.9
phellandrene	0.3	0.3	0.2	0.2	0.3	0.3	0.1	0.1	t	t
α-terpinene	0.3	0.5	0.6	0.8	0.3	0.6	0.3	0.6	0.4	0.8
limonene	4.0	4.5	1.9	2.1	3.1	3.5	0.9	1.0	0.5	0.5
γ-terpinene	0.5	0.8	1.0	1.5	0.6	1.0	0.7	1.1	0.8	1.5
p-cymene	0.3	0.4	0.2	0.3	0.5	0.6	0.2	0.2	0.2	0.3
terpinolene	0.2	0.2	0.3	0.4	0.2	0.3	0.2	0.3	0.2	0.4
Oxygenated monoterpenes	*19.7*	*20.5*	*17.7*	*20.1*	*19.4*	*20.5*	*17.0*	*19.2*	*12.8*	*12.2*
1,8-cineole	12.4	13.0	11.0	12.6	13.7	14.5	10.4	11.3	5.2	4.4
3-octanone	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	t	t
trans-sabinene hydrate	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.5	0.1	t
citronellol	0.9	0.8	0.6	0.5	0.7	0.6	0.3	0.2	1.6	1.7
linalool	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.1	t
cis-sabinene hydrate	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.3	0.1	t
terpinen-4-ol	1.1	1.7	1.7	2.5	1.2	1.7	1.1	1.9	1.0	1.5
citronellyl acetate	1.3	1.2	0.8	0.7	1.2	1.0	0.4	0.4	3.5	3.6
α-terpineol	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.1	t
α-terpinyl acetate	2.8	2.7	2.6	2.7	1.5	1.6	3.6	4.0	1.0	0.9
Sesquiterpenes	*17.9*	*14.7*	*17.4*	*13.6*	*16.2*	*12.3*	*18.2*	*14.0*	*24.9*	*24.0*
α-bergamotene*	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.4
β-caryophyllene	2.5	2.3	5.3	4.5	1.7	1.4	3.9	3.2	5.9	6.0
allo-aromadendrene	0.1	0.1	0.2	0.1	t	t	0.2	0.1	1.3	1.2
(E)-β-farnesene	9.8	7.5	6.5	4.8	8.4	6.0	9.9	7.2	8.8	7.8
α-humulene	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.1	0.3	0.2
germacrene D	1.1	0.9	1.1	0.8	1.2	0.9	1.0	0.9	0.5	0.7
β-bisabolol	0.3	0.2	0.1	0.1	0.2	0.2	t	0.1	t	t
nerolidol*	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.3	0.3
ledol	1.4	1.5	0.8	0.8	1.2	1.2	0.3	0.4	4.1	4.3
spathulenol	0.3	0.4	0.3	0.3	0.6	0.6	0.2	0.3	0.3	0.3
α-muurolol	0.5	0.3	0.7	0.4	0.6	0.4	0.7	0.4	1.2	1.0
τ-cadinol	1.2	0.7	1.8	1.0	1.5	0.9	1.5	0.9	1.4	1.2
Mean Ess. Oil Yield (%)	0.99	0.97	0.99	0.94	0.58	0.56	0.64	0.61	0.18	0.15

[1]Column FFAP: N=Nomikiana; A=Agia Marina; F=Frangokastello; C=Cournas; T=Tavronitis.
Com. = comminuted fruits distilled for 3 h. Entire = entire fruits distilled for 5 h. t= trace (< 0.05%). *correct isomer not determined

due to the size of the unit and the separator and it can not be excluded that a part of the oil can not be completely recovered from walls and tubes of the separating device.

Comparing the mean oil composition of the populations as determined by the laboratory experiments (Table II) with the results obtained by analysis of the pilot still produced oil (Table I), the composition seems comparable in that all the compounds found in the lab distilled oil were present in the pilot still produced oil as well, largely in comparable percentages. The occurrence of β-bisabolene (0.09%), viridiflorol (0.14%) and an eudesmol isomer (0.09%) exclusively in the pilot still produced oil, could indicate that when collecting a larger amount of plant material without the careful cleaning and de-dusting measures applied as to the fruits subjected to laboratory distillation, small particles of flowers or leaves might be present in the material to be distilled, contributing to minor alterations of the oil composition.

Nevertheless, the results obtained seem to show that when exactly the same method is followed as in laboratory distillations, provided that the still is adequately built regarding the materials used and the technical construction, a comparable oil quantity and quality should be obtained in case of this plant material.

Inter-Population Variation: A considerable variation in oil yield and composition (Table II) was found between Cretan populations. Yields ranged from 0.15% (T) to 1.0% (A). The yield characteristics of A and N seem quite similar. Both high yielding populations are located in the south of the island and live in quite similar environments, that is very close to the sea along a riverbed, the third southern population (F), however, does not exhibit such high yield, probably due to its position more inland.

Statistically, all populations varied significantly from each other in the quantitative composition of the oils. The main components in all oils were sabinene, ranging from 16.6% in the Frangokastello (F) population to 29.0% in the Cournas (C) population, followed by 1,8-cineole, ranging from 5.2% in Tavronitis (T) to 13.7% in F population, and α-pinene ranging from 0.4% in T to 8.9% in Nomikiana (N). In the sesquiterpenoid fraction of the oils as well, differences were found between the major components; (E)-β-farnesene, the main sesquiterpene, ranged from 6.5% in Agia Marina (AM) to 9.9% in T and β-caryophyllene varied between 1.7% in F and 5.9% in T.

From the above results it can be concluded, that the oil yield and composition of Cretan *V. agnus castus* fruits varied considerably between populations in a relatively confined geographical situation. To generally compare between fruit oil yields of various national origins with the investigated Cretan oil does not seem possible. However, the mean and maximum oil yields obtained from the investigated Cretan populations compared roughly to several reports (13,14,19) but were lower than reported for Yugoslavian (15) and Italian (18) oils. The present yield results obtained from the Tavronitis population (0.17%) were the lowest recorded up to date.

Regarding the composition of the oils, significant differences were found in their quantitative composition. These findings show that also the comparison between the *V. agnus castus* fruit oil composition of different national origins is rather difficult and that substantial differences, in especially quantitative composition, might be due to inter population variation alone.

Comminution of the Fruits: Considering the commonly accepted opinion that tough materials such as fruits with hard seed covers nearly always require grinding to release a substantial amount of oil (23,24), it is interesting to note that the comminution of *V. agnus castus* fruits does not seem to influence the oil yield to the same extent as usually reported for plant materials which synthesize or store their oils in subcutaneous cavities, passages or ducts. Considering that during hydrodistillation of such subcutaneous oils the process of hydrodiffusion of the volatiles to the surface is a very slow process, the results obtained are somewhat unusual for such tough material. Our results showed, that in case of the investigated species, a five-hour distillation of entire fruits yields a comparable amount of oil compared to comminuted fruits subjected for three hours of distillation. Regarding the compositional differences, as can be seen in Table II, monoterpenoid content was elevated at the expense of the sesquiterpenoids when the fruits were left entire, however, no dramatic differences were noted.

Acknowledgments

The authors wish to thank Dr. A. Nikolaidis of the Mediterranean Agronomic Institute of Chania, Crete, Greece for making this study possible, and Mr. Ch. Psaroudakis from "Wild Herbs of Crete," Chania, Crete, Greece for the pilot still distillation facilities offered.

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