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Annals of Biological Research, 2013, 4 (2):308-312 (http://scholarsresearchlibrary.com/archive.html)



# Variations in essential oils of *Vitex agnus castus* fruits growing in Qum, Khorasan and Tehran in Iran

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## ABSTRACT

It is judicious to be reminiscent of the environmental conditions of plant growth being a primary factor affecting the components and amount of constituents in essential oils making the pharmacological action of essential oils uncertain. The wide range of distribution for Vitex agnus castus (VAC) in Iran made it a suitable candidate for assessing the chemical profiles of samples collected from three different places. The GC-MS analysis of the essential oils obtained by the hydrodistillation of the plant materials, led to identification and quantification of 30, 7, and 18 compounds representing 74.95, 82.53 and 84.41 percent of total oil for Qum, Khorasan and Tehran samples, respectively. All samples were rich in monoterpenes including monoterpene hydrocarbons (42.84-64.48%), monoterpene alcohols (~0.7-8%) and sesquiterpenes (9.45-31.33%). A diterpene compound (3.11%) was found only in Tehran samples. The variations in presence or absence and also the identity of some compounds were quite evident in the VAC fruits.

Keywords: Vitex agnus castus, Verbenaceae, essential oil, GC-MS analysis, Iran

## INTORDUCTION

Following on from recent advancements, in a time of increased considerations to natural products as a safe herbal remedies, it is as important as ever to gather detailed documentations of the latter in different aspects. *Vitex agnus castus* (Verbenaceae) commonly known as chaste berry has a widespread use and holds a high reputation in herbal medicine among the ancients, due to its effectiveness in management of several different disorders such as its usage in prevention and treatment of the pre-menstrual syndrome symptoms like depression, irritability, anxiety, mastalgia, fatigue, and headache [1-3]. The merit of the traditional use of *Vitex agnus castus* (VAC) has been supported by the isolation and identification of several different biologically active chemical constituents, including polyphenols (flavonoids), iridoids, diterpenoids, tannins, and essential oils extracted from VAC fruits [4-9]. A large number of literatures have been published on screening the bioactivity and pharmacological activities of different classes of the compounds present in the plant [10-15]. In a recent study conducted by Sarikurkcu et al., essential oil and different solvent extracts of VAC fruits were analyzed for their antioxidant activity that exhibited excellent activity for water extract of the plant containing higher amounts of phenolic compounds. Low urease- (32.0%) and chymotrypsin- (31.4%) inhibitory activity, and moderate (41.3%) anti-inflammatory

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activities for aerial parts of VAC extract [16,17]. Menopausal balance in humans with two essential oils extracted from leaves and berries of the VAC showed widely divergent response to use of the oil, mirroring contraindications to the use of herb [18]. Not surprisingly, components and amount of constituents in VAC essential oils could be variable according to diversity of the growing conditions of these genus distributed all over the world. Differences in contents of the VAC essential oil may cause variation in achieving the ideal pharmacological action of the essential oil. Hence, it is judicious to have a deeper insight to chemical profiles of the VAC essential oils with differences in environmental conditions of plant growth. In order to have the predictability and reproducibility in designing an efficient natural medicine containing VAC essential oil, there is a need to assess the chemical profile of VAC growing in different places. Herein, we tried to study the variations in components of the VAC essential oils of three samples growing in different soil conditions, temperature and at different altitudes in Iran.

# MATERIALS AND METHODS

#### Plant materials

Fruits of VACs that were grown widely at different altitudes from Qum with dry desert climate  $(34^{\circ}47'58''N \text{ latitude} and 51^{\circ}05'07''E \text{ longitude})$  Khorasan with semi-desert climate  $(36^{\circ}21'06''N \text{ latitude} and 56^{\circ}52'42''E \text{ longitude})$  and Tehran with semi-arid desert climate  $(35^{\circ}41'46''N \text{ latitude} and 51^{\circ}25'23''E \text{ longitude})$  in Iran were collected during the month of July. The identities of the samples were confirmed by anatomical examination in comparison with the herbarium specimen retained in the School of Pharmacy, Tehran University of Medical Sciences, Iran.

#### Essential oil extraction

The essential oils of each collected sample (50 g) were extracted by hydrodistillation for 4 h, using a Clevenger-type apparatus. The extracted essential oils exhibited light yellow color that were dried over anhydrous sodium sulphate and stored in the refrigerator (4  $^{\circ}$ C).

#### **GC-MS** analysis

The essential oils were analysed by GC–MS using a Shimadzu GCMS-QP5050A gas chromatograph mass spectrometer, HP-5MS capillary column (30 m x 0.25 mm diameter x 0.25  $\mu$ m thick). Operating conditions were as follows: carrier gas, helium with a flow rate of 1 mL/min; column temperature, 5 min at 60 °C, 60-230 °C at 2 °C/min and finally 3 min at 280 °C; injector temperature, 240 °C; volume injected, 1  $\mu$ L of the oil in n-hexane (0.1%); split ratio, 1:67 at ionization potential of 70 eV. Moreover, ion source and interdace tempretures were 200 and 250 °C, correspondingly, and mass range was from m/z 43-455.

#### **Identification of components**

The constituents of the essential oils were identified by their retention indices under temperatureprogrammed conditions for n-alkanes and the oil on a DB-1 column under the same chromatographic conditions. The identification of compounds was based on direct comparison of the retention indices and mass spectral data with those for the standards and by computer matching with the Wiley 229, Nist 107, Nist 21 Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [19-22]. For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors, where the FID detector condition was set on a duplicate of the same column applying the same operational conditions.

#### RESULTS

The GC-MS analysis of the essential oil of VAC fruits collected from three different places around Qum, Khorasan and Tehran led to the identification and quantification of approximately 30, 7, and 18 compounds, respectively. These compounds accounted for the 74.95%, 82.53% and 84.41% of the total components present in Qum, Khorasan and Tehran VAC fruits essential oils. All samples were composed predominantly of monoterpenes including monoterpene hydrocarbons (42.84-64.48%), and monoterpene alcohols (0.78-8.60%). There were about 9.45-31.33% sesquiterpene and 0-3.11% diterpene compounds; the percentage of compounds from VAC fruit essential oils collected from Qum, Khorasan and Tehran are presented in Table 1.

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Inneterpene         42.84         64.48         61.4           Pinene         932         12.05         16.21         18.3           abinene         965         3.50         -         4.20           Pinene         978         -         -         0.55           Pinene         982         -         13.15         5.66           fyrcene         983         1.45         -         -           - Phellandrene         100         0.37         -         0.33           - Cymene         101         0.18         -         -           - Phellandrene         105         0.07         -         2.55           ornyl acetate         127         0.16         -         -           - Terpinyl acetate         133         16.57         12.11         16.3           inabol         108         0.22         6.50         0.66           erpinene-4-ol         117         0.29         2.10         0.50           c-Terpineol         117         0.27         -         -           esquiterpene         133         9.45         7.70           is - β - Farnesene         142         0.13         -	Compounds	RI *	Percent (%) composition at different alti		
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- Selinene       148       0.30       -       0.33         - Bisabolene       148       0.05       -       -         - Selinene       149       0.35       -       -         - Cadinene       150       0.09       -       -         - Cadinene       150       0.08       -       1.12         - Cadinene       150       0.08       -       1.12         - Cadinene       155       -       -       4.20         alustrol       156       0.46       -       -         alustrol       156       1.18       -       -         'iridiflorol       156       11.18       -       -         pathulenol       160       -       -       1.33         - Eudesmol       161       2.43       -       3.44         - Bisabolol       164       0.07       -       -         - Farnesyl acetate       181       1.02       -       0.60	Allo aromadendrene	144	0.37	-	-
- Bisabolene       148       0.05       -         - Selinene       149       0.35       -         - Cadinene       150       0.09       -         - Cadinene       150       0.09       -         - Cadinene       150       0.08       -       1.12         cermacrene B       155       -       -       4.20         alustrol       156       0.46       -       -         aryophyllene oxide       155       1.03       -       -         füridiflorol       156       11.18       -       -         pathulenol       160       -       -       1.33         - Eudesmol       161       2.43       -       3.44         - Bisabolol       164       0.07       -       -         - Farnesyl acetate       181       1.02       -       0.60	Trans - β - Farnesene	145	0.39	-	-
- Selinene       149       0.35       -       -         - Cadinene       150       0.09       -       -         - Cadinene       150       0.08       -       1.12         Germacrene B       155       -       -       4.20         alustrol       156       0.46       -       -         alustrol       156       1.03       -       -         Griyophyllene oxide       155       1.03       -       -         Viridiflorol       156       11.18       -       -         pathulenol       160       -       -       1.33         - Eudesmol       161       2.43       -       3.44         - Bisabolol       164       0.07       -       -         - Farnesyl acetate       181       1.02       -       0.60	- Selinene	148	0.30	-	0.35
- Cadinene       150       0.09       -       -         - Cadinene       150       0.08       -       1.12         - Cadinene       150       0.08       -       1.12         - Cadinene       150       0.08       -       1.12         - Cadinene       155       -       -       4.20         alustrol       156       0.46       -       -         alustrol       155       1.03       -       -         Ciridiflorol       156       11.18       -       -         pathulenol       160       -       -       1.33         - Eudesmol       161       2.43       -       3.44         - Bisabolol       164       0.07       -       -         - Farnesyl acetate       181       1.02       -       0.60	- Bisabolene	148	0.05	-	-
- Cadinene       150       0.08       -       1.12         iermacrene B       155       -       -       4.20         alustrol       156       0.46       -       -         iaryophyllene oxide       155       1.03       -       -         irridiflorol       156       11.18       -       -         pathulenol       160       -       -       1.33         - Eudesmol       161       2.43       -       3.44         - Bisabolol       164       0.07       -       -         - Farnesyl acetate       181       1.02       -       0.60	- Selinene	149	0.35	-	-
ermacrene B     155     -     4.20       alustrol     156     0.46     -       aryophyllene oxide     155     1.03     -       iridiflorol     156     11.18     -       pathulenol     160     -     -       Eudesmol     161     2.43     -       - Bisabolol     164     0.07     -       - Farnesyl acetate     181     1.02     -       0     0     3.11	- Cadinene	150	0.09	-	-
alustrol       156       0.46       -       -         aryophyllene oxide       155       1.03       -       -         iridiflorol       156       11.18       -       -         pathulenol       160       -       -       1.33         - Eudesmol       161       2.43       -       3.44         - Bisabolol       164       0.07       -       -         - Farnesyl acetate       181       1.02       -       0.60	Cadinene	150	0.08	-	1.12
aryophyllene oxide     155     1.03     -       aryophyllene oxide     155     1.03     -       iridiflorol     156     11.18     -       pathulenol     160     -     -       160     -     -     1.33       - Eudesmol     161     2.43     -       - Bisabolol     164     0.07     -       - Farnesyl acetate     181     1.02     -       0     0     3.11	ermacrene B	155	-	-	4.20
iridifioral       156       11.18       -       -         pathulenol       160       -       -       1.33         - Eudesmol       161       2.43       -       3.44         - Bisabolol       164       0.07       -       -         - Farnesyl acetate       181       1.02       -       0.60	alustrol	156	0.46	-	-
naminal     160     -     1.33       pathulenol     160     -     1.33       - Eudesmol     161     2.43     -     3.44       - Bisabolol     164     0.07     -     -       - Farnesyl acetate     181     1.02     -     0.60	aryophyllene oxide	155	1.03	-	-
- Eudesmol         161         2.43         -         3.44           - Bisabolol         164         0.07         -         -           - Farnesyl acetate         181         1.02         -         0.60           iterpene hydrocarbons         0         0         3.11	iridiflorol	156	11.18	-	-
- Bisabolol 164 0.07	pathulenol	160	-	-	1.33
- Farnesyl acetate 181 1.02 - 0.60	- Eudesmol	161	2.43	-	3.44
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	Tanool	205	-	-	3.11
otal 74.95 82.53 84.4	otal		74.95	82.53	84.41

# Table 1: Chemical compositions of the essential oils of Vitex agnus castus fruits collected from different places.

\* RI: retention indices to C8-C22 n-alkanes.

In all three samples, monoterpene hydrocarbons were the main component where  $\alpha$ -pinene,  $\alpha$ -terpinyl acetate, limonene and  $\beta$ -pinene were some of the main components of these VAC fruits essential oils.  $\alpha$ -Pinene possessed highest percentages of 18.90 in essential oil collected from Tehran followed by Khorasan (16.21%) and Qum (12.05%). In the case of limonine samples of VAC essential oil from Khorasan has highest percentages (23.01%) followed by Tehran (12.50%) and Qum (8.49%). Moreover,

 $\alpha$ -terpinyl acetate was maximum (16.81%) in essential oil collected from Tehran followed by Qum (16.57%) and Khorasan (12.11%) samples.

Additionally, there was a monoterpene hydrocarbon, arthole, (0.50 %) that was present only in the VAC essential oil from Tehran. p-Cymene and bornyl acetate were present only in VAC fruits collected from Qum and  $\beta$ -phellandrene with sabinene were present only in Tehran and Qum samples. Other components of the monoterpene hydrocarbons comprised low portions of the essential oils, as it has been indicated in Table 1.

Among the monoterpene alcohols, linalool and terpinene-4-ol were detected in all samples. A minute amount of  $\alpha$ -terpineol was detected in the sample from Qum, where  $\alpha$ -terpineol was absent in Khorasan and Tehran samples. In the case of sesquiterpenes compounds,  $\beta$ -Caryophyllene was present in all samples as the major component with varying quantities of 11.93, 9.45, and 7.70 %. Germacrene B (4.20%) and Sapthunelol (1.33%) were found only in plants collected from Tehran, while,  $\alpha$ -humelene (1.64%), viridiferol (11.18%), caryophyllene oxide (1.03%) were present only in essential oils collected from Qum. However,  $\beta$ -eudesmol was not present in Khorasan essential oil samples. A diterpene hydrocarbon, manool, was only identified in the sample from Tehran (3.11%).

#### DISCUSSION

The chemical profiles of the essential oils of the fruits of three samples of VAC growing in different conditions, temperature and at different altitudes were quite different of some Vitex spp [22]. In a study conducted by Males et al. leaves and flowers of VAC essential oils from Dalmatia, Croatia were investigated by GC and GC-MS. They showed principal constituents of the monoterpenoids were limonene, cineole, sabinene, and  $\alpha$ -terpineol, while the major sesquiterpenes were  $\beta$ -caryophyllene,  $\beta$ gurjunene, cuparene, and globulol [21]. Elsewhere, two VAC essential oils were assessed in a survey by Lucks et al.; one was from the ripe fruit of the plant in 'Wild Herbs of Crete' and the other was obtained from the aerial parts (leaves) of the plants in the Pacific Institute of Aromatherapy, Turkey. The findings of the study revealed that the leaf oil contained a greater amount of  $\alpha$ -pinene, 1,8-cineole and  $\beta$ farnesenethan the fruit oil, but the fruit oil contains slightly more sabinene than the leaf oil [18]. Nevertheless, similar results have also been reported by other workers on other Vitex spp where a clear influence of growing place of the plants on the essential oils in VAC and the variations in presence or absence and also the identity of some compounds were quite evident in these samples. Accordingly, these data showed the relative differences in the chemical compositions of the VAC essential oils from the viewpoint of quantitative and qualitative considering the indispensable role of these components in achieving pharmacological effects of the VAC essential oils.

#### CONCLUSION

The findings of the study are in line with published data on variations of composition and amount of essential oils produced by other plants growing in different geographical and environmental conditions. Concisely, developing an efficient natural medicinal agent is reliant to the better understanding of the relation between the constituents in essential oils and performance of these components individually and in combination with each other leading to a superior pharmacological action.

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