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ANALYSIS OF THE ESSENTIAL OILS OF *VITEX AGNUS-CASTUS* AND ITS VARIETY *ALBA*, CULTIVATED IN EGYPT.

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ABSTRACT

The essential oils of *Vitex agnus-castus* and its variety *alba* Weston cultivated in Zagazig-Egypt were analysed using GC and GC/MS. In this investigation, comparative study of the oils of the fruits, leaves and flowers of both plants were investigated and compared with each other as well as testing their antimicrobial activity. About 31 components were identified. The major components were 1,8-cineol, sabinene, α-pinene, isocaryophyllene, β-caryophyllene and bicyclogermacrene.

INTRODUCTION

Vitex agnus-castus L. chaste tree (family Verbenaceae) is an aromatic shrub or small tree (10-20 ft), leaves are 5-7 lanceolate or elliptic, acuminate, nearly entire, cymes dense, sessile or sub sessile. The flowers are violet while the flowers of the var. *alba* are whitish[1].

Vitex agnus-castus endorsed as a normalizing herb for female sex hormones. It is especially benificial during monopausal changes and used to regulate hormones involved in menstrual cycle[2]. *V. agnus castus* is valuable also in treating other problems that can be linked to the menstrual cycle such as migraine and acne. The berries are taken to increase breast milk production[3].

The importance of *V. agnus-castus* has been greatly increased specially after the discovery of its potential activity as female sex hormones regulator[3].

Previous studies[4-11] of the volatile oil of *Vitex agnus-castus* (fruits, leaves and flowers) growing in Egypt and abroad resulted in the isolation and/or identification of its major constituents, viz α-pinene, sabinene, β-phellandrene, 1,8 cineol, β-pinene, β-caryophyllene, spathulenol, α-terpeneol, borneol, and camphor with an exception of very low percentage of 1,8-cineol (0.15%) in the plant studied in the Netherlands[10].

Reviewing the previous literature of the oil of *Vitex agnus-castus* growing in Egypt[6] revealed the identificating of about 17 components which constitute about 55-76% of the oil for different organs. Nothing has been reported about the variety (*alba*).

In this study, the volatile oils of the fruits, leaves and flowers of *Vitex agnus-castus* and its variety (*alba*) were analysed. The qualitative and quantitative differences of their oils are recorded. The antimicrobial and antifungal activities of these oils were tested.

EXPERIMENTAL

The leaves and flowers of *V. agnus castus* and its variety *alba* were collected in June 1998 from Zagazig University's gardens while the fruits were collected in September of the same year.

The two plants were kindly identified by Prof. Dr. Nabil El-Hadidi, prof. of plant taxonomy, Faculty of Science, Cairo University. A voucher specimens were deposited at the Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

Preparation of oils:

The freshly collected parts of both plants were subjected to hydrodistillation, following the E.P. method[12]. The percentage in each organ was determined as shown in (Table 1).

Analysis and identification:

GLC analysis was performed on a Hewlett Packard 5890 GLC, fitted with a flame ionization detector with the following conditions : injector 250°C, detector 280°C, initial temp. 50°C for 2 min. then increased to 200°C at rate 5°C/min, nitrogen as carrier gas, flow rate 2 ml/min split ratio (1: 20). Column capillary DB-5 dimensions 30 m x 0.25 mm, film thickness 0.25 μm.

GC/MS Amicro masstrio 2000 was used operated at 70 eV. The operating conditions were similar to those used in GLC analysis except the capillary column 30 m x 0.32 mm, with helium as carrier gas, flow rate 2 ml/min split ratio (1:20). The individual components of the oils were identified by their retention times and by comparison of their mass spectra with those given in the literature[14,15].

Antimicrobial activity :

The essential oils under investigation were tested for their antimicrobial activity following the disc agar diffusion method[13] using 30 - 50 μg of 10% oil in DMF. The activity of the six oils were tested against *Bacillus subtilus* as a gram positive bacilli and *Staphylococcus aureus* as a gram positive cocci, *Pseudomonas aeruginosa* and *Escherichia coli* as gram negative bacilli, Candida albicans as fungi. All of them are standard strains obtained from stock cultures of the Department of Microbiology, Faculty of Pharmacy, Zagazig University. Standard antibiotic discs of Erythromycine, Gentamycin, Ceftriaxone and Nystatin were used for comparison. The results are summarized in Table (4).

RESULTS AND DISCUSSION

The essential oils of the leaves, flowers and fruits of both *Vitex agnus-castus* and *V. agnus castus var alba* were prepared by hydrodistillation of fresh organs. Organs of *V. aganus-castus* showed higher yields of the essential oils than those of *V. agnus var. alba* (Table 1).

Table (1): The Percentage Yield Of The Volatile Oil

The plant	Fruit	Flowers	Leaves
Vitex agnus-castus	0.75	0.30	0.45
Vitex agnus-castus var. alba	0.45	0.20	0.30

GC/MS analysis of the fruit oil of *V. agnus castus* allowed the identification of 13 components out of 15 ones contributing to nearly 85% of the oil (Table 2) while in the case of the flower oil 27 components out of 36 were identified, nearly 71% and of the leaf oil 27 components out of 36 about 92% of the oil. For *V. agnus castus* var. *alba* the number of identified compounds and their collective relative percentage were as follows: fruit oil; 28 out of 35 (90%); flower oil 17 out of 25 (65%) and leaf oil 29 out of 39 (92%). It is worth to note that most of the unidentified compounds (Table 2 and 3) are high boilers, revealing molecular ion peaks 270 or more and their spectra couldn't be matched with any of the available published data.

Comparing the major components of the oils of the three organs, viz fruits, flowers, leaves, of *V. agnus castus* (Table 2) it was noticed that in all, such components could be arranged in a decreasing order as follows: 1,8-cineol, sabinene, α-pinene, β-terpinyl acetate, bicyclogermacrene, β-caryophyllene, and isocaryophyllene.

On the other hand, the three oils of variety *alba* showed more or less similar quantitative composition as that in the type species, the quantitative sequence of their major components varied. The fruit oil is dominated by sabinene then 1,8-cineol, isocaryophyllene, α-pinene and β-caryophyllene, the flower oil: isocaryophylene then sabinene, 1,8-cineol, β-caryophyllene and α-pinene while the dominant components in the leaf oil are 1,8-cineol then sabinene, isocaryophyllene, β-terpinyl acetate, α-pinene then, β-caryophyllene.

The collective data on the composition of the fruit, flower and leaf oils of *V. agnus castus* are significantly different from those previously reported for oils obtained from locally cultivated species[5,6].

Some of the major components identified in the present study like sabinene, β-terpinyl acetate, isocaryophyllene, β-caryophyllene, bicyclogermacrene, spathulenol, viridiflorol and others were not reported in the previous studies. In stead, several components were mentioned[5,6] couldn't be traced in the present one such as: β-pinene, dipentene, α-terpinene, camphor, borneol, neryl acetate, geranyl acetate, isoeugenol, eugenol and others.

Also, the collective data obtained from Croatia and the Netherlands[9-11], show the absence of some major components such as: β-terpinyl acetate, isocaryophyllene, bicyclogermacrene, 4-terpineol and others. While several components were mentioned, couldn't be traced in the present study such as: limonene, β-phellandrene, Eβ-farnesene, caryophyllene oxide, α- terpinyl acetate, β-germacrene, alloaromodendrene, cuparene and others.

The real difference is the great variation in the percentage of 1,8-cineol which is the major component. In this study the percentage of 1.8 cineol ranges from 18 to 34.5% while found as a mixture with limonene (12-36%) in the oils of plants growing wild in Croatia[9,11] and 0.15% in the fruit oil studied in the Netherlands[10].

From this comparison it was evident that there is a considerable qualitative and quantitative differences between the composition of the oils of *V. agnus castus* in this study and those previously reported which may be attributed to ecological variations.

From Table (4) it is shown that all the essential oils from different organs of the two plants have slight different activity against gram positive bacilli bacteria and no activity against gram negative and fungi.

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Table (2): The percentage of the volatile components identified in the oils of *Vitex agnus castus* and its variety "*alba*"

No	Compound	*Vitex agnus castus*			*Var. alba*		
		Fruits	Flowers	Leaves	Fruits	Flowers	Leaves
1	α-pinene	13.02	4.83	6.77	10.33	4.70	5.45
2	Sabinene	16.66	8.11	17.19	23.21	10.88	23.97
3	β-Myrcene	2.08	1.30	1.48	1.83	0.71	1.25
4	α-Phellandrene	0.76	0.23	-	0.50	0.10	0.05
5	1,8 cineol	31.30	17.92	34.34	16.52	8.73	29.99
6	Cis ocimene	-	0.15	0.16	0.10	-	-
7	δ-Terpinene	-	0.20	0.20	0.71	0.16	0.10
8	Cis-sabinene hydrate	-	-	0.32	0.50	-	0.60
9	α- Terpinolene	-	0.12	0.09	0.26	-	0.34
10	Trans-sabinene hydrate	-	-	0.34	0.41	-	0.17
11	Linalool	-	0.29	0.45	-	-	0.29
12	Cis-p-menth-2-en-1-ol	-	0.08	0.23	0.17	-	0.17
13	Unidentified	-	0.32	1.03	0.22	-	0.45
14	4-Terpineol	1.11	2.19	2.98	3.57	2.21	3.57
15	α-Terpineol	-	1.63	3.74	0.90	-	1.25
16	Citronellol	-	-	0.46	-	-	0.25
17	Δ Elemene (Delta)	-	0.07	0.06	0.20	-	0.28
18	β-Terpenyl acetate	5.35	5.32	8.00	2.50	2.57	6.54
19	Citronellyl acetate	-	-	-	0.61	-	0.04
20	β-Bourbonene	-	-	-	0.44	0.40	0.04
21	δ- Gurjunene	0.36	-	0.09	-	-	0.20
22	Isocaryophyllene	3.19	7.35	2.30	10.64	14.59	5.52
23	β- Cedrine	-	0.31	0.10	0.14	-	-
24	α- Caryophyllene	-	0.09	0.05	0.17	-	0.02
25	β-Caryophyllene	4.50	4.40	1.84	7.67	6.28	4.21
26	β-Cubebene	-	-	-	1.55	1.27	0.49
27	Bicyclo germacrene	4.99	6.03	2.25	2.22	1.93	2.36
28	Ledol	-	-	0.24	0.14	-	0.09
29	Spathulenol	1.02	1.59	3.00	2.25	3.14	1.80
30	Viridiflorol	0.60	2.29	3.01	0.93	1.28	1.18
31	τ-cadinol	-	2.30	1.77	0.14	1.55	0.83
32	Unidentified	-	1.60	0.80	-	-	0.12
33	Unidentified	-	1.95	-	0.14	0.45	0.09
34	Unidentified	-	4.04	0.42	0.14	0.55	0.16
35	Unidentified	-	3.04	0.39	-	-	-
36	Unidentified	-	7.78	0.78	2.29	8.99	1.65
37	Unidentified	-	-	0.48	1.45	5.31	1.00
38	Unidentified	0.51	6.46	1.94	2.32	9.41	1.62
39	Unidentified	-	-	0.65	-	2.19	0.90
40	Thunbergol	-	4.00	1.03	1.47	5.24	0.72
41	Unidentified	-	2.97	0.65	0.55	5.20	0.50
42	Unidentified	14.43	-	-	-	1.31	0.09
% Total identified		84.94	70.8	92.39	90.08	65.74	91.77

Table (3) : GC/MS analysis of the oils of *Vitex agnus-castus* and its variety "*alba*"

No.	Compound	Rt	M+	B.P.	Major Peaks
1	α-pinene	4.94	136	93	91,79,77,41,121,105,53,136
2	Sabinene	5.80	136	93	91,77,41,79,51,136,121
3	β-Myrcene	6.33	136	41	93,69,91,79,53
4	α-Phellandrene	6.61	136	93	91,77,136,92,41,51
5	1,8-cineol	7.27	154	43	81,93,108,71,55,139
6	Cis ocimene	7.85	136	93	91,79,41,77,53,67,121,105
7	δ-Terpinene	8.06	136	93	91,77,136,121,41,105,65
8	Cis-sabinene hydrate	8.26	154	43	71,93,81,111,55,121,139
9	α- Terpinolene	8.88	136	121	93,136,91,79,77,105,53
10	Trans-sabinene hydrate	9.12	154	43	71,93,81,55,111,121,139
11	Linalool	9.28	154	71	43,93,55,80,121,136
12	Cis -p-menth-2-en-1-ol	9.80	154	43	69,71,79,93,111,55,81,121,139,125
13	Unidentified	11.01	154	59	81,43,93,67,96,136,55,121
14	4- Terpineol	11.32	154	71	43,111,93,55,67,86,81,77
15	α-Terpineol	11.74	154	59	93,121,43,136,81,67,55
16	Citronellol	13.01	156	41	69,67,81,55,95,123,109,138
17	Δ Elemene (Delta)	15.99	204	121	93,41,107,79,67,136,53,161,189
18	β-Terpenyl acetate	16.16	196	43	121,93,136,79,59,67
19	Citronellyl acetate	16.32	198	43	81,67,95,123,55,138,109
20	β-Bourbonene	17.20	204	81	123,80,41,161,91,105,53
21	δ- Gurjunene	17.92	204	105	41,119,91,161,133,55,79,189,147
22	Isocaryophyllene	18.16	204	41	93,69,79,133,105,55,119,161,147,189
23	β- Cedrine	18.76	204	69	41,91,55,77,120,133,161,105
24	α- Caryophyllene	18.96	204	93	41,80,121,67,107,53,147,161
25	β-Caryophyllene	19.29	204	41	91,105,79,69,133,161,55,147,189
26	β-Cubebene	19.68	204	161	105,41,91,119,81,133,55,147
27	Bicyclo germacrene	20.08	204	121	93,41,107,79,67,53,161,133,147,189
28	Ledol	21.71	222	111	41,122,55,107,93,81,67,147,161,189
29	Spathulenol	21.93	220	43	91,79,119,69,105,55,131,147,159,205
30	Viridiflorol	22.52	222	43	109,122,81,69,95,55,149,133,161,189
31	τ-cadinol	23.47	222	161	43,105,81,119,95,134,55,204,189
32	Unidentified	26.94	286	151	43,136,55,119,131,105,81,91,145,69
33	Unidentified	28.85	272	109	41,93,119,150,135,55,81,187,257,204
34	Unidentified	29.65	286	131	43,55,119,145,105,91,69,159,187,79,173 ,201,271
35	Unidentified	29.96	286	187	145,43,157,128,119,71,55,91,172,200
36	Unidentified	30.17	n.d	191	43,135,121,109,55,69,95,81,149,161
37	Unidentified	30.62	270	119	189,43,105,133,71,55,91,145,159
38	Unidentified	30.99	n.d.	191	80,119,43,136,71,55,107,93,175
39	Unidentified	31.39	272	41	107,95,120,55,79,67,135,189
40	Thunbergol	32.70	290	107	95,43,121,71,55,81,135,191,147,161,175 ,257,272
41	Unidentified	33.28	272	95	95,107,43,121,55,71,81,189,135,257
42	Unidentified	35.35	n.d.	43	55,69,109,95,81,151,119,169,135,191

Table (4): Results of antimicrobial screening of the volatile oils

Microorganism	Inhibition zone in mm								Ceftri-axone	Nystatin
	1	2	3	4	5	6	Erythro-mycin	Gentamycin		
Gram -ve :										
Pseudomonas aeruginosa	-	-	-	-	-	-	12	20	21	-
Escherchia coli	-	-	-	-	-	-	15	19	20	-
Gram +ve rods :										
Bacillus subtilus	14	12	15	8	15	14	28	17	15	-
Cocci :										
Staphlococcus aurus	-	-	-	-	-	-	18	13	15	-
Fungi :										
Candida albicans	-	-	-	-	-	-	-	-	-	22

* Sample 1,2,3 *V. agnus-castus;* Sample 4,5,6 variety "*alba*"

REFERENCES

1. Hortus third, Aconcise Dictionary of Plants Cultivated in United States and Canada, Staff of the L.H. Bailey Hortorium, Cornell University, Macmillan Publishing Collier, p1162-1163 (1976).
2. The Green Pharmacy, New discoveries in herbal remedies for common diseases and conditions from the world's foremost authority of healing herbs by James A. Duke, p 324-325 (1997).
3. The Encyclopedia of Medicinal plants "by Andrew Chevallier, p 149 (1996).
4. Gildemeister and Hoffmann, Fr.: "Die Atherischen Ole" Vol. VI, 610. Akademic-Verlag, Berlin (1961).
5. El-Moghazi, A.M., Halim, A.F., Goomaa, C.S. and El-Sayed E.A.: paper presented at the XVIth Egyptian conference of pharmaceutical sciences, Dec. 1975, Cairo, Egypt.
6. A.M. El-Shamy, M.Y. Haggag and S.M. El-Zalabani. **Journal of African Medicinal Plants** Vol. 6, p. 35 - 42 (1982).
7. Kartnig, Th., **Z. Phytother.,** 7, 119-122 (1986).
8. Laakso, I., Ekundajo, O., Oguntimein, B., and Hiltumen, R. **Pharm. Week Bl. Sci.** Ed. 9, 238 (1987).
9. Kustrak, D., Kuffinec, J., and Blazevic, N., **Planta Medica** 58, Suppl. 1 A 681 (1992).
10. J.H. Zwaving and R. Bos, **Planta Medica.,** 62, 83 - 84 (1996).
11. Males, Z., Blazevic, N. and Antolic, A., **Planta Medica.**, 64, 286-287 (1998).
12. Egyptian Pharmacopoeia, (Arabic text), general organization for Governmental Printing Affairs, p. 31 (1984).
13. Balows, A. ; Hauster, W.J. ; Herrmann, K.L. ; Isenberg, H.D. and Shadomy, H.J. "Manual of Clinical Microbiology" 5th ed. American Society for Microbiology, Washington, D.C., p. 1059 (1991).
14. Adams, R.P., Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publ. Corp., Caral Stream, Illinois (1995).
15. Masada, Y., "Analysis of essential oils by gas chromatography and mass spectrometry" John Wiley, New York (1976).

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تحليل الزيوت الطيارة لثمار وزهور وأوراق نبات فيتكس أجنس كاستس (كف مريم) مع نوعه (ألبا) والمزروعة فى مصر .

إحسان محمود عبدالعزيز أبوزيد

قسم العقاقير – كلية الصيدلة – جامعة الزقازيق – الزقازيق – مصر

فى هذا البحث تم اجراء دراسة مقارنة بين مكونات الزيوت الطيارة لأجزاء نبات الفيتكس اجنس كاستس من العائلة الفربينية (ثمار وأزهار وأوراق النبات) مع نوعه "ألبا" باستخدام كروماتوجرافيا الغاز المتصل بمطياف الكتلة ، ووجد أن هناك اختلاف كيفى وكمى بين محتويات كل منهما وكذلك تم اختبار فاعلية الزيوت ضد الميكروبات والفطريات فوجد أن له بعض التأثير على بعض أنواع البكتريا الموجبة وليس لهم جميعاً أى تأثير على أنواع البكتريا السالبة والفطريات.