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ESSENTIAL OILS OF CERTAIN CULTIVARS OF *ANNONA SQUAMOSA* L.; GROWING IN EGYPT.

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ABSTRACT The composition of the hydrodistilled essential oils obtained from the leaves of two cultivars of *Annona squamosa* L. (Balady and Abdel-Razik cultivars) were analyzed by gas chromatography-mass spectroscopic technique (GC-MS). The study resulted in the identification of 18 and 26 components, comprising 89% and 92.7% of the oils of the Balady and Abdel-Razik cultivars, respectively. Results showed evident qualitative and quantitative differences between the two oils. Both oils were rich in sesquiterpene hydrocarbons (40.3% and 42.2%, respectively). Sesquiterpene alcohols were higher in the Balady (35.0 %), than in Abdel-Razik (10.9 %) cultivar, while monoterpene alcohols were found in a higher percentage in Abdel-Razik (21.4 %), than in the Balady cultivar (9.5 %). The two oils exhibited remarkable wide spectrum antibacterial effects, but no antifungal activity.

INTRODUCTION

The genus *Annona* (*ca* 120 species) belongs to the family Annonaceae[1]. Several *Annona* species are grown for their edible fruits, as they are of high nutrition value, and for production of edible seed oils[2]. In traditional medicine, many members of this genus are used as diuretics, antitumors and for treating gonorrhea, as well as insecticides [3,3,4]. Previous chemical studies of this genus have concentrated mainly upon the alkaloidal constituents [2,5]. Recently, interest in *Annona* plants has become worldwide due to the isolation of Annonaceous acetogenins, that have a remarkable antitumor, pesticidal, antimalarial and anthelmintic activities [1,6-9].

Annona squamosa L. (commonly referred to as the custard apple) is a fruit tree native to Central America, and is cultivated through the tropics[1,2]. Different organs of this plant have been used in folk medicine for the treatment of acute dysentery, depression and spinal marrow disease[10]. The ethanolic extract of the leaves and stems of *A. squamosa* is reported to have anticancer activity[5]. Regarding the chemical constituents, alkaloids[2,5] as well as acetogenins[9,11,12] have been reported in *A. squamosa*.

In Egypt, there are four species of *Annona*, of which only *A. squamosa* contains essential oil, to which its pleasant smell is attributed [3,13]. The essential oils of the seed, root and, and fruit peel of this species have been studied [2]. The leaf essential oil has also been studied [13], however, some 21 compounds (representing only less than 70% of the oil constituents) have been identified from *A. squamosa* L. leaf without specifying the studied cultivar. The present study reports the chemical composition of the essential oils of the leaves of two *A. squamosa* cultivars (Balady and Abdel-Razik), growing in Egypt, using more advanced GC-MS facilities. Thus, the effect of cultivar variation on the production of certain oil constituents is studied. In addition the antibacterial and antifungal activities of both oils have been investigated, to find out their potential future in pharmaceutical and/or industrial uses.

Experimental

Plant material

The leaves of *Annona squamosa L.* cultivars (Balady and Abdel-Razik) were collected from El-Kassasin Horticulture Research station, Ismailia in May 1999 and identification was confirmed by Dr. Talat A. Abou Sayed Ahmed, Prof. of Pomology, Horticulture Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. Voucher specimens are deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

Preparation of oils

The fresh leaves of the two *A. squamosa* cultivars (Balady and Abdel-Razik) were subjected to hydrodistillation and the percentage of the oil for each cultivar was determined following the E.P. method [14].

Analysis and identification

GC-MS analysis was performed on a Varian 3400 gas chromatograph equipped with a fused silica column (DB5, 30m x 0.25 mm.; i.d., 0.25 mm); Carrier gas : He at 40 cm^3 / sec.; Oven temp. program; 45°c, 3 min.; 45-160°c, 4 °c / min., 160°c, 10 min.; 160-260°c, 10°c / min.; 260°c, 10 min., Injector : split 1:100, 250°c; Detector: FID, at 300°c. The capillary column was directly coupled with a quadrupole mass spectrometer (Finnigan MAT SSQ 7000). EI-MS spectra were recorded at 70 eV. Individual components of the oils were identified by their retention indices [15-19] and by comparison of their mass spectra with those given in the literature [20,21]. Kovats retention indices "RI"[22] were calculated using co-chromatographed standerd n-alkanes (C_8-C_{22}). Results are shown in table 1and 2.

Antimicrobial and antifungal activities

The disc diffusion method [23] was employed to evaluate the antimicrobial and antifungal activities of the two *A. squamosa* cultivars. Four Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis and Bacillus bronchoseptica*) and

Table 1: Composition of the essential oils of the leaves of the Balady and Abdel - Razik cultivars Of *Annona squamosa* .

No	Compounds	RI DB5	M^+ and major MS ions m/z	%Composition	
				Bal.*	Abd.*
1	α-Terpinene	1022	**136**,105,93,77,66,51	0.2	--
2	*(z)*-β-Ocimene	1047	**136**,121,105,93,79,67,53	2.5	14.2
3	*(E)*- β -Ocimene	1055	**136**,121,107,93,67,55	0.5	--
4	*cis*-Sabinene hydrate	1114	**n.d.**,136,121,107,93,79,53	3.6	13.9
5	*trans*- Sabinene hydrate	1118	**n.d.**,136,121,107,93,77,69,53	0.5	3.2
6	Pinocarveol	1146	**n.d.**,134,119,115,91,79,55	1.8	--
7	*(E)*- β -Terpineol	1159	**154**,136,121,107,93,79,68,53	0.9	2.7
8	*(z)*- β -Terpineol	1160	**154**,136,121,105,93,79,67,53	--	0.1
9	α -Terpineol	1177	**n.d.**,136,121,107,93,81,67,59	2.7	0.7
10	Dihydrocarvacrol	1212	**n.d.**,136,121,107,93,79,67,55	--	0.8
11	Linalyl butyrate	1228	**n.d.**,154,136,121,107,93,71,55	0.5	2.3
12	*cis*-Anethole	1245	**148**,121,117,105,91,79,55	--	0.1
13	*trans*-Anethole	1246	**148**,134,121,117,105,91,79,55	--	0.1
14	Linalyl acetate	1269	**n.d.**,136,121,105,93,79,59	--	0.6
15	Bornyl acetate	1284	**196**,136,121,108,95,67,55	--	0.4
16	δ-Elemene	1346	**204**,189,161,136,121,105,93, 55	--	1.7
17	α-Ylangene	1371	**204**,161,133,119,105,93,55	--	1.0
18	β-Cubebene	1386	**204**,161,147,133,119,105,91, 55	11.2	0.9
19	β-Elemene	1390	**204**,189,175,161,147,133,105, 93,53	3.6	2.3
20	α -Gurjunene	1405	**204**,189,175,161,147,133,105, 91,55	--	0.4
21	β -Caryophyllene	1414	**204**,189,161,147,121,105,93, 53	2.7	1.0
22	α -Himachalene	1443	**204**,189,175,161,147,133,105, 93,55	4.0	--
23	δ-Murolene	1470	**204**,189,175,161,147,133,105, 91,55	3.8	--
24	Germacrene D	1474	**204**,189,161,147,133,119,91,55	15.0	18.6
25	Germacrene B	1494	**204**,189,161,147,133,121,93,55	--	7.1
26	δ -Cadinene	1503	**204**,189,161,147,133,119,91,55	--	7.2
27	δ -Elemene	1551	**204**,189,161,147,133,121,93,53	--	2.0
28	Cadinol(TAU)	1597	**222**,204,189,161,147,133,105, 93,55	--	1.8
29	α-Eudesmol	1645	**222**,204,189,161,149,145,119, 105,91,67	29.4	6.5
30	α -Cadinol	1653	**222**,204,189,161,148,137,105, 95,58	5.6	2.6
31	Cedren-13-ol acetate	1921	**n.d.**,202,187,173,159,145,132, 119,105,91,55	0.5	0.5

Molecular ion peaks are in bold face; **n.d.**, mol.ion was not detected;
* **Bal.**, Balady cultivar; **Abd.**, Abdel-Razik cultivar

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four Gram-negative (*Klebsella pneumonia, Pseudomonas aeruginosa, Escherichia coli and Proteus vulgaris*) bacteria beside two fungi (*Candida albicans* and *Aspergillus niger*) were used in this study. The employed organisms were isolated, identified and cultured on nutrient agar and the fungi were cultured on Sabouroud dextrose agar. Sterile paper discs (6mm diameter) were impregnated with the individual oils (20µl / disc). The oil-impregnated discs were applied gently to the surface of the inoculated plates. The plates were then, incubated at 35 °C (18 hr.) for bacteria and 25 °C (48 hr.) for fungi. The observed inhibition zones were measured and compared against standerd antibiotic discs (Oxoid) as references. The employed bacteria, fungi and antibiotics as well as the results are shown in (Table 3).

Result and Discussion

Hydrodistillation of the fresh leaves of the Balady and Abdel-Razik cultivars of *Annona squamosa* L. yielded 0.20 % v/w and 0.25 % v/w essential oils, respectively. Both oils are pale yellow in color, lighter than water and possess a strong agreeable aromatic odor. Analysis of the volatile oils by GC-MS resulted in identification of 18 and 26 compounds comprising 89% and 92.7 % of the oils of Balady and Abdel-Razik cultivars, respectively. All the unidentified components occurred in trace amounts. Table 1 shows a list of the constituents identified in the two cultivars. The components are arranged in order of elution from a DB5 column. The general elution sequence is confirmed by literature Kovats retention indices [15-19]. Positive identifications are based on comparison with reported literature mass spectral data [20-21].

The obtained results revealed clear qualitative and quantitative differences between the components of the two oils (Table 1). The major constituents of the Balady cultivar are α-eudesmol (29.4%), germacrene D (15.0 %) and β-cubebene (11.2%), whereas, Abdel Razik cultivar is characterized by the presence of high percentages of germacrene D (18.6 %), cis-sabinene hydrate (13.9 %) and (z)-β-ocimene (14.2 %).

Both oils are rich in sesquiterpene hydrocarbons (Table 2), they constituted 40.3% and 42.2% of the oil of the Balady and Abdel Razik cultivars, respectively. The major sesquiterpene hydrocarbons in the leaf oil of Balady cultivar are β-cubebene (11.2 %), β-elemene (3.6 %), β-caryophyllene (2.7 %), α-himachalene (4.0 %), δ-murolene (3.8 %) and germacrene D (15 %), while β -elemene (2.3 %), germacrene D (18.6 %), germacrene B (7.1 %) and δ-cadinene (7.2 %) constituted the major sesquiterpene hydrocarbons in Abdel-Razik cultivar.

On the other hand sesquiterpene alcohols occur in a higher percentage (35.0 %) in the Balady than in Abdel-Razik cultivar (10.9 %). The main sesquiterpene alcohol of the Balady cultivar is α eudesmole (29.4 %), which was also the major component of the oil. The leaf oils of both cultivars showed a trace amount of sesquiterpene alcohol esters (0.5 %).

Table2: Relative terpene contents of the leaf essential oil of Balady and Abdel- Razik cultivars *of A.squamosa L.*

No	Terpene contents	% composition	
		Balady	Abdel.Raqzik
1-	Monoterpene hydrocarbons	3.2	14.2
2-	Monoterpene alcohols	9.5	21.4
3-	Monoterpene alcohol esters	0.5	3.3
4-	Phenolic ethers	--	0.2
5-	Sesquiterpene hydrocarbons	40.3	42.2
6-	Sesquiterpene alcohols	35.0	10.9
7-	Sesquiterpene alcohol esters	0.5	0.5

The percentage of monoterpene alcohols together with their esters is higher in Abdel-Razik cultivar than the Balady cultivar (Table 2), being 24.7% and 10 %, respectively. cis-Sabinene hydrate (13.9%), trans-sabinene hydrate (3.2 %) and (E)- β-terpineol (2.7 %) are the major terpene alcohols in Abdel – Razik cultivar while, in the Balady cultivar cis-sabinene hydrate (3.6 %), pinocarveol (1.8 %) and α-terpineol (2.7 %) are the major terpene alcohols. The Balady cultivar showed only linalyl butyrate (0.5 %) as a monotenpene alcohol ester, but the esters in Abdel-Razik cultivar are linalyl butyrate (2.3 %), linalyl acetate (0.6 %) and bornyl acetate (0.4 %).

Monoterpene hydrocarbons constituted 3.2 % and 14.2 % in the Balady and Abdel-Razik cultivars, respectively (Table 2).The monoterpene hydrocarbons of the Balady cultivar are α-terpinene (0.2 %), (Z)-β-ocimene (2.5 %) and (E)-β-ocimene (0.5 %), but in Abdel-Razik cultivar they are represented only by (Z)-β-ocimene (14.2 %).

The phenolic ethers occur in trace amounts in Abdel-Razik cultivar only (0.2%).

Subsequently, it was evident that there is a considerable qualitative and quantitative difference between the two essential oils of the leaves of *A. squamosa* Balady and Abdel-Razik cultivar. In addition, qualitative and quantitative differences were found in our results from those previously reported on the oil of *A. squamosa*[13]. However, no indication of a specific cultivar used in that published study, and hence the presence of a third cultivar or different variety of *A. squamosa* is possible.

Table 3 shows the antibacterial activities of the two oils against several bacteria (4 Gram-negative and 4 Gram-positive) and two fungi. Both oils showed a broad-spectrum antibacterial activity against all the studied bacteria, compared to the standard antibiotic (amikacin). Whereas, no antifungal activity was exhibited by both oils against the tested fungi.

The results revealed that the oil of *A. squamosa* Balady cultivar showed a marked antibacterial activity against Gram-negative bacteria and very strong antibacterial activity against Gram-positive bacteria. On the other hand, that of Abdel-Razik cultivar showed a moderate antibacterial

Table 3: Antimicrobial activity of the leaf essential oils of Balady and Abdel - Razik cultivars of *A. squasma* L.

Microorganisms	Diameter of zone of inhibition in mm			
	Volatile oil of cultivars (20 ul/disc)		Standard antimicrobial agents	
	Balady cultivar	Abdel Razik cultivar	Amikacin (30 ug/disc)	Nystatin (100 ug/disc)
Gram negative:				
Klebsella pneumonia	11	8	13	----
Pseudomonas aeruginosa	13	7	14	----
Escherichia coli	20	17	22	----
Proteus vulgaris	12	10	17	----
Gram positive				
Staphylococcus aureus	28	25	21	----
Staphylococcus epidermidis	30	28	24	----
Bacillus subtilis	25	22	20	----
Bacillus bronchseptica	27	19	23	----
Fungi				
Candida albicans	-----	-----	-----	16
Aspergillus niger	-----	-----	----	18

*These results are the average of three readings.

activity against Gram-negative bacteria and a strong antibacterial activity against Gram-positive bacteria as compared with amikacin. These antibacterial activities may be attributed to the presence of the high contents of alcoholic compounds in both oils. This is considered as the first report about these antibacterial activities of these oils.

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الزيوت الطيارة لبعض أصناف القشطة (الأنونا سكواموزا) التى تنمو فى مصر

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فى هذة الدراسة تم تحليل مكونات الزيوت الطيارة المقطرة من أوراق صنفى القشطة البلدى وعبد الرازق باستخدام كروماتوجرافيا الغاز– مطياف الكتلة وتم التعرف على ١٨ , ٢٦ مركب بنسبة ٨٩% ,٧و٩٢% فى كلا من الصنفين البلدى و عبد الرازق على التوالى. وأظهرت هذة الدراسة فروقا كيفية و كمية واضحة بين الزيوت الطيارة للصنفين ووجد أن كلا منهما غنيا بالهيدروكربونات السيسكوتربينية (٣و٤٠% بالصنف البلدى , ٢و٤٢% بالصنف عبد الرازق) بينما وجدت السيسكوتربينات الكحولية بنسبة أعلى فى الصنف عبد الرازق(٣٥%) وبنسبة أقل فى الصنف البلدى(٩و١٠%). أما المونوتربينات الكحولية فقد وجدت بنسبة أعلى فى الصنف عبد الرازق (٤و٢١%) مقارنة بالصنف البلدى (٥و٩%). وقد أظهر كلا من الزيتين تأثيرات ملحوظة مضادة للبكتيريا بينما لم يظهر لهما تأثير مضاد للفطريات.