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ON THE CHEMICAL, BOTANICAL AND CHEMOTAXONOMICAL EVALUATION OF THE GENUS *CITRUS**

Part III : A Comparative Study of Essential Oil Components In The Leaf Oils of *Citrus deliciosa* Ten. and *Citrus reticulata* Blanco grown in Egypt

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ABSTRACT

The aroma from the leaves of *Citrus deliciosa* Ten. and *Citrus reticulata* Blanco cultivated in Egypt were analysed using GC-MS. Eighteen components were identified in each species. The leaves of the investigated species showed 0.4% v/w and 0.3% v/w oil, respectively. Methyl N-methylanthranilate was the major component in *C. deliciosa* Ten. (41.4%) while, α-Pinene was the major one in the leaf oil of *C. reticulata* Blanco (36.2%). The proportion of the terpene hydrocarbons and the oxygenated components were 53.9% and 44.84% in the leaf oil of *C. deliciosa* Ten., and 65.43% and 22.8% in the leaf oil of *C. reticulata* Blanco, respectively. Each of the prepared oils showed a remarkable antimicrobial and / or antifungal effects.

INTRODUCTION

The genus *Citrus* (Family Rutaceae) is commonly grown in the Mediterranean region and well known for its popular fruits and essential oils[1]. The species *Citrus deliciosa* Ten. is the major mandarin in the Mediterranean countries[1]. Obvioulsy, there have been many studies on the volatile oil components of leaves, flowers and peels of different species among the genus, and many volatile principles have been identified and reported elsewhere[2-7]. The fruit, the flower and the leaf oils of *Citrus reticalata* Blanco grown in Egypt have been previously studied by several authors [2-5,8]. Mandarin oil prepared from *C. reticulata* var mandarin is produced for industrial purposes mainly in Southern Italy and Sicily, Spain, Algeria and Cyprus[2,9]. This oil is used mainly for flavouring beverages, liquors, sweets and bitter chocolates[9]. In addition the terpeneless oil of *C. reticulata* is not frequently used in perfumery[9].

In previous works,[10,11] simultaneous phytochemical and botanical investigations were carried out to reveal the botanical characteritics and to provide a chemotaxonomic marker for identification of *C. deliciosa* Ten.

In the present study, the volatile oils prepared from the leaves of *C. deliciosa* Ten. and the closely related species *C. reticulata* Blanco were analysed. The qualitative and quantitative differences of their oils composition have been recorded. Moreover, the antimicrobial and antifungal activities of these oils were determined to fined out their potential future in pharmaceutical and / or industrial uses .

EXPERIMENTAL

Plant Material:

The leaves of *C. deliciosa* Ten. and *C. reticulata* Blanco (Rutaceae) were collected in April 1996 from local farms and from the Agrarian Reform farms at El-Sharkia Governorate, Egypt. The two species were kindly identified by Prof. Dr. Abdalla M.A. Mohsen, Prof. of Horticulture, Faculty of Agriculture, Zagazig University. A voucher specimen is on deposite at the Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

Preparation of Oils:

The leaves of *C. deliciosa* Ten. and *C. reticulata* Blanco were subjected to hydrodistillation and the percentage of the oil for each species was determined following the E.P. method[12].

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), J & W P/N : 122 - 5032; 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, 300°C. The capillary column was directly coupled with a quadrupole mass spectrometer (Finnigan MAT SSQ 7000). EI-MS were recorded at 70 eV.

Antimicrobial activity:

The antimicrobial and antifungal activities of the essential oil of both species were carried out, adopting

* Part I: polymethoxyflavones of *Citrus deliciosa* Ten., Natural product Sciences, 2 (2), 106-114 (1996). Part II: macro- and microscopphological study of the leaf and stem of *Citrus deliciosa* Ten., Bull. Fac. Pharm., Cairo Univ., Submitted for publication.

the disc agar diffusion method[13] using the available microorganisms isolated and identified by the staff of Microbiology department, Faculty of Pharmacy, Zagazig University. Standard antimicrobial and antifungal discs (Oxoid) are used as references. The bacteria were cultured on neutrient agar, while the fungi were cultured on Sabouround dextrose agar. Paper discs of 6 mm diameter, were impregnated with 25 µl / disc of each oil. The impregnated discs were gently applied on the surface of the inoculated plates. Then, the plates were incubated at 35°C (24 hr) for bacteria and 25°C (48 hr) for fungi. The observed zones of inhibition were measured and compared against the standard discs of antibiotics (Oxoid). Table (2) represents the obtained antimicrobial and antifungal results.

RESULTS AND DISCUSSION

Hydrodistillation of the leaves of *C. deliciosa* Ten. and *C. reticulata* Blanco yielded 0.4% v/w and 0.3% v/w essential oils, respectively. The oil of *C. deliciosa* separates in two portions, one is lighter than water and one is heavier than water[2] which were combined, while the oil of *C. reticulata* was clearly lighter than water; both oils possess a strong agreable aromatic odour. The oil of *C. deliciosa* shows a distinct bluish fluorescence, especially when diluted with alcohol indicating the presence of methyl-N-methylanthranilate in a high percentage[2].

Identification of components in each oil was achieved by interpretation of their mass spectra as well as comparing these data with those available in literature [2,8, 14-17]. The results of qualitative and quantitative analysis of both species are given in table(1).

Eighteen components were identified in each of the investigated species representing 98.74% and 88.23% from the leaf oil constituents of *C. deliciosa* and *C. reticulata*, respectively. As a result, the main constituent of *C. deliciosa* leaf oil was found to be methyl-N- methylanthranilate (41.4%), this compound could not be detected in *C. reticulata* but, α-pinene was the major constituent in the leaf oil of *C. reticulata* (36.2%).

Furthemore, terpene hydrocarbons constitute up to 53.9% and 65.43% in *C. deliciosa* and *C. reticulata*, respectively. The identified common constituents in both species are α-thujene, α-pinene, d-limonene and γ-terpinene. On the other hand, sabinene, β-pinene and P-cymene are detected only in *C. deliciosa* while, myrcene, α-phellandrene, cis- and trans - ocimene are identified in *C. reticulata*. The analysis also showed that γ-Terpinene is the major terpene hydrocarbon in *C. deliciosa* (24.2%) while, α-pinene is the major one in *C. reticulata* (36.2%).

The oxygenated constituents reache up to 44.84% and 22.8% in the two investigated species *C. deliciosa* and *C. reticulata*, respectively. Terpinen-4-ol acetate and α-terpineol are the only common oxygenated members in the two oils. Lavendulol, methyl salicylate, thymol methylether, thymol, 4-methoxy-3-acetoxy styrene, sabinyl acetate, methyl anthranilate and methyl-N- methylanthranilate are detected in *C. deliciosa* but, the identified oxygenated components in *C. reticulata* are menth-2-en-1-ol, lanalool, citronellal, terpinen-4-ol, citronellol, neryl acetate, farnesol and farnesal. The major oxygenated constituent in the leaf oil of *C. deliciosa* is methyl-N-methylanthranilate (41.4%) but, linalool (13.5%) is the major one in *C. reticulata* leaf oil. Subsequently, the presence of methyl-N-methylanthranilate in such a high percentage in the leaf oil of *C. deliciosa* can be considered as a useful chemotaxonomic marker for the discrimination of the two studied species.

Finally, it was evident that there is a considrable qualitative and quantitative differences between the two essential oils of the leaves of *C. deliciosa* and *C. reticulata*. The obtained results besides the previously reported chemical constituents of the leaves (Polymethoxyflavones as chemotaxonomic markers) and the botanical characteristics of *C. deliciosa* Ten. provide firm, unambiguous identification of this species from other closely related species (e.g. *C. reticulata*).

Concerning the antimicrobiol activity, both oils showed a higher gram +ve antimicrobial activity against *Bacillus subtilis* in comparison with those of tetracycline and amoxycillin (Table 2). A remarkable antifungal activity against *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* was recored for oils of both species in comparison with that of nystatin which showed an activity against *Aspergillus flavus* and *Candida albicans* only (Table 2).

The obtained antifungal activity may suggest the use of the oils and or leaves of *C. deliciosa* and *C. reticulata* as a protective and safe ingredient against the reported common toxicity by *Aspergillus flavus* [18].

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Table 1: The relative composition of the leaf essential oils of *C. deliciosa* Ten. and *C. reticulata* Blanco.

Component	R_t**	M^+**	B.P.**	Major peaks	Relative %	
					C.d**	C.r**
* α-Thujene	7.28	136	93	121, 105, 91, 77, 65	3.2	2.5
* α-Pinene	7.46	136	93	121, 105, 91, 77	6.1	36.2
* Sabinene	9.24	136	93	121, 107, 91, 69	4.4	-
* β-Pinene	9.53	136	93	121, 107, 91, 69	1.0	-
* Myrcene	9.58	136	93	121, 107, 79, 69	-	4.3
* α-Phellandrene	10.37	136	93	121, 105, 91, 77, 65	-	7.8
* P-Cymene	11.11	134	119	117, 91, 77, 65	5.3	-
* d-Limonene	11.37	136	68	121, 107, 93, 79, 53	9.6	4.3
* Cis-Ocimene	11.40	136	93	121, 105, 91, 79, 77, 65, 53	-	0.5
* Trans-Ocimene	12.12	13.6	93	121, 105, 91, 79, 77, 67, 55, 53	-	8.03
* γ-Terpinene	12.31	136	93	121, 105, 91, 77, 65, 51	24.2	1.8
* Menth-2-en-1-ol	12.59	154	71	139, 121, 111, 93, 81, 55	-	0.8
* Terpinee-4-ol acetate	13.42	n.d**	93	136, 121, 105, 79, 55	1.6	1.5
* Linalool	14.33	n.d**	71	139, 121, 109, 93, 80, 55	-	13.5
* Citronellal	16.17	154	69	136, 121, 111, 95, 81, 55	-	2.3
* Lavendulol	17.13	154	69	137, 123, 111, 93, 84, 53,	0.6	-
* Terpinen-4-ol	17.18	154	71	136, 111, 93, 86, 55	-	2.8
* Methyl salicylate	17.37	152	120	137, 92, 65, 53	0.2	-
* α-Terpineol	17.50	n.d**	59	136, 121, 93, 81, 67	0.5	0.5
* Citronellol	19.03	156	69	138, 123, 109, 95, 81, 55	-	0.5
* Thymol methylether	19.04	164	149	134, 119, 105, 91, 77	0.2	-

Table 1: Cont.

Component	R_t**	M^+**	B.P.**	Major peaks	Relative %	
					C.d**	C.r**
* Thymol	21.22	150	135	115, 104, 91, 77	0.2	-
* 4-Acetoxy-3-methoxy styrene	21.55	n.d**	150	135, 107, 91, 77, 51	0.1	-
* Sabinyl acetate	22.28	n.d**	91	150, 135, 119, 105, 77, 51	0.03	-
* Methyl anthranilate	23.25	151	119	134, 128, 109, 91, 81, 69, 55	0.01	-
* Neryl acetate	24.23	n.d**	69	136, 121, 107, 93, 80, 53	-	0.5
* Methyl-N-methyl-anthranilate	27.13	165	165	150, 132, 116, 105, 91, 77, 63	41.4	-
* γ-elemene	28.33	204	121	189,175,161,147,136,107,93,79,67	0.1	-
* Farnesol	31.11	222	69	204,189,161,134,119,95,82,55	-	0.1
* Farnesal	34.44	218	93	190, 133, 120, 105, 79, 67, 55	-	0.3

** R_t, retention time; M^+, molecular ion peak; B.P., base peak; C.d., *C. diliciosa*; C.r., *citrus reticulata*, n.d. not detected.

Table 2: The antimicrobial activity of the essential oils of the leaves of *Citrus deliciosa* Ten. and *C. reticulata* Blanco

Microorganisms	Diameter of inhibition zone (mm).						
	The tested oils		The standard antimicrobial agent				
	C.d* 25ul/disc	C.r* 25ul/disc	GM* 10µg/disc	TE* 30µg/disc	AK* 30µg/disc	AMX* 25µg/disc	NS* 100µg/disc
Bacillus subtilis (Gram+ve)	14	16	20	11	27	15	-
Escherichia coli (Gram-ve)	-	-	18	15	18	23	-
Staphylococcus aureus (Gram+ve)	8	-	18	23	14	22	-
Aspergillus niger (Fungi)	17	17	-	-	-	-	-
Aspergillus flavus (Fungi)	17	17	-	-	-	-	14
Candida albicans (Fungi)	10	15	-	-	-	-	13

* C.d., *C. deliciosa*; C.r., *C. reticulata*; GM., Gentamycin; TE., Tetracycline; AK., Amikacin; AMX., Amoxycillin; NS., Nystatin.

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دراسة مقارنة لمكونات الزيوت الطيارة لأوراق نبات سيترس ديليسيوزا ونبات سيترس رتيكيولاتا

محمود محمد عبدالعال
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سبق للباحث الإشتراك فى دراسة كيميائية وعقاقيرية لنبات يوسفى البحر الأبيض المتوسط (سيترس ديليسيوزا) وتم مناقشة هذه النتائج للإستفادة منها فى التعرف على هذا النبات .

وفى هذا البحث تم إجراء دراسة مقارنه بين مكونات الزيوت الطيارة لهذا النبات مع نظيرتها لنبات سيترس رتيكيولاتا بإستخدام كروماتوجرافيا الغاز المتصل بمطياف الكتلة ووجد أن هناك إختلاف كيفى وكمى بين محتويات كلا منهما . وقد تم مناقشة إدماج نتائج هذا البحث مع النتائج السابقة لتحديد طريقة أكيدة لتمييز نبات سيترس ديليسيوزا عن الأنواع الأخرى.