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Volatile Oils and Pectins from *Citrus aurantifolia* (Lime) and *Citrus limonia* (Lemon)

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Volatile oils were prepared by steam distillation from leaves and peels of both *Citus aurantifolia* (lime) and *Citrus limonia* (lemon), and also by cold pressing both of their fruit peels. The percentage yield of oil for steam distilled lime peel and leaf were considerably lower than for lemon peel and leaf and lower yet for the cold pressed peel material from both plants. Nineteen components were identified in the oils by TLC and GC. Yields of pectin from the pulp of lime and lemon fruits were about 50% higher using a 0.3% ammonium oxalate solution for extraction rather than a water extraction. Both pectins afforded galacturonic acid, glucose, arabinose and xylose on acid hydrolysis, but the pectin from lime fruit pulp contained more galacturonic acid than the pectin from the lemon fruit pulp, and thus is of higher quality.

INTRODUCTION

Lime and lemon trees are important fruit crops in Egypt, and their leaves and other nonfruit plant parts represent a major available resource. We chemically analyzed plant parts other than the fruits to determine their potential use to man. In a previous study we investigated the coumarins in leaves because these compounds are of possible medicinal use.[1] We now extend the study to the volatile oils in the leaves and peels of these two species because such oils have potetial use in the perfume industry.[2] The pectin in the juice-free fruit pulp was investigated since previous studies only examined pectin in the peels[3–6], and since pectin is of importance in the food industry[6] and recently has been used in the treatment of diarrhoea in children.[7]

EXPERIMENTAL

Plant materials: Leaf and fruit samples of *Citrus aurantifolia* (lime) and *Citrus limonia* (lemon) were obtained from fruiting trees cultivated at the Agricultural Crops Research Institute, Giza, Egypt in November 1986–87. The

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plants were identified by Dr. M.H. El-Hadidy, and herbarium specimens are deposited in the Agricultural Crops Research Institute.

Authentic reference compounds: Linalol, nerol, geraniol, terpeniol, farnesol, citronellol, citral, citronellal, thymol, carvacrol, eugenol, linalyl and geranyl acetates, methyl anthranilate, limonene, α- and β-pinene, camphene, *p*-cymene, galacturonic acid, glucose, arabinose, and xylose were obtained from the Natural Product Department, National Research Centre, Dokki, Cairo, Egypt.

Special reagents and instruments: Aniline hydrogen phthalate color reagent[8]: 1.66 g of *o*-phthalic acid and 0.9 mL of aniline (redistilled from zinc) dissolved in 48 mL of 1-butanol, 48 mL of ethyl ether, and 4 mL of water. Eluting agent for developed spots from the pectin hydrolyzates was 0.7 N HCl in 80% ethanol (prepared by mixing 29 mL of 36% HCl with 420 mL of 95% ethanol, then adjusting the volume to 600 mL with water).[9] A Clevenger's apparatus was used for the preparation of the volatile oils. Other equipment included: a Shimadzu UV 240 spectrometer; Varian gas chromatograph 3700; and a Beckman DU spectrophotometer.

Preparation, determination, and identification of the volatile oils: Volatile oils were prepared by steam distillation according to Guenther[10] from the leaves and peels of both *Citrus* species; the oils were also prepared from peels of both species by cold pressing. The determination of the volatile oil content was done following the Egyptian Pharmacopia steam distillation method[11] by using 100 g of the sample, 200 mL of water, and 100 mL of glycerin; distillation was continued for 3 h, and the percentage yield of the oils is recorded in Table-1. Some oil components were identified using comparisons with standards by TLC on silica gel G plates with ethyl acetate-petroleum ether 15 : 85 as a solvent system and vanilin-sulfuric acid as a spray reagent. GC was also employed for analysis of the steam-distilled oils. All results are recorded in Tables 2 and 3.

Extraction, determination and identification of the pectin: Extraction of pectin from the lime and lemon juice-free fruit pulps was carried out using two methods.[12] (I) with a water extraction and (II) with a 0.3% ammonium oxalate solution extraction:

Method I: The yellow layer was peeled from the rind of both lime and lemon fruits, then each of the juice-free pulp (250 g) was covered with water (500 mL), and the mixture was boiled for 2 h with frequent addition of water to maintain a volume just sufficient to maintain a constant volume. Finally, each mixture was filtered hot through cheese cloth and with pressing. The filtrates were allowed to cool, then twice of its volume of 95% ethanol was added to each, and the precipitated pectin was filtered. For both samples the precipitate was allowed to dry at room temperature and weighed.

Method II: 250 g of juice-free lime and lemon pulp were each extracted twice with a 0.3% ammonium oxalate solution (2 × 500 mL); each 1000 mL of extract was boiled for 2 h, and the boild extracts were filtered hot. The two filtrates were allowed to cool, and then each was treated with twice its volume of acidified ethanol (12 mL of conc. HCl / 1L); the precipitates were washed several times with 70% ethanol until free from acid. Finally, the precipitates were washed with

95% ethanol, and allowed to dry at room temperature. The percentage yields of pectin are recorded in Table-4.

TABLE-1
PERCENTAGE OF VOLATILE OILS IN PLANT PARTS OF LIME AND LEMON TREES

Citrus species	Oil per cent V/Fresh W for		
	peel		leaves
	steam distilled	cold pressed	steam distilled
C. aurantifolia (lime)	1.5	0.5	0.75
C. limonia (lemon)	5.0	2.5	1.20

Each figure is the average of 3 determinations, and the percentages are calculated with reference to fresh weight.

TABLE-2
TLC RESULTS FOR EXAMINATION OF THE VOLATILE OILS PREPARED FROM PLANT PARTS OF LIME AND LEMON TREES

Spot No	R_f value	Standard	detected in			detected in		
			peel		leaf	peel		leaf
			cold pressed	steam distilled	steam distilled	cold pressed	steam distilled	steam distilled
1.	0.97	Limonene	+	+	+	+	+	+
2.	0.80	Linalyl acetate	+	±	±	+	±	±
3.	0.75	Citronellal	+	+	+	+	±	+
4.	0.74	Geranyl acetate	+	±	±	+	±	±
5.	0.67	Methyl anthranilate	+	−	−	+	−	−
6.	0.59	Citral	+	±	+	+	±	+
7.	0.47	Linalol	+	+	+	+	+	+
8.	0.35	Terpineol	+	+	+	+	+	+
9.	0.31	Farnesol	+	+	−	+	+	−
		Geraniol	+	+	+	+	+	+
		Nerol	+	+	+	+	+	+
		Citronellol	+	+	+	+	+	+
10.	0.22*	Unidentified	+	+	−	+	+	−
11.	0.12	Unidentified	+	+	−	+	+	−
12.	0.05	Unidentified	+	+	−	−	−	−

+ = present − = absent ± = traces

TABLE-3
GC RESULTS* FOR VOLATILE OILS OF PLANT PARTS OF LIME AND LEMON TREES

Peak No.	Retention time (min)	Compound	Lime oils		Lemon oils	
			% Peel oil	% Leaf oil	% Peel oil	% Leaf oil
1.	0.88	Unknown	0.13	0.02	0.10	0.02
2.	1.27	α-Pinene	12.64	4.11	26.90	3.95
3.	1.96	Camphene	4.02	—	0.87	—
4.	2.93	β-Pinene	13.40	0.14	18.29	9.72
5.	4.03	D-Limonene	9.28	32.07	9.58	20.05
6.	5.50	*p*-Cymene	0.91	0.28	0.60	0.29
7.	6.63	Unknown	0.38	—	—	—
8.	7.17	Unknown	0.52	—	0.02	—
9.	8.25	Unknown	1.44	0.08	—	0.01
10.	9.11	Unknown	1.06	—	0.34	—
11.	10.01	Citronellal	2.17	2.64	0.60	2.16
12.	10.98	Unknown	0.20	1.17	0.30	1.23
13.	11.74	Linalol	1.52	3.97	1.19	1.50
14.	12.41	Unknown	3.43	—	—	—
15.	12.89	Unknown	3.03	0.97	—	—
16.	14.12	Nerol	5.00	1.48	2.67	1.24
17.	15.62	Terpeniol	4.08	12.63	1.27	18.93
18.	16.48	Citronellol	5.26	21.39	7.00	22.95
19.	17.63	Geraniol	2.74	10.38	3.55	3.20
20.	18.21	Unknown	2.56	0.01	1.38	0.02
21.	18.71	Unknown	1.76	—	1.66	—
22.	19.95	Unknown	1.23	—	1.15	—
23.	21.67	Unknown	1.58	—	0.84	—
24.	22.86	Thymol	1.09	0.50	2.66	0.23
25.	23.02	Unknown	0.39	—	—	—
26.	23.73	Unknown	1.43	—	—	—
27.	24.21	Unknown	0.85	—	—	—
28.	25.58	Unknown	1.13	—	0.66	—
29.	26.65	Unknown	0.86	—	0.69	—
30.	27.07	Carvacrol	1.07	0.38	1.33	0.12
31.	27.87	Unknown	2.10	0.19	1.77	—
32.	28.70	Eugenol	5.87	0.70	8.55	0.21
33.	29.57	Unknown	0.96	—	—	—
34.	30.31	Unknown	1.75	—	1.75	—
35.	32.35	Unknown	2.32	—	—	—

Peak No.	Retention time (min)	Compound	Lime oils		Lemon oils	
			% Peel oil	% Leaf oil	% Peel oil	% Leaf oil
36.	34.61	Unknown	< 0.01	—	—	—
37.	36.37	Unknown	0.22	—	0.97	—
38.	39.67	Unknown	0.58	—	—	—
39.	44.05	Unknown	0.33	—	0.02	—
Total number of peaks detected			39	19	29	17

*The identified compounds represent the standards availed for the GC work.

TABLE-4
PERCENTAGE OF PECTIN CONTENT IN THE JUICE-FREE FRUIT PULP OF LIME AND LEMON TREES

Citrus species	Percentage of pectin by	
	Method I (water only)	Method II (aqu. oxalate)
C. aurantifolia (lime)	10.42	17.44
C. limonia (lemon)	9.64	16.14

Identification of the pectin monomers was done after acid hydrolysis[12] of the pectin (carried out by refluxing the pectin with 100 mL 1N HCl for 3 h); the obtained monomers were identified by comparing with standards by Whatmann 3 MM paper chromatography using *n*-butanol-acetic acid-water 12 : 3 : 5 as a solvent system and aniline hydrogen phthalate as a spray reagent. The percentage of each monomer was determined by combined paper chromatography-spectrophotometry[9]: 5 µl (representing 50 µg) of each of the pectin hydroylzate samples was spotted at 3.0 cm along one edge of a Whatmann 3 MM paper and *n*-butanol-pyridine-water 6 : 4 : 3 was used as a solvent system. The paper was allowed to dry for 6 h, then it was dipped into 150 mL of aniline hydrogen phthalate solution (color reagent); the paper was air-dried, then heated at 105°C for 10 min to develop the spots. Each individual spot was cut into smaller strips and placed in test tubes contaning 4 mL of the eluting agent (0.7 N HCl in 80% ethanol), and the absorbance of each solution was determined at 390 nm. The amount of each sugar was determined based on a standard curve, and the results of the percentage of the hydrolyzates are recorded in Table-5.

RESULTS AND DISCUSSION

The percentage yield of volatile oil from the lime fruit peels prepared by steam distillation is 3 times higher than when prepared by cold pressing in the case of lime fruit, and 2 times higher in the case of lemon fruit peels (Table-1). The volatile oil in lemon peel is more than 3 times higher than in lime peel and leaves of both species have considerably less oil content (Table-1). GC analysis shows that D-limonene is a major constituent in leaf oils along with terpeniol and citronellol, while α- and β-pinene are the major constituents of peel oils (Table-3). The percentage yields of pectin from lime and lemon fruit pulp were higher when the ammonium oxalate solution was used for extraction rather than water because

TABLE-5
PERCENTAGES OF HYDROLYSATES FROM LIME AND LEMON PECTINS

Monomers from the pectins	Percentage	
	C. aurantifolia	*C. limonia*
Galacturonic acid	85.12	76.50
Glucose	6.30	8.12
Arabinose	3.25	7.23
Xylose	5.32	8.14

ammonium oxalate solubilizes protopectin by breaking its calcium-magnesium bridges thus allowing a soluble form without significant decomposition[12] (Table-4). The percentage of galacturonic acid was about 85% for lime pulp and only about 75% for lemon pulp (Table-5). Since good quality pectin is expressed as having an 85% or better content of galacturonic acid[6], the pectin obtained from lime fruit pulp is of higher quality than that of lemon fruit pulp.

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