Malaysian Journal of Science (**24**): 109-111 (2005)

Identification of Essential Oil Composition of Peel and Fruits of *Citrus hystrix* DC.

M. F. Safian[1], N. A. Mohamad Ali[2], N. Yury[1] and Z. Zainal Ariffin[1]

[1]Faculty of Applied Sciences, UiTM, 40450 Shah Alam, Malaysia
[2]Medicinal Plants Division, Forest Research Institute of Malaysia, Kepong, 52109 Kuala Lumpur, Malaysia

ABSTRACT The essential oils isolated by hydrodistillation from the peel and fruits of fresh *Citrus hystrix* DC, were identified by GC-Kovats retention indices and GC-EI-MS. Twenty six 26 compounds have been identified from the peel and thirty seven 37 compounds have been identified from the fruit oils. The major compounds contained in peel oil were β–pinene (27%), limonene (24.7%) and sabinene (13.8%) while α– terpeneol (15.8%), β–pinene (15.1%) and limonene (9.1%) were the major compounds of fruit oil.

INTRODUCTION

Citrus hystrix DC is a member of the Rutaceae family alternatively known as 'limau purut' in Malay, 'Wild lime' in English, 'Kaffir Lime' in Danish and 'som makrut' in Thailand. The *Citrus hystrix* tree grows best in the tropical region with high exposure to the sun. The juice of *Citrus hystrix* is sour, slightly bitter and highly aromatic [1]. In this study the constituents of essential oils of *Citrus hystrix* are identified by gas chromatography and Kovats retention indices, however further confirmations of the components were done by EI-MS [2] . The Kovats retention, KI relates the retention of the sample component to the retention of straight-chain saturated hydrocarbons that elute before and after the sample component [3, 4]. The Kovats index can be expressed as;

$$KI = 100\left[\frac{\log (t_x - t_m) - \log (t_n - t_m)}{\log (t_{n+1} - t_m) - \log (t_n - t_m)}\right] + 100n$$

Where, t_x is the retention time of the sample component,
t_m is the retention time of mobile phase,
t_n is the retention time of the saturated hydrocarbon containing n carbons elute before the sample component,
t_{n+1} is the retention time of the saturated hydrocarbon which contains n+1 carbon and that just elutes after the sample component.

MATERIAL AND METHOD

Plant Material

The fruits of *Citrus hystrix* were collected from Hulu Langat, Selangor, Malaysia. Collection of the plant materials was carried out on a dry, warm sunny day. The fruits were distilled immediately after harvesting to avoid loss of volatile components due to evaporation or decomposition and resinification.

Extraction Procedure

About 5 kg of sample were peeled off and washed. The lime peel and fruit were cut into small pieces. Both the samples were preceded to hydrodistillation in essential oil extractor for about eight hours. The oily layers obtained were separated and dried over anhydrous sodium sulphate.

Quantitative and Qualitative Analysis of Essential Oils

The essential oil samples were injected on a Shimadzu GC 14A gas chromatograph equipped with a flame ionization detector using BP-1 fused silica column (25m × 0.22 mm i.d.) The samples were injected using pressure-controlled helium as carrier gas at a flow rate of 1 ml/min. The injector and detector temperatures were maintained at 250 ^{0}C. The oven temperature was

programmed from 60 ^{0}C for 10 minutes, then to 230 ^{0}C at the rate of 3 ^{0}C/min and the final temperature was held for 1 minute. Peak areas and retention times were measured by electronic integration.

The analysis of the essential oil by GC-MS was performed using a Hewllet-Packard GC-MSD Series II. A BP-1, (30 m × 0.25 mm) column was employed, with helium as carrier gas, at a flow rate of 1ml/min. Column temperature was initially at 60 ^{0}C for 10 minutes, then gradually increased to 230 ^{0}C at a rate of 3^{0}C/min and the final temperature was held for I minute. EI mass spectra were measured at 70 eV ionization voltages with the ion source temperature of 250 ^{0}C.

Identification of the compounds

The chemical constituents of the oils were identified by calculating their Kovats retention indices with the use of a series of standard compounds of n-alkanes (C_7-C_{25}) and compared them with literature data [5]. Further identification was confirmed by comparison of their mass spectra with those from the Wiley mass spectral data base. The component concentrations were obtained directly from GC peak without applying correction factors.

RESULTS AND DISCUSSION

The chemical constituents identified in the peel oils of *'Citrus hystrix* species are presented in Table I. About 26 compounds were identified and constituted 99 % of the total oil. The major compounds were β– pinene (27.0%) limonene (24.7%), terpinene-4-ol (5.7%), sabine (13.8 %) and citronellal (7.4%). Other monoterpenes present were α-terpineol (3.1%), α-pinene (2.1%), citronellol (2.1%), γ-terpinene (1.9%) and cis-linalool oxide (1.9%).

The fruit oil was found to contain about 37 compounds and representing 99% of the oil (Table 2). The major compounds were α-terpineol (15.8%), β-pinene (15.1%), limonene (9.1%), cis-linalool oxide (8.8%), terpinen-4-ol (6.6%) terpinolene (5.4%), trans-linalooi oxide (5%) and γ-terpinene (4.3%). There were more than ten compounds found in fruit oil, which were not contained in peel oil. The compounds were ρ-cymene (4.3%), δ-3-carene (1.7%), β–phellandren (1.4%), α-fencyl alcohol (1.1%), and some minor compounds which constituted about 4 % of the total composition.

Table 1. Percentage composition of the peel oil of *Citrus hystrix* DC

Compound	KI	Composition (Area %)	Method of Identification
α – thujene	925	0.2	KI, MS
α -pinene	932	2.1	KI, MS
Camphene	944	0.2	KI, MS
Sabinene	968	13.8	KI, MS
β -pinene	974	27	KI, MS
Myrcene	985	1.6	KI, MS
α - phellandren	995	0.2	KI, MS
α - terpinene	1009	1.1	KI, MS
Limonene	1027	24.7	KI, MS
γ - terpinene	1055	1.9	KI, MS
cis-linalool oxide (furanoid)	1064	1.9	KI, MS
trans- Linalool oxide (furanoid)	1076	0.9	KI, MS
Terpinolene	1081	0.6	KI, MS
linalool	1088	2.	KI, MS
Isopulegol	1123	0.2	KI, MS
Citronellal	1138	7.4	KI, MS
p-menth-8-en-1-ol	1149	0.1	KI, MS
Terpinen-4-ol	1164	5.7	KI, MS
α- terpineol	1175	3.1	KI, MS
citronellol	1216	2.1	KI, MS
Citronellyl acetate	1337	0.2	KI, MS
Geranyl acetate	1364	0.2	KI, MS
β – elemene	1381	0.3	KI, MS
β - caryophyllene	1406	0.3	KI, MS
α - humulene	1468	0.3	KI, MS

Table 2. Percentage composition of the fruit oil of *Citrus hystrix* DC

Compound	KI	Composition (Area %)	Method of Identification
α -pinene	931	2.3	KI, MS
Camphene	944	1.1	KI, MS
Sabinene	965	2.3	KI, MS
β -pinene	970	15.1	KI, MS
Myrcene	985	1.1	KI, MS
α - phellandren	995	0.8	KI, MS
δ -3-carene	1004	1.7	KI, MS
α - terpinene	1009	2.3	KI, MS
ρ-cymene	1013	4.2	KI, MS
β - phellandren	1021	1.4	KI, MS
Limonene	1025	9.1	KI, MS
Trans- β- ocimene	1041	t	KI, MS
γ terpinene	1055	4.3	KI, MS
cis-linalool oxide (furanoid)	1064	8.8	KI, MS
trans- Linalool oxide (furanoid)	1077	5	KI, MS
Terpinolene	1082	5.4	KI, MS
linalool	1089	0.8	KI, MS
Isopulegol	1120	0.8	KI, MS
Citronellal	1130	1.1	KI, MS
p-menth-8-en-1-ol	1141	0.5	KI, MS
Isoborneol	1148	1.1	KI, MS
Terpinen-4-ol	1163	6.6	KI, MS
α- terpineol	1180	15.8	KI, MS
γ - terpineol	1184	2.1	KI, MS
citronellol	1216	0.5	KI, MS
neryl acetate	1345	0.1	KI, MS
Geranyl acetate	1370	0.5	KI, MS
β – elemene	1383	0.1	KI, MS
β - caryophyllene	1407	0.3	KI, MS
β-bergamotene	1430	t	KI, MS
α - humulene	1465	0.1	KI, MS
β -guaiene	1478	0.1	KI, MS
α-muurolene	1484	0.1	KI, MS
δ-cadinene	1511	1	KI, MS

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