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Volatile aroma compounds in *Citrus hystrix* oil

(Sebatian aroma meruap dalam minyak *Citrus hystrix*)

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Key words: *Citrus hystrix*, aromatic compounds, GC, GC-MS analysis

Abstrak

Minyak pati *Citrus hystrix* (limau purut) diekstrak daripada daun dan kulit buah yang segar. Kemudiannya dianalisis menggunakan kromatografi gas (KG) dan gabungan kromatografi gas dengan spektrometer jisim (KG-SJ). Sebanyak 41 sebatian aroma dikenal pasti, merangkumi 95% daripada kandungan minyak; tertakluk pada kaedah pengekstrakannya. Sebatian utama minyak daun limau purut ialah ***l***-sitronelal (61.73%) manakala ***l***-limonena, sabinena dan ß-pinena terdapat dalam jumlah yang banyak dalam minyak kulit buah. Tiga sebatian baru iaitu *p*-mentan-8-ol (dihidro-α-terpineol), guaiol, dan 4-*p*-mentena, telah dikenal pasti hadir dalam minyak C. *hystrix.*

Abstract

The essential oil of *Citrus hystrix* was prepared from fresh leaves and peels and analysed by means of capillary GC and GC-MS. Forty-one components were identified, accounting ca. 95% of the oil, depending on the method of preparation. The leaf oil contains ***l***-citronellal (61.73%) as a major compound whereas ***l***-limonene, sabinene and ß-pinene are predominant in the peel oil. This is the first time *p*-menthan-8-ol (dihydro-α-terpineol), guaiol, and 4-*p*-menthene is reported in *C. hystrix* oil.

Introduction

Citrus hystrix which is called ‘limau purut’ in Malaysia, ‘jeruk purut’ in Indonesia and ‘som makrut’ in Thailand, belongs to the Rutaceae family. It is originated in Asia and cultivated throughout the warmer parts of the world (Burkill 1966). In Malaysia, this plant is usually planted in the house garden together with other plants such as lemon grass, ginger, galanga and fragrant pandan. Its pear-shaped fruit with highly wrinkled skin of intense green colour was traditionally used among the Malaysian folks for washing their hairs and other parts of the body in bathing ceremony called ‘mandi berlimau’, and in certain occasion was used in getting rid of evil spirit. Currently, the *C. hystrix* peel is used as perfume in shampoos and its leaves are often used as a food flavouring ingredient in making ‘laksa asam’ sauce, curries, cakes, satay and tomyam soup, an Asean delicacy (Wong 1992; Muhammad Nor 1992a).

A survey of the literature reveals that local *C. hystrix* oil was recently studied in brief by Wong (1992) and the production of natural flavour in liquid and powdered form had been formulated (Muhammad Nor 1992a,b). This paper reports a detailed examination of the leaf and peel oil and the differences in aroma constituents are discussed.

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Materials and methods

Materials

C. hystrix fruits and leaves were obtained from MARDI Research Station, Jerangau, Terengganu.

Isolation of volatile components

C. hystrix leaves (5 kg) were steam distilled for about 6 hrs using previously reported method (Muhammad Nor and Norhanim 1981; Muhammad Nor 1992a). Alternatively, the leaves and peels were extracted for 3 hrs in a modified Likens-Nickerson (1964) apparatus using 50 mL distilled dichloromethane (CH_2Cl_2). The CH_2Cl_2 extract was dehydrated using anhydrous Na_2SO_4 and subjected to low temperature-high vacuum concentration (MacLeod and Coppock 1976).

Gas chromatography (GC) analysis

One microlitre solution of *C. hystrix* oil in 250 μL CH_2Cl_2 was on-column injected (0.2 μL) into HP 5890II GC instrument equipped with a bonded phase fused silica capillary column (Ultra 1, 50 m x 0.32 mm i.d x 0.52 mm), electronic pressure controller (EPC) and a (FID). Operating condition: Injector and detector temperatures were 220 °C and 250 °C respectively; oven temperature was initially kept at 70 °C for 3 min and then programmed to 220 °C at 4 °C/min. Carrier gas He linear velocity was 37.6 cm/min and the peak areas were calculated by a HP 3396B integrator. For reference purposes, Kovats indices were obtained from temperature programmed GC, using the homologous series of C_6-C_{18} *n*-alkanes as standard, by linear interpolation (Kovats 1965).

Gas chromatography – mass spectrometer (GC-MS) analysis

A HP 5890II model gas chromatograph equipped with the same column as GC analysis was directly interfaced to the ion source of a HP 5971A mass spectrometer. Working condition: GC as above with He column head pressure 35 kPa, MS: transfer line temperature 280 °C, ion source temperature 182 °C. MS spectral identification was based on comparison of the reference compounds from Wiley library.

Odour pot assessment (OPA)

The components of the essential oil were separated in a capillary column (Ultra 1, 50 m x 0.32 mm i.d x 0.52 μm) in a HP 5890II gas chromatograph as described above. The instrument was equipped with a TCD, where the odour of the eluted substances was assessed by sniffing after the detection (De Pooter et al. 1985; MacLeod and Ames 1986; Muhammad Nor 1991).

Results and discussion

The essential oil was obtained in a fairly high yield (*ca*. 2.5%) by steam distillation of fresh leaves and collected on the top of distillate. Alternatively, *ca*. 1.8% oil was isolated by Likens-Nickerson (1964) extraction with distilled dichloromethane. Isolation of the peel oil using Likens-Nickerson extraction methods afforded *ca*. 2.0% oil.

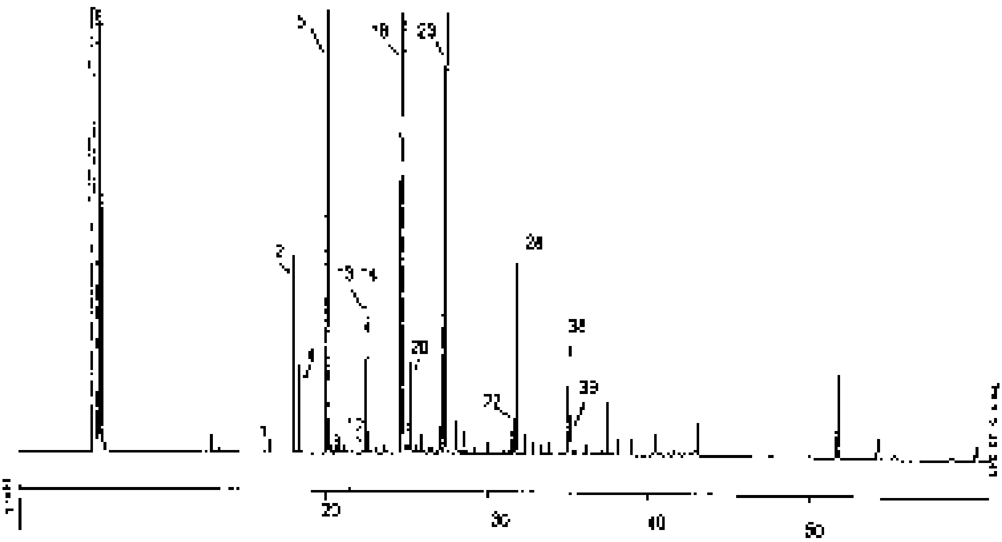
Aromatic compounds of *C. hystrix* oil are shown in *Table 1*, and GC chromatogram (FID) of the leaf oil is shown in *Figure 1*. Analysis of the oil by GC, GC-MS, Kovats indices and odour pot assessment (OPA) resulted in the identification of 41 compounds (comprising *ca*. 95% of the total volatiles). More than 32 components could be positively identified by GC-MS alone without further purification. The other nine compounds need to be first isolated by column fractionation on silica gel, with hexane-diethyl ether elution, prior to GC-MS and with the help of Kovats indices and OPA (De Pooter et al. 1985; MacLeod and Ames 1986; Muhammad Nor 1991).

Among the components detected, ***l***-citronellal (61.73%), β–citronellol (13.43%) and limonene (5.90%) were predominantly found in the leaf oil. This is in contrast with other citrus leaf oils, which are usually rich in β-pinene, sabinene,

Table 1. Composition (in %) of *C. hystrix* oil prepared by different methods

Peak	Component	Kovats Index	Fresh leaves, steam distillation	Fresh leaves, Likens-Nickerson extraction	Peels, Likens-Nickerson extraction	Odour evaluation
1	α-pinene	942	0.11	0.05	1.72	Fresh, turpentine
2	sabinene	976	1.60	2.03	20.13	Fresh, greeny
3	ß-pinene	980	0.11	0.05	23.45	Fresh, turpentine
4	ß-myrcene	986	0.72	0.56	0.98	Weakly lemon-like
5	*l*-limonene	1005	5.90	6.78	11.78	Lemon-like
6	*p*-cymene	1010	0.07	tr	0.28	Lemongrass
7	Δ-3-carene	1025	0.17	tr		
8	cis-ocimene	1030	0.19	0.04	0.05	Mint, breeze
9	2,6-dimethyl-5-heptenal	1037	0.08	tr		Melon-like
10	γ-terpinene	1057	tr	0.11	0.56	Lemon-like
11	trans-linalool oxide	1062	tr		0.04	
12	*p*-menthan-8-ol*	1075	tr		0.13	
13	α-terpinolene	1087	0.52	tr	0.25	
14	linalool	1092	0.96	1.56	1.82	Floral, sweet
15	epoxy-linalool oxide	1103	tr		0.11	
16	2,6 dimethyl-5-heptenol	1113	tr		0.13	Melon-like
17	*d*-citronellal	1120	0.02	tr	tr	Aldehydic
18	*l*-citronellal	1125	61.73	72.45	12.56	Aldehydic
19	*iso*-pulegol	1133	0.94	1.22	0.54	
20	terpinen-4-ol	1155	0.09	tr	4.13	Lemon-like
21	*l*-α-terpineol	1177	0.22	tr	1.25	Weakly lemon-like
22	*trans*-sabinene hydrate	1180	0.42	tr	0.88	
23	ß-citronellol	1182	13.43	10.34	3.34	Floral
24	geraniol	1201	0.43	1.23		Fruity, floral
25	nerol	1215	0.44	tr		
26	*p*-menthan-3,8-diol	1300	0.53	tr		
27	4-*p*-menthene*	1330	0.25		0.23	
28	citronellyl acetate	1334	2.02	1.22	1.67	Fruity, floral
29	neryl acetate	1341	0.01	tr		
30	geranyl acetate	1361	tr	0.12	0.88	Fruity, floral
31	eugenol	1368	tr			Spicy, clove-like
32	trans-caryophyllene	1403	tr			Spicy
33	ß-elemene	1436	tr			
34	α-muurolene	1463	tr			
35	ß-bisabolene	1493	tr		1.23	
36	δ-cadinene	1507	tr	tr	0.57	
37	elamol	1517	tr	tr	tr	
38	nerolidol	1532	1.24	tr	0.23	
39	guaiol*	1536	0.23	tr	0.10	
40	α-bergamotene	1550	0.01	0.56		Sweety spicy
41	caryophyllene oxide	1553	tr		0.12	Floral

tr: trace level
*new identified compound



Retention Time (min)

Figure 1. GC charomatogram of ***Citrus hystrix*** *leaf oil on Ultra 1 column*

γ-terpinolene and limonene (Kamiyama and Amaha 1972). Higher amount of citronellal indicated that this oil is slightly similar to the citronella oil which consisted of more than 55% citronellal. Because of this, Akihama (1985) has described the characteristic aroma of *C. hystrix* as a blend of citronella oil and lime oil, and its aroma is then classified as aldehydic, citrus like and slightly warm with floral odour (Muhammad Nor 1992b). Also, higher citronellal content in the leaf oil is in contrast with the results obtained by Wong (1992) who claimed that citronellal content was fairly low (18.5%). This difference might be due to the method of extraction used, planting location, and cultivation practices. However, compared to the oil of foreign origin, the citronellal content of the local leaf oil is at par with the Siamese origin (Sato et al. 1990).

The peel oil is predominated in β-pinene, sabinene and limonene *(Table 1);* and slightly similar to the peel oil previously reported (Sato et al. 1990). Although its citronellal content is much lower compared to leaf oil, its potent aroma had influenced the aroma of the oil. Because of the predominance of ß-pinene, sabinene and limonene in the peel oil, its aroma was described as warm spicy with lemon-like odour. On the other hand, the limonene content of this oil is much lower compared to the other citrus oils which contained more than 95% limonene. Hence, this oil is more soluble in alcohol, the fact that its hydrocarbon content is less than 50%. The absence of 2,6-dimethyl-5-heptenal in the peel oil had also decreased the floral/fruity odour of the oil as this compound exhibited a very strong melon-like character and possessed a water threshold at 1 ppb (Sato et al. 1990).

There were a few compounds not normally found in most citrus oils were identified in this oil, *e.g.*: 2,6-dimethyl-5-heptenal, *p*-menthan-3,8-diol (*cis* and *trans*-), and 2,6-dimethyl-5-heptenol. The presence of 2,6-dimethyl-5-heptenal and 2,6-dimethyl-5-heptenol confirmed the previous result (Sato et al. 1990). However, *p*-menthan-3,8-diol was only found in local oil and confirmed the result obtained by Wong (1992). This is the first time menthan-8-ol (dihydro-α-terpineol) (Peak 12), 4-*p*-menthene (Peak 27) and guaiol (Peak 39) reported present in the *C. hystrix* oil (leaf and peel).

Conclusion

Steam distillation of *C. hystrix* leaves yield *ca*. 2.5% oil. The leaf oil contains 61.73% ***l*** -citronellal whereas only 12.56% of this compound was found in the peel oil. The higher aldehyde content makes these oils totally different from other citrus oils which are predominant in limonene content. GC and GC-MS analysis revealed that 41 constituents were identified in the leaf oil and 29 components found in the peel oil. The results also indicated that 2,6-dimethyl-5-heptenal and 2,6-dimethyl-5-heptenol were identified in the leaf oil but not in the peel. It is also reported that three new compounds, *i.e*. *p*-menthan-8-ol (dihydro-α-terpineol) (Peak 12), 4-*p*-menthene (Peak 27), and guaiol (Peak 39) were present in *C. hystrix* oil.

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