

Identification of the aroma-active constituents of the essential oils of Water Dropwort (*Oenanthe javanica*) and 'Kacip Fatimah' (*Labisia pumila*)

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Abstract: The chemical constituents of the essential oils from the leaves of *Oenanthe javanica* (Water Dropwort) and *Labisia pumila* ('Kacip Fatimah') were established by GC-FID and GC-MS analysis. A total of 41 compounds were each identified in oils of both of 'Kacip Fatimah' and Water Dropwort. The compounds obtained from the oil of Water Dropwort were richer in sesquiterpenes such as α -Copaene (18.3%), Z-Caryophyllene (0.34%) α -Cuprenene (0.40%) and Cembrene-type diterpenes such as Incensole (26.4%) and Cembrenol (0.45%). However, essential oil obtained from 'Kacip Fatimah' was predominated with sesquiterpenes alcohols such as Longiborneol (4.83%), Geranyl linalool (3.90%) and T-Cadinol (24.78%). Other significant constituents of the oil from 'Kacip Fatimah' were the diterpenes alcohol, Isoabienol (7.89%), β -Santalol acetate (9.35%) and 1, 4-trans-6-methoxyiso-calamene (13.75%).

Keywords: 'Kacip Fatimah' (*Labisia pumila*), Water Dropwort (*Oenanthe javanica*), volatiles, essential oil, constituents

Introduction

Water Dropwort (*Oenanthe javanica*) is a perennial herb with a distinctive aroma and taste. It is cultivated in marshy places of Asia and Australia. It belongs to the Umbelliferae family along with coriander, caraway, fennel and cumin. The stems and leaves are used in salad or as a seasoning in soups and stews in Korea. Water Dropwort is known to have an antimutagenic effect against aflatoxin B1 due to its flavonoids and the capability to remove heavy metals, such as Cadmium and Plumbum in polluted water (Won and Hyung, 2005).

Water Dropwort has creeping stolons and long, threadlike, white rootlets. The erect, slender, hollow and green stems range from 4 inches to 5 feet high. The deep green leaves, elicit an odor like carrot tops and resembles celery in shape and size. The herb has tiny, white, fragrant flowers which are formed in compound umbels of 10 to 25 blooms (Huopalahti and Linko, 1983). The plant grows wild in freshwater marshes and swampy fields, and along ditches, canals, and streams in many Asian countries. The tops are eaten raw in salads or as a garnish similar to parsley. The young stems and leaves are also steamed with rice or boiled and chopped as greens. There are many oriental recipes that include this vegetable (Park *et al.*, 1996).

On the other hand, the *Labisia pumila* is a smaller

herbaceous under shrub that roots from the stem with few leaves pointing upwards. The root is tough and woody with long primary roots and few secondary roots (Mashita, 2005). The tip of the leaf is pointed with a base that is tapered or rather broad rounded. Three varieties of 'Kacip Fatimah' are found in Malaysia. They are *Labisia pumila* var *alata*, *L. pumila* var *pumila* and *L. pumila* var *lanceolata*. It is important to ensure that the right variety is used in each case thus each variety commands a different use. Species identification is made difficult by the lack of difference in the leaves and petioles between var. *alata* and var. *pumila*. Thus, an efficient method of authentication needs to be developed. The leaf has a slight odor and taste. The whole leaf is about 5 to 35 cm long and 2 to 8 cm wide finely toothed with numerous veins (Stone, 1998). It is of a dark green color on adaxial and lighter green on the abaxial. Flowers on the shrub are very small, generally white or pink, in spike like panicle of small clusters. They range from 6 to 30 cm long with sepals, petals and stamens. The petals wrap around and enclose the stamens. The fruit are about 5 cm in diameter and are either bright red or purple (Zaizuhana, 2006).

Labisia pumila which is popularly known as 'Kacip Fatimah'. It has been used by many generations of the Malay women to induce and facilitate childbirth as well as a post-partum medicine (Shahrim, 2006). 'Kacip Fatimah' (*Labisia pumila*) has been widely

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used by the traditional practitioners as a remedial for involution of birth channel, delay fertility and to regain body strength (Zakaria and Mohd, 1994). 'Kacip Fatimah' is also used to reduce excessive gas in the body, treat flatulence, dysentery, dysmenorrhea, gonorrhoea and "sickness in the bones". Apart from the aforementioned uses, the extract from the plant is also used as an energy drink (Asiaah, 2007). The objective of this study is to identify the key chemical constituents of the essential oils of Water dropwort (*Oenanthe javanica*) and 'Kacip Fatimah' (*Labisia pumila*) using liquid solvent extraction.

Materials and Methods

Preparation of fresh herbs

Fresh leaves of herbs (Water Dropwort (Pasar Borong Sri Kembangan, Selangor) and 'Kacip Fatimah' (Jalan Chow Kit, Kuala Lumpur) were washed in cold tap water to discard the impurities. They were manually milled in a Panasonic extractor (Model Panasonic, EX 4384, 1990).

Isolation of fresh herbs

Air-dried fresh leaves of the herbs (500 g each) were ground and subjected to solvent, diethyl ether (Merck Sdn Bhd, Petaling Jaya, Malaysia) distillation for 2 hours, using a Clevenger-type apparatus. The extract (400 ml) was isolated and dried over anhydrous sodium sulphate (Merck Sdn Bhd, Petaling Jaya, Malaysia) (Lasekan *et al.*, 2007). Dried extract was concentrated (45°C at 900 rpm) using rotary evaporator (Model Heidolph/ Laborata 400/4B/G1, 2006) to 1 ml and stored at 4°C until analysis.

Gas Chromatography –FID (GC-FID) analysis

The extracts (1 µl) were analysed using an Agilent 6890 gas chromatography (Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a DB-5 capillary column (30 m x 0.32 mmID x 0.25 µm thickness film; Agilent J & W Scientific, Folsom, CA, USA). The GC injection port was equipped with 0.75 mm i.d. liner (Supelco, Belleforita, PA, USA) to minimize peak broadening. Helium was used as the carrier gas at a constant flow rate of 0.8 ml/min. The oven temperature was raised from 35°C to 200°C at a rate of 2°C/min. The injector and detector (FID) temperature were kept at 200°C and 250°C respectively.

Gas Chromatography –Mass Spectrometry (GC-MS) analysis

The qualitative identification of aroma-active constituent of the essential oils of the herbs was

performed with (Agilent 6890 Gas Chromatography, Palo Alto, CA, USA, Corp, MI, USA). 1 µl of essential oil was injected into the GC injection port at 200°C and maintained there for 2 min for full desorption. Separation of analytes was achieved with DB-5 capillary column (30 m x 0.32 mmID x 0.25 µm thickness film; Agilent J&W Scientific, Folsom, CA, USA). The same temperature programming as described for GC was adopted. The transfer line temperature was 250°C and helium was used as the carrier gas at a flow rate of 1.4 ml/min. The mass spectrometer was operated in scan mode from m/z 35 to 40 at 20 scans/s, with 70 eV electron ionization at 200°C. The constituents of the volatile oils were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C₈-C₂₀) and the oil on a DB-5 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal references mass spectra library, NIST (National Institute of Standards and Technology) and confirmed by comparison of their retention indices with those reported in the literature (Schwarz *et al.*, 1996; Joulain *et al.*, 1998; König *et al.*, 2004; Pichette *et al.*, 2006; Akbarzadeh *et al.*, 2006; Moussaieff *et al.*, 2008).

Quantification

The concentrations of the compounds were calculated using the reported equation of Lee *et al.* (2005) as:

$$\text{Conc (mg/g)} = \frac{\text{Weight of extract without solvent} \times \text{GC peak area \%100 (mg)}}{\text{Weight of Herbs (g)}}$$

Results and Discussion

Greenish-yellow oils with yields of 0.21% and 0.11% were obtained from the *Oenanthe javanica* (Water Dropwort) and *Labisia pumila* ('Kacip Fatimah') respectively. The chemical constituents of the oils are presented in Tables 1 and 2 respectively. A total of 41 compounds were identified in the Water Dropwort and these comprised mainly of terpenes (26.85%), oxygenated sesquiterpenes (20.90%) and monoterpenes (1.55%). Other constituents of the oil were oxygenated compounds such as phenols, monoterpene alcohols, esters, aldehydes and ketones. Similarly, 41 aroma-active compounds were identified in 'Kacip Fatimah' and these included an array of terpenes (14%) and a few numbers of monoterpenes (0.08%) and sesquiterpenes (0.23%) (Table 2). There were some miscellaneous compounds such as Nonadecane, Methyl elaidate, Methyl stictate and (2,E)-Nonenol acetate. Also, a number of acid

Table 1. Aroma-active constituents of the essential oil of Water Dropwort (*Oenanthe javanica*)

Peak	Compounds	Linear Retention Indices ^a	Concentration (mg/g)	Concentration (%)	Methods of Identification
1	α -Pinene ^{b,c}	930.00 ^{b,c,i}	0.002	0.001	MS, RI
2	β -Pinene ^{b,c}	972.00 ^{b,c,i}	0.109	0.510	MS, RI
3	Hexylacetate ^{b,c}	1006.92 ^{b,i}	0.004	0.002	MS, RI
4	(E)-2-Octanal ^b	1030.79 ^{b,i}	0.119	0.560	MS, RI
5	Limonene ^{c,d}	1031.00 ^{c,d,i}	0.026	0.120	MS, RI
6	2,6,6-Trimethyl-cyclohex-2-enone ^b	1045.45 ^{b,i}	0.003	0.010	MS, RI
7	α -Terpineol ^b	1199.29 ^{b,i}	0.218	1.030	MS, RI
8	α -Octanide ^c	1208.45 ^{c,i}	0.042	0.190	MS, RI
9	n-Nonylacetate ^b	1283.52 ^{b,i}	3.773	17.79	MS, RI
10	Pregejerec ^{b,c,d}	1288.00 ^{b,c,d,i}	0.119	0.560	MS, RI
11	Ethyl decanoate ^c	1374.29 ^{b,i}	0.005	0.020	MS, RI
12	β -Lonal ^{b,c}	1395.78 ^{b,c,i}	0.389	1.830	MS, RI
13	Citronellyl ^{c,d,e}	1426.51 ^{c,d,e,i}	0.369	1.740	MS, RI
14	β -Ionone epoxide ^c	1455.07 ^{c,i}	0.024	0.110	MS, RI
15	Cis-Geranyl acetone ^b	1457.40 ^{b,i}	0.041	0.190	MS, RI
16	Germacrene D ^{b,c}	1475.00 ^{b,c,i}	0.082	0.390	MS, RI
17	3-Methyl-4-(2,6,6-trimethylcyclohex-2-enyl) ^{b,c}	1496.00 ^{b,c,i}	2.132	10.90	MS, RI
18	α -Copaene ^b	1510.55 ^{c,i}	3.871	18.25	MS, RI
19	Myristicin ^d	1519.00 ^{d,i}	0.088	0.410	MS, RI
20	4-Desmethyl caryophyll-8(14)-en-5-one ^{b,c}	1523.61 ^{b,c,i}	0.131	0.620	MS, RI
21	α -Cuprenene ^b	1555.00 ^{b,i}	0.085	0.400	MS, RI
22	β -Sesquiphelladiene ^{c,d}	1561.00 ^{c,d,i}	0.087	0.410	MS, RI
23	n-Decyl butanoate ^{b,c}	1561.78 ^{b,c,i}	0.207	0.970	MS, RI
24	Nerolidyl acetate ^{b,c}	1564.00 ^{b,c,i}	0.109	0.510	MS, RI
25	(Z)-Caryophyllene ^{b,c}	1578.08 ^{b,c,i}	0.073	0.340	MS, RI
26	Allo cedrol ^{b,c,d}	1615.00 ^{b,c,d,i}	0.095	0.450	MS, RI
27	Alismol ^{b,c}	1619.41 ^{b,c,i}	0.107	0.500	MS, RI
28	Trans-cadina-1(6)-4-diene ^{b,c}	1658.00 ^{b,c,i}	0.082	0.390	MS, RI
29	7-Acetoxyelemene-1,3-dien-8-ol ^c	1837.89 ^{c,i}	0.037	0.170	MS, RI
30	10-epi-ltaliene ether ^c	1856.00 ^{c,i}	0.087	0.410	MS, RI
31	5-Hydroxymarsapellyl acetate ^b	1860.17 ^{b,i}	0.107	0.500	MS, RI
32	Methyl palimatio-leatec ^c	1879.08 ^{c,i}	0.154	0.730	MS, RI
33	(Z)-Methyl Heptadec-10-enoate ^{c,d}	1933.79 ^{c,d,i}	0.113	0.530	MS, RI
34	(E)-15-16-Bisnorlabda-8(17),12-diene-14-al ^d	1948.09 ^{d,i}	0.022	0.100	MS, RI
35	Phytol ^c	2034.19 ^{c,i}	1.142	5.380	MS, RI
36	Aphidicol-16-ene ^c	2113.14 ^{c,i}	1.201	5.660	MS, RI
37	Cembrenol ^{b,c,g}	2132.81 ^{b,c,i}	0.095	0.450	MS, RI
38	Syn-capalol ^{b,h}	2164.97 ^{b,i}	0.067	0.320	MS, RI
39	Incensole ^{b,c}	2193.39 ^{b,c,i}	5.597	26.39	MS, RI
40	(E)-Phytolacetate ^b	2223.00 ^{b,i}	0.159	0.750	MS, RI
41	Capalol ^{b,c}	2281.96 ^{b,c,i}	0.037	0.170	MS, RI
	Monoterpenes		0.329	1.550	
	Terpenes		5.696	26.85	
	Susquiterpenes		4.433	20.90	
	Miscellaneous		12.96	63.02	
	TOTAL		21.42	100.00	

^a Retention indices measured on DB-5 column.

^b Daniel *et al.*, 2007.

^c Wilfried *et al.*, 2006.

^d Schwarz *et al.*, 1996.

^e Moussaoui *et al.*, 2008.

^f NIST, National Institute of Standards and Technology.

^g Sylvestre *et al.*, 2006.

^h Semmani, 2006.

Table 2. Aroma-active constituents of the essential oil of 'Kacip Fatimah' (*Labisia pumila*)

Peak	Compounds	Linear Retention Indices ^a	Concentration (mg/g)	Concentration (%)	Methods of Identification
1	6-methyl-2-heptanol ^b	798.62 ^{b,e,i}	0.005	0.190	MS, RI
2	Ethylbenzene ^{b,c,d}	844.69 ^{b,c,i}	0.004	0.150	MS, RI
3	(3-E)-Heptanal ^b	877.00 ^{b,i}	0.002	0.080	MS, RI
4	β -Ocimene ^{b,c}	1050.00 ^{b,i}	0.006	0.230	MS, RI
5	(2-E)-Nonenol acetate ^b	1187.00 ^{b,i}	0.004	0.150	MS, RI
6	1-Octen-3-yl-propanoate ^b	1225.00 ^{b,i}	0.002	0.150	MS, RI
7	Neral ^b	1251.41 ^{b,i}	0.002	0.080	MS, RI
8	Dehydroeosinin ^{b,c}	1321.79 ^{b,i}	0.002	0.080	MS, RI
9	Ethyl decanoate ^{b,c}	1374.29 ^{b,i}	0.005	0.190	MS, RI
10	β -Copaene ^b	1430.00 ^{b,i}	0.006	0.230	MS, RI
11	3-Methyl-4-(2,6,6-trimethyl-cyclohex-2-enyl) ^{b,c}	1474.07 ^{b,i}	0.008	0.310	MS, RI
12	Isobornyl isovalerate ^{b,c}	1516.48 ^{b,i}	0.006	0.230	MS, RI
13	β -Vetivene ^b	1525.00 ^{b,i}	0.007	0.270	MS, RI
14	2- α -Acetoxy-8-one ^{b,c,d}	1525.00 ^{b,c,i}	0.101	3.870	MS, RI
15	Artmisia triene ^b	1564.00 ^{b,i}	0.001	0.040	MS, RI
16	Longiborneol ^{b,f}	1567.00 ^{b,i}	0.126	4.830	MS, RI
17	T-Cadinol ^{b,c}	1608.17 ^{b,i}	0.647	24.78	MS, RI
18	3-Iso-Thujanol ^b	1625.00 ^{b,i}	0.002	0.080	MS, RI
19	Citronellyl pentanoate ^{b,c}	1626.00 ^{b,c,i}	0.007	0.270	MS, RI
20	Allo-Aromadendrene epoxide ^f	1646.00 ^{f,i}	0.008	0.310	MS, RI
21	(Z)- α -Santalolacetate ^{b,c}	1669.00 ^{b,i}	0.009	0.340	MS, RI
22	8-Cedron-13-ol-acetate ^{b,c}	1679.00 ^{b,i}	0.009	0.340	MS, RI
23	1-4-trans-6-Methoxyiso-calamene ^b	1690.00 ^{b,i}	0.009	0.340	MS, RI
24	Azulene ^b	1722.00 ^{b,c}	0.359	13.75	MS, RI
25	(E)-Isovalencenol ^{c,f}	1773.00 ^{b,i}	0.009	0.340	MS, RI
26	Musk ambrette ^g	1801.00 ^{g,i}	0.010	0.380	MS, RI
27	β -Santalol acetate ^b	1815.69 ^{b,i}	0.244	9.380	MS, RI
28	Hexadecanol ^{b,c}	1865.00 ^{b,i}	0.007	0.270	MS, RI
29	6-Acetoxy-cudesi-4-(15)-en-7b-ol ^b	1878.60 ^{b,c,i}	0.154	5.900	MS, RI
30	(Z)-g-curcuml-2-methylbutyrate ^{c,d}	1902.39 ^{c,i}	0.001	0.040	MS, RI
31	Nonadecane ^{c,g}	1906.45 ^{b,d,i}	0.117	4.480	MS, RI
32	β -Cedrene epoxide ^{c,d}	1961.00 ^{c,i}	0.007	0.270	MS, RI
33	(Z)-Methylheptadec-10-enoate ^{b,c,h}	1974.60 ^{b,c,i}	0.003	0.110	MS, RI
34	Methyl Eaiadate ^{c,d}	2206.00 ^{c,d,i}	0.165	6.320	MS, RI
35	Geranyl Linalool ^{b,c}	2008.00 ^{b,c,i}	0.102	3.900	MS, RI
36	Geranyl Linalool (Z,Z) ^b	2008.00 ^{b,c,i}	0.009	0.340	MS, RI
37	Isoabienol ^b	2113.97 ^{b,i}	0.206	7.890	MS, RI
38	Incensole ^b	2193.00 ^{b,i}	0.011	0.420	MS, RI
39	(E)-Phytol acetate ^c	2223.00 ^{c,i}	0.117	4.420	MS, RI
40	Methyl Stearate ^{c,d}	2324.00 ^{c,d,i}	0.003	0.110	MS, RI
41	Methyl Strictate ^g	2430.00 ^{g,i}	0.009	0.340	MS, RI
	Monoterpenes		0.002	0.080	
	Terpenes		3.577	13.74	
	Susquiterpenes		0.006	0.230	
	Miscellaneous		10.62	85.26	
	TOTAL		11.12	100.00	

^a Retention indices measured on DB-5 column.

^b Daniel *et al.*, 2007.

^c Wilfried *et al.*, 2006.

^d Schwarz *et al.*, 1996.

^e Moussaoui *et al.*, 2008.

^f NIST, National Institute of Standards and Technology.

^g Sylvestre *et al.*, 2006.

^h Semmani, 2006.

ⁱ GC/MS Library: Terpenoids and Related Constituents of Essential Oils, 2006, retrieved from <http://www.massfinder.com/mfiterpenoids.html>.

derivatives ((3,E)- Heptanol, 6-Methyl-2-heptanol, Methyl stearate) were identified in 'Kacip Fatimah' oil.

The essential oil constituents of the two herbs consisted very limited number of monoterpenes. For instance, Water Dropwort has α -pinene, β -pinene, γ -terpineol and limonene. On the other hand, 'Kacip Fatimah' has β -Ocimene (0.23%) and Neral. These monoterpenes have previously been reported in leaves of *Macaranga* species (Eupharbiaceae) (Jurgens *et al.*, 2005), *Bubonium graveolens* flowers of *Chloranthus spicatus* (Wilfried *et al.*, 2006). Apart from the above named sources, the monoterpenes are found in nearly all essential oil and have a structure of 10 carbon atoms and at least one double-bond. The 10 carbon atoms are derived from two isoprene units. It has also been reported (<http://www.essentialoils.co.za/components>) that these monoterpenes have anti-inflammatory, antiseptic, antiviral and antibacterial therapeutic properties.

Also present in the oils of the two herbs is the Cembrene-type diterpenes Incensole and Cembrenol. Incensole (Figure 1A) a predominant diterpenes compounds (26.4%) of Water Dropwort has been identified in resin of *Boswellia carterii* (Hamm *et al.*, 2005) and the neuroprotective properties of Incensole and its acetylated form Incensole-acetate has been reported (Moussaieff *et al.*, 2008). Another important diterpenes identified in 'Kacip Fatimah' oil is Isoabienol a diterpene alcohol previously identified in the oil of *Juniperus communis* (Adam *et al.*, 2010). Isoabienol (7.89%) is one of the major components of the essential oil of 'Kacip Fatimah' (Table 2).

A significant quantitative and qualitative difference in the sesquiterpenes of the oils from Water Dropwort and 'Kacip Fatimah' was noticed (Tables 1 and 2). In fact, the essential oil obtained from Water Dropwort was richer in sesquiterpenes such as α -Copene (18.23%), Z-Caryophyllene (0.34%), α -Cuprenene (0.40%), β -Sesquiphelladiene (0.41%) and Germacrene D (0.39%). In contrast, the oil from 'Kacip Fatimah' has more sesquiterpene alcohols such as Longiborneol with a characteristic strong moss woody aroma and Cendron-13-ol. However, the most prominent sesquiterpenes components of the oil from 'Kacip Fatimah' were 1,4-Trans-6 methylisocalamene (13.75%) β -Santalol acetate (9.35%) and T-Cadinol (24.78%). 1,4-Trans-6-methylisocalamene was previously reported in the leaf volatiles of *Macaranga* species (Jurgens *et al.*, 2005). On the other hand, the non-acetylated form of β -Santalol has been identified in the oil of Sandalwood (Buchbauer *et al.*, 2001) and it has also been used as fragrance ingredients (Bhatia *et al.*, 2008) and possibly as

viral inhibitor (Buchbauer *et al.*, 2001). T-Cadinol (24.78%) which is the most prominent constituents of the 'Kacip Fatimah' was recently reported as having smooth muscles relaxing properties in rat aorta (Claesom *et al.*, 2009). T-Cadinol was reported to relax contractions induced by 60 mM K^+ in a concentration dependent fashion.

Meanwhile, the presence of T-Cadinol has earlier been reported in the volatile compounds from leaf-buds of *Populus nigra* (Jerkovic and Mastelic, 2003), *Strawberry guava* (Pino *et al.*, 2001) and fruits of *Cinnamomum zeylanicum* (Jayaprahasga *et al.*, 1997), 'Kacip Fatimah' has been used over this past century for the treatment and management of menstrual irregularities and post-partum complication by the traditional Malay women (Zakaria and Mohammad, 1994; Zaizuhana *et al.*, 2006). It is probable that T-Cadinol (Figure 1B) and some other terpenoid constituents of the oil from this herb might be playing significant roles in the therapeutic properties of 'Kacip Fatimah'. It is imperative therefore, that more research studies should investigate the role if any of the key essential oil constituents of 'Kacip Fatimah' in the management of post-partum complication.

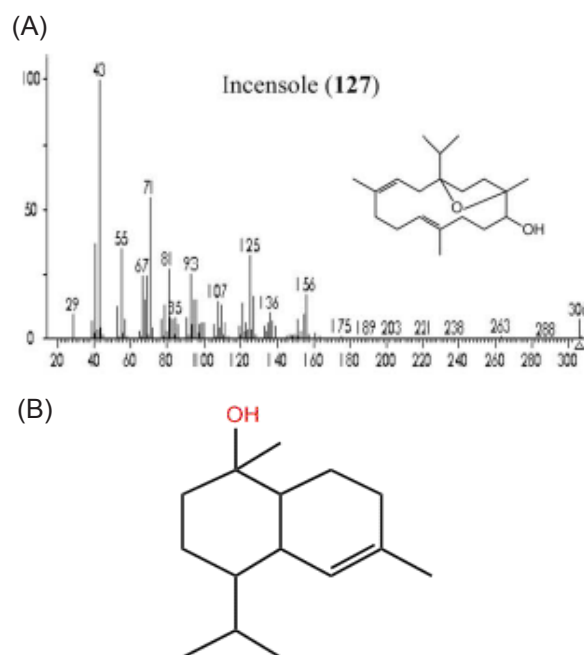


Figure 1. Mass spectra of Incensole (A) (Hamm *et al.*, 2005) and the structure of T-Cadinol (B) (<http://www.pherobase.net/database/kovats/kovats-detail-t-cadinol.php>)

Conclusion

The chemical composition of the essential oils obtained from the leaves of *Oenenthe javanica* and *L. pumila* revealed 41 aroma-active compounds made up of limited number of monoterpenes (α -pinene,

β -pinene, limonene, γ -terpineol and β -ocimene). A significant quantitative and qualitative difference was noticed in the sesquiterpenes obtained from the two herbs. While the essential oil obtained from Water Dropwort was predominated with sesquiterpenes such as α -Copaene, (Z)-Caryophyllene, α -Cuprene, β -Sesquiphelladiene and Germacrene D. The essential oil of 'Kacip Fatimah' consisted more sesquiterpene alcohols such as Longiborneol, 8-Cendron-13-ol and T-Cadinol. Significant presence of diterpenes such as Incensole, Cembrenol and Isoabienol were noticed in the two herbs.

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