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Short Communication

Quantitative analysis of some volatile components in *Mimusops elengi* L.

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Abstract

Dried pikul flower (*Mimusops elengi* L., Sapotaceae) is used in many recipe′s of Thai traditional medicine i.e. cardiotonic and stomachic. In this study, fresh and dried pikul flowers were investigated. The odour of pikul flower, even when it was dried, is very strong and characteristic. The constituents of volatile oils in fresh and dried pikul flowers extracted by ether were analysed by gas chromatography-mass spectrometry. 2-Phenylethanol, 4-hydroxybenzenemethanol and cinnamyl alcohol were mainly found in fresh flower, 10.49, 8.69 and 6.17%, respectively. Whereas those mainly found in dried flowers were long chain carboxylic acid ester and (Z)-9-octadecenoic acid, 5.37 and 4.71% of ether extract, respectively.

An analytical method simultaneously determining benzyl alcohol, 2-phenylethanol and methyl paraben was developed by using the GC-FID method. The percent recoveries were 91.66, 104.59 and 105.28%, respectively. The intraday variations (% RSD) were 7.22, 6.67 and 1.86%; and the interday variation were 3.12, 2.52 and 3.55%, respectively. Detection limits were 0.005, 0.014 and 0.001 ppm, and quantitation limits were 0.015, 0.048 and 0.003 ppm, respectively. Benzyl alcohol, 2-phenylethanol and methyl paraben content of dried flowers (9 samples from various drug stores in Thailand and one sample from China) were 6.40-13.46, 17.57-196.57 and 27.35-355.53 ppm, respectively.

Keywords: *Mimusops elengi,* ether extract, dried and fresh flowers

1. Introduction

Mimusops elengi is a medium tree growing in Southeast Asia. Leaves are glossy, dark green and wavy, oval shaped, 5-10 cm long and 2-5 cm wide (Bunyapraphatsara, 1998). Flowers are cream-colored, hairy and scented. Dried pikul flowers are used in many recipe′s of Thai traditional medicine for cardiotonic and stomachic (The Institute of Thai Traditional Medicine, 2006). Some physicochemical properties (Aromdee *et al.*, 2005) of dried pikul flowers and its antioxidant activities (Rattanadon *et al.*, 2006) were also reported. In this study, fresh and dried pikul flowers were investigated. The odour of pikul flower, even when it was dried, is very strong and characteristic. In order to establish the standard of dried pikul flowers, the existence of volatile constituents were determined in order to examine the quality of dried pikul flowers purchased from traditional herbal drug stores from different parts of Thailand. It was found that the specimens from various sources and the degree of dryness gave various constituents of volatile oil.

2. Experimental

Samples were obtained from the North (Chiang Rai), South (Songkhla), central (Bangkok), and Northeast (Khon Kaen) of Thailand, and one sample was obtained from China (Nanning). A sample of fresh flowers was collected at Khon Kaen University campus. One portion was extracted fresh, other parts were air dried, and sampling was carried out weekly for extraction over four weeks to determine the contents of selected volatile components. Authentic flowers and the plant were dried and stored at the Faculty of Pharmaceutical Sciences, Khon Kaen University, Herbarium no. BR001-002. Purchased samples were examined and

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compared with the authentic flowers. Gas chromatography was employed for identification using a GC-MS (Trace Ultra Italy, MS was a DSQ, USA), with a TR-5 column of 30 m x 0.25mm x 0.25m film thickness. The injector temperature 290°C in a split mode 1:100. Following temperature programme was applied, from 60-180°C with a ramp rate of 3°C.min^{-1} and from 180-280°C with a ramp rate 10°C.min^{-1}. The carrier gas was He with a flow rate of 1mL.min^{-1}. The temperatures of the ion source and the interface were 220° and 275°C, respectively. The conditions were modified from Wong and Teng (1994). Aliphatic alkanes (C10-C23) were used for linear retention index (LRI) determination. The interpretation was carried out by comparing the MS spectra to the MS Library (NIST), with the LRI data from the literature (Davies, 1990), and three authentic standards. Quantitative determination was carried out by a GC-FID (Hewlet Packard HP 6890). The carrier gas was nitrogen with 2 mL. min^{-1} flow rate. n-Hexadecane was used as an internal standard. Validation of the method was carried out according to the ICH guideline (2007).

2.1 Sample Preparation

1. GC-MS: Three grams of finely ground dried flowers were sonicated in 10 mL of ether for 1 hour and allowed to stand overnight. Then the mixture was filtered and dried under nitrogen. The residue was reconstituted with 300 mL of ether and centrifuged. The supernatant, 1 μL, was injected into the GCMS.

2. GC-FID: Three grams of sample were extracted with ether thrice (12, 5, and 5 mL). The solvent was evaporated under nitrogen to dryness. Ethyl acetate 200 μL and 100 μL diluting solvent (100 μL n-hexadecane in 100 mL ethyl acetate) were added to the residue and mixed well.

2.2 Standard preparation

1. Stock standard solutions: Stock standard solutions of benzyl alcohol, 2-phenylethanol and methyl paraben in ethyl acetate at the concentrations of 100, 10 and 1 μL or μg .mL^{-1} were prepared.

2. Standard solutions: Stock standard solution was aliqouted accordingly to contain various concentrations of benzyl alcohol, 2-phenylethanol and methyl paraben as shown in Table 1. Internal standard was added to contain 0.33 μL.mL^{-1}.

3. Results and Discussion

The main constituents in fresh and dried pikul flowers were shown in Table 2. Some of the volatile components in the flowers were not detected in dried flowers. In the Thai

Table 1. Preparation of standard solutions

Standard solutions	Benzyl alcohol μL.mL^{-1}	2-Phenylethanol μL.mL^{-1}	Methyl paraben μg.mL^{-1}
Standard solution 1	0.33	0.50	0.53
Standard solution 2	0.67	1.50	1.07
Standard solution 3	1.00	2.50	1.60

Table 2. Some main constituents of fresh and dried pikul flowers

Component	Retention time (min)		LRI	Relative amount (%)		Identification
	Fresh flower	Dried flower		Fresh flower	Dried flower	
Benzyl alcohol	7.89	7.91	1040	0.79	0.38	MS*, LRI**Authentic standard
2-Phenylethanol	10.72	10.73	1119	10.49	3.29	MS, LRI, Auth. std
3-Phenyl-2-propene-1-ol	18.46	18.43	1308	6.17	0.53	MS, LRI
4-Hydroxybenzenemethanol	20.24	20.25	1354	8.69	0.41	MS, LRI
Methyl 4-hydroxybenzoate	24.67	24.78	1462	2.69	1.74	MS, LRI Auth. std
2-Butyl phenol	31.20	-	1632	1.74	-	MS, LRI
Hexadecanoic acid	42.02	42.02	1968	2.43	0.07	MS, LR
Long chain carboxylic acid	-	42.29	1980	-	5.37	MS, LR
Unidentified		44.92	2150	-	6.65	MS, LR
(Z)-9-Octadecanoic acid	-	45.02	2158	-	4.71	MS, LR

* MS= mass spectrum, ** LRI= linear retention index

Herbal Recipe′, dried flowers were used. Thus it is possible that these volatile compound were not the main active ingredients in the therapeutic activities. However, those left over compounds i.e. alcohols and long chain fatty acids are good natural antiseptics and good preservatives for drug formulations. In the quantitative determination of benzyl alcohol, 2-phenylethanol and methyl paraben, the validation parameters were evaluated according to the ICH guidelines. The percent recoveries were 91.66, 104.59 and 105.28%, respectively (Table 3). Detection limits were 0.005, 0.014 and 0.001 ppm, respectively, and quantitation limits were 0.015, 0.048 and 0.003 ppm, respectively. The intraday variations (% RSD) were 3.12, 2.52 and 3.55% and the interday variation were 7.22, 6.67 and 1.86%, respectively (Table 4). In the quantitative determination of these components, benzyl alcohol was found only in three samples. Whereas in the crispy dried samples, as obtained, none of these components was found as shown in Table 5 (Samples G and I). When the flowers were air dried at room temperature, the contents of these components decreased gradually, and benzyl alcohol and 2-phenylethanol diminished faster (Table 6 and Figure 1).

In this study, compositions of ether extract of dried pikul flowers varied vastly. Degree of dryness, conditions and time length of storage influence the remain of these compounds.

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Table 3. Percent recovery, detection limit (DL), and quantitation limit (QL) of Benzyl alcohol, 2-Phenylethanol and Methyl paraben by standard addition method

Compound	% Recovery	DL (ppm)	QL (ppm)
Benzyl alcohol	91.66	0.005	0.015
2-Phenylethanol	104.59	0.016	0.048
Methyl paraben	105.28	0.001	0.003

Table 4. Intraday and interday variation

compound	Intraday variation			Interday variation		
	slope	sd	% RSD	slope	sd	% RSD
Benzyl alcohol	15.27	0.48	3.12	14.32	1.03	7.22
2-Phenylethanol	5.42	0.14	2.52	5.10	0.34	6.67
Methyl paraben	1.36	0.05	3.55	1.33	0.02	1.86

Table 5. Benzyl alcohol, 2-Phenylethanol and Methyl paraben in dried pikul flowers

Sample	Concentration (ppm)		
	Benzyl alcohol	2-Phenylethanol	Methyl paraben
A	13.46	196.57	232.54
B	-	98.23	204.00
C	10.54	41.70	91.44
D	-	109.60	63.86
E	6.40	108.77	355.53
F	-	17.57	-
G	-	-	-
H	-	178.85	144.41
I	-	-	-
J	-	87.03	27.35

Table 6. Contents of Benzyl alcohol, 2-Phenylethanol and Methyl paraben in air-dried samples over four weeks.

week	Benzyl alcohol		2-Phenylethanol		Methyl paraben	
	ppm	% left	ppm	% left	ppm	% left
1	25.30	100	35.99	100	942	100
2	19.06	75.33	22.98	63.85	881	99.48
3	12.98	51.30	16.41	45.60	861	91.39
4	8.46	33.44	12.51	34.75	577	61.21

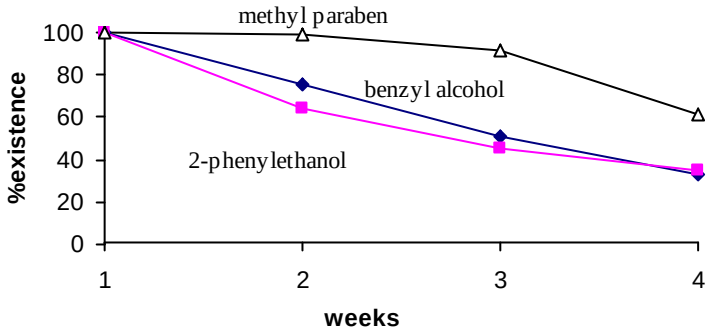

Figure 1. Effect of drying time on some active components of pikul flower.

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