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ANALYSIS OF ODOR CONSTITUENTS OF *Melodorum fruticosum* FLOWERS BY SOLID-PHASE MICROEXTRACTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Odor components are one of the essential properties emitted from flowers. They are often pleasant to the sensory system of humans. The identification of these components plays a role in analyzing the aromatic characteristics and flavors of flowers while also serving as a significant starting point. Gas chromatography-mass spectrometry (GC-MS) is a useful technique that has been employed to investigate the volatile components of flowers [1–4]. Solid-phase microextraction (SPME), introduced recently by Pawliszyn et al. [5], is a fast, efficient, solvent-free alternative to conventional sample extraction techniques. Due to its sensitivity, reproducibility, and high concentration capability, SPME has been widely used for extracting the volatile components from flowers and other parts of plants [6–8]. *Melodorum fruticosum* Lour. belongs to the family of Annonaceae, commonly known as Devil Tree, White Cheesewood, and Lamduan (Thai) [9]. This is widely distributed throughout Indochina and Thailand. *M. fruticosum* Lour. flowers are solitary, pale yellow, and scented. The strong scents of these flowers trigger tropically complex olfactory signals that attract pollinators and natural enemies of herbivores. Thus, the identification of the volatile odors is a prerequisite for further applications of this species. The purpose of this research was to identify the volatile constituents released from fresh *M. fruticosum* Lour. flowers obtained by the extraction of SPME with three different fibers. This is the first time that the relative composition of the flower obtained using all extraction methods is reported and discussed.

As the results indicate, different odor volatiles were detected among the three SPME fibers with the SPME method. The affinity of SPME fibers extracting the analytes was based on the "like dissolve like" concept and thickness of selected fibers. Three different SPME fibers of PDMS, PDMS-DVB, and CAR-PDMS were selected to extract the odor constituents of *M. fruticosum* Lour. flowers. Using PDMS fiber, 37 odor constituents were identified. The majority of the constituents, approximately 85.23%, were represented by the dominant components β-phellandrene (8.98%), *p*-methylanisole (7.02%), δ-cadinene (4.18%), germacrene B (3.81%), and bicyclogermacrene (3.62%). Forty-six odor constituents representing 83.59% were identified by PDMS-DVB fiber. The principal scent volatiles were found to be β-phellandrene (17.19%), *Z*-β-ocimene (9.07%), linalool (7.38%), δ-cadinene (4.15%), and *p*-methylanisole (3.01%). Using the CAR-PDMS fiber, we identified 26 components (80.91%), the major scents being *p*-methylanisole (18.04%), *Z*-β-ocimene (10.42%), β-phellandrene (7.24%), linalool (4.52%), and α-phellandrene (4.09%). The volatiles extracted by the SPME method with PDMS-DVB and CAR-PDMS fibers showed higher amounts of monoterpenes than sesquiterpenes compared to those extracted by PDMS fiber. As can be seen, the PDMS-DVB fiber has much better extraction efficiency than PDMS and CAR-PDMS fibers. Most constituents extracted by PDMS-DVB were superior to those of the other fibers under the same conditions due to the intermediate polarity of PDMS-DVB fiber. It demonstrated the best technique for trapping the key scent constituents of *M. fruticosum* Lour. flowers with different polarities whereby most components were a group of hydrocarbon and oxygenated monoterpenes. PDMS fiber is recommended for extraction of only nonpolar components, which showed better extraction efficiency than the CAR-PDMS fiber, which extracted both polar and nonpolar compounds due to a mutual potential effect of adsorption to and distribution of the stationary phase [10, 11]. The different fibers have significant effects on the percentage composition of the odor volatiles in fresh *M. fruticosum* Lour. flowers obtained by SPME methods. As can be seen, the content of *p*-methylanisole was higher using SPME with CAR-PDMS fiber than those using PDMS and PDMS-DVB fiber, whereas β-phellandrene showed opposite results. It is noted that the SPME technique is more sensitive for volatile components, which played a significant role as the key scent in *M. fruticosum* Lour. flower.

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TABLE 1. Chemical Constituent of *M. fruticosum* Flowers with the Percentage of Content Extracted by SPME with Three Different Fibers

Constituents	LTRI	Content (%)		
		PDMS	PDMS-DVB	CAR-PDMS
Ethyl acetate	800			1.72 ± 0.01
Methoxymethylvinyl ether	811			1.12 ± 0.01
Ethyl butyrate	885			1.41 ± 0.02
Ethyl crotonate	890			1.86 ± 0.06
Ethyl isovalerate	900			1.76 ± 0.04
α-Thujene	935			1.18 ± 0.02
α-Pinene	940	1.44 ± 0.02	0.98 ± 0.01	1.52 ± 0.02
Sabinene	981	1.65 ± 0.06	1.58 ± 0.02	1.89 ± 0.02
β-Pinene	979	1.24 ± 0.04	0.74 ± 0.02	0.98 ± 0.06
Myrcene	995	1.88 ± 0.05	2.09 ± 0.01	2.57 ± 0.04
δ-2-Carene	1000	1.20 ± 0.01	0.62 ± 0.03	1.76 ± 0.02
α-Phellandrene	1006	2.18 ± 0.02	2.05 ± 0.04	4.09 ± 0.03
α-Terpinene	1019		0.47 ± 0.02	1.45 ± 0.02
p-Methylanisole	1025	7.02 ± 0.02	3.01 ± 0.03	18.04 ± 0.16
p-Cymene	1032		1.15 ± 0.02	
β-Phellandrene	1040	8.98 ± 0.03	17.19 ± 0.02	7.24 ± 0.04
δ-3-Carene	1047		0.67 ± 0.01	
Z-β-Ocimene	1050	2.43 ± 0.06	9.07 ± 0.01	10.42 ± 0.02
γ-Terpinene	1065	1.29 ± 0.04	0.59 ± 0.02	
Methyl benzoate	1086		0.56 ± 0.02	
Linalool	1100	2.93 ± 0.04	7.38 ± 0.02	4.52 ± 0.03
allo-Ocimene	1142			2.85 ± 0.02
neo-allo-Ocimene	1150			2.37 ± 0.03
α-Humulene	1460	1.34 ± 0.08		
2-Methoxy-*p*-cresol	1196	1.35 ± 0.02	1.23 ± 0.03	
E-Piperitol	1205		0.41 ± 0.04	
Pulegone	1240	1.42 ± 0.03	0.64 ± 0.02	3.04 ± 0.02
Methyl geranate	1330	1.21 ± 0.01	0.73 ± 0.02	
δ-Elemene	1339	1.55 ± 0.02	0.43 ± 0.01	
α-Cubebene	1354		0.75 ± 0.03	
Neryl acetate	1360	1.27 ± 0.03	0.77 ± 0.04	
α-Copaene	1384	1.42 ± 0.05	0.70 ± 0.01	2.95 ± 0.01
β-Elemene	1395	2.90 ± 0.01	1.85 ± 0.01	0.95 ± 0.02
Z-Caryophyllene	1412	1.80 ± 0.03	0.14 ± 0.01	1.38 ± 0.02
γ-Elemene	1438	2.93 ± 0.02	1.06 ± 0.02	
2-Methyl butyl benzoate	1440	2.02 ± 0.02	1.74 ± 0.02	
E-Muurola-3,5-diene	1457		0.89 ± 0.04	
E-Cadina-1(6),4-diene	1482	1.42 ± 0.02	0.69 ± 0.02	
γ-Gurjunene	1489		0.89 ± 0.03	1.47 ± 0.02
Germacrene D	1495	2.69 ± 0.01	0.77 ± 0.02	
β-Selinene	1499		0.68 ± 0.02	
E-Muurola-4(14),5-diene	1504	1.31 ± 0.03	0.73 ± 0.01	
Bicyclogermacrene	1507	3.62 ± 0.04	2.38 ± 0.01	1.23 ± 0.04
α-Muurolene	1509	1.49 ± 0.08	0.90 ± 0.04	
γ-Cadinene	1515	1.64 ± 0.04	1.40 ± 0.12	
δ-Cadinene	1531	4.18 ± 0.05	4.15 ± 0.05	1.14 ± 0.02
E-Cadina-1(2),4-diene	1536	1.30 ± 0.06	0.68 ± 0.02	
α-Cadinene	1542	1.42 ± 0.01	0.74 ± 0.03	
Z-Sesquisabinene hydrate	1547		0.68 ± 0.02	
Elemol	1552	1.41 ± 0.02	0.75 ± 0.03	
Germacrene B	1568	3.81 ± 0.03	1.60 ± 0.04	
epi-α-Cadinol	1652	2.13 ± 0.01	2.69 ± 0.05	
epi-α-Muurolol	1660	2.52 ± 0.02	2.89 ± 0.02	
Z-Methyl jasmonate	1669	1.73 ± 0.02	0.57 ± 0.02	
Benzyl benzoate	1764	1.63 ± 0.03	1.09 ± 0.05	
Total number of volatile components		37	46	26

LTPRI: linear temperature program retention index.

Plant Material and Chemicals. Aerial portions of *M. fruticosum* Lour. flowers at the flowering stage were collected from Mae Fah Luang University, Chiang Rai Province located in the northern part of Thailand in April 2009. Voucher herbarium specimens (QBG No. 41461) of the plant were identified and deposited at the Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai, Thailand. Mixtures of C_8 to C_{22} *n*-alkanes were purchased from Merck (Darmstadt, Germany).

Solid-phase Microextraction (SPME). The SPME apparatus with an SPME fiber assembly holding 1.0 cm fused-silica fibers was purchased from Supelco, Bellefonte, PA, USA. A 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane-divinylbenzene (PDMS-DVB), and 75 μm carboxen-polydimethylsiloxane (CAR-PDMS) fiber were selected to extract the odor volatiles of *M. fruticosum* Lour. flowers in this study. All fibers were mounted in a manual SPME holder and preconditioned for 2 h in a GC injection port set at 250°C. For each extraction, 25 g of fresh *M. fruticosum* Lour. flowers were picked and immediately placed into a 250 mL headspace bottle sealed with a silicone septum and a Teflon cap. The sample bottle was equilibrated at room temperature around 25°C for 30 min. By insertion through the septum of the sample bottle, the fiber was then exposed to the sample headspace for 30 min prior to desorption of the volatiles into the splitless injection port of the GC-MS instrument for 30 min. Extraction of each fiber was performed in triplicate.

Gas Chromatography-Mass Spectrometry (GC-MS). The volatile constituents of fresh *M. fruticosum* Lour. flowers obtained from the SPME extracts with three fibers were analyzed using a Hewlett Packard model HP6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA). It was equipped with an HP-5MS (5% phenylpolymethylsiloxane) capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; Agilent Technologies, USA) interfaced to an HP model 5973 mass-selective detector. The oven temperature was initially held at 50°C and then increased by 2°C/min to 180°C. The injector and detector temperatures were 250 and 280°C, respectively. Purified helium was used as the carrier gas at a flow rate of 1 mL/min. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 29–300. The electron multiplier voltage was 1150 V. The ion source and quadrupole temperatures were set at 230°C and 150°C, respectively. Identification of volatile components was performed by comparison of their Kovats retention indices, relative to C_8-C_{22} *n*-alkanes, and comparison of the mass spectra of individual components with the reference mass spectra in the Wiley 275 and NIST 98 databases. The relative quantity of the components of all samples was determined directly from the GC peak areas. The percentage composition of *M. fruticosum* Lour. flowers extracted by SPME with three different fibers is shown in Table 1.

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