

ANALYSIS OF AROMA VOLATILES FROM FRESH LEAVES OF *PLECTRANTHUS AMBOINICUS* USING SOLID – PHASE MICROEXTRACTION (SPME) TECHNIQUE

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Abstract: This research presents first report on the analysis of aroma volatile compositions from the fresh leaves of *P. amboinicus* by using headspace solid-phase microextraction–gas chromatography/mass spectrometry (HS-SPME-GC/MS) technique. Four different SPME fibers of PDMS, PDMS-DVB, CAR-PDMS, and DVB-CAR-PDMS were selected to extract the odor constituents of the fresh leaves of *P. amboinicus*. The DVB-CAR-PDMS fiber had much better extraction efficiency than the other. Using DVB-CAR-PDMS fiber, thirty-two aroma volatile constituents of the plant were identified. The majority of the constituents, approximately 87.38%, were thymol carbamate (30.26%), globulol (21.77%), 2-(1Z)-propenylphenol (10.92%), santalol (9.99%), 2-(7Z)-bisaboladien-4-ol (7.58%), Z-isoeugenol (1.96%), *allo*-ocimene (1.56%), *neoiso*-menthol (1.21%), Z-jasmone (1.11%), and 8- α -11-elemodiol (1.02%).

1. Introduction

Plectranthus amboinicus (synonyms: *Coleus amboinicus* Lour., *Coleus aromaticus* Benth., and *Plectranthus aromaticus* Roxb [1]) is commonly known in Thai as Niam Huu Suea. The plant belongs to the family Lamiaceae (Labiatae) [2]. *P. amboinicus* is probably the plant most used both for food and medicinal purposes for treatment of sore throat, cough, headache, digestive disorders in children, and insect bites [3, 4]. While there have been several studies reported on the chemical composition of *P. amboinicus* including analysis of essential oil [5, 6] and characterisation of bioactive linalin (5,6-dihydroxy-7,4-dimethoxyflavone) [1], there have been no reports on the chemical composition of fresh leaves of this plant. We report here the chemical composition of aroma volatiles from fresh leaves of the plant that was identified by using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) technique.

2. Materials and Methods

2.1 Plant Materials

Fresh leaves of *P. amboinicus* were collected from Phitsanulok, Thailand in June, 2011. The plant were identified and deposited at CMU herbarium, Faculty of Science, Chiang Mai University, Chiang Mai,

Thailand. Mixtures of C₈ to C₃₀ *n*-alkanes were purchased from Merck (Darmstadt, Germany).

2.2 Solid-Phase Microextraction (SPME)

The SPME apparatus with an SPME fiber assembly holding 1.0 cm fused-silica fiber was purchased from Supelco, Bellefonte, PA, USA. A 100 μ m polydimethylsiloxane (PDMS), 75 μ m carboxen-polydimethylsiloxane (CAR-PDMS), 65 μ m polydimethylsiloxane-divinylbenzene (PDMS-DVB), and 50/30 μ m divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) fibers were selected to extract the aroma volatiles of fresh *P. amboinicus* leaves 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, 10 g of chopped fresh *P. amboinicus* leaves were immediately placed into a 1000 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.

2.3 Gas Chromatography-Mass Spectrometry

The volatile compositions of fresh *P. amboinicus* leaves obtained from the SPME extracts with four fibers were identified 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 $\times$ 0.25 mm i.d., film thickness 0.25 μ m; 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 °C 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

Table 1: Chemical constituents of four fibers from fresh leaves of *P. amboinicus*

Chemical compound	%RA ^a				RI ^b
	A ^c	B ^d	C ^e	D ^f	
2E,4E-Hexadienol		1.79			916
2E-Heptanal		0.17			954
<i>trans</i> -Pinene	0.99				975
<i>cis</i> -Pinene		2.39			986
Z- β -Ocimene	0.26		0.30		1037
β -Phellandrene		1.55	0.41	1.15	1029
E- β -Ocimene	0.47	0.10			1050
γ -Terpinene				tr ^g	1059
<i>p</i> -Mentha-2,4(8)-diene	0.14			0.15	1088
dehydro-Sabina ketone	0.28			0.27	1120
<i>allo</i> -Ocimene	1.56			0.97	1132
<i>neo-allo</i> -Ocimene		2.54	1.01	11.26	1144
2-1Z-Propenyl phenol	10.92		11.49		1150
Santalone	9.99			6.26	1180
<i>p</i> -Menthyl acetophenone		15.25			1182
neiso-Menthol	1.21				1186
γ -Terpineol		7.62	6.38		1199
<i>iso</i> -dehydro Carveol		tr			1214
Citronellyl formate	tr				1273
<i>iso</i> -Menthyl acetate	0.20			tr	1305
<i>cis</i> -2,3-Pinenediol	0.68				1320
8-hydroxy-neo-Menthol	tr				1330
Z-Jasmone	1.11				1392
Z-Isoeugenol	1.96				1407
Thymol carbamate	30.26	10.63	12.61	8.01	1472
γ -Cadinene		0.11			1513
δ -Cadinene			tr	tr	1523
Zonarene	tr	0.21		0.10	1529
Elemol	tr				1549
<i>trans</i> -Dauca-4(11),7-diene				tr	1557
Germacrene B	tr				1561
Globulol	21.77				1590
Carotol	tr				1594
Guaiol	tr				1600
2-(7Z)-Bisaboladien-4-ol	7.58	10.75	11.98	11.27	1619
Eremoligenol	tr				1631
2E-Hexenyl phenyl acetate	tr				1636
α -Eudesmol	0.15				1653
α -Muurolol		tr		tr	1646
α -Cadinol	0.29				1654
Intermedeol	0.66				1666
5-neo-Cedranol	0.12				1685
8- α -11-Elmodiol	1.02			0.31	1747
2,2-dimethyl-7- <i>sec</i> -butyl-2H,5H-pyrano[4,3- <i>b</i>]-pyran-5-one	0.20				1802
14-Hydroxy- δ -Cadinene			0.38		1803
Vetivonic acid	0.14				1811

^a%RA, Relative area (raw peak area relative to total peak area)

^bRI, programmed temperature retention indices as determined on HP-5MS column using a homologous series of *n*-alkane (C₈-C₃₀) as external standard and H₂ as carrier gas

^cA, DVB-CAR-PDMS fiber assembly

^dB, CAR-PDMS fiber assembly

^eC, PDMS-DVB fiber assembly

^fD, PDMS fiber assembly

^gtr, Trace (<0.1%)

Kovats retention indices, relative to C₈-C₃₀ *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 fresh *P. amboinicus* leaves extracted by SPME with four different fibers is shown in Table 1.

3. Results and Discussion

From the analysis results, different aroma volatiles were detected among the four SPME fibers with the SPME method. Four different SPME fibers of DVB-CAR-PDMS, CAR-PDMS, PDMS-DVB, and PDMS were selected to extract the volatile constituents of fresh *P. amboinicus* leaves. Major compounds analyzed by using SPME with the four fibers were thymol carbamate, and 2-(7Z)-bisaboladien-4-ol. Thirty-two volatile constituents representing 92.39% were identified by DVB-CAR-PDMS fiber. The major volatiles were thymol carbamate (30.26%), globulol (21.77%), 2-(1Z)-propenylphenol (10.92%), santalone (9.99%), and 2-(7Z)-bisaboladien-4-ol (7.58%). Using CAR-PDMS fiber, we identified fourteen constituents (53.23%), the main volatiles were *p*-methylacetophenone (15.25%), 2-(7Z)-bisaboladien-4-ol (10.75%), thymol carbamate (10.63%), γ -terpineol (7.62%), and *neo-allo*-ocimene (2.54%). Nine odor constituents approximately 44.62% of chromatographable components were analyzed by PDMS-DVB. The dominant components were thymol carbamate (12.61%), 2-(7Z)-bisaboladien-4-ol (11.98%), 2-(1Z)-propenyl phenol (11.49%), γ -terpineol (6.38%), and *neo-allo*-ocimene (1.01%). In addition, fourteen aroma volatiles were identified by PDMS fiber. The principal compounds approximately 28.71% were 2-(7Z)-bisaboladien-4-ol (11.27%), thymol carbamate (8.01%), santalone (6.26%), β -phellandrene (1.15%), and *allo*-ocimene (0.97%). As can be seen, the analysis of volatiles from fresh *P. amboinicus* leaves by the SPME method with DVB-CAR-PDMS fiber showed the highest amounts of components. The affinity of SPME fibers to extract the aroma compounds was based on the concept of "like dissolves like" and the thickness of selected fibers. DVB-CAR-PDMS fiber has intermediary polarity and it is the highly-crosslinked stationary phase. In this work it showed to provide to better trap aroma compounds with different polarities and by far showed the best performance. CAR-PDMS and PDMS-DVB fibers are recommended for polar volatile compounds and for extraction of low molecular weight compounds. PDMS fiber is preferred for extraction of nonpolar analytes [7]. These results were shown in Table 1. Therefore the DVB-CAR-PDMS fiber has much better extraction efficiency than CAR-PDMS, PDMS-DVB, and PDMS fibers. The different fibers

show significant effects on the percentage composition of the odor volatiles in fresh *P. amboinicus* leaves obtained by SPME methods. It is noted that the SPME technique is more sensitive for volatile components, which played a significant role as the key aroma volatiles in fresh *P. amboinicus* leaves.

4. Conclusions

In this study the aroma volatiles of the fresh *P. amboinicus* leaves were analyzed by using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) technique. Four different SPME fibers of DVB-CAR-PDMS, CAR-PDMS, PDMS-DVB, and PDMS were selected and used to extract the volatile constituents of the plant. DVB-CAR-PDMS fiber showed the better extraction efficiency than CAR-PDMS, PDMS-DVB, and PDMS fibers. The major components found in all four different fibers were thymol carbamate and 2-(7Z)-bisaboladien-4-ol.

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