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Determination of Volatile Compounds from Hoy a carnosa Flowers (Asclepiadaceae)(การวิเคราะห์หาสารประกอบที่ระเหยได้จากดอก Hoya carnosa (Asclepiadaceae))

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การวิเคราะห์หาสารประกอบที่ระเหยได้จากดอก *Hoya carnosa* (Asclepiadaceae)

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บทคัดย่อ

การวิเคราะห์สารระเหยง่ายจากดอก *Hoya carnosa* (L. f.) R. Br. (Asclepiadaceae) ทำโดยการใช้เทคนิคการสกัด 3 วิธี ได้แก่ hydrodistillation, headspace trapping และ solid phase microextraction สารที่สกัดได้นำมาวิเคราะห์และพิสูจน์เอกลักษณ์ด้วยเครื่องแก๊สโครมาโทกราฟีชนิด GC-FID และ GC-MS สารประกอบที่ตรวจพบได้แก่ methyl butanal, ethylbenzene, *o*-xylene, *p*-xylene, benzaldehyde, 6-methyl-5-heptan-2-one, benzyl alcohol, linalool, 2-nonen-1-ol, phenylethylalcohol และ 2-(*E*)-nonenal

กุญแจคำ

ดอก *Hoya carnosa*, สารระเหยง่าย, แก๊สโครมาโทกราฟีชนิด GC-FID, แก๊สโครมาโทกราฟีชนิด GC-MS

Original Article

Determination of Volatile Compounds from *Hoya carnosa* Flowers (Asclepiadaceae)

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Abstract

The volatile components from *Hoya carnosa* (L. f.) R. Br. flowers (Asclepiadaceae) were determined by three extraction methods: hydrodistillation, headspace trapping and solid-phase microextraction. The extracts were analyzed and identified by GC-FID and GC-MS. The components presently identified were methyl butanal, ethylbenzene, *o*-xylene, *p*-xylene, benzaldehyde, 6-methyl-5-heptan-2-one, benzyl alcohol, linalool, 2-nonen-1-ol, phenylethylalcohol and 2-(*E*)-nonenal.

Key words

Hoya carnosa flowers, Volatile components, GC-FID, GC-MS

Introduction

Floral fragrances have been widely integrated in modern life, including medicine, aromatherapy, foods and cosmetics. The rapid expansion of the fragrance industry worldwide has been driven by many demands for all natural fragrances. Many scientists still survey natural sources for novel fragrance compounds (1).

Several hundred essential oil and fragrance compounds have been identified from various fragrant flowers. Most of these compounds are terpenes, esters, alcohols, aldehydes, ketones and alkanes (2). Many essential oils and single fragrance compound as well show therapeutic properties. For example, linalool has sedative property on human upon inhalation (3). Linalool, linalyl acetate, benzaldehyde, neroli oil, α-terpineol and isoeugenol are able to decrease the motility of the test animals upon inhalation (4). Therefore, separation and analysis of floral fragrances are very important and useful (1).

Hoya, the wax plant, is a genus of tropical plants in the Asclepiadaceae family and comprises around 200 different species (5). They are widely known for their attractive flowers in color ranging from purest white, pink, yellow, green, purple, red and brown and for their powerful characteristic fragrance. The old favorite is *Hoya carnosa* (L. f.) R. Br. which is native to South East Asia. Its sap is the color of maple-syrup and its taste is also similar. It is odorless during the day but its fragrance is overpowering at night (6). There have been some reports on the constituents isolated from the stem (7) and caules (8) of *H. carnosa*. Hence, the aim of this research was to study the volatile constituents of *H. carnosa* flowers.

The fragrance compounds are traditionally obtained from plants by distillation with steam, distillation per se, expression and extraction (9). Generally, they are obtained from flowers by solvent extraction, hydrodistillation and headspace sampling. Organic solvent extraction has long been used for isolation of volatile compounds from natural

products. However, the disadvantages of solvent extraction are time-consuming and the presence of toxic organic solvent residues after extraction. Hydrodistillation is also the conventional technique that has been widely used. Headspace sampling is a gas extraction technique. Headspace trapping is the dynamic headspace in which volatiles are trapped by adsorption and desorption with a solvent or by heating (10). The solid phase microextraction (SPME) is a solvent-free technique which is well established in headspace analysis. Volatile compounds are directly absorbed onto an adsorbent-coated fused-silica fibre and then desorbed directly into a gas chromatography injection port (11).

In this study, hydrodistillation, headspace trapping and SPME were used in order to compare the volatile compounds extracted from these three different methods.

Materials and Methods

Apparatus

A simple laboratory apparatus which consisted of a 1000 ml steam generator flask, a distillation flask, a condenser and a receiving flask was used to perform hydrodistillation.

The headspace trapping technique was performed using a portable headspace device. The sampling tube was activated charcoal type NIOSH from Draeger Sicherheitstechnik Gmbh and 9 volt pump from ASF Thomas Menningen.

The solid-phase microextraction (SPME) method was performed using a SPME device which contained a SPME holder 5-7330 and fiber phase 100 μm polydimethylsiloxane (PDMS) fiber assemblies from Supelco.

The analysis was performed using a high resolution gas chromatography HRGC 8000 series equipped with a Rtx-5 crosslinked 5% diphenyl 95% dimethylpolysiloxane column (30 m x 0.25 mm id, x 0.25 μm film thickness) from Restex Inc, MFC 800 controller and a flame ionization detector (FID). Software was Chromcard for window 1.17 from Fison. GC-MS analysis was performed on a gas chromatography-mass spectrometer QP-5000 operated in the positive electron impact (EI) mode, with a cryogenic oven temperature device from Shimadzu. The column was HP-5 MS, 5% phenyl methylsiloxane column (30 m x 0.25 mm id, x 0.25 μm film thickness) from Hewlett-Packard.

Reagents

The n-paraffin hydrocarbons used for Kovats retention index identification were HPLC grade, comprising n-hexane, n-heptane, n-octane, n-nonane, n-decane, n-undecane, n-dodecane, n-tridecane, n-tetradecane, n-pentadecane and n-hexadecane. Trichloromethane and magnesium sulphate were analytical grade.

Sample preparation

A sample of 9.0 gram of *Hoya carnosa* flowers was cut immediately prior to hydrodistillation or extraction with headspace trapping or SPME technique.

Procedure

Hydrodistillation. The flower sample was put into a 1000 ml distillation flask and the hydrodistillation was performed as usual for 3 hours. After hydrodistillation, the volatile components were extracted with dichloromethane and the remaining water was removed with magnesium sulphate. Afterwards, the organic extract was concentrated and the remaining dichloromethane was removed by an argon stream. An amount of 0.2 μl of the extract was injected to GC and GC-MS.

Headspace trapping technique. The flower sample was placed in a well-closed container which contained a headspace device. Fragrance compounds were collected by trapping tube analysis for 4 hours at room temperature and eluting with 0.5 ml dichloromethane. An amount of 0.2 μl of the extract was injected to GC and GC-MS.

Solid phase microextraction technique (SPME). The flower sample was placed in a 40-ml screw cap vial, then the vial was sealed with a teflon-coated silicone rubber septum and kept for 12 hours. A fiber was inserted through the septum into the vial and exposed to the headspace at 25+5°C for 4 hours. The fiber was immediately inserted into injector port of the GC instrument. SPME sampling was splitless mode, the splitter being opened 1 min after the SPME assembly was inserted and the fiber thermally

desorbed. Before sampling, fiber was conditioned for 1 hour in the GC injection port at 220°C.

Gas chromatography (GC). The extracts from 3 extraction methods were analysed by a HRGC 8000 series gas chromatography. The carrier gas was nitrogen (100 kPa). The injector port and detector temperature were 220 and 250 °C, respectively. The oven temperature was programmed as follows: 40°C held for 10 min, then increased to 150 °C at a rate of 1°C min^{-1}, kept for 30 min and finally increased to 250°C at a rate of 50°C min^{-1}.

Gas chromatography-Mass spectrometry (GC-MS). All samples were analysed by GC-MS QP 5000 with helium as carrier gas at a flow rate of 1 ml min^{-1}. The ionizing energy was 70 eV, the scan mass range was 41-400 amu and the interface temperature was 280°C. The oven temperature program was the same as GC.

Identification of volatile components. The identification of volatile components was performed by comparison of their Kovats retention indices (KI) which were calculated by using the homologues series of n-paraffin hydrocarbon (C_6-C_{16}) and by comparison of their mass spectra with those of reference spectra of the NIST mass spectra libraries (on-line)

Results and Discussion

The chromatograms of the extracts from hydrodistillation (A), headspace trapping (B) and SPME (C) are shown in Figure 1. SPME is a very clean method which avoids using toxic solvents so the chromatogram is not interfered by the solvent.

The qualitative composition of *H. carnosa* flowers obtained by three extraction methods were compared. The composition of *H. carnosa* flowers and KI were listed in Table 1. The results showed qualitative differences of volatile components. Hydrodistillation process yielded more volatile compounds than the headspace technique. Headspace trapping and SPME took shorter time than hydrodistillation but some volatile compounds have not been found. This is partly due to the nature of flowers that store the volatiles produced in the daytime and release them at night. Hydrodistillation can extract the volatiles in the cell of picked flowers but headspace sampling adsorbs the volatile compounds in the gas phase. Further studies should be performed to investigate whether there is any difference in the volatile components between picked flowers and living flowers or the time of sample collection.

Volatile compounds presently identified in *H. carnosa* flowers were the hydrocarbons ethylbenzene, o-xylene and p-xylene, the aldehydes methyl butanal, benzaldehyde, and 2-nonenal, the monoterpene D-limonene, the ketone 6-methyl-5-heptan-2-one, and the alcohols benzyl alcohol, phenylethylalcohol, 2-nonen-1-ol and linalool. Linalool was the main component present in the volatiles.

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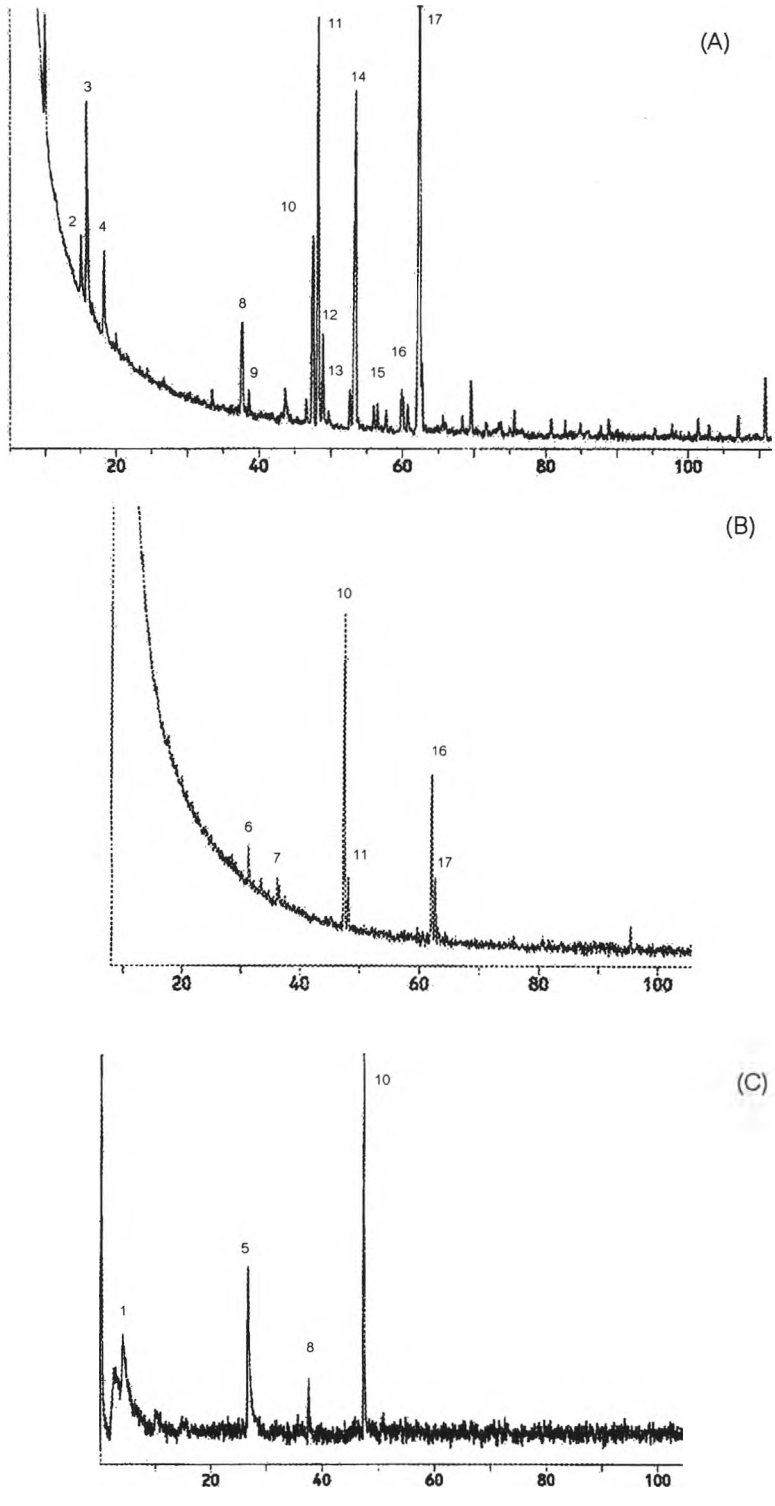

Figure 1. Comparison of gas chromatograms of the extracts from hydrodistillation (A), headspace trapping (B) and solid-phase microextraction (C) of *Hoya carnosa* flowers (numbers correspond to Table 1)

Table 1. Volatile components and KI on 5% phenyl methylsiloxane column of *Hoya carnosa* flowers extracted by hydrodistillation, headspace trapping and solid-phase microextraction.

Peak No.	Volatile components	Retention time (min)	KI	Volatile components found		
				Hydrodistillation	Headspace trapping	SPME
1.	Methyl butanal	4.12	672			✓
2.	Ethylbenzene	14.94	874	✓		
3.	*o*-Xylene	15.81	882	✓		
4.	*p*-Xylene	18.26	905	✓		
5.	Benzaldehyde	26.72	970			✓
6.	6-Methyl-5-heptan-2-one	31.29	996		✓	
7.	D-Limonene	36.55	1036		✓	
8.	Benzyl alcohol	37.62	1044	✓		✓
9.	nid	38.62	1050	✓		
10.	Linalool	47.50	1108	✓	✓	✓
11.	2-Nonen-1-ol	48.32	1114	✓	✓	
12.	Phenylethylalcohol	48.95	1119	✓		
13.	nid	52.68	1146	✓		
14.	nid	53.58	1151	✓		
15.	2-(*E*)-Nonenal	56.02	1169	✓		
16.	nid	62.13	1209		✓	
17.	nid	62.68	1214	✓	✓	

nid: not identified

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