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# A STUDY ON THE CHEMICAL CONSTITUENTS OF GERANIUM OIL

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**Abstract:** In this paper, the chemical constituents of geranium oil, collected from Rong Jiang, Guizhou, were studied by means of capillary gas chromatography-mass spectrometry. Over 40 constituents were separated, of which 31 components, constituting 95.07% of the oil, have been identified. The main ingredients are β-citronellol (24.73 %), garaniol (8.79%), β-guaiene (8.97 %), *p*-menthone (7.19%) and linalool (4.16%), etc.

〔Key Words〕 geranium oil; capillary gas chromatography-mass spectrometry

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## Introduction

*Pelargonium graveolens* (family: Geraniaceae) is a perennial plant and is widely cultivated in many parts of the world including China, France, Egypt, la Réunion Island, Algeria, Morocco, former Soviet Union, Japan, etc. In addition to the major production areas including Yunnan and Sichuan provinces in China, some parts of Guizhou province (Guiyang, Wangmo and Rongjiang) also cultivate this herb (1). Geranium oil is mainly extracted from freshly harvested aerial parts of the two major Pelargonium species (*Pelargonium graveolens* and *Pelargonium roseum*). The harvesting time depends on the geographical regions and climate. Generally it is harvested and processed between July and October in China. The yield of the essential oil is in the range of 0.1-0.3%. The color of the oil is from light yellow to deep yellow with a mixture of rosy-, geranyl- and minty- scents. Geranium oil has a highly added monetary value in cosmetic industries (2). In order to provide scientific evidence to support the assessment of the quality of geranium oil and to help development of high oil content of subgroups, we used capillary gas chromatography-mass spectrometry (GC-MS) to identify and quantify the major components in geranium oil from Rongjiang. This study provides an analytic method for monitoring the quality and manufacturing process of geranium oil.

## 1. General

The chemical composition of geranium oil is very complicated as it is a mixture of monoterpenes, sesquiterpenes, aromatic/alicyclic/aliphatic compounds, and many other compounds. In the past, the quality assessment of geranium oil depended on the conventional physical and chemical methods, which are slow, inaccurate and composition of the oil could not be determined. Gas chromatography (GC) uses gas as a mobile phase and the organic polymer as a stationary phase equipped with various detectors to separate and detect the volatile compounds. Mass spectrometry (MS) detection is used in this study as it is a powerful tool to elucidate the structure of the organic compounds (3).
Identification of the compounds was done by matching their retention times, fragmentation patterns, and mass spectra with an on-line library. Quantification was performed by using peak area of each specific peak divided by the total eluted peak areas. In comparison to the traditional methods, GC-MS is not only fast, sensitive and accurate, but also has special uses for identification and quantification of each component.

**2. Sample Preparation and Physical Characteristics**

Freshly harvested leaves and stems of *P. graveolens* were air-dried slightly and cut into small pieces. A steam distillation method was used to obtain geranium oil with a yield of 0.15% and with the following physical characteristics: light yellow color; $d^{20}_{4} = 0.895$; $n^{20}_{D} = 1.465$ ι 1.473; and $[\alpha]^{20}_{D} = -7^{o}\ 30'$ ι $-10^{o}\ 16'$ .

**3. Instrumentation**

A Hewlett Packard GC (HP 5892 Series II) equipped with a MS detector (HP 5989A) was used in this study. The specifications are: mass range (m/z), 10-1000 amu (extendable to 2000 amu); sensitivity, EI (electronic ionization) source—50 pg of hexachlorobenzene with s/N > 20, CI (chemical ionization) source—100 pg of benzophenone with s/N > 10; scan rate, up to 2000 mau/s; resolution > 2500; electron energy, 10-250 eV; and mass axis stability < 0.1 amu/8 hrs.

**4. Experimental**

4.1 GC column and running conditions
A 5% phenyl-methylpolysiloxane fused silica capillary column (30 m x 0.25 mm) was used with the following running conditions: carrier gas: helium with a flow rate of 50 mL/min; inlet pressure: 12.0 kPa; injector temperature, 240 ºC; the oven temperature was programmed from 50 ºC, increased to 125 ºC at 25 ºC/min, then increased to 250 ºC at 10 ºC/min and maintained isothermally for 7 min; injection volume, 1 μL (diluted in anhydrous ethyl alcohol); and split ratio, 50:1.

4.2 MS parameters
Mass spectrometric analysis was carried out with the following operating conditions: source temperature, 250 ºC; ionization mode, EI; ionization voltage, 70 eV; resolution 2500;

temperature of transfer line (direct interface), 280 ºC; scanning speed, 0.9 s/whole scan; and scan range, 40-500 *mlz.*

## 5. Results and Discussion

By using the GC-MS method, more than 40 peaks have been separated and 31 of them have been identified from geranium oil (Figure 1); the relative content of each peak was calculated based on the respective peak area (Table 1). As seen in Table 1, the

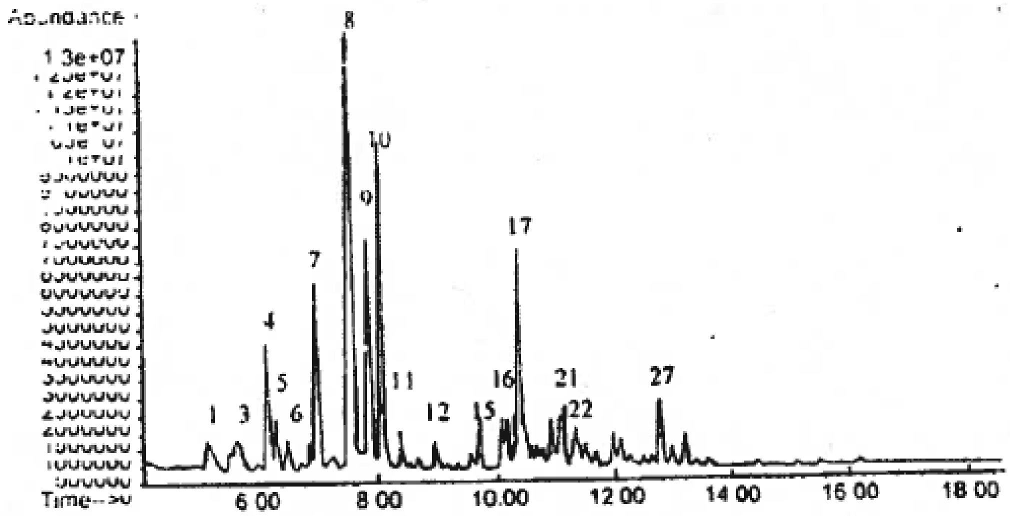


Figure 1: GC-MS spectrum of geranium oil

major compounds were β-citronellol, geraniol, citronellyl acetate, β-guaiene, *p*-menthone and linalool; other compounds were in lower concentrations. Previous studies (4,5) have shown that the major components were geraniol and β-citronellol with concentrations of 10-20% and 30-40%, respectively along with their corresponding esters, geraniol formate and citronellyl formate. It is noted that the concentrations of geraniol and β-citronellol in this study were slightly lower than those in other studies; however, the geranium oil in this study contained higher concentrations of β-guaiene, *p*-menthone, linalool and rose oxide, which produces mixed scents of orange- and rose- blossoms, and mint flavor. In addition, the esters in this product were mainly citronellyl acetate, citronellyl propionate and geraniol acetate instead of citronellyl formate and geraniol formate. Furthermore, this oil also contained relatively high concentrations of β-myrcene, 1-β-pinene, calarene, γ/δ-cadinene, α-elemene and caryophyllene. Although they are not extremely high, they play an important role in the scent of oil, which also contribute to the unique scent of this oil.

As the constituents of plant oils in both compositions and concentrations depend on plant parts, geographical locations, climates, agricultural practices, harvesting time and extract methods, GC-MS is a very useful tool in exploring sources, modification of scents of products, renovation of manufacturing process and mentoring quality of products.

References:

1. Yuan Jiamo et al. Guizhou Aromatic Plants. Guiyang: Guizhou Science & Technology Press, 1990, 49.
2. Fujimaki Masao et al (Chinese translation by Xia Yun). Aromatic Science. Beijing: Light Industry Press, 1987, 385-393.
3. Yoshiro Masada. Analysis of essential oil by gas chromatography and mass spectrometry, Tokyo: Hirokawa Publishing Company Inc. 1975.
4. Heller SR, Milne GWA. EAP/NIH mass spectral database, Washington: US Government Printing Office, 1978.
5. Jennings WG et al. Qualitative analysis of flavour and fragrance volatiles by gas chromatography, New York: Academic Press, 1980.

Table 1. Constituents of Geranium Oil

| Peak # | Chemical Names | Chinese Names | Formulae | MW | Content (%) |
|---|---|---|---|---|---|
| 1 | β-Myrcence β-Myrcene | β-香叶烯 | $C_{10}H_{16}$ | 136 | 1. 96 |
| 2 | δ-4-Carene | δ-4-蒈烯 | $C_{10}H_{16}$ | 136 | 0. 64 |
| 3 | Linaluol Linalool | 芳樟醇 | $C_{10}H_{18}O$ | 154 | 4. 16 |
| 4 | Rose oxidi Rose oxide | 玫瑰醚 | $C_{10}H_{18}O$ | 154 | 2. 86 |
| 5 | 2-Propenal, 3-(dimethylamino)-3-ethoxy- | 3-(二甲胺基)-3-乙氧基-2-丙烯醛 | $C_7H_{13}O_2N$ | 143 | 0. 82 |
| 6 | σ-3-Carene | σ-3-蒈烯 | $C_{10}H_{16}$ | 136 | 1. 58 |
| 7 | P-Menthone | p-薄荷酮 | $C_{10}H_{18}O$ | 154 | 7. 19 |
| 8 | β-Citronellol | β-香茅醇 | $C_{10}H_{20}O$ | 156 | 24. 73 |
| 9 | Geraniol | 香叶醇 | $C_{10}H_{18}O$ | 154 | 8. 19 |
| 10 | Citronellyl acetate | 香茅醇乙酸酯 | $C_{12}H_{22}O_2$ | 198 | 9. 97 |
| 11 | 1-β-Pinene | 1-β-蒎烯 | $C_{10}H_{16}$ | 136 | 1. 08 |
| 12 | Citronellyl Propionate | 香茅醇丙酸酯 | $C_{13}H_{24}O_2$ | 212 | 2. 31 |
| 13 | α-Methylphenethylamine ıine | α-甲基-苯乙胺 | $C_9H_{13}N$ | 135 | 0. 19 |
| 14 | α-Ylangene | α-依兰烯 | $C_{15}H_{24}$ | 204 | 0. 57 |
| 15 | Calarene | 白菖油萜 | $C_{15}H_{24}$ | 204 | 1. 65 |
| 16 | Caryophyllene | 石竹烯 | $C_{15}H_{24}$ | 204 | 1. 62 |
| 17 | β-Guaiene | β-愈创木烯 | $C_{15}H_{24}$ | 204 | 8. 97 |
| 18 | α-Humulene | α-葎草烯 | $C_{15}H_{24}$ | 204 | 0. 68 |
| 19 | N-Methylphenethylamine ırmine | N-甲基-苯乙胺 | $C_9H_{13}N$ | 135 | 1. 59 |
| 20 | α-Cubebene | α-荜澄茄烯 | $C_{15}H_{24}$ | 204 | 0. 79 |
| 21 | γ-Cadinen γ-Cadinene | γ-杜松烯 | $C_{15}H_{24}$ | 204 | 1. 76 |
| 22 | α-Elemene | α-榄香烯 | $C_{15}H_{24}$ | 204 | 1. 75 |
| 23 | δ-Cadinen δ-Cadinene | δ-杜松烯 | $C_{15}H_{24}$ | 204 | 2. 17 |
| 24 | Geranyl acetate | 香叶醇乙酸酯 | $C_{12}H_{20}O_2$ | 196 | 1. 13 |
| 25 | Propanoic acid, 2-phenylethyl ester | 丙酸-2-乙酸苯乙酯 | $C_{11}H_{14}O_2$ | 192 | 1. 41 |
| 26 | N-methyl-2-hydroxytyramine ine | N-甲基-2-羟基酪胺 | $C_9H_{13}NO$ | 151 | 1. 02 |
| 27 | Cyclohexene, 1-methyl-4-isopropyl- | 1-甲基-4-异丙基-环已烯 | $C_{10}H_{18}$ | 138 | 2. 07 |
| 28 | Tricyclene | 三环萜 | $C_{10}H_{16}$ | 136 | 1. 03 |
| 29 | 2-Heptanamine, 5-methyl- | 5-甲基-2-庚胺 | $C_8H_{19}N$ | 129 | 0. 23 |
| 30 | 2-Hexanamine, 4-methyl- | 4-甲基-2-己胺 | $C_7H_{17}N$ | 115 | 0. 66 |
| 31 | Isomer of 2-Heptanamine, 5-methyl- | 5-甲基-2-己胺 | $C_8H_{19}N$ | 129 | 0. 29 |

Amount of 31 identified peaks (%) in total analysis based on peak area : 5. 07

β-Myrcene



**Translator's notes:**

Some typographical errors have been found and corrected in this translation, such as Geraniaceaue has been revised to Geraniaceae, Linaluol to Linalool, etc. In addition, a number of revised chemical names (shaded in greenish blue color) have been added to Table 1 based on the information provided (chemical names, empirical formulae and molecular weight). However, the chemical name of peak #31, at the very bottom of Table 1, cannot be determined as its chemical name, Chinese name and empirical formula are not corresponding to its molecular mass. That being said, based on the MW and the proposed molecular formula of $C_8H_{19}N$ (might be mistakenly typed as $C_7H_{19}N$), this peak is likely to be an isomer of peak #29, 5-methyl-2-heptanamine.