Preparation and Chemical Analysis of Volatile Oil in Two Different leaf type of Ficus hirta Vahl.

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Abstract. To analyze the chemical constituents of volatile oil in two different leaf types of Ficus hirta Vahl. Steam distillation was used to collect the different leaf types of Ficus hirta Vahl., the volatile chemical components of extracts were analyzed using GC-MS, and the relative contents of various chemical components in the volatile oils of the two medicinal materials were calculated by area normalization method. The volatile oils of the Ficus hirta Vahl. of three-lobed leaf type and five-lobed leaf type contained 38 and 39 compounds, respectively. The main components are coumarins, organic acids, aldehydes, esters, alcohols, etc. In addition, there are some Hydrocarbons, phenols, ketones, and the chemical composition and relative content of volatile oils of the two have large differences. The method can quickly and easily identify the chemical constituents of the volatile oils of two different leaf Ficus hirta Vahl., and there are significant differences in the chemical constituents in the volatile oils of the Ficus hirta Vahl. of three-lobed leaf type and five-lobed leaf type.

1. Introduction
It is found that the basic source of the medicinal materials of the Ficus hirta Vahl.is belongs to the family Moraceae[1]. Ficus hirta Vahl. is a common medicinal plant in South China, mainly distributed in the southern and southwestern parts of China. Its roots and fruits can be used as medicine [2-3]. At present, Ficus hirta Vahl. is mainly planted artificially, which is favored by people because of its medicine food homology and rich biological activity[4]. Modern pharmacological studies have shown that Ficus hirta Vahl. has the functions of improving digestion and respiratory system, improving immune function, anti-tumor, anti-oxidation and anti-inflammatory [5-9]. Folks often use odor to evaluate the quality of the Ficus hirta Vahl., so volatile components, such as volatile oil, are the research hotspots of the Ficus hirta Vahl. Studies have shown that the volatile oil of the Ficus hirta Vahl. contains flavonoids, organic acids, coumarins, etc. [10]. There are many forms of leaves in Ficus hirta Vahl., such as three-lobed leaves and five-lobed leaves. Although they have different leaf forms, they both belong to Ficus hirta Vahl.. However, the scent of the three-lobed Ficus hirta Vahl. and the five-lobed Ficus hirta Vahl. are different, and it is speculated that the chemical constituents in the volatile oil are substantially different. In order to study the difference of chemical composition
between the two types of *Ficus hirta Vahl.*, gas chromatography-mass spectrometry (GC-MS) was used to analyze them, so as to provide basis for the development and utilization of *Ficus hirta Vahl.*

2. Instruments, Materials and Reagents

2.1. Instruments

DSQ II Gas Chromatography-Mass Spectrometry was from Seymour Fisher Company, USA; Sartorius MA35M moisture measuring instrument was purchased from Beijing Sartorius Scientific Instruments Co., Ltd.; YP 3001N electronic balance was purchased from Shanghai Jing Branch Instrument Co., Ltd.; Sartorius One Over Ten-thousand Analytical Balance was from Beijing Sartorius Scientific Instruments Co., Ltd.; DFT-200 Portable High Speed Universal Crusher was provided by Wenling Linda Machinery Co., Ltd.

2.2. Materials and Reagents

Three-lobed leaf type *Ficus hirta Vahl.* and the five-lobed leaf type *Ficus hirta Vahl.*, aged about 4 years, was collected by Heyuan city Jinyuan green life co., ltd. in the GAP industrial planting demonstration base of *Ficus hirta Vahl.* in Heyuan city, Guangdong province. It was certified as genuine by Lin Zhiyun senior experimenter of Department of Traditional Chinese Medicine, Guangdong University of Pharmacy; Cyclohexane is of analytical grade, which was purchased from Tianjin Zhiyuan Chemical Reagent Co., Ltd.

3. Methods and Results

3.1. Extraction and Sample Preparation of Volatile Oil

Pulverizing freshly picked three-lobed leaf type *Ficus hirta Vahl.* and the five-lobed leaf type *Ficus hirta Vahl.*, taking 300g each of medicinal materials with the same amount after water content is converted. Place each material in a 5000ml round bottom flask, add five times the amount of water and soak for 30 minutes, and then extract for 1.5 hours. The collected distillate was added with 6ml cyclohexane for extraction, transferred to a separatory funnel, the upper oil phase was collected, blow to the remaining 2ml with nitrogen.

3.2. GC-MS Conditions

3.2.1. Gas chromatography conditions. The GS system used chromatographic separation was achieved by using a fused capillary column DM-5MS, length (30m×0.25mm×0.25um). The inlet temperature was 250°C, the initial column temperature was kept at 60 °C for 3min, and it was raised to 180 °C at 10°C/min for 8min, and then raised to 230 °C at 15°C/min for 5min. The carrier gas was high purity helium with a flow rate of 1.0 mL / min. The volume sample is 1.0μl.

3.2.2. Mass spectrometry conditions. The interface temperature between chromatography and mass spectrometry is 230 °C, Electron bombardment source is used as ionization mode, The monitoring method is full scanning, The scanning range is 35 - 500, The ionization energy is 70eV, Solvent delay 4 min, The ion source temperature is 250 °C.

3.3. Results

GC-MS was used to analyze the volatile oil extracts of two different leaf type in *Ficus hirta Vahl.*, and the total ion chromatogram of them was obtained, as shown in figure 1,2. The initial identification results of the target components were obtained by INST2005 mass spectrometry, and the relative contents of the components were determined by peak area normalization. The results of chemical composition analysis of two different leaf type in Ficus hirta Vahl are shown in table 1,2. The results of comparative analysis on the chemical constituents of volatile oil of two kinds of leaves were shown in table 3.
Figure 1. GC-MS total ion chromatogram of *Ficus hirta* Vahl. of three-lobed leaf type

Figure 2. GC-MS total ion chromatogram of *Ficus hirta* Vahl. of five-lobed leaf type
### Table 1. GC-MS Analysis Results of Chemical Constituents of Volatile Oil of *Ficus hirta Vahl.* of three-lobed leaf type

| Number | Retention (min) | Compound Description | Molecular Formula | Molecular Weight | Relative molecular content (%) |
|--------|-----------------|----------------------|-------------------|------------------|-------------------------------|
| 1      | 11.82           | trans-2,4-Decadien-1-al | C_{10}H_{18}O     | 152              | 19.81                         |
| 2      | 24.28           | Palmitic acid         | C_{16}H_{32}O_{2} | 256              | 17.13                         |
| 3      | 27.02           | Linoleic acid         | C_{18}H_{34}O_{2} | 280              | 12.90                         |
| 4      | 29.70           | Hexacosane            | C_{26}H_{54}      | 366              | 4.60                          |
| 5      | 28.94           | Heptacosane           | C_{27}H_{56}      | 380              | 4.15                          |
| 6      | 27.95           | Henicosane            | C_{29}H_{44}      | 296              | 3.20                          |
| 7      | 20.83           | Dibutyl phthalate     | C_{16}H_{22}O_{4} | 278              | 3.04                          |
| 8      | 26.11           | Xanthyletine          | C_{14}H_{16}O_{3} | 228              | 2.21                          |
| 9      | 26.51           | Pentagonosine         | C_{25}H_{52}      | 352              | 1.84                          |
| 10     | 16.67           | 7-tert-butyl-3,3-dimethyl-2H-inden-1-one | C_{15}H_{20}O | 216              | 1.52                          |
| 11     | 18.71           | 1-naphthylethanol     | C_{12}H_{14}O_{2} | 172              | 1.28                          |
| 12     | 28.89           | Psoralen              | C_{11}H_{16}O_{2} | 186              | 1.25                          |
| 13     | 15.89           | (E)-oct-2-enal        | C_{8}H_{14}       | 222              | 1.25                          |
| 14     | 23.98           | 1-O-butyl 2-Octyl benzene-1,2-dicarboxylate | C_{20}H_{30}O_{4} | 334              | 1.24                          |
| 15     | 16.12           | Epoxystearic acid     | C_{16}H_{22}O_{3} | 268              | 1.08                          |
| 16     | 13.78           | Cycloheptasiloxane, tetradecamethyl- | C_{16}H_{22}O_{5} | 518              | 1.15                          |
| 17     | 27.31           | Z-(13, 14-Epoxy)tetradec-11-en-1-ol acetate | C_{16}H_{22}O_{3} | 268              | 1.08                          |
| 18     | 4.82            | 2-Pentan-2-yloxirane  | C_{7}H_{14}O      | 114              | 0.81                          |
| 19     | 9.44            | trans-2-Nonenal       | C_{9}H_{18}O_{2}  | 140              | 0.75                          |
| 20     | 7.73            | (E)-oct-2-enal        | C_{9}H_{16}O_{2}  | 126              | 0.71                          |
| 21     | 6.49            | 2-Pentylfuran         | C_{9}H_{16}O_{2}  | 138              | 0.71                          |
| 22     | 16.34           | 3,3,5,6,7-pentamethyl-2H-inden-1-one | C_{14}H_{16}O_{2} | 202              | 0.66                          |
| 23     | 6.31            | octamethyleneclotetrasiloxane | C_{16}H_{22}O_{5} | 296              | 0.60                          |
| 24     | 21.80           | Linoleoyl Chloride    | C_{16}H_{32}O_{2} | 298              | 0.56                          |
| 25     | 8.51            | Nonanal               | C_{9}H_{18}O_{2}  | 142              | 0.55                          |
| 26     | 14.32           | 2,4-Di-tert-butylphenol | C_{16}H_{20}O_{2} | 206              | 0.48                          |
| 27     | 21.98           | 1-Alpha-linolenoylglycerol | C_{21}H_{36}O_{4} | 352              | 0.43                          |
| 28     | 6.65            | Octa-2,4-diene        | C_{8}H_{14}       | 110              | 0.41                          |
| 29     | 9.75            | DL-Menthol            | C_{10}H_{16}O_{2} | 156              | 0.40                          |
| 30     | 30.64           | Ethyl iso-allocholate | C_{26}H_{42}O_{5} | 436              | 0.39                          |
| 31     | 17.33           | 7-Methoxy-1,3-benzodioxole-5-carboxaldehyde | C_{16}H_{18}O_{5} | 212              | 0.32                          |
| 32     | 18.36           | 13-Epimanoic acid     | C_{20}H_{22}O_{2} | 290              | 0.31                          |
| 33     | 22.34           | 3-Ethyl-5-(2-ethylbutyl)octadecane | C_{22}H_{44}O_{2} | 366              | 0.30                          |
| 34     | 12.46           | 2-Pentadec-12-ynoxyoxane | C_{20}H_{36}O_{2} | 308              | 0.30                          |
| 35     | 17.05           | 9-Hexylheptadecane    | C_{25}H_{48}      | 324              | 0.28                          |
| 36     | 29.18           | Rac cis-9,10-Epoxyxystearic Acid | C_{18}H_{34}O_{2} | 298              | 0.28                          |
| 37     | 18.24           | 3,5-Di-tert-butyl-4-hydroxybenzaldehyde | C_{15}H_{22}O_{2} | 234              | 0.27                          |
| 38     | 27.52           | (Z)-2-(9-Octadecenoxyloxy)ethanol | C_{20}H_{40}O_{2} | 312              | 0.23                          |
Table 2. GC-MS Analysis Results of Chemical Constituents of Volatile Oil of *Ficus hirta Vahl.* of five-lobed leaf type

| Number | Retention (min) | Compound | Molecular Formula | Molecular Weight | Relative Molecular Content (%) |
|--------|----------------|----------|-------------------|------------------|-------------------------------|
| 1      | 24.31          | Palmitic acid | C_{16}H_{32}O_{2} | 256              | 34.57                         |
| 2      | 26.94          | Linoleic acid | C_{18}H_{34}O_{2} | 280              | 9.66                          |
| 3      | 28.5           | Psoralen     | C_{15}H_{16}O_{2} | 186              | 8.10                          |
| 4      | 18.5           | Cedrenol     | C_{17}H_{30}O     | 220              | 7.14                          |
| 5      | 11.47          | trans-2,4-Decadien-1-al | C_{10}H_{10}O_{2} | 152              | 2.62                          |
| 6      | 26.1           | Xanthyletine | C_{14}H_{22}O_{3} | 228              | 2.24                          |
| 7      | 31.1           | Hexacosane   | C_{26}H_{54}      | 366              | 1.78                          |
| 8      | 15.88          | (1S,4aR,8aR)-7-Isopropylidene-1,4a-dimethyldecahydro-1-naphthalenol | C_{15}H_{26}O | 222              | 1.69                          |
| 9      | 6.28           | Oct-1-en-3-ol | C_{8}H_{16}O     | 128              | 1.48                          |
| 10     | 20.82          | Diisobutyl phthalate | C_{13}H_{22}O_{4} | 278              | 1.34                          |
| 11     | 17.83          | (3E,7E)-3,7-Dimethyl-10-(propan-2-ylidene)cyclodeca-3,7-dienone | C_{15}H_{19}O_{3} | 218              | 1.26                          |
| 12     | 23.97          | 1-O-butyl 2-O-octyl benzene, 1,2-dicarboxylate | C_{20}H_{30}O_{4} | 334              | 1.19                          |
| 13     | 13.71          | 4-(1-hydroperoxy-2,2-dimethyl-5-methylidenecyclohexyl)pent-3-en-2-one | C_{14}H_{22}O_{3} | 238              | 1                             |
| 14     | 19.09          | 3, 5, 6, 7, 8, 8a-hexahydro-4, 8a-dimethyl-6-(1-methyl vinyl)-2 (1H) Naphthone | C_{13}H_{22}O | 218              | 0.97                          |
| 15     | 16.65          | β-Guaiene    | C_{15}H_{24}      | 204              | 0.94                          |
| 16     | 7.7            | (E)-oct-2-enal | C_{8}H_{16}O     | 126              | 0.83                          |
| 17     | 9.98           | Methyl salicylate | C_{7}H_{14}O_{3} | 152              | 0.79                          |
| 18     | 6.47           | 2-Pentylfuran | C_{10}H_{14}O     | 138              | 0.77                          |
| 19     | 28.93          | 3-ethyl-5-(2-ethylbutyl)octadecane | C_{26}H_{54} | 366              | 0.77                          |
| 20     | 29.69          | Nonahexacontanoic acid | C_{60}H_{132}O_{2} | 998              | 0.75                          |
| 21     | 9.15           | 1,3-Dimethyl-5-(2-methylenoxy)benzene | C_{18}H_{18}O_{2} | 138              | 0.67                          |
| 22     | 14.31          | 2,4-Di-tert-butylphenol | C_{13}H_{18}O | 206              | 0.65                          |
| 23     | 9.41           | Non-2-enal   | C_{8}H_{16}O     | 140              | 0.62                          |
| 24     | 17.68          | Aromadendrene oxide | C_{13}H_{22}O | 220              | 0.6                           |
| 25     | 30.84          | Oleamide     | C_{19}H_{30}NO   | 281              | 0.6                           |
| 26     | 12.13          | 4-methoxy-1-methylbicyclo[2.2.2]octane | C_{19}H_{20}O | 154              | 0.59                          |
| 27     | 13.55          | Paenol       | C_{19}H_{22}O | 166              | 0.59                          |
| 28     | 15.73          | β-Ionone     | C_{13}H_{22}O | 192              | 0.52                          |
| 29     | 16.11          | (−)-α-Cedrene | C_{15}H_{26} | 204              | 0.52                          |
| 30     | 8.49           | Nonanal      | C_{9}H_{16}O | 142              | 0.45                          |
| 31     | 22.34          | Tetraetracontane | C_{44}H_{90} | 618              | 0.45                          |
| 32     | 17.35          | (1R,3Z,7Z,11S)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene (8R,9S,13S,14S,17S)-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[α]phenanthren-17-ol | C_{18}H_{22}O | 220              | 0.43                          |
| 33     | 26.08          | Linoleyl Chloride | C_{16}H_{31}ClO | 652              | 0.42                          |
| 34     | 27.31          | DL-Menthol   | C_{10}H_{18}O | 280              | 0.42                          |
| 35     | 9.74           | 7-Hexadecenal (Z) | C_{16}H_{30}O | 156              | 0.4                           |
| 36     | 17.04          | Pentan-2-ylcyclopropane | C_{8}H_{16} | 238              | 0.39                          |
| 37     | 4.79           | Methylcicosan-7,10,13-trienoate | C_{19}H_{26}O | 112              | 0.38                          |
| 38     | 18.03          | 2-Pent-2-enylfuran | C_{8}H_{12}O | 320              | 0.34                          |
| 39     | 6.63           | 2-Pent-2-enylfuran | C_{8}H_{12}O | 136              | 0.27                          |
Table 3. Analysis of Chemical Composition Types of Volatile Oil in Two Different leaf types of *Ficus hirta Vahl.*

| chemical component | species( three-lobed leaf type) | species( five-lobed leaf type) |
|--------------------|--------------------------------|--------------------------------|
| coumarin           | 3                              | 4                              |
| terpenoids         | 1                              | 6                              |
| Organic acids      | 3                              | 3                              |
| alcohols           | 4                              | 2                              |
| phenolic           | 2                              | 2                              |
| ethers             | 0                              | 1                              |
| aldehyde           | 6                              | 5                              |
| ketones            | 2                              | 3                              |
| esters             | 5                              | 4                              |
| hydrocarbon        | 6                              | 5                              |
| other              | 6                              | 4                              |

4. Discussion

In this study, it was suggested that the odor of three-lobed leaves *Ficus hirta Vahl.* and five-lobed leaves *Ficus hirta Vahl.* were different due to the different components of volatile oil. According to the results of the research, there are 38 and 39 compounds in them respectively, among which the main components are coumarins, organic acids, aldehydes, esters, alcohols, etc. In addition, some hydrocarbons, phenols, ketones are still present, and they have same chemical components which were trans-2,4-decadien-1-ol, palmitic acid, linoleic acid, hexacosane, psoralen, Xanthyletine, (E)-oct-2-enal, 2-pent-2-enylfuran, nonanal, DL-Menthol, etc. There were 3 kinds of essential oils with relative content over 5.0 % in *Ficus hirta Vahl.* of three-lobed leaf type, and the contents were trans-2,4-decadien-1-ol (19.81%), palmitic acid (17.13%) and linoleic acid (12.90%). There were 4 kinds of essential oils with relative content of over 5.0 % in *Ficus hirta Vahl.* of five-lobed leaf type, and the contents were palmitic acid (34.57%), linoleic acid (9.66%), psoralen (8.10%) and Cedrenol (7.14%) from high to low.

Psoralen is one of the main active components of *Ficus hirta Vahl.*, which has antibacterial, antiviral, anticoagulant, tumor inhibition, immune regulation and other functions [11], and accounts for 8.10% of the volatile oil of *Ficus hirta Vahl.* of five-lobed leaf type. However, only 1.25% of the volatile oil in the *Ficus hirta Vahl.* of three-lobed leaf type was found. Studies have shown that psoralen can protect and activate chondrocytes, resist the expression of matrix metalloproteinase secreted by synovial cells, and effectively reduce sodium iodiacetate (MIA) induced osteoarthritis (OA) [12]. Palmitic acid, also known as palmitic acid, is a saturated fatty acid, widely exists in nature, almost all oils and fats contain a number of palmitic acid components. Liu yan et al [13], found that palmitic acid may activate ERK pathway through c5a-c5ar and induce inflammatory response of microglia cells, and C5aR antagonist has a neuroprotective effect in inflammatory injury diseases of the nervous system. Linoleic acid is called an essential fatty acid because it cannot be synthesized by the body itself or is rarely synthesized and must be obtained from food. Linoleic acid can reduce blood cholesterol and prevent atherosclerosis.

Generally speaking, the difference of planting origin, plant year and harvest time of medicinal herbs may cause differences in the chemical composition of volatile oil. However, the samples of *Ficus hirta Vahl.* in this experiment, all the *Ficus hirta Vahl.* are from the same planting area, with the same planting year and harvest time. Based on the analysis of the chemical constituents of the essential oils of *Ficus hirta Vahl.*, it is found that the components and contents of *Ficus hirta Vahl.* with two different leaves are quite different, and it is pointed out that in the future development and utilization of *Ficus hirta Vahl.* with different leaves type, it is necessary to distinguish *Ficus hirta Vahl.* with different leaves, so as to ensure the efficacy of drugs.
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6. References
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