

Chemical Composition Analysis of Extracts from *Ficus Hirta* Using Supercritical Fluid

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Abstract: *Ficus hirta* was extracted by supercritical carbon dioxide. The volatile chemical components of extracts were analyzed using gas chromatography-mass spectrometry (GC-MS). The percentage of products extracted by Supercritical Fluid Extraction(SFE) was 2.5%. Nineteen volatile compounds were identified. The main volatile components were Elemicin, Psoralen, Palmitic acid, Bergapten, α -Linolenic acid, Medicarpin, Retinoic Acid, Maackiain, and Squalene. The method is simple and quick, and can be used for the preliminary analysis of chemical constituents of supercritical extracts of *Ficus hirta*.

1. Introduction

Hairy fig (*Ficus hirta* Vahl.) is shrub or small tree, which belongs to the family of Moraceae. It mainly distributes in the temperate and subtropical zones of Asia, especially in southern China^[1]. The root of hairy fig, known as Cantonese ginseng, has a pleasantly fragrance of coconut milk and has been used for the treatment of hepatitis and improving fatigue resistance by Hakka people in China^[2]. Some research revealed that the roots of hairy fig exhibited bioactivities of hepatoprotective^[3], antitumor^[4], and antibacterial^[5]. It has many functional constituents, including flavonoids, coumarins and saponins, such as psoralen, bergapten, luteolin, apigenin, vitexin and 3,5,4'-trihydroxy-3,7-dimethoxyflavone^[6-9]. Wei et al. analyzed the *Ficus hirta* ethanol extract with



Gas chromatography-mass spectrometry(GC-MS) method^[10]. Li et al. analyzed the Ficus hirta steam distillation liquid with GC-MS method^[11]. Liu et al. analyzed the Ficus hirta ethanol extract and steam distillation liquid with GC-MS method^[12]. Lin et al. analyzed the Ficus hirta ether extract and steam distillation liquid with GC-MS method^[13]. No supercritical extraction of Ficus hirta and chemical composition analysis of its supercritical extracts have been reported. In this paper, GC-MS technique was used to analyze the chemical composition of supercritical extracts of Ficus hirta, aiming to provide the theoretical basis for its development and utilization.

2. Instruments and Reagents

2.1 Instruments

SFE-5(32) Supercritical extraction device (Guangzhou Lvheyuan Biotechnology Co., Ltd.); TRACE DSQ Gas chromatography-mass spectrometry (ThermoFinnigan); YP 3001N Electronic balance (Shanghai Precision Scientific Instrument Co., Ltd.); DFT-200 Portable high speed universal grinder(Wenling Lingda Machinery Co., Ltd.); Sartorius One Over Ten-thousand Analytical Balance (Sartorius Scientific Instruments (Beijing) Co., Ltd.).

2.2 Reagents

Ficus hirta (Provided by Heyuan Jin Yuan green life Co. Ltd., identified by Professor Liu Jizhu (Guangdong Pharmaceutical University) as the dried root of Moraceae Ficus hirta Vahl.); Ethyl acetate (Tianjin Zhiyuan Chemical Reagent Co., Ltd.); Carbon dioxide (Food grade).

3. Methods

3.1 Supercritical Fluid Extraction

Firstly, Ficus hirta were broke into pieces (20 mesh). Weigh accurately 2000.0g of the coarse powder, put into the extraction kettle. The extraction pressure was 28 MPa, the extraction temperature was 45°C, the CO₂ flow rate was 30 L/h, the separation pressure was 6 MPa, the temperature of separation kettle I was 55°C, and the extraction time was 2 h. The extract was yellow semi-solid oil with an extraction rate of 2.5%.

3.2 Test solution

Taking 400 mg of the supercritical extracts, accurately weighed, in a 5 mL volumetric flask, add ethyl acetate to volume and mix well, filter and discard the initial filtrate, preserved the successive filtrate in liquid vial, as a test solution for GC-MS analysis.

3.3 GC-MS Analysis

3.3.1 Gas Chromatography Conditions

The GC system used a fused capillary column DM-5MS, length 30m, internal diameter 0.25mm, and film thickness 0.25μm. Ultra-high purity helium (99.99%) was used as the carrier gas at a constant flow rate of 1mL/min. The column oven temperature was programmed at 50°C under a holding time of

2 min, while the injection temperature was set at 250°C. The temperature program increased at a rate of 12°C/min to 240°C under a holding time of 22 min. The injection mode was splitless. Injection volume was 1.0 µL.

3.3.2 Mass Spectrometry Conditions

The acquisition mode was operated at 1000 scan speed with 1.0 s event time. Sample was run in the 15-380 amu range and the total ion chromatogram obtained was auto-integrated using ChemStation.

4. Results

4.1 Component Analysis of The Supercritical Extracts

The supercritical extracts of the *Ficus hirta* were analyzed by GC-MS to obtain the total ion chromatogram (Figure 1). The initial identification results of the target components were obtained by automatic retrieval of the NIST mass spectrometry, and the relative contents of the components were determined according to the peak area normalization method. According to the results of GC-MS analysis, there were nineteen compounds identified in the *Ficus hirta* supercritical extracts and ten compounds with relative content of more than 1.0%. The results were shown in Table 1.

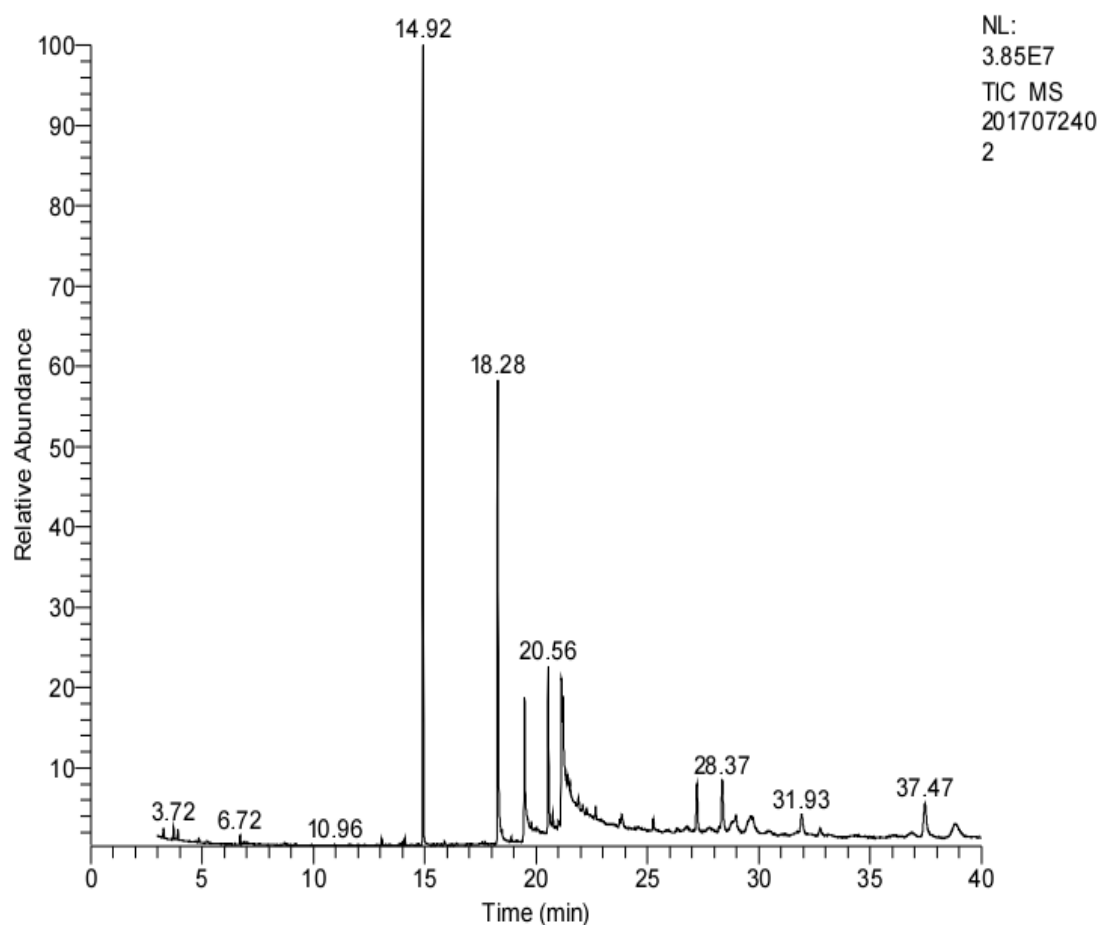


Figure 1. The total ion chromatogram of the *Ficus hirta* supercritical extracts

Table 1. Component analysis of the *Ficus hirta* supercritical extracts

Number	Retention time (min)	Compound	Molecular formula	Relative molecular mass	Relative content (%)
1	3.27	Toluene	C ₇ H ₈	92	0.33
2	3.72	Hexanal	C ₆ H ₁₂ O	100	0.49
3	14.12	Tricyclo[6.3.0.0(2,4)]undec-8-ene, 3,3,7,11-tetramethyl-	C ₁₅ H ₂₄	204	0.28
4	14.92	1,2,3-trimethoxy-5-(2-propenyl)-Benzene	C ₁₂ H ₁₆ O ₃	208	23.11
5	18.28	7H-Furo[3,2-g][1]benzopyran-7-one	C ₁₁ H ₆ O ₃	186	18.90
6	19.49	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	8.34
7	19.80	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	0.34
8	20.55	7H-Furo[3,2-g][1]benzopyran-7-one, 4-methoxy-	C ₁₂ H ₈ O ₄	216	6.85
9	20.76	2H,8H-Benzo[1,2-b:5,4-b']dipyrans-2-one, 8,8-dimethyl-	C ₁₄ H ₁₂ O ₃	228	0.93
10	21.15	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	17.45
11	21.54	Cyclopropanecarboxylic acid	C ₂₅ H ₄₂ O ₂	374	0.57
12	21.91	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308	0.61
13	22.68	[1,1'-Biphenyl]-4,4'-diamine, 3,3',5,5'-tetramethyl-	C ₁₆ H ₂₀ N ₂	240	0.58
14	23.87	2,2',4,4',6-Pentamethyldiphenylsulfone	C ₁₇ H ₂₀ O ₂ S	288	1.06
15	25.28	Dibenz[a,c]cyclohexane,	C ₁₈ H ₂₀ O ₃	284	0.84

2,4,7-trimethoxy-

16	28.37	6H-Benzofuro[3,2-c][1]benzopyran-3-ol, 6a,11a-dihydro-9-methoxy-, (6aR-cis)-	C ₁₆ H ₁₄ O ₄	270	3.85
17	28.98	Retinoic acid	C ₂₀ H ₂₈ O ₂	300	1.75
18	31.94	6a,12a-Dihydro-6H-(1,3)dioxolo(5,6)benzofuro(3,2-c)chromen-3-ol	C ₁₆ H ₁₂ O ₅	284	2.41
19	37.47	Squalene	C ₃₀ H ₅₀	410	4.14

4.2 Bioactivity Analysis of the Main Constituents of the Supercritical Extracts

Five compounds with relative content of more than 5% were identified in the *Ficus hirta* supercritical extracts. The content of compounds was arranged from high to low as: Elemicin (23.11%), Psoralen (18.90%), α -Linolenic acid (17.45%), Palmitic acid (8.34%), Bergapten (6.85%), which consisted the main components of the *Ficus hirta* supercritical extracts. Zhao et al. studied the volatile oil components and antioxidant activity of *Dalbergia pinnata* fragrance ingredient. It was found that Elemicin is the main component of the volatile oil from *Dalbergia pinnata* fragrance ingredient and the total volatile oil appears better antioxidant activity^[14]. Tang et al. studied on *Acorus tatarinowii* Schott for cerebral diseases. The experiment showed the Elemicin, β -asarone and α -asarone of *Acorus tatarinowii* Schott which can pass through the rat's blood brain barrier (BBB) have direct effect on brain. *Acorus tatarinowii* Schott is a good herb for cerebral diseases on which the Elemicin, β -asarone and α -asarone have effect all together^[15]. Psoralen showed strong antibacterial activity against gram positive bacteria and *Staphylococcus aureus*^[16]. Psoralen also showed dose-dependent antitumor activity, which can significantly induce the apoptosis of tumor cells^[17]. It can inhibit the bone metastasis of breast cancer cells in vivo, inhibit the growth of breast cancer cells, and regulate the function of osteoblasts and osteoclasts of tumor bearing mice in the bone microenvironment^[18]. α -Linolenic acid is an essential fatty acid for the body to play its normal physiological function, which can modulate blood lipids, anti-thrombosis, regulating blood sugar and blood pressure, anti-tumor, anti-inflammatory, inhibiting allergic reactions, enhance intelligence and protect eyesight and so on^[19]. Xiao found that Bergapten could promote the osteogenic differentiation of bone marrow mesenchymal stem cells into osteoblasts in the mice in vivo. It is beneficial to bone formation, so as to prevent and treat osteoporosis^[20]. Huang et al. studied that Bergapten could suppresses lung cancer cells secreting soluble interleukin-2 receptor by inhibiting the binding and the transcriptional activity of STAT3 on the promoter of IL2RA^[21]. In addition, there were also relatively high content of components such as Medicarpin (3.85%), Maackiain (2.41%) and Squalene (4.14%) in the *Ficus hirta* supercritical extracts. Li et al. found that Medicarpin could inhibit or kill human's liver cancer cells under the cultured condition^[22]. Maackiain is a novel antiallergic compound that suppresses transcriptional upregulation of the histamine H1 receptor and interleukin - 4 genes^[23]. Squalene is an important bioactive

substance, which has anti-cancer, anti-tumor, anti-fatigue, anti-infection, immune and antioxidant effects, and has been widely used in medicine, food, cosmetics industry and other fields^[24].

5. Discussion

Nineteen compounds in the samples of supercritical extracts were identified using GC-MS, accounting for 92.83% of the total chemical constituents. The main components were long chain organic acids (25.79%), coumarins (25.75%) and phenylpropanoids (23.11%). In addition, there were some unsaturated hydrocarbons, sulfones, isoflavones, dihydroflavones, terpenoids and so on. In the premise of the *Ficus hirta* from the same origin, the results of the *Ficus hirta* supercritical extracts were compared with those of the subcritical extracts, the ethanol extracts and the volatile oil extracted by the steam distillation. It was found that the relative content of Elemicin extracted by supercritical fluid extraction was the highest.

In this paper, GC-MS method was used to analyze the supercritical extracts of the *Ficus hirta*. In order to analyze the chemical constituents more detailed, other analytical methods, eg, High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS), should be used.

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