

Analysis of Chemical Constituents in *Ficus Hirta* Vahl. by LCMS-IT-TOF and GC-MS.

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Abstract. Objective: Analysis the chemical constituents from the roots of *Ficus hirta*. **Methods:** The *Ficus hirta* Vahl. were extracted with 75% ethanol. Qualitative analysis of ethanol extracts was carried out by using high performance liquid chromatography-tandem mass spectrometry (LCMS-IT-TOF) and gas chromatography- mass spectrometry (GC-MS). **Result:** Twenty compounds were identified by LCMS-IT-TOF. Nine compounds were identified by GC-MS. Psoralens and bergapten were identified by LCMS-IT-TOF and GC-MS. **Conclusion:** LC-MS-TOF and GC-MS have identified 27 compounds, including 9 flavonoids, 6 coumarins, 3 organic acids, 3 organic alcohols, 2 organic esters, 1 terpene, 1 polyphenol, 1 alkaloid and 1 anthraquinone, which laid a foundation for further study of *Ficus hirta* Vahl. and its compound preparations.

1. Introduction

Ficus hirta Vahl. is the dry root of *Ficus hirta* of Moraceae plants, sweet flavor, neutral in nature, has the effect of promoting dampness and relaxing tendons, strengthening the spleen and invigorating the lung. It is a traditional Chinese herbal medicine used in the south of the five ridges and mainly used in the treatment of spleen deficiency and edema, hepatitis, rheumatism and arthralgia, tuberculosis and cough. Studies have shown that *Ficus hirta* Vahl. has the functions of immune regulation[1], antibacterial[2], anti-inflammatory, analgesic, liver protection[3], antioxidant, apoptosis induction[4] and anti radiation[5]. In addition, Cheng[6] et al. found that *Ficus hirta* Vahl. can inhibit the production of NO induced by lipopolysaccharide in rats. However, the active components of the ethanol extracts of *Ficus hirta* Vahl. are not clear. In order to ensure the safety and effectiveness of the product, the determination of its active ingredients is particularly important. In this study, LCMS-IT-TOF and GC-MS were used to separate and analyze the chemical components in the extract of *Ficus hirta* Vahl., and to identify the chromatographic peak qualitatively, so as to provide theoretical basis for the development and utilization of *Ficus hirta* Vahl..



2. Instruments, Samples and Reagents

2.1. Instruments

LCMS-IT-TOF mass spectrometer(Shimadzu, Kyoto, Japan), equipped with standard electrospray ionization (ESI), Mass Hunter, Qualitative Analysis and photo-diode array(PDA); TRACE DSQ GC-MS(ThermoFinnigan, USA); BS423S One thousandth electronic balance(Sartorius); CP225D One in 100,000 electronic balance(Sartorius); HWS26 Electro-Thermostatic Water Bath(Shanghai yiheng Scientific Instruments Co., Ltd); crusher DFT-200(Wenling Linda Machinery Co., Ltd, Zhejiang); IKA -RV8 V Rotary Evaporator(IKA, Germany).

2.2. Materials and Reagents

Ficus hirta Vahl. provided by Heyuan Jinyuan green life Co., Ltd, identified by Professor Liu Jizhu, teaching and research Office of traditional Chinese medicine, Guangdong Pharmaceutical University as the dry root of *Ficus hirta* of *Ficus* of Moraceae; Psoralen standard(purity \geq 98%, Guangzhou Qiyun Biotechnology Co., Ltd); Apigenin standard(purity \geq 98%, Guangzhou Qiyun Biotechnology Co., Ltd); ultrapure water(A.S. Watson Group Ltd); Formic acid(Tianjin kemio Chemical Reagent Co., Ltd); methanol(Thermo Fisher Scientific); Acetonitrile(Thermo Fisher Scientific);ethyl alcohol absolute(AR, Tianjin Damao Chemical Reagent Factory), CO₂ (Guangzhou Zhenyu weiou Trading Co., Ltd).

3. Methods and Results

3.1. Preparation of Test Solution

The 200g of *Ficus hirta* Vahl. powder was passed 16 mesh sieve and refluxed two times with 6 times 75% ethanol, 3h per time. The filtrate was combined and concentrated. Ethanol was recovered under reduced pressure. Then the filtrate was diluted with methanol to 200 mL, and filtered again to get the subsequent filtrate. The subsequent filtrate was filtered by 0.22 μ m membrane and stored in a small liquid phase bottle for LCMS-IT-TOF and GC-MS analysis as test solution.

3.2. LCMS-IT-TOF Chromatography and Mass Spectrometry Conditions

3.2.1. LCMS-IT-TOF chromatographic condition. Chromatographic column: YMC-Pack ODS-A C18 column(250 \times 4.6 mm, 5 μ m); mobile phase: 0.1% formic acid-water solution(A)/acetonitrile(B); gradient: 10 min, 90:10(V(A) V(B)); 30 min, 80:20(V(A) V(B));60 min, 60:40(V(A) V(B)); 80 min, 30:70(V(A) V(B)), 90 min, 20:80(V(A) V(B)); 100 min, 0:100(V(A) V(B));100 min, 95:5(V(A) V(B)); flow rate: 1.00 mL \cdot min⁻¹; column temperature: 35 $^{\circ}$ C \pm 5 $^{\circ}$ C; detection wavelength:300 nm and 350 nm.

3.2.2. LCMS-IT-TOF mass spectrometry conditions. Mass spectrometry conditions: positive and negative ion modes; scanning range: m/z 50~600; ESI source spray voltage:1.50 KV; the ionization temperature: 200 $^{\circ}$ C; detector voltage: 1.65KV; Separator voltage: 8.5 KV; Pressure in TOF zone: 1.4 \times 10⁻⁴ Pa; TOF temperature: 40 $^{\circ}$ C; Ion accumulation time: 10 ms.

3.3. GC-MS Chromatography and Mass Spectrometry Conditions

3.3.1. GC-MS gas phase conditions. Chromatographic column:30.00 m \times 0.25 mm \times 0.25 μ m DM-5MS capillary column; injector temperature: 250 $^{\circ}$ C, Interface temperature: 230 $^{\circ}$ C, Helium was used as carrier gas, flow rate: 1.0 mL \cdot min⁻¹, Column pressure:80 kp, splitless injection mode, Injection volume: 1.0 μ L; Temperature program: column temperature: 40 $^{\circ}$ C, constant temperature for 2 min, The temperature is raised to 70 $^{\circ}$ C by the program of 10 $^{\circ}$ C \cdot min⁻¹, and then to 280 $^{\circ}$ C by the program of 10 $^{\circ}$ C and constant temperature for 10 min.

3.3.2. GC-MS mass spectrometry conditions. Electron impact ion source(EI), the ionization temperature:200 $^{\circ}$ C, Electron multiplier voltage:520, mass range:15-380 amu, total ion scanning,

Scanning gap:1.0 s, Scanning rate: 1000 amu · s⁻¹.

3.4. Results

3.4.1. LCMS-IT-TOF analysis of ethanol-extracts from *Ficus hirta* Vahl. LCMS-IT-TOF was used to detect the chemical components of *Ficus hirta* Vahl., The total ion chromatogram is shown in Figure 1, 2. The obtained mass spectrogram was retrieved by Metlin mass spectrometry database and Chemsipider database, and the analysis results are shown in Table1.

Table 1. Identification results of *Ficus hirta* Vahl.

peak	T _R	Formula	m/z	MS/MS fragments	Compound
1	19.128	C ₁₅ H ₁₄ O ₆	291.0806[M+H] ⁺	165,139,123	Epicatechin
2	19.433	C ₁₆ H ₁₈ O ₉	355.1010[M+H] ⁺	289,163,145	Chlorogenic acid
3	24.850	C ₁₅ H ₁₄ O ₅	275.0887[M+H] ⁺	149,139,207	(-)(2R,3R)Epiafzelechin
4	26.573	C ₁₇ H ₁₈ O ₉	389.0823[M+Na] ⁺	227,185,153	Psoralenoside
5	27.355	C ₁₈ H ₂₀ O ₁₀	419.0943[M+Na] ⁺	257,213,185	Methoxypsoralenoside
6	29.220	C ₂₁ H ₂₁ NO ₆	384.1450[M+H] ⁺	178,164,136	Hydrasine
7	32.152	C ₁₇ H ₂₀ O ₉	391.1000[M+Na] ⁺	229,185,149	4,5-dihydrogenpsoralenoside
8	33.333	C ₂₁ H ₂₀ O ₁₀	431.0946[M-H] ⁻	283,311,341	Pelargonidin 7-glucoside
9	33.592	C ₂₁ H ₂₂ O ₉	417.1171[M-H] ⁻	255,256	Aloin A
10	33.692	C ₁₅ H ₁₂ O ₄	257.0791[M+H] ⁺	239,147,137	Isoliquiritigenin
11	34.367	C ₂₁ H ₂₀ O ₁₀	433.1133[M+H] ⁺	364,337,283	Vitexin
12	35.103	C ₁₈ H ₂₂ O	399.1304[M+H] ⁺	319,263,237	Picraquassioside A
13	44.430	C ₁₁ H ₁₀ O ₄	205.0525[M-H] ⁻	161,187	Isoeugenitol
14	49.938	C ₁₅ H ₁₀ O ₆	287.0570[M+H] ⁺	269,153,135	Kaempferol
15	52.560	C ₁₁ H ₆ O ₃	187.0402[M+H] ⁺	159,143,131	Psoralen
16	54.975	C ₁₅ H ₁₂ O ₅	273.0757[M+H] ⁺	189,153,147	(±)-Naringenin
17	56.062	C ₁₅ H ₁₀ O ₅	271.0660[M+H] ⁺	243, 187,163	Apigenin
18	60.505	C ₁₂ H ₈ O ₄	217.0501[M+H] ⁺	202,174,115	Bergapten
19	70.257	C ₁₄ H ₁₂ O ₃	229.0861[M+H] ⁺	211,187,145	Resveratrol
20	80.110	C ₁₈ H ₃₀ O ₂	279.2333[M+H] ⁺	261,173,145	Pinolenic Acid

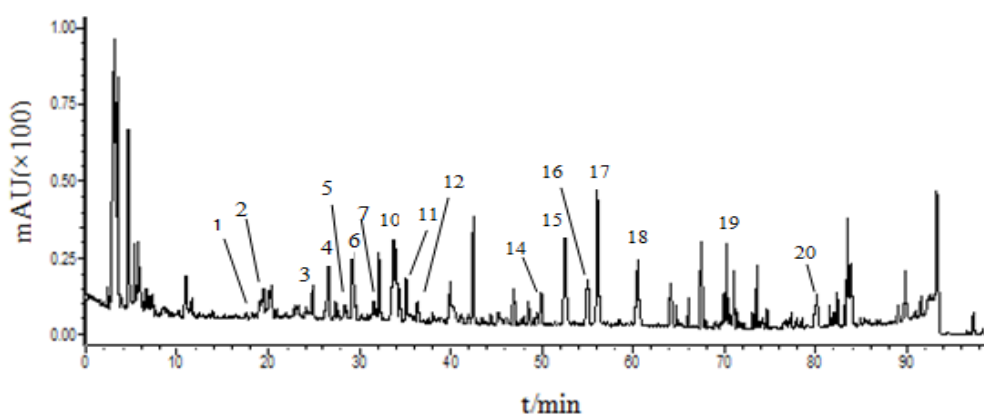


Figure 1. LCMS-IT-TOF positive ion chromatogram of *Ficus hirta* Vahl.

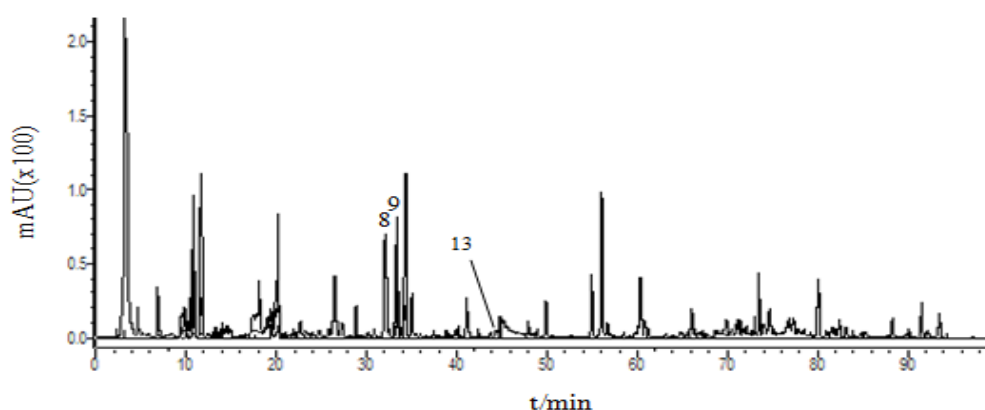


Figure 2. LCMS-IT-TOF anion chromatogram of *Ficus hirta* Vahl.

According to the results, in the positive ion mode, the m/z is $[M+H]^+$ or $[M+Na]^+$. In the anion ion mode, the m/z is $[M-H]^-$. Coumarin contains keto carbonyl, and the m/z of its fragments are $[M+H-CH_3]^+$, $[M+H-CO]^+$ and $[M+H-2CO]^+$, etc. The mass spectrum of peak 15 is shown in Figure 3.

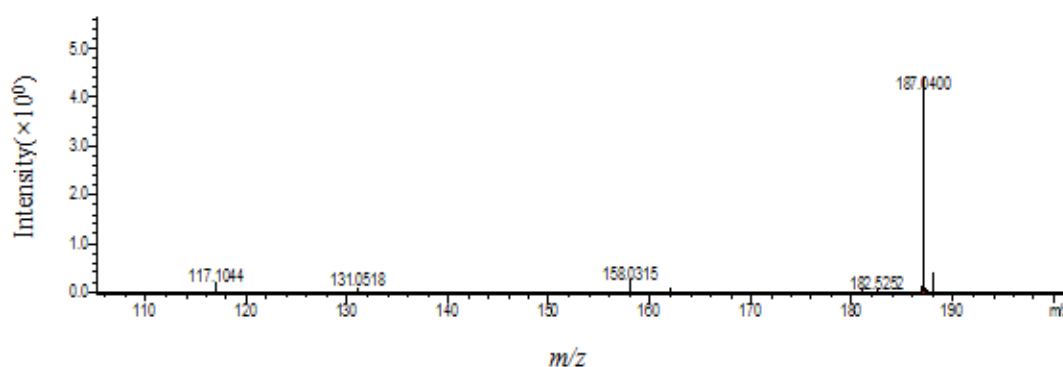


Figure 3. MS spectrum of peak of psoralen (m/z 187).

The fragmentation process of psoralen by mass spectrometry is shown in Figure 4. The $[M+H]^+$ of peak 15 is 187. Its relative molecular mass is 186. The m/z of fragment $[M+H-CO]^+$ is 159. The m/z of fragment $[M+H-2CO]^+$ is 131. The substance can be identified as psoralen.

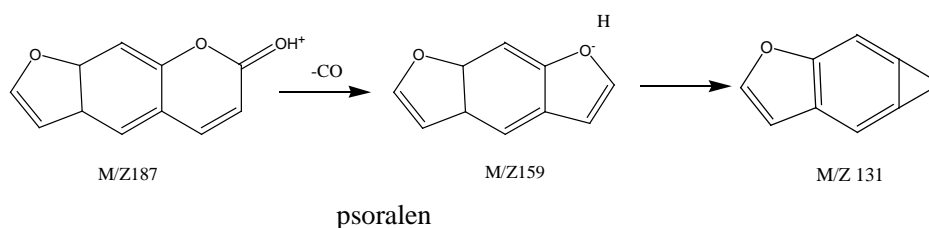


Figure 4. Proposed MS-MS fragmentation of psoralen.

3.4.2. GC-MS analysis of ethanol-extracts from *Ficus hirta* Vahl. GC-MS was used to detect the chemical components of the extract of *Ficus hirta* Vahl., The total ion chromatogram is shown in Figure 5. The content was calculate by the area normalization method of gasification products. The analysis and identification results is shown in Table 2.

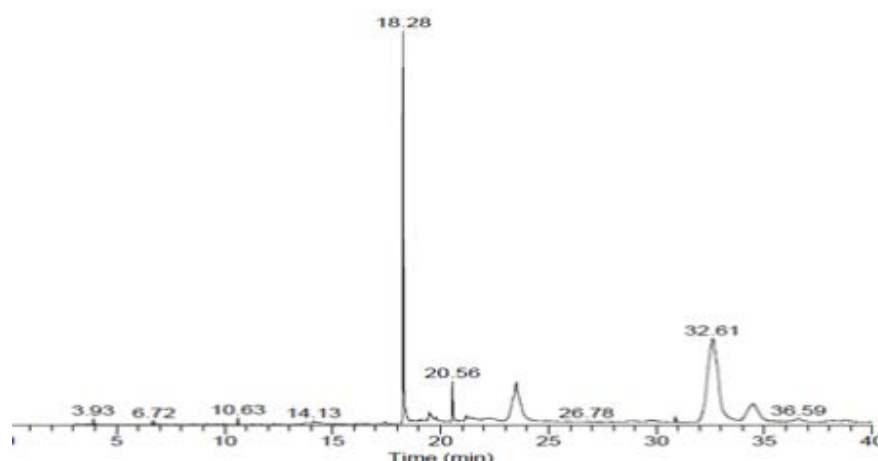


Figure 5. GC-MS total ion chromatogram of *Ficus hirta* Vahl.

Table 2. Identification results of *Ficus hirta* Vahl.

peak	T _R	Formula	Compound	relative content/%
1	10.630	C ₁₁ H ₂₀ O ₂	2-ethylhexyl ester-2-Propenoic acid	0.46
2	18.280	C ₁₁ H ₆ O ₃	Psoralen	25.56
3	19.480	C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid	0.74
4	19.560	C ₂₆ H ₄₄ O ₅	Ethyl iso-allocholate	0.92
5	20.550	C ₁₂ H ₈ O ₄	Bergapten	2.88
6	21.200	C ₁₉ H ₃₆ O	2-Methyl-Z, Z-3,13-octadecadienol	1.20
7	23.530	C ₂₉ H ₄₈ O	Stigmasterol	3.53
8	32.600	C ₂₉ H ₅₀ O	22,23-dihydro- Stigmasterol	32.57
9	41.690	C ₃₀ H ₄₈ O	Amyrin	7.90

According to the results in Table 2, The ethanol extracts of *Ficus hirta* Vahl. include coumarins, flavonoids, sterols and other compounds. The compounds with higher content were 22, 23-dihydro-stigmasterol (32.57%), Psoralen (25.46%), Amyrin (7.90%), Stigmasterol (3.53%), Bergapten (2.88%), and so on.

4. Discussion

The analysis of chemical components of traditional Chinese medicine includes separation, analysis and identification, such as TLC, HSCCC, HPLC and HPCE. However, the chemical components of traditional Chinese medicine are complex, so the above methods are difficult to identify and analyze. The GC-MS technology combines the advantages of mass spectrometry and chromatography, and mass spectrometry has high sensitivity and specificity, which is very suitable for the analysis of complex samples such as traditional Chinese medicine[7]. In this study, the components of the extracts of *Ficus hirta* Vahl. were separated and analyzed, and 27 compounds were identified, including flavonoids, coumarins, organic acids, terpenes, polyphenols, alkaloids, anthraquinones and sterols. The active substance kaempferol could regulate the activity of pro-inflammatory enzymes and the expression of inflammation related genes, which has obvious anti-inflammatory effect[8]. Epicatechin significantly reduced the level of inflammatory factors released by RAW264.7 cells induced by LPS, and reduced the inflammatory response after cerebral hemorrhage[9]. Geranium could inhibit the migration, invasion and proliferation of gastric cancer cells[10]. Ibuprofen could regulate NF-κB signal pathway, control the expression of cytokines, and partially inhibit the release of NO and PGE2[11]. Psoralen inhibited the expression of inflammatory factors in human periodontal ligament cells induced by Pg-LPS, and could be used for the treatment and prevention of periodontitis[12]. Bergamot lactone significantly inhibited the recruitment of neutrophils and macrophages from zebrafish to the injured

site, and promoted the clearance of neutrophils and macrophages from the wound site[13]. Apigenin inhibited the secretion of IL-10 and TNF- α and play an anti-inflammatory role[14].

It can be seen from the total ion chromatogram of *Ficus hirta* Vahl. that there are some chromatographic peaks with good response, and their mass can not be found in the database of known chemical components, which indicates that there are still a lot of unknown components of *Ficus hirta* Vahl. to be further studied.

5. References

- [1] Zhang Z C, Liu J H, Pan Y A. Effects of *Ficus hirta* Vahl. and astragalus extract on cellular immunity of immunosuppressed mice [J]. *Chinese folk medicine*, 2017, **26** (05): 58-59.
- [2] Chen Q, Ye S X, Yu J. Antibacterial Activity of RADIX FICI HIRTAE by Chromotest Microassay[J]. *Medicinal Plant*, 2012, **3** (9): 13-16.
- [3] Wang M, He R R, Li Y F, et al. Protective effect of water extract from *Ficus hirta* Vahl. on restrained stress liver injury [J]. *Chin Hosp Pharm J*, 2015, **35**(6): 522-525.
- [4] Zeng Y W, Liu X Z, Lv Z C, et al. Effects of *Ficus hirta* Vahl. (Wuzhimaotao) extracts on growth inhibition of HeLa cells[J]. *Exp. Toxicol. Path.* 2012, **64**(7-8): 743-749.
- [5] Wang X P, Duan L J, Huang X, et al. Protective effect of aqueous extract of *Ficus hirta* Vahl. on DNA damage of mouse bone marrow cells induced by - (60)Coy ray [J]. *Chinese Modern Applied Pharmacy*, 2011, **28** (04): 284-287.
- [6] Cheng J, Yi X, Wang Y, et al. Phenolics from the roots of hairy fig (*Ficus hirta* Vahl.) exert prominent anti-inflammatory activity[J]. *J. Funct. Foods*, 2017, **31**:79-88.
- [7] Wang Y M, Zhang S J, Luo G A, et al. Study on phenylethanolic glycosides in Cistanche deserticola and its substitutes by LC/ESI-MS/MS [J]. *Acta Pharma Sinica*, 2000, **35** (11): 839-842.
- [8] Devi K P, Malar D S, Nabavi S F, et al. Kaempferol and inflammation: From chemistry to medicine[J]. *Pharmacol. Res.* 2015, **99**: 1-10.
- [9] Ruan H S, Mou J Z. Effect of epicatechin on lipopolysaccharide induced secretion of inflammatory factors in RAW264.7 cells [J]. *Chinese Journal of ETMF*, 2017, **23**(4): 159-163.
- [10] Liang S, Jiang Z C, Feng J, et al. Inhibition of human gastric cancer cell migration and invasion by carmine radish Geranium [J]. *Food industry technology*, 2017, **38** (22): 1-4+10
- [11] Wang Y. Study on the anti-inflammatory effect of isoeugenol in vivo and in vitro [D]. *Shandong university*, 2016.
- [12] Li X T, Zhou W, Song Z C. Anti-inflammatory effects of psoralen and isopsoralen on human periodontal cells [J]. *Shanghai Jiaotong Univ. (Sci)*, 2018, **38** (2): 128-132.
- [13] Yang Y, Zheng K, Mei W, et al. Anti-inflammatory and proresolution activities of bergapten isolated from the roots of *Ficus hirta* Vahl. in an in vivo zebrafish model[J]. *Biochem. Biophys. Res. Commun.* 2018, **496** (2): 763-769.
- [14] Palacz-Wrobel M, Borkowska P, Paul-Samojedny M, et al. Effect of apigenin, kaempferol and resveratrol on the gene expression and protein secretion of tumor necrosis factor alpha (TNF- α) and interleukin-10 (IL-10) in RAW-264.7 macrophages[J]. *Retour Au Numéro*, 2017, **93**: 1205-1212.