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Identification of chemical constituents in cuscuta chinensis using HPLC-ESI/Q-TOF MS/MS

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ABSTRACT

AIM: To identify the chemical constituents in Cuscuta Chinensis. **METHODS:** A High-performance liquid chromatography (HPLC) method combined with quadrupole-time-of-flight-mass spectrometry (QTOF-MS) has been developed for precise separation and structural identification of components in Cuscuta Chinensis. Mass spectrometry was performed in reflective time-of-flight using electron spraying ionization in negative mode. Accurate mass measurements (less than 5 ppm error) for molecular ions and characteristic fragment ions could represent reliable identification criteria for these compounds. **RESULTS:** Combination of MS and the information provided by the part of the standard, and 22 components are identified, such as:quercetin-3-O-(2 "-O-α-rhamnose -6"-O-malonyl)-beta -D-glucoside; kaempferol-3-O-of β-the D-apiose - (1 → 2) - [-alpha-L-rhamnose - (1→6)]-beta-D-glucoside; 6-O-(trans) - (2 → 1) p-coumaroyl-β-D-fructofuranosyl-alpha-D-pyran-glucosidase; the kaempferol -7 - rhamnosidase; kaempferol-3-β -D-glucuronide;Apigenin;4-caffeoyl-5-coumaroylquinic acid; Kaemoferol-3-arabofuranoside; quercetin-3-O-beta-D-galactose - (2 →1)-of β-the D-celery glycoside;Dicaffeoylquinic acid; Hyperin;Quercitin;quercetin-3-glucuronide;Isorhamnetin;coumaroyl-dicaffeoylquinic acid; Chlorogenic acid; Kaemoferol-3, 7-O-diglucuronide; Quercetin;Quercltrin-2"-gallate; Quercetin-3, 7-α-L-dirhamnoside; Stigmasterol;Kaemoferol. **CONCLUSION**: It is for the first time to identify the estrogenic constituents in Cuscuta Chinensis by HPLC/MS. And this finding could be valuable for the further research of Cuscuta Chinensis.

KEYWORDS

Quadropole-time-of-flight-mass-spectrometry;
Cuscuta Chinensis;
Chemical constituents.

INTRODUCTION

Cuscuta Chinensis, the seed of *Cuscuta chinensis* Lam. (Convolvulaceae), have been widely used as Chinese herbal medicine to treat *yang*-deficiency in the kidney and liver for more than 2000 years from

FULL PAPER

ShenNongBenCaoJing, a famous ancient Chinese medicinal literary. Cuscuta Chinensis have multiple benefits to human health[1, 3]. With the deepening study of phytoestrogms, the research of Cuscuta chinensis are mostly concentrated on estrogen receptor gene[4, 7]. At present, Cuscuta chinensis is still limited by lack of knowledge on Active Components, thus hindering its clinical application.

Because of TOF-MS has several distinctive properties on top of regular MS, including fast acquisition rates, high accuracy in mass measurements and a large mass range, the combination of LC and ESI-TOF-MS allow us to obtain a powerful in the quantitative and qualitative analysis of molecules in complex matrices by reducing the matrix interferences. In this paper, chemical constituents of Cuscuta Chinensis were identified by HPLC-ESI/Q-TOF MS/MS.

METHODS

Instrumentation

Agilent 1100 HPLC system (including quaternary gradient pump, vacuum degasser, automatic sampling, temperature me, DAD detector); Agilent 6500 Series Quadrupole Time-of-Flight LC/MS (Q-TOF) system; Equipment operation and data processing system using chemical HPLC-3D workstations. Milli-Q ultrapure water for preparation. TDL80-2B Dove licensing centrifuge; vortex mixer XW-80A; Beckman Coulter Allegra 64R Centrifuge.

Drugs

Cuscuta chinensis were purchased from drug market. The plant materials were identified by Professor Delian Zhang (College of Pharmacy, Harbin University of Commerce, Harbin); Standard: Chlorogenic acid (110753-200413), Hyperin (111521-200303), Quercitin (111538-200504), Kaempferol (110861-200808), Quercetin (081-9003) were obtained from the National Institute for the Control of Phar-maceutical and Biological Products(Beijing, China); acetonitrile (chromatography pure, Shandong Yu Wang), methanol (chromatography pure, Shandong Yu Wang), formic acid (chromatography pure, Shanghai Career), ultra pure water.

Preparation of the test sample

The dried seeds of Cuscuta Chinensis (300 g) were extracted with 8 times of ethanol (95%) solution or water solution respectively under reflux for 3 h, thrice. The solution was filtered with paper. The filtrate was evaporated to dryness *in vacuo* and the ethanol and water extracts of Cuscuta Chinensis were obtained. Ethanol extract and water extract of Cuscuta Chinensis were diluted with water into the suitable concentration, respectively.

Preparation of standard solutions

Chlorogenic acid (3.0 mg), hyperoside (2.4 mg), quercitroside (3.6 mg), kaempferide (3.7 mg) and quercetin (5.5 mg) were dissolved in 80% methanol, and made up to 10 mL in a volumetric flask with 80% methanol. The sample solution was filtered through 0.45 μm organic membranes prior to use.

HPLC-MS/MS analysis

Chromatography was performed on a 4.6 mm i.d.×250 mm Waters Symmetry Shield™ RP 1.8 μm column (Waters Corp, Milford, USA) using an Agilent 1100 series HPLC system. The column was maintained at 30 °C, and subsequently, a gradient of 0.3% formic acid solution (solvent A), methanol (solvent B) and methyl (solvent C) was used as TABLE 1. 20 μL aliquot of each sample was injected onto the column. The eluent was detected at wavelength of 328 nm and introduced to the mass spectrometry directly, i.e. without a split.

TABLE 1 : Eluotropic procedure of HPLC system

Time	Methyl%	Methanol%	0.3%	Flow rate
0	12	0	88	0.3
40	16	0	84	0.3
60	20	0	80	0.3
80	0	45	55	0.4
105	0	50	50	0.4
120	0	68	32	0.4
125	12	0	88	0.3
135	12	0	88	0.3

The Mass spectrometry was carried out on an Agilent 6520 series Q-TOF equipped with Electrospray Ionization source (ESI), which data were acquired in positive and negative mode and conditions of MS analysis were as follows: drying gas (N_2) flow-rate, 10.0 L/min; drying gas temperature, 350 °C; nebulizing gas

(N_2) pressure, 25 psi; capillary voltage, 8000 V. To ensure low ppm mass accuracy, the mass spectrometric data was collected in full scan mode with a range of *m/z* from 100 to 1200.

RESULTS AND DISCUSSION

Identification of compounds in cuscuta chinensis by HPLC-TOF-MS /MS

The mass spectrometry-total ion current chromatograph were performed with both positive and negative ion mode. By comparison, a better total ion current chromatograph with high sensitivity were obtained in negative ion mode, so negative ion mode was more suitable for identification of compounds in serum samples.

Based on molecular ion peak and the first and second order fragment ion, compared with some reference substances, or the literature[8], the identification of the chemical components was established. 22 components were were identified with their retention times, fragment ions and MS data.(The results are shown in Figure 1 and TABLE 2).

Secondary ion mass spectrometry analysis of the 22 compounds was as follows: Peak 1 was Quercetin—3-O-, 2"-O-α-rhamnosy-6"-O-malony [- β - D-glucoside (MW696), *m/z* 695 [M- H]$^-$, m/z533 [M-H-glc]$^-$; Peak 2 was Kaemoferol-3-O-β-D-aplosyl-[1→2] -[-α-L-rhamnosy-[1'→6]-β-D-glucoside (MW276), *m/z* 275 [M- H]$^-$, m/z359 [M-H-glc-rha]$^-$;

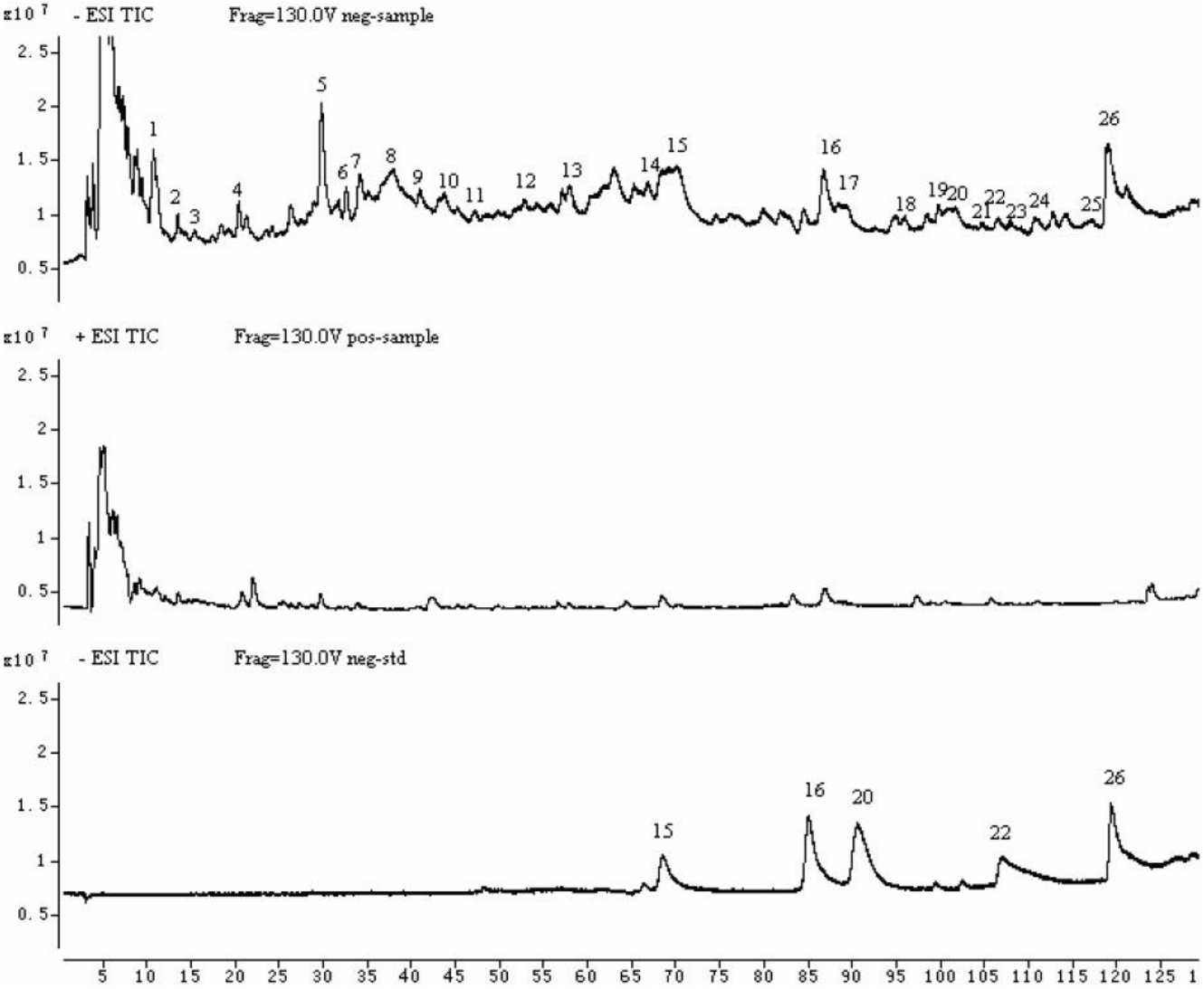

Figure 1 : the mass spectrometry-total ion current chromatograph in positive and negative ion mode for the aqueous extraction of Cuscuta chinensis Lam. and reference substances

FULL PAPER

TABLE 2 : Identification of Chemical composition of the peaks

Peak NO. of characteristic peaks	Retention time(min)	negative mode (m/z) Character fragmentation MS MS/MS	M.W.	Compound presumed
1	10.76	695.2134 533.1871	696	Quercetin--3-O-（2“-O-α-rhamnosy-6”-O-malony）-β-D-glucoside
2	13.46	725.2170 359.0983	726	Kaemoferol-3-O-β-D-aplosyl-（1? 2）-[-α-L-rhamnosy-（1? 6）]-β-D-glucoside
3	15.29	587 461 325	588	unknown
4	20.33	503 341	504	unknown
5	29.78	487.1678 341.0885 325.0930	488	6-O-(E)-P-coumaroyl)–β-D-fructofuranosyl-(2? 1)-α-D-glucopyranoside
6	32.53	431.1977 325.0935	432	Kaemoferol-7-rhamnosy
7	34.21	443 183 325	444	unknown
8	37.86	501 367 957	502	unknown
9	41.06	461.1102 182.9910	462	Kaemoferol-3-β-D-glucuronide
10	43.66	269.1399 182.9896 137.0266	270	Apigenin
11	47.17	499.1805 452.9227	500	4-caffeoyl-5-coumaroylquinic acid
12	52.81	417.1203 195.0520	418	Kaemoferol-3-arabofuranoside
13	57.84	595.1400 149.0461	596	Quercetin-3-O-β-D–apiofuranosyl -(1? 2)-β-D- galactoside
14	65.46	515.1417 211.0466	516	Dicaffeoylquinic acid
15	68.37	463.0982 353.1245	464	Hyperin
16	86.82	447.1073 895.1957	448	Quercitin
17	88.44	477.1070 353.0888	478	Querciturone
18	95.97	631.1687 316.9482	316	Isorhamnetin
19	99.79	661.1808 353.0884	662	coumaroyl- dicaffeoylquinic acid
20	101.16	353.0896 191.0560	354	Chlorogenic acid
21	104.67	609.1262 384.9358	610	Kaemoferol-3,7-O-diglucuronide
22	106.65	301.0361	302	Quercetin
23	107.87	599.2110 447.2425	600	Quercltrin-2”-gallate
24	110.77	593.1337 301.0356	594	Quercetin-3, 7-α-L-dirhamnoside
25	117.17	411.2005 397.2047	412	Stigmasterol
26	119.15	285.0642 571.0921	286	Kaemoferol

Peak 5 was 6-O-(E)-P-coumaroyl)–β-D-fructofuranosyl-(2’!1)-α-D-glucopyranoside (MW488), *m/z* 487 [M- H]$^-$, m/z341 [M-H-146]$^-$, m/z325 [M-H-146-16]$^-$; Peak 6 was Kaemoferol-7-rhamnosy (MW462), *m/z* 461 [M- H]$^-$ m/z325 [M-H-106]$^-$; Peak 9 was Kaemoferol-3-β-D-glucuronide (MW432), *m/z* 431 [M-H]$^-$; Peak 10 was Apigenin (MW500), *m/z* 499 [M- H]$^-$, m/z183 [M-H-86]; Peak 11 was 4-caffeoyl-5-coumaroylquinic acid (MW432), *m/z* 431 [M-H]$^-$, m/z453 [M-H-COOH]$^-$; Peak 12 was Kaemoferol-3-arabofuranoside (MW418), *m/z* 417 [M -H]$^-$, m/z195 [M-H-149-73]$^-$; Peak 13 was Quercetin-3-O-β-D–apiofuranosyl -(1→2)-β-D- galactoside (MW596), *m/z* 595 [M-H]$^-$; Peak 14 was Dicaffeoylquinic acid (MW516), *m/z* 515 [M-H]$^-$, the same as the literature[8]; Peak 15 was Hyperin (MW464), *m/z* 463 [M -H]$^-$, consistent with the standard control; Peak 16 was Quercitin (MW448), *m/z* 447 [M-H]$^-$ m/z895 [2M-H]$^-$, consistent with the standard control; Peak 17 was Querciturone (MW478),

m/z 477 [M - H]$^-$, m/z353 [M-H-124]$^-$; Peak 18 was Isorhamnetin (MW316), m/z631 [2M-H]$^-$, the same as the literature[8]; Peak 19 was coumaroyl-dicaffeoylquinic acid (MW662), *m/z* 661 [M-H]$^-$ the same as the literature[8]; Peak 20 was Chlorogenic acid (MW354), *m/z* 353 [M-H]$^-$, consistent with the standard control; Peak 21 was Kaemoferol-3, 7-O-diglucuronide (MW610), *m/z* 609 [M-H]$^-$; Peak 22 was Quercetin (MW302), *m/z* 301 [M-H]$^-$ consistent with the standard control; Peak 23 was Quercltrin-2''-gallate (MW600), *m/z* 599 [M - H]$^-$; Peak 24 was Quercetin-3, 7-α-L-dirhamnoside (MW594), *m/z* 593 [M " H]"; Peak 25 was Stigmasterol (MW412), *m/z* 411 [M - H]$^-$, m/z397 [M-H-CH_3]$^-$; Peak 26 was Kaemoferol (MW286), *m/z* 285 [M - H]$^-$, m/z571 [2M-H]$^-$, consistent with the standard control.

DISCUSSION

In the above research, an HPLC-ESI/Q-TOF MS/MS method was established for the identification of multiple chemical components in Cuscuta chinensis. 22 chemical constituents were identified. at the same time, there has certain guiding significance for the study of clinical pharmacology. Therefore, HPLC-ESI/MS can quickly achieve qualitative chemical composition of traditional Chinese medicine, and it also provides with a new way of thinking and the depth of Chinese medicine chemical composition and widely study.

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