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Identification and Characterization of Constituents in Si-Wu-Tang by Liquid Chromatography Connected with Time of Flight Mass Spectrometry and Ion Trap Mass Spectrometry

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A combination of high performance liquid chromatography time of flight mass spectrometry (HPLC-TOF/MS) and high performance liquid chromatography coupled with diode array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI-MS/MS) as a powerful method to profile and identify non-target components in traditional Chinese medicine (TCM) was developed. Si-Wu-Tang (SWT), a popular traditional Chinese medicine, was studied using the combinative method as for an application. Based on the mass spectra, UV spectra and retention time, 21 compounds were identified or tentatively characterized. This study represents the first detailed investigation of the components of Si-Wu-Tang with HPLC-TOF/MS technique. The identification and structural elucidation of the chemical constituents provided essential data for further quality control and pharmacological mechanism research of Si-Wu-Tang. With the introduction of the combinative HPLC-MS technique analytical system to traditional Chinese medicine, we expected that approach would be useful for the screening and characterization of non-target compounds in other famous traditional Chinese medicine.

Key Words: Constituent characterization, HPLC-DAD-ESI-MS/MS, HPLC-TOF/MS, Traditional Chinese medicine, Si-Wu-Tang.

INTRODUCTION

Si-Wu-Tang (SWT) is a traditional Chinese medicine (TCM) ancient formulae widely used for the treatment of women's disease in China and Asia (Japanese name, Shimotsu-to)[1]. The formula has been used for the the treatment of cutaneous pruritus, chronic inflammation[2], anaemia[3] and other diseases. A more recent study demonstrated that the SWT formula can be integrated as an alternative therapy within Western medicine[4]. Si-Wu-Tang is composed of four herbal medicines, Radix Angelica sinensis, Rhizoma Chuanxiong, Radix Paeoniae Alba and Radix Rehmanniae Preparata. Utilizing an HPLC-ESI-MS method to determine 12 compounds including phenolic acids, phthalides and terpene glycoside had been reported[5]. Meanwhile, essential oils and part of extracts from SWT were investigated[6,7]. Apparently, only qualitative analysis of these compounds is not sufficient for the comprehensive quality control and further study since SWT is a complex system. So a new method is required to extend the previous study.

High performance liquid chromatography time of flight mass spectrometry (HPLC-TOF/MS) has become a suitable technique for the accurate mass measurement and high full-scan analysis[8]. The combination of HPLC-TOF/MS and HPLC-DAD-ESI-MS/MS represents a novel and powerful method for the analysis of the complex systems[9]. This strategy has been applied in the analysis of herbs[10]. However, the application of this combinative technique on the complex traditional Chinese medicine was rarely reported.

In this work, an approach to identify the main constituents in SWT by combining HPLC-TOF/MS and HPLC-DAD-ESI-MS/MS would be described. The results proved that the established method could provide helpful chemical information for quality control and pharmacological mechanism research of SWT. Also, it is expected to be accepted as an effective and reliable pattern for qualitative analysis of non-target constituents in the traditional Chinese medicine systems.

EXPERIMENTAL

An Agilent 1200 series HPLC linked with a diode array detector (DAD) and a micrOTOF-Q II mass spectrometer (Bruker Daltonics, CA, USA) equipped with an ESI ion source was used to carry out the assay. For the MS/MS detection, a LCQ Advantage MAX instrument of thermo Finnigan (Thermo Electron Corporation, San Jose, CA, USA) was applied. Data were processed by Data Analysis 4.0.

Preparation of samples: Crude drugs controlled by Chinese Pharmacopoeia (Part I, 2010) were supplied by the Tianjin Tasly Pharmaceutical Co. Ltd (Tianjin, China). Powders of crude drugs compounded (1 g Radix Angelica sinensis, 1 g Rhizoma Chuanxiong, 0.8 g Radix Paeoniae Alba and 0.8 g Radix Rehmanniae Preparata) were boiled with 70 % alcohol on an electric heater for 1 h. The decoction was filtered under vacuum. The residue was re-extracted in the same way. The filtrates were evaporated to dryness at 55 ºC *in vacuo*. The evaporated residue was dissolved with 70 % methanol into a volumetric flask. The final volume of the extracting solution was set to 50 mL. Reference standards were also dissolved with 70 % methanol. Prior to use all samples were filtered through a 0.22 μm membrane filters.

Instrumentation and chromatographic condition: The chromatographic separation was carried out on a Phenomenex C_{18} column (250 mm × 4.6 mm, 5 μm). The column temperature was maintained at 30 ºC. The mobile phase consisted of water-formic acid (100:0.1, v/v) (A) and acetonitrile (B) with gradient elution. The DAD detector scanned from 200-600 nm and the samples were detected at 280 nm. The flow rate was 1.0 mL/min and the sample volume injected was 10 μL. The HPLC eluent was introduced into mass spectrometer in a postcolumn splitting ratio of 1:1. The TOF/MS analysis was performed in both negative and positive ion mode using full scan mode and the mass range was set at 100-1200 Da. The conditions of the ESI source were: drying gas (N_2) flow rate, 6.0 L/min; drying gas temperature, 180 ºC; nebulizer, 0.8 Bar; collision cell RF, 200 Vpp; capillary, 3500 V. The fragment ions were obtained using collision energy of 45 eV for MS/MS experiments.

RESULTS AND DISCUSSION

Qualitative identification of multi-components: The total ion chromatograms (TIC) of SWT obtained by HPLC-TOF/MS in both negative and positive ion mode were presented in Fig. 1 (a) and (b). The compounds could be classified into three groups (phenolic acids, terpene glycosides and phthalides) according to their chemical structures and the exact identification of each group was outlined below. Their structure was provided in Fig. 2.

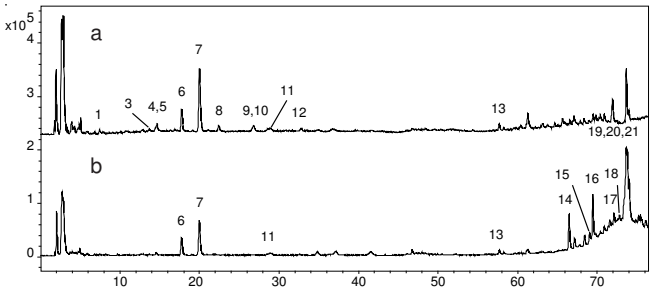

Fig. 1. HPLC-TOF/MS total ion chromatogram (TIC) of SWT in negative (a), positive (b) ion mode

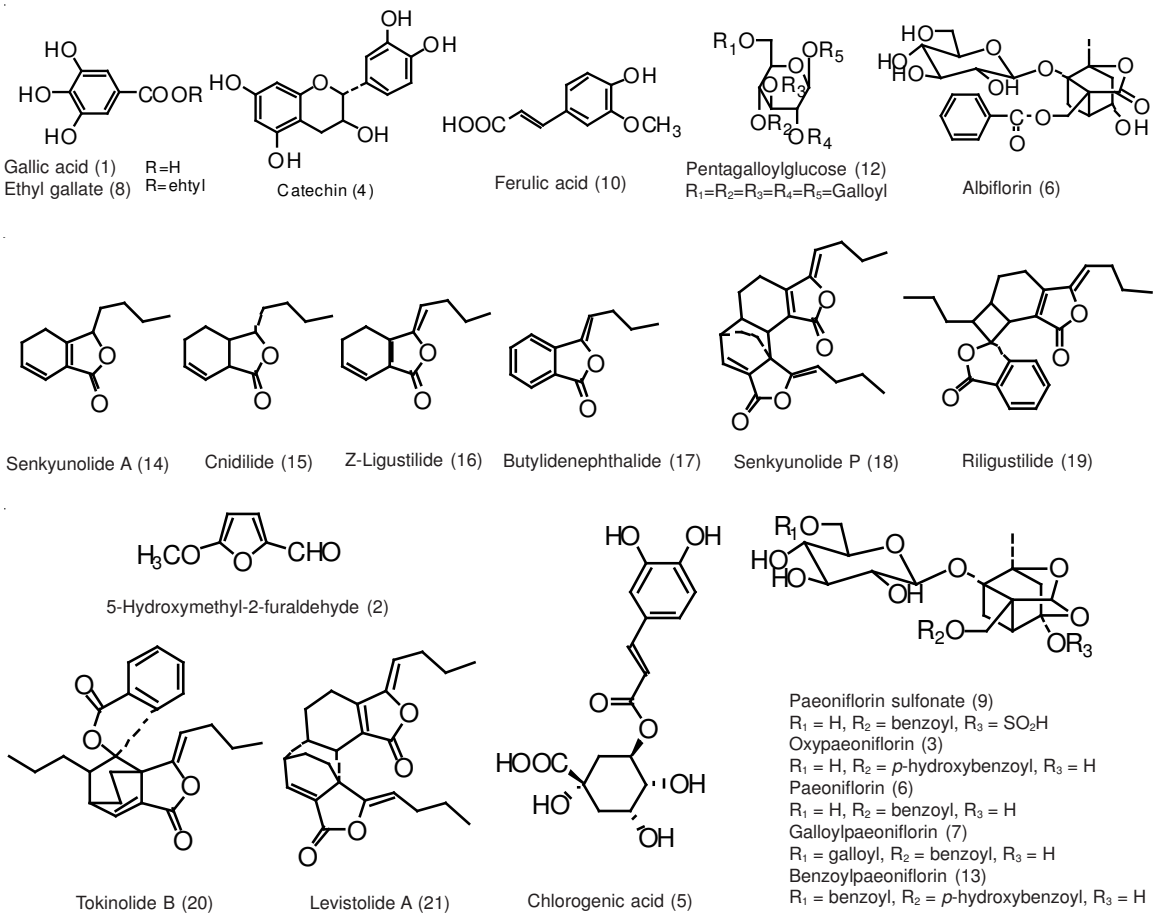

Fig. 2. Chemical structures of the constituents identified from Si-Wu-Tang

Phenolic acids: Seven organic acids (compounds **1**, **2**, **4**, **5** and **10** were unambiguously identified by comparing with the standards) were identified in SWT, including gallic acid (**1**), 5-hydroxymethyl-2-furaldehyde (**2**), catechin (**4**), chlorogenic acid (**5**), ethyl gallate (**8**), ferulic acid (**10**) and pentagalloylglucose (**12**). The characteristic fragment ions of compounds **1**, **4** and **8** were produced by the loss of small groups from the compounds as CO_2, H_2O and CO. Peak 5 gave an accurate mass (m/z 353.0863, Table-1) determined as an elemental composition $C_{16}H_{17}O_9$, which derived from Rhizoma Chuanxiong. By comparison with authentic standard, it was identified as chlorogenic acid. Product ion 169 $[M-H-CO]^-$ was observed in the MS/MS spectrum of precursor ion 197$[M-H]^-$ (Table-1). Compound **8** was tentatively identified as ethyl gallate compared with literature data[11]. Compound **12** displayed successive losses of one or two gallic acid group (170 Da) suggesting that it might be several gallic acid units in its structures. Compared with the literature about Radix

TABLE-1
IDENTIFICATION RESULTS OF SWT BY HPLC-DAD-ESI-MS/MS

Peak No.	t_R (min)	Measured mass (m/z)	Formula	λ_{max} (nm)	Characteristic ion fragments[b] (m/z)		Identification	Crude drug[d]
					MS	MS/MS[c]		
1[a]	6.1	169.0136	$C_7H_6O_5$	216, 268	169$[M-H]^-$	125$[M-H-CO_2]^-$	Gallic acid	3
2[a]	8.7	149.0215	$C_6H_6O_3$	285	149$[M+Na]^+$	–	5-Hydroxymethyl-2-furaldehyde	4
3	13.6	495.1497	$C_{23}H_{28}O_{12}$	260	495$[M-H]^-$	451$[M-H-CO_2]^-$, 333$[M-H-Glc]^-$	Oxypaeoniflorin	3
4[a]	14.1	289.0706	$C_{15}H_{14}O_6$	280	289$[M-H]^-$	271$[M-H-H_2O]^-$, 245$[M-H-C_2H_4O]^-$	Catechin	3
5[a]	14.1	353.0863	$C_{16}H_{18}O_9$	240, 325	353$[M-H]^-$	191[M-H-caffeoyl]$^-$, 179$[CA-H]^-$	Chlorogenic acid	2
6[a]	17.4	479.1548	$C_{23}H_{28}O_{11}$	235, 275	503$[M+Na]^+$	381$[M+Na-BA]^+$, 341$[M+Na-Glc]^+$	Albiflorin	3
					525$[M+HCOO]^-$	479$[M-H]^-$, 357$[M-H-BA]^-$ 283$[BA+Glc-H]^-$		
7[a]	19.7	479.1550	$C_{23}H_{28}O_{11}$	235, 275	503$[M+Na]^+$	–	Paeoniflorin	3
					525$[M+HCOO]^-$	479$[M-H]^-$, 449$[M-H-HCOH]^-$ 327$[M-H-HCOH-BA]^-$		
8	21.5	197.0451	$C_9H_{10}O_5$	270	197$[M-H]^-$	169$[M-H-CO]^-$	Ethyl gallate	3
9	25.8	543.1161	$C_{23}H_{28}O_{13}S$	235, 278	543$[M-H]^-$	497[M-H-HCOOH]$^-$, 421$[M-H-BA]^-$ 375[M-H-HCOOH-BA]$^-$ 259[M-H-BA-Glc]$^-$	Paeoniflorin sulfonate	3
10[a]	25.4	193.0501	$C_{10}H_{10}O_4$	320	–	–	Ferulic acid	1, 2
11	28.4	631.1641	$C_{30}H_{32}O_{15}$	224, 280	633$[M+H]^+$	–	Galloylpaeoniflorin	3
					631$[M-H]^-$	613$[M-H-H_2O]^-$, 491$[M-H-BA-H_2O]^-$ 313[GA+Glc-H-H_2O]$^-$, 169$[GA-H]^-$		
12	32.1	939.1075	$C_{41}H_{32}O_{26}$	225, 280	939$[M-H]^-$	769$[M-H-GA]^-$, 617[M-H+H_2O-2GA]$^-$	Pentagalloylglucose	3
13	57.4	583.1804	$C_{30}H_{32}O_{12}$	240	585$[M+H]^+$	463$[M+H-BA]^+$, 319[M+H-BG]$^+$ 267[M+H-aglycone-BA]$^+$ 197[M+H-BA-BG]$^+$	Benzoylpaeoniflorin	3
					629$[M+HCOO]^-$	583$[M-H]^-$, 553[M-H-HCHO]$^-$		
14[a]	65.0	193.1216	$C_{12}H_{16}O_2$	280	193$[M+H]^+$	175$[M+H-H_2O]^+$, 147[M+H-H_2O-CO]$^+$	Senkyunolide A	1, 2
15	66.9	195.1382	$C_{12}H_{18}O_2$	–	195$[M+H]^+$	177$[M+H–H_2O]^+$, 149[M+H-H_2O-CO]$^+$ 125$[M+H-C_5H_{10}]^+$	Cnidilide	2
16[a]	68.0	191.1073	$C_{12}H_{14}O_2$	280, 325	191$[M+H]^+$	173$[M+H-H_2O]^+$, 163[M+H-CO]$^+$ 155$[M+H-2H_2O]^+$, 145[M+H-H_2O-CO]$^+$	Z-Ligustilide	1, 2
17	71.0	189.0903	$C_{12}H_{12}O_2$	265, 320	189$[M+H]^+$	128[M+H-H_2O-CO-CH_3]$^+$, 115[M+H-H_2O-CO-C_2H_4]$^+$	Butylidenephthalide	1, 2
18	71.4	383.2221	$C_{24}H_{30}O_4$	230, 278	383$[M+H]^+$	355$[M+H-CO]^+$, 191$[C_{12}H_{15}O_2]^+$	Senkyunolide P	1, 2
19	72.4	381.2041	$C_{24}H_{28}O_4$	280	381$[M+H]^+$	363$[M+H-H_2O]^+$, 191$[C_{12}H_{15}O_2]^+$	Riligustilide	1, 2
20	72.5	381.2057	$C_{24}H_{28}O_4$	278	381$[M+H]^+$	191$[C_{12}H_{15}O_2]^+$	Tokinolide B	1, 2
21[a]	72.7	381.2059	$C_{24}H_{28}O_4$	275	381$[M+H]^+$	191$[C_{12}H_{15}O_2]^+$	Levistolide A	1, 2

[a]Compared with reference compounds. [b]'–' in the 'positive ions' or 'negative ions' column means no mass spectrum signals. [c]BA = benzoic acid, Glc = glucose, CA = caffeic acid, GA = gallic acid, BG = glucosyl group with benzoate group on C-6. [d]Crude drug: 1. Radix Angelica sinensis, 2. Rhizoma Chuanxiong, 3. Radix Paeoniae Alba, 4. Radix Rehmanniae Preparata.

Paeoniae Alba[12], it was regarded as pentagalloylglucose. However, ferulic acid (**10**), which was derived from Radix Angelica sinensis and Rhizoma Chuanxiong was identified by comparing with reference standard, showed good UV responses but no signals in MS/MS spectrum. 5-Hydroxymethyl-2-furaldehyde (**2**) showed similar character.

Terpene glycosides: Six terpene glycosides were identified in SWT, including oxypaeoniflorin (**3**), albiflorin (**6**), paeoniflorin (**7**), paeoniflorin sulfonate (**9**), galloylpaeoniflorin (**11**) and benzoylpaeoniflorin (**13**). The characteristic ions of terpene glycosides were mainly formed by the losses of benzoic acid (BA) and glucose (Glc)[13]. Compared with literature data[14], compound **9** was identified as paeoniflorin sulfonate. Fragmentation in the negative mode MS/MS spectrum focused on the precursor ion $[M + H]^-$ (m/z 543.1161, formula $C_{23}H_{28}O_{13}S$) showed peaks corresponding to the successive loss of a HCOOH (m/z 497, $[M-H-HCOOH]^-$), a benzoic acid (m/z 421, $[M-H-BA]^-$ and m/z 375, $[M-H-HCOOH-BA]^-$) and a glucose group (m/z 259, $[M-H-BA-Glc]^-$).

Phthalides: Quasi-molecule ions $[M + H]^+$ were found for phthalides in SWT, including senkyunolide A (**14**), cnidilide (**15**), Z-ligustilide (**16**), butylidenephthalide (**17**), senkyunolide P (**18**), riligustilide (**19**), tokinolide B (**20**) and levistolide A (**21**), whose fragments were usually generated by the losses of H_2O (18 Da), CO (28 Da) and their combination. The structures of compounds **14** and **16** were identified as senkyunolide A and Z-ligustilide compared with authentic standard. The tandem mass spectrometry fragmentation behaviours of compounds **15**, **17** and **18** reported previously were referred to in this study[13]. Compound **19**, **20** and **21** shared similar mass spectra ions $[M + H]^+$ at m/z 381.2060 and the probable formula of $C_{24}H_{28}O_4$ as well as gave the product ion at m/z 191, suggesting that they were phthalide dimmers with the same molecular mass of 380 Da. By comparison with authentic standard, compound **21** was identified as levistolide A and the other two were tentatively assigned to be riligustilide and tokinolide B by the comparison with reported data[14].

Conclusion

In this study, a reliable and powerful analytical method by using a combination of HPLC-TOF/MS and HPLC-DAD-ESI-MS/MS for rapid screening and identification of non-target multi-components in Si-Wu-Tang (SWT) was established. The extracts were separated on a C_{18}-HPLC column using gradient elution. As a result, total 21 compounds from SWT were simultaneously identified based on retention time, UV and MS spectra compared with those of authentic compounds or literature data. Their accurate molecular masses as well as their fragmentation patterns were determined, which provided essential data for quality control and pharmacological mechanism research.

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REFERENCES

1. Q. Hao, J. Wang, J. Niu, P. Zhao and Y. Cui, *Acta Chin. Med. Pharm*., **36**, 10 (2008).
2. Y. Dai, P.PH. But, Y.P. Chan, H. Matsuda and M. Kubo, *Biol. Pharm. Bull*., **25**, 1175 (2002).
3. Q.D. Liang, Y. Gao, H.L. Tan, P. Gou, Y.F. Li, Z. Zhou, W. Tan, Z.C. Ma, B.P. Ma and S.Q. Wang, *Biol. Pharm. Bull*., **29**, 1378 (2006).
4. J.F. Cheng, Z.Y. Lu, Y.C. Su, L.C. Chang and R.Y. Wang, *J. Clin. Nurs*., **17**, 2588 (2008).
5. H.J. Zhang, P. Shen and Y.Y. Cheng, *J. Pharm. Biomed. Anal*., **34**, 705 (2004).
6. Y.P. Tang, M. Zhu, S. Yu, Y.Q. Hua, J.A. Duan, S.L. Su, X. Zhang, Y. Lu and A.W. Ding, *Molecules*, **15**, 341 (2010).
7. H.P. Zeng, T.T. Wang, W. Chen, C.Y. Wang, D.F. Chen and J.G. Shen, *Bioorg. Med. Chem*., **16**, 5109 (2008).
8. J.L. Zhou, L.W. Qi and P. Li, *J. Chromatogr. A*, **1216**, 7582 (2009).
9. M. Yang, J.H. Sun, Z.Q. Lu, G.T. Chen, S.H. Guan, X. Liu, B.H. Jiang, M. Ye and D.A. Guo, *J. Chromatogr. A*, **1216**, 2045 (2009).
10. Y.Y. Zhang, Q. Wang, L.W. Qi, X.Y. Qin and M.J. Qin, *J. Pharm. Biomed. Anal*., **56**, 304 (2011).
11. Q. Tan, W.H. Wang, P. Song, L.M. Chen, J.J. Zhu, Z.M. Wang and J.C. Cui, *China J. Chin. Mater. Med*., **36**, 687 (2011).
12. L. Yang, S.J. Xu, R.T. Tian, P.S. Xie and Z.T. Wang, *Acta Pharm. Sin*., 42, 71 (2007).
13. L.L. Chen, J. Qi, Y.X. Chang, D.N. Zhu and B.Y. Yu, *J. Pharm. Biomed. Anal*., **50**, 127 (2009).
14. T. Yi, K.S. Leung, G.H. Lu, K. Chan and H. Zhang, *Chem. Pharm. Bull*., **54**, 255 (2006).