Asian Journal of Chemistry; Vol. 25, No. 14 (2013), 7840-7842

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14652

Identification and Characterization of Anthraquinones in *Cassia tora* L. by Liquid Chromatography Connected with Time of Flight Mass Spectrometry and Ion Trap Mass Spectrometry

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(*Received*: 8 October 2012; *Accepted*: 20 July 2013) AJC-13822

A combination of HPLC-TOF/MS and HPLC-DAD-ESI-MS/MS as a powerful method to profile and identify multi-components in herb medicine was developed. *Cassia tora* L., a popular traditional Chinese medicine, was studied using the combinative method as an application. Based on the mass spectra, UV spectra and retention time, 13 anthraquinones were identified or tentatively characterized. It is the first time that the systematic analysis of the anthraquinones compounds, the main bioactive components, derived from *Cassia tora* L. was provided. This identification and structural elucidation of the chemical constituents provided essential data for further quality control and pharmacological mechanism research. 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 compounds in other herb medicines.

Key Words: Anthraquinones, *Cassia tora* L., HPLC-DAD-ESI-MS/MS, HPLC-TOF/MS.

INTRODUCTION

Cassia tora L., a worldwide herbal medicine listed in the Chinese Pharmacopoeia[1], is a small shrub growing as common weed in Asian countries. Because of the favourite flavor, most of *Cassia tora* L. was conventionally consumed as a healthy tea beverage in China[2]. The bioactive constituents of this herb are anthraquinones, which have medical value as an antimicrobial, antidiuretic, antidiarrhoeal, antioxidant, antimutagenic and hypolipidemic[3,4]. Utilizing an HPLC-MS method to determine five anthraquinones had been reported[5]. However, there have been few studies dealing with the systematic analysis of anthraquinones in *Cassia tora* L.. And the accurate mass data and structural features of anthraquinones have not been investigated. 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[6]. The combination of HPLC-TOF/MS and high performance liquid chromatography coupled with diode array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI-MS/MS) represents a novel and powerful method for the analysis of the herbal medicine[7]. In this study, full-scale qualitative information of anthraquinones from *Cassia tora* L. acquired by combinative HPLC-MS technique was provided, which would be a valuable reference for the further study and development of this herb and its related products.

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.

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 (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 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 experiments[2].

Extract preparation: *Cassia tora* L. controlled by Chinese Pharmacopoeia (Part I, 2010) was supplied by Tianjin Tasly Pharmaceutical Co. Ltd. (Tianjin, China). Weigh 0.5 g of the herb into 10 mL volumetric flask. The weighted powder was suspended in methanol-water (25:75, v/v) and extracted in an ultrasonic water bath for 0.5 h and then made up to volume.

RESULTS AND DISCUSSION

Most of the anthraquinones compounds gave abundant signal response in both positive and negative ion mode, including eight phenolic compounds (**6-13**) and five phenolic glycosides (**1-5**). Adduct ions $[M + Na]^+$ and $[M + HCOO]^-$ could be observed in their MS spectra besides $[M + H]^+$ and $[M-H]^-$. Compound **6**, **9**, **11-13** were deduced by comparing their retention time and MS spectra with authentic compounds while other anthraquinones comparing with literature date[4,8-10]. Eight phenolic compounds showed similar fragmentation patterns such as losses of CH_3 (15 Da), H_2O (18 Da), CO (28 Da) and their combination. The ions at m/z 316 and 298 in positive mode were recorded in the mass spectrum of compound **6** (m/z 331.0796, formula $C_{17}H_{14}O_7$), which were assumed to be a fragment derived from the molecular ion resulting in $[M + H\text{-}CH_3]^+$ and $[M + H\text{-}CH_3\text{-}H_2O]^+$, respectively. The ion at m/z 314 obtained in the mass spectrum of negative mode was due to the loss of a methyl group leading to $[M\text{-}CH_3]^-$. Compared with reference standard, compound **6** was identified as aurantio-obtusin. Product ions of five phenolic glycosides demonstrated the presence of a glucose group linked to the anthraquinones skeleton. Fragmentation in the positive mode MS/MS spectrum focused on the compound **4** precursor ion $[M + H]^+$ (m/z 619.1602, formula $C_{27}H_{32}O_{15}$) showed peaks corresponding to the successive loss of glucose groups (m/z 435, $[M + H\text{-}162]^+$ and m/z 273, $[M + H\text{-}162\text{-}162]^+$). The total ion chromatograms obtained by HPLC-TOF/MS in both negative and positive ion mode were presented in Fig. 1(a-b). The detail structure information was listed in Table-1 and the anthraquinones structures were provided in Fig. 2.

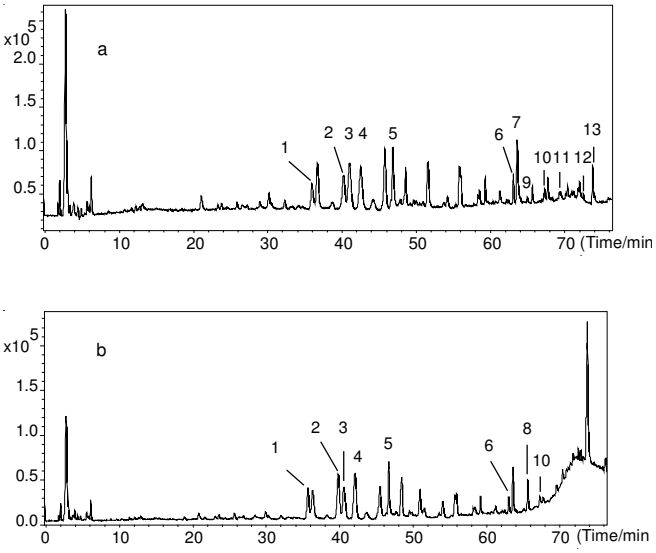

Fig. 1. HPLC-TOF/MS total ion chromatogram of *Cassia tora* L. in negative (a) and positive (b) ion mode

ACKNOWLEDGEMENTS

The project was supported by National Key Subject of Drug Innovation (Grant No. 2013ZX09402-202).

TABLE-1
IDENTIFICATION OF ANTHRAQUINONES BY HPLC-TOF/MS AND HPLC-DAD-ESI-MS/MS

Peak No.	Measured mass (m/z)	Formula	λ_{max} (nm)	Characteristic ion fragments[b] (m/z)		Identification
				MS	MS/MS[c]	
1	943.2657	$C_{39}H_{52}O_{25}$	275, 395	943$[M+Na]^+$ 965$[M+HCOO]^-$	925$[M+Na\text{-}H_2O]^+$, 671$[Aglycone+H]^+$ –	Cassiaside B_2
2	943.2648	$C_{39}H_{52}O_{25}$	280, 388	943$[M+Na]^+$ 965$[M+HCOO]^-$	925$[M+Na\text{-}H_2O]^+$, 671$[Aglycone+H]^+$ –	Cassiaside C_2
3	421.1119	$C_{20}H_{20}O_{10}$	258, 275	421$[M+H]^+$ 419$[M-H]^-$	259$[M+H\text{-}Glc]^+$ –	Cassiaside
4	619.1602	$C_{27}H_{32}O_{15}$	280	597$[M+H]^+$, 619$[M+Na]^+$ 595$[M-H]^-$, 641$[M+HCOO]^-$	435$[M+H\text{-}Glc]^+$, 273$[M+H\text{-}2Glc]^+$ –	Rubrofusarin-6-O-β-D-gentiobioside
5	619.1605	$C_{27}H_{32}O_{15}$	275	597$[M+H]^+$, 619$[M+Na]^+$ 595$[M-H]^-$, 641$[M+HCOO]^-$	435$[M+H\text{-}Glc]^+$ –	CassiasideC
6[a]	331.0796	$C_{17}H_{14}O_7$	230, 285	331$[M+H]^+$ 329$[M-H]^-$	316$[M+H\text{-}CH_3]^+$, 298$[M+H\text{-}CH_3\text{-}H_2O]^+$ 314$[M\text{-}H\text{-}CH_3]^-$	Aurantio-obtusin
7	315.0501	$C_{16}H_{12}O_7$	235, 285, 320, 430	315$[M-H]^-$	–	1-Desmethylaurantio-obtusin
8	359.1105	$C_{19}H_{18}O_7$	230, 285, 360	359$[M+H]^+$	344$[M+H\text{-}CH_3]^+$, 326$[M+H\text{-}CH_3\text{-}H_2O]^+$	Chryso-obtusin
9[a]	283.0252	$C_{15}H_8O_6$	258, 431	283$[M-H]^-$	239$[M\text{-}COOH]^-$, 211$[M\text{-}COOH\text{-}CO]^-$	Rhein
10	345.0960	$C_{18}H_{16}O_7$	240, 285, 315	345$[M+H]^+$ 343$[M-H]^-$	330$[M+H\text{-}CH_3]^+$, 312$[M+H\text{-}CH_3\text{-}H_2O]^+$ 328$[M\text{-}H\text{-}CH_3]^-$	Obtusin
11[a]	269.0462	$C_{15}H_{10}O_5$	285, 325, 440	269$[M-H]^-$	241$[M\text{-}H\text{-}CO]^-$	Emodin
12[a]	253.0502	$C_{15}H_{10}O_4$	285, 430	253$[M-H]^-$	225$[M\text{-}H\text{-}CO]^-$	Chrysophanol
13[a]	283.0603	$C_{16}H_{12}O_5$	286,433	283$[M-H]^-$	240$[M\text{-}H\text{-}CH_3\text{-}CO]^-$	Physcion

[a]Compared with reference compounds. [b]'–' in the 'Characteristic ion fragments' means no mass spectrum signals. [c]Glc = glucose.

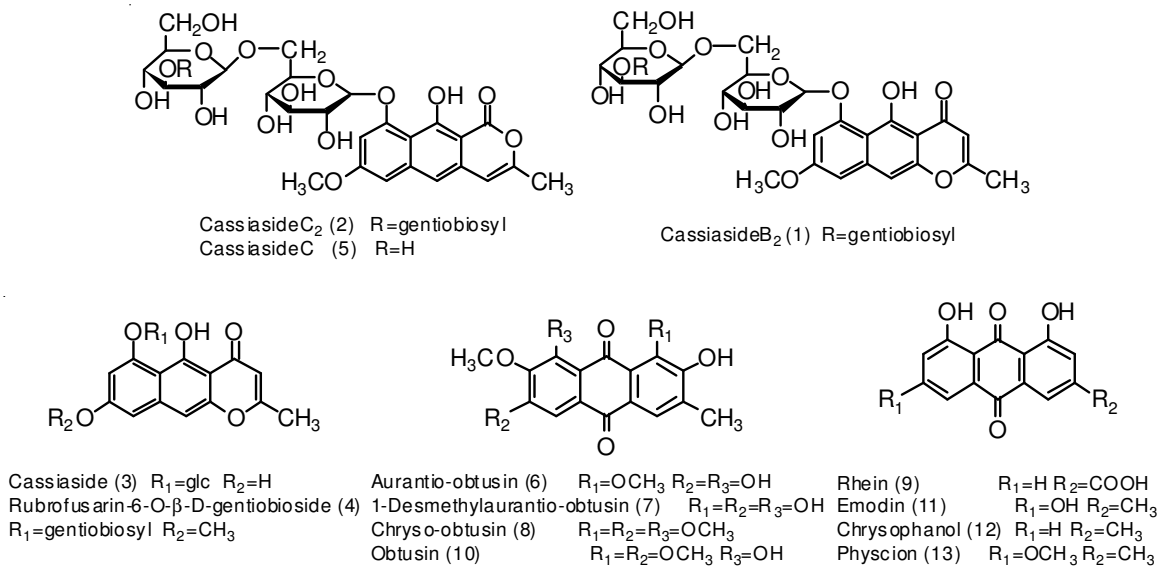

Fig. 2. Chemical structures of anthraquinones

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