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Characterization of flavonoids in the extract of *Sophora flavescens* Ait. by high-performance liquid chromatography coupled with diode-array detector and electrospray ionization mass spectrometry

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Abstract

A method coupling high-performance liquid chromatography (HPLC) with diode-array detector (DAD) and electrospray ionization mass spectrometry (ESI) was established for the separation and characterization of flavonoids in *Sophora flavescens* Ait. Based on the chromatographic separation of most flavonoids present in *S. flavescens* Ait., a total of 24 flavonoids were identified. Fourteen compounds were unambiguously identified comparing experimental data for retention time (t_R), UV and MS spectra with those of the authentic compounds: 3′,7-dihydroxy-4′-methoxy-isoflavone (**13**), trifolirhizin (**14**), kurarinol (**18**), formononetin (**19**), 7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**22**), maackiain (**21**), isoxanthohumol (**23**), kuraridine (**26**), kuraridinol (**27**), sophoraflavanone G (**30**), xanthohumol (**31**), isokurarinone (**33**), kurarinone (**35**) and kushenol D (**38**), and additional 10 compounds were tentatively identified as kushenol O (**10**), trifolirhizin-6″-malonate (**15**), sophoraisoflavanone A (**20**), norkurarinol/kosamol Q (**24**), kushenol I/N (**25**), kushenol C (**28**), 2′-methoxykurarinone (**29**), kosamol R (**32**), kushecarpin A (**34**) and kushenol A (**37**) by comparing experimental data for UV and MS spectra with those of literature. Furthermore, fragmentation pathways in positive ions mode of 24 flavonoid compounds of types of flavanone, flavanonol, flavonol, chalcone, isoflavone, isoflavanone and ptercocarpane were summarized. Some common features, such as $CH_3\cdot$, H_2O, CO, CO_2, C_3O_2 and C_2H_2O losses, together with Retro-Diels–Alder fragmentations were observed in the prenylated flavonoids in *S. flavescens* Ait. The loss of the lanandulyl chain was their characteristic fragmentation, which might help deducing the structure of unknown flavonoid compounds. The present study provided an approach to rapidly characterize bioactive constituents in *S. flavescens* Ait.

Keywords: HPLC-DAD-ESI/MS^2; Flavonoids; Characterization; *Sophora flavescens* Ait

1. Introduction

Traditional Chinese medicines (TCMs) and their preparations have been extensively used to prevent and cure human disease for thousands of years in many oriental countries. Because of its low toxicity and effective therapeutical performance, TCMs have attracted considerable attention in many fields [1]. But, the quantity and quality of safety and efficacy data on the herbal medicines are far from sufficiency to satisfy the criteria needed to support its use world-wide [2–4]. The main reasons lie in the lack of the reliable and acceptable strategy for the quality evaluation of TCMs. In general, a few markers or pharmacological active components are employed for evaluating the quality and authenticity of TCMs. This determination, however, does not give a complete picture of herbal products, because multiple constituents may work 'synergistically' and be responsible for its therapeutic effects. Consequently, the fingerprint technique, which could offer integral characterization of a complex system with a quantitative degree of reliability, has been widely applied for studying the consistency of herbal products [5–9]. Never-

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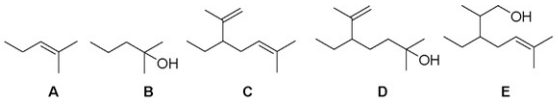
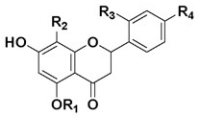
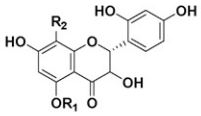


Flavanone



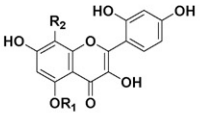
No.	Compound	R_1	R_2	R_3	R_4
18	Kurarinol	Me	D	OH	OH
22	7,4'-Dihydroxy-5-methoxy-8-(γ γ-dimethylallyl)-flavanone	Me	A	H	OH
23	Isoxanthohumol	Me	A	H	OH
24	Norkurarinol / Kosamol Q	H	D / E	OH	OH
29	2'-Methoxykurarinone	Me	C	OMe	OH
30	Sophoraflavanone G	H	C	OH	OH
32	Kosamol R	Me	C	OH	H
33	Isokurarinone	H	C	OMe	OH
35	Kurarinone	Me	C	OH	OH
37	Kushenol A	H	C	OH	H

Flavanonol



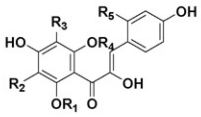
No.	Compound	R_1	R_2
25	Kushenol I /N	Me	C

Flavonol



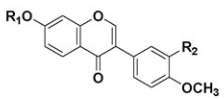
No.	Compound	R_1	R_3
28	Kushenol C	H	C

Chalcone



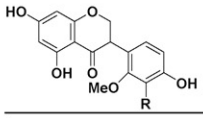
No.	Compound	R_1	R_2	R_3	R_4	R_5
26	Kuraridine	H	C	H	Me	OH
27	Kuraridinol	Me	H	D	H	OH
31	Xanthohumol	Me	H	A	H	H
38	Kushenol D	Me	H	C	H	OMe

Isoflavone



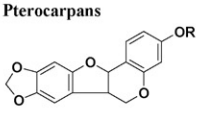
No.	Compound	R_1	R_2
10/11	Kushenol O	β-D-xyl 16 β-D-glu	H
13	3',7-Dihydroxy-4'-methoxy isoflavone	H	OH
19	Formononetin	H	H

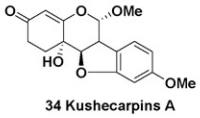
Isoflavanone



No.	Compound	R
20	Sophoraisoflavanone A	A

Pterocarpans





34 Kushecarpins A

No.	Compound	R
14	Trifolirhizin	-β-D-Glu
15	Trifolirhizin6''-malonate	-6-O-Malonyl-β-D-glu
21	Maachiain	H

Fig. 1. Structure of flavonoids identified in *Sophora flavescens* Ait.

theless, this strategy could only reflect the unitary character of chemical ingredients lacking for the structure information about the constituents of TCMs.

Sophorae Radix (Kushen in Chinese), the dried root of *Sophora flavescens* Ait. (Leguminosae), is a well known TCM widely spread in China, Japan and Korea [10]. It has been applied frequently in traditional medicines as an antipyretic, diuretic, anthelmintic and atomachic [11,12], and for the treatment of diarrhea, gastrointestinal hemorrhage and eczema [13]. Phytochemical studies revealed that it mainly contains quinolizidine alkaloids and prenylated flavonoids [14–19]. During the past work, pharmacological and clinical research was focused on its alkaloids. Nevertheless, it has been proved that the existence of lavandulyl radical could intensify the biologic activity of flavonoids [20–22]. Prenylated flavonoids from the roots of *S. flavescens* have a variety of biological and chemical activities, and have the meaning of the comprehensive evaluation of its quality. Thus, in order to more precise control the quality of *S. flavescens*, on the basis of its quantitative and qualitative analysis, a reliable method for identification of the major constituents in *S. flavescens* is indispensable. In our previous paper [23], we applied two chromatographic conditions to establish chromatographic fingerprints of alkaloids and flavonoids constituents of *S. flavescens*, respectively, and main alkaloids were identified by comparing their retention time (t_R) values with those of authentic standards. However, characterization of the flavonoids in *S. flavescens* is proposed to be a challenge for the difficulty of obtaining authentic standards. Hence, a simple and rapid analytical method for characterization and identification of the flavonoids in *S. flavescens* Ait. is of enormous interest.

During the past decade, HPLC and coupled techniques, especially DAD and MS, have been proved to be a powerful approach for the rapid identification of the constituents in natural materials because DAD and MS as a sensitive detector could provide abundant structural information and thus facilitate the structural identification of unknown compounds. A number of papers concerned applying LC-ESI/MS analyses of constituents in TCMs and its formulae have been reported [24–30]. In this paper, we described a method on identifying flavonoids in *S. flavescens*. Using an HPLC-DAD-ESI-MS/MS technique, a total of 24 flavonoids (listed in Fig. 1) including 10 flavanones, 1 flavananol, 1 flavonol, 4 chalcones, 3 isoflavones, 1 isoflavanone and 4 pterocarpans were identified or tentatively characterized. The MS, MS^2, and UV data obtained were applied to produce a library that allowed the complete identification of characteristic peaks in chromatographic fingerprint of *S. flavescens*.

2. Experiment

2.1. Chemicals and materials

Sophorae Radix samples purchased from Tian-yi-tang Pharmaceutical Co. (Shenyang, China) was identified by Professor Qi-Shi Sun in Shenyang Pharmaceutical University. The voucher specimens were deposited in the Department of Pharmaceutical Analysis, Shenyang Pharmaceutical University, Shenyang, China. The air-dried samples were smashed into powder and stored in exsiccator.

Kurarinol, kuraridin, kuraridinol, kushenol D, isoxanthohumol, kurarinone and isokurarinone were kindly supplied by the Prof. Chul-Sa Kim in Biology Resource Department of Koti Industry University (Japan). Xanthohumol were purchased from J&K Chemical Ltd. (Beijing, China). 3′,7-

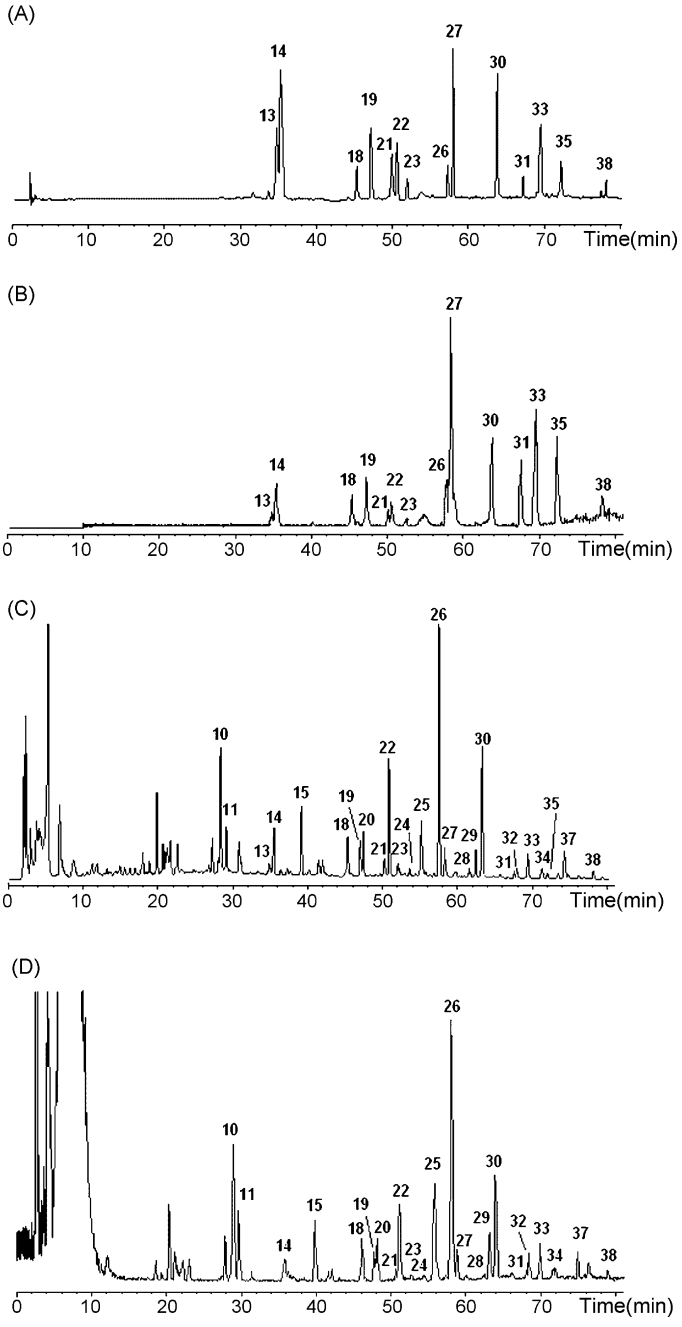


Fig. 2. Simultaneous HPLC-UV and HPLC-ESI-MS chromatograms of flavonoids standard compounds and *S. flavescens* Ait. extracts: (A) HPLC-UV chromatogram at 280 nm; (B) HPLC-MS total ion current chromatogram of the flavonoid standards; (C) HPLC-UV chromatogram at 280 nm; (D) HPLC-MS total ion current chromatogram of the *Sophora flavescens* Ait. extracts.

Table 1
Peak assignments for analysis of flavonoids in *Sophora flavescens* Ait.

Number	t_R (min)	$[M+H]^+$ (*m/z*)	$[M+Na]^+$ (*m/z*)	$UV_{\lambda_{max}}$ (nm)	Identity
10	28.41	563	585	258, 306	Kushenol O[b] [31]
11	29.20	563	585	258, 306	
13	34.63	285	307	258, 308 (sh)	3′,7-Dihydroxy-4′-methoxy-isoflavone[a]
14	35.25	447	469	211, 287, 310	Trifolirhizin[a]
15	39.22	533	555	213, 284, 310	Trifolirhizin-6′′-o-malonate[b] [32]
18	45.42	457	479	232, 289	Kurarinol[a]
19	47.09	269	291	260, 308 (sh)	Formononetin[a]
20	47.52	371	393	230, 291, 330 (sh)	Sophoraisoflavanone A[b] [33]
21	50.21	285	–	213, 286, 310	Maachiain[a]
22	50.64	355	377	228, 290, 321 (sh)	7,4′-Dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone[a]
23	52.13	355	377	287	Isoxanthohumol[a]
24	53.90	443	–	292, 338 (sh)	Norkurarinol/kosamol Q[b] [34,18]
25	55.29	455	477	290, 333	Kushenol I/N[b] [31]
26	57.66	439	461	246, 392	Kuraridine[a]
27	58.42	457	479	245, 388	Kuraridinol[a]
28	61.97	439	–	270, 308 (sh)	Kushenol C[b] [35]
29	62.59	453	475	287	2′-Methoxykurarinone[b] [36]
30	63.45	425	447	208, 292, 337 (sh)	Sophoraflavanone G[a]
31	67.83	355	–	246, 382	Xanthohumol[a]
32	68.09	423		286, 321	Kusamol R[b] [18]
33	69.60	439	–	295, 318, 340 (sh)	Isokurarinone[a]
34	71.88	319	–	206, 247, 309	Kushecarpins A[b] [18]
35	72.49	439	–	213, 289, 320 (sh)	Kurarinone[a]
37	74.34	409	–	242, 295, 340 (sh)	Kushenol A[b] [18]
38	78.95	453	–	255, 385	Kushenol D[a]

[a] Identified by comparing experiment data with those of standard compounds.
[b] Identified by comparing with literature.

Dihydroxy-4′-methoxy-isoflavone, trifolirhizin, formononetin, maackiain, 7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone and sophoraflavanone G were isolated from the roots of *S. flavescens* Ait. by author. Their structures were fully characterized on the basis of their spectral data. The purities were above 97% as determined by HPLC-DAD at four different wavelengths (203, 254, 280 and 320 nm).

HPLC grade acetonitrile was purchased from Tedia (Fairfield, OH, USA). Acetic acid and methanol were of analytical grade from Shenyang Chemical Reagent Co. Ltd. (Shenyang, China). Deionized water used for HPLC analysis and sample extraction procedure was purified by a milli-Q academic water purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation and chromatographic condition

Analyses were performed using an Agilent 1100 HPLC system (Agilent Technologies, Willington, USA) equipped with a quaternary solvent delivery system, an on-line degasser, an autosampler, a column temperature controller and photodiode-array detector coupled with an analytical workstation. The chromatographic separation was carried out on a Hypersil ODS_2 column (5.0 μm, 200 mm × 4.6 mm, Yilite Co., Dalian, China). The mobile phase consisted of acetonitrile (A) and water containing 0.3% acetic acid (B). A gradient program was used as follows: 0–42 min, linear change from A–B (6:94, v/v) to A–B (32:68, v/v); 42–80 min, linear change to (70:30, v/v). A 10 min re-equilibration time was used between HPLC runs. The flow rate

Table 2
ESI-MS^2 product ions for the $[M+H]^+$ of flavanones (compounds **18**, **22**–**24**, **29**, **30**, **32**, **33**, **35**, **37**) in the *S. flavescens* Ait.

	18	**22**	**23**	**24**	**29**	**30**	**32**	**33**	**35**	**37**
$[M+H]^+$	457	355	355	443	453	425	423	439	439	409
$[M+H–H_2O]^+$	439	–	–	425	–	407	405	–	421	391
$[M+H–2H_2O]^+$	421	–	–	407	–	–	–	–	–	–
$[M+H–CH_3\cdot]^+$	–	–	–	–	438	–	–	–	–	–
$[M+H–CH_3\cdot–CO]^+$	414	–	–	–	410	–	–	–	–	–
$[M+H–lanandulyl]^+$	315	299	299	301	329	–	299	315	315	285
$[M+H–lanandulyl–H_2O]^+$	–	–	–	283	–	–	–	–	297	267
$[M+H–lanandulyl–CH_3\cdot–CO]^+$	–	–	256	–	–	–	–	–	–	–
$^{1,3}A^+$	321	235	235	307	303	289	303	289	303	289
$^{1,3}A^+–H_2O$	303	–	–	289	–	–	–	–	–	–
$^{1,3}A^+$–lanandulyl	179	179	179	165	179	165	179	165	179	165

was 1.0 mL/min with 20% of the eluent being splitted into the inlet of the mass spectrometer and the column temperature was maintained at 35 °C. The UV spectra were recorded between 190 and 460 nm for peak characterization, and the detection wavelength was set at 280 nm.

The mass spectra were acquired using an Agilent 1100 series SL ion trap mass spectrometer equipped with an electrospray ionization (ESI) interface. Nitrogen (N_2) was used as the sheath and auxiliary gas, and helium (He) was used as the collision gas. The ESI-MS spectra were acquired in the positive ion mode recorded over a mass range of *m/z* 150–800. Capillary voltage was 3000 V. Drying gas temperature was set at 350 °C with a gas flow rate of 10.0 L/min and nebulising pressure was of 35 psi.

2.3. Sample preparation

2.5 g of Kushen was extracted with 20-fold mass of 50% methanol solution at room temperature for 20 min using sonication, then centrifuged at 4000 × *g* for 10 min. The extraction was repeated twice. The supernatant fluid was combined and then concentrated under reduced-pressure and dried. The residue was diluted to 25 mL with 50% methanol. The sample solution was filtered through a 0.45 μm membrance filter. Twenty microliters was injected for HPLC–MS^2 analysis.

3. Results and discussion

3.1. Optimization of extract procedure

As we know that ethanol and methanol were preferred solution for extraction of flavonoids in nature materials. The Kushen powder samples were extracted by ultrasonication for 20 min with 30% methanol, 50% methanol, 70% methanol, methanol, 30% ethanol, 50% ethanol, 70% ethanol and 95% ethanol, respectively. The areas and numbers of 'common peaks' were used as response to evaluate the extraction solvent, and the results indicated that 50% aqueous methanol was the most suitable solution. Ultrasonic extraction was compared with refluxing. It was found that both extraction methods provided the similar chromatograms of Kushen samples, but ultrasonication was of high efficiency and easy to operate. Based on the results of test above, the extraction procedure was further optimized by investigating the influence of following factors as solvent volume, extraction time and frequency. Finally, the optimum extraction procedure was established as ultrasonic extraction by 20-fold mass of 50% aqueous methanol for two times, each time for 30 min.

3.2. Optimization of chromatographic condition

To obtain LC chromatograms with good resolution of adjacent peaks within a reasonably short analysis time, different mobile phase compositions were screened, and acetonitrile–0.3% aqueous acetic acid was chosen as the most suitable eluting solvent system. Based on the UV absorption maxima of most flavonoids recorded by HPLC-DAD, the monitoring wavelength was chosen at 280 nm. For MS analysis the positive ion mode of ESI was selected in the present study, as it provided extensive information of most flavonoids in *S. flavescens*. Under the optimised HPLC and MS/MS condition 38 peaks gained baseline separation.

Table 3
ESI-MS^2 product ions for the $[M+H]^+$ of flavanonol (compound **25**), flavonol (compound **28**) and chalcones (compounds **26**, **27**, **31**, **38**) in the *S. flavescens* Ait.

	25	**28**	**26**	**27**	**31**	**38**
$[M+H]^+$	455	439	439	457	355	453
$[M+H–H_2O]^+$	437	421	–	439	–	–
$[M+H–H_2O–CO]^+$	409	–	–	–	–	–
$[M+H–CH_3\cdot]^+$	–	–	424	–	–	–
$[M+H–lanandulyl]^+$	331	315	–	315	299	329
$^{1,3}A^+$	303	289	303	321	235	303
$^{1,3}A^+–H_2O$	–	–	–	303	–	–
$^{1,3}A^+–CO$	–	–	–	293	–	–
$^{1,3}A^+–CH_3\cdot$	288	–	–	–	–	–
$^{1,3}A^+$–lanandulyl	179	165	179	179	179	179

3.3. MS analysis and identification

In this study, a total of 24 compounds were characterized. Fourteen of them were unambiguously identified by comparing retention times (t_R), UV and MS data with those of the reference standards (shown in Fig. 2A and B), meanwhile the possible structures of another 10 peaks in the chromatograms of *S. flavescens* extracts were tentatively characterized on the basis of literature [18,31–36] and analyzing their fragmentation pathways. The HPLC/MS chromatograms and data for samples were shown in Fig. 2C and D and Table 1, respectively.

Most of the compounds exhibited quasi-molecular ions of $[M+H]^+$, adducted ions of $[M+Na]^+$, $[2M+Na]^+$ and $[2M+H]^+$ in positive ions mode MS spectra. The $[M+H]^+$

Table 4
ESI-MS^2 product ions for the $[M+H]^+$ of isoflavones (compounds **10**, **13**, **19**) and isoflavanone (compound **20**) in the *S. flavescens* Ait.

	10	**13**	**19**	**20**
$[M+H]^+$	563	285	269	371
$[M+H–$β-D-xyl **16** β-D-glu$]^+$	269	–	–	–
$[M+H–H_2O]^+$	–	–		353
$[M+H–CH_3\cdot]^+$	–	270	254	–
$[M+H–2CO]^+$	–	–	213	–
$[M+H–H_2O–CO]^+$	–	239	–	325
$[M+H–H_2O–2CO]^+$	–	211	–	–
$[M+H–lanandulyl]^+$	–	–	–	315
$[M+H–CO_2–C_2H_2O]^+$	–	–	–	285
$[M+H–C_3O_2–C_2H_2O]^+$	–	–	159	–
$[M+H–lanandulyl–H_2O–CO]^+$	–	–	–	269
$[M+H–2C_3O_2]^+$	–	–	–	235
$^{1,3}A^+$	–	137	137	153
$[M+H–CH_3\cdot–2C_3O_2]^+$	–	–	118	–
$[M+H–$ring B$]^+$	–	–	–	179

ions were selected for collision-induced dissociation (CID) fragmentation to produce MS/MS spectra. Tables 2–5 displayed the characteristic fragments observed for each compound obtained by MS^2 from the $[M+H]^+$ ions of the seven types of flavonoids, and typical MS/MS spectra for each type of flavonoids are shown in Fig. 3. Some common features, such as H_2O, $CH_3\cdot$, CO, CO_2, C_2H_2O and C_3O_2 losses were observed in the present study, consistent with the literature [37]. It was demonstrated that CO and CO_2 losses from $[M+H]^+$ were attributed to the structure of the C-ring, and loss of $CH_3\cdot$ suggested the existence of $-OCH_3$ group. Compounds **13**, **15**, **18**, **20**, **24–28**, **30**, **32**, **34–35** and

Table 5
ESI-MS^2 product-ions for the $[M+H]^+$ of pterocarpans (compounds **14**, **15**, **21**, **34**) in the *S. flavescens* Ait.

	14	**15**	**21**	**34**
$[M+H]^+$	447	533	285	319
$[M+H–H_2O]^+$	–	515	–	301
$[M+H$-glu/glu-malonyl$]^+$	285	285	–	–
$[M+H–C_6H_6O_2]^+$	–	–	175	–
$[M+H–C_6H_4O–C_2H_2O]^+$	–	–	151	–
$[M+H–C_6H_4O–C_2H_2O–CO]^+$	–	–	123	–

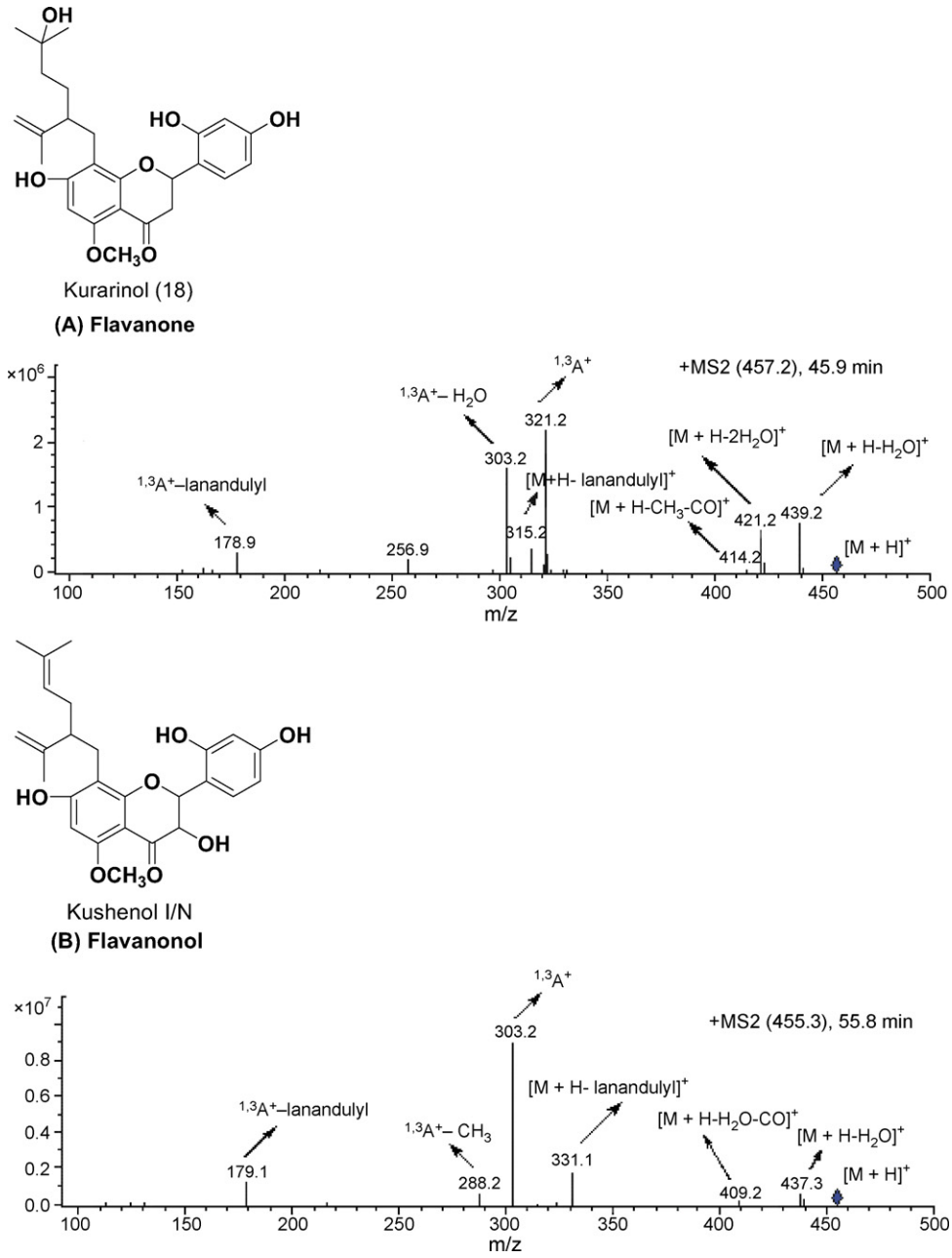


Fig. 3. Typical ESI-MS/MS spectra of seven types of flavonoids in positive ions mode: (A) typical ESI-MS/MS spectra of flavanone; (B) typical ESI-MS/MS spectra of flavanonol; (C) typical ESI-MS/MS spectra of flavonol; (D) typical ESI-MS/MS spectra of chalcone; (E) typical ESI-MS/MS spectra of isoflavone; (F) typical ESI-MS/MS spectra of isoflavanone; (G) typical ESI-MS/MS spectra of pterocarpan.

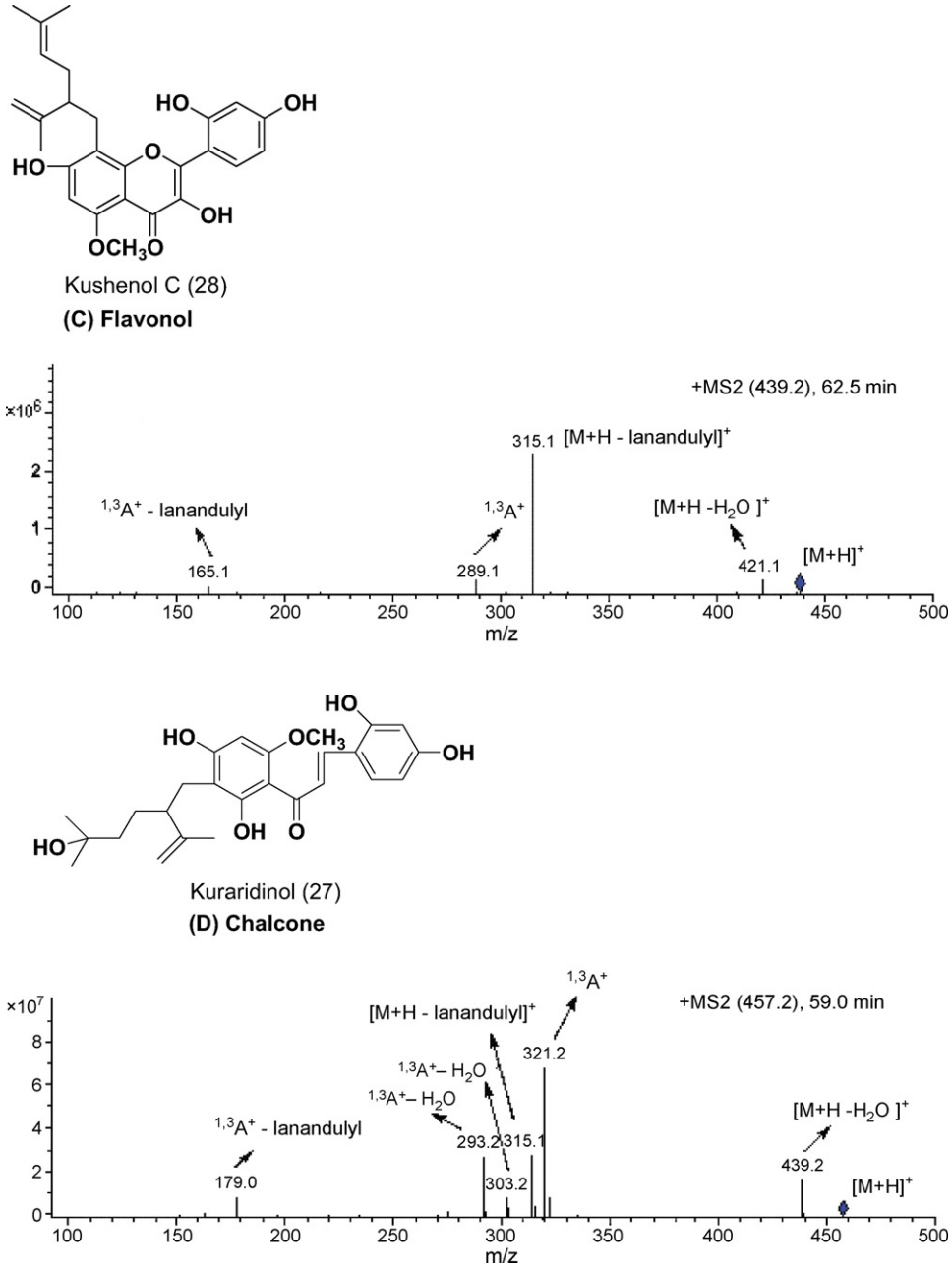
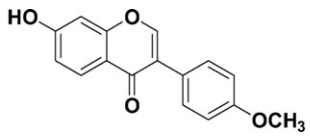


Fig. 3. (*Continued*)

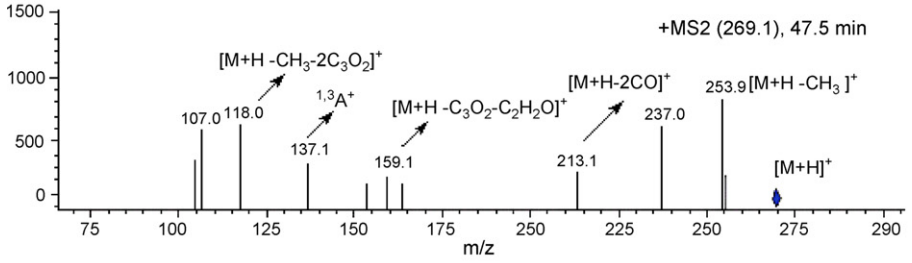
37 provided significant $[M+H–H_2O]^+$ fragments in production spectra. Except **13**, **15** and **20**, these compounds had some common characteristic in their structures that either 2′-OH/6′-OH/3-OH or B/D/E lateral was present in their constitutional formulae. So it was proposed that H_2O losses form the $[M+H]^+$ ion of compounds **8**, **24**, **26**, **27**, **30**, **32**, **35** and **37** were probably attributed to lateral B/D/E or the segmentation of 3-H and 6′-OH/2′-OH, of compound **25** being the segmentation of 2-H and 3-OH, and of compound **28** being the fragmentation of 2′-H and 3-OH. Previous study reported that $[M+H–H_2O]^+$ fragments could be considered a diagnostic feature for the presence of two OH groups in *ortho* positions [38]. In the present work, we found that this neutral loss was also associated with the specific location of the hydroxyl groups. However, the compounds **13** and **20** that had no structural features talked above also underwent the loss of H_2O. Thus, the $[M+H–H_2O]^+$ fragment could aid in deducing the location of hydroxyl substitution, but must be used with caution.

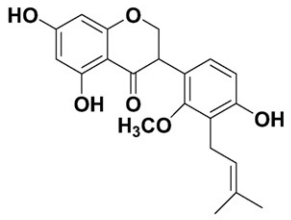
Retro-Diels–Alder (RDA) fragmentation reaction was observed undergoing in flavanones, flavanonols, flavonols, chalcones, isoflavones and isoflavanones but not in any pterocarpans. Besides regular MS fragmentation patterns discussed above, prenylated flavonoids in *S. flavescens* Ait. were of characteristics of losing the lanandulyl chain by the fragmentation



Formononetin (19)

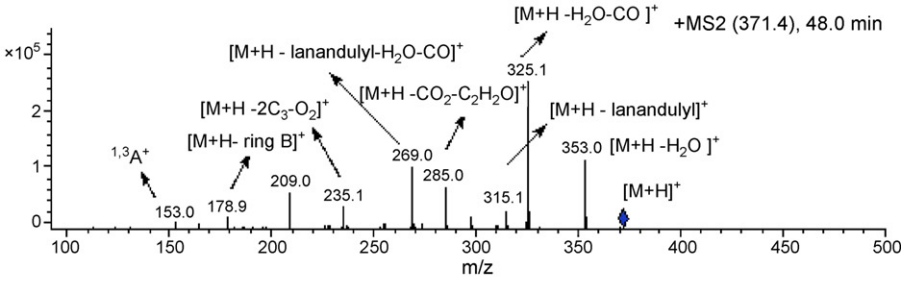
(E) Isoflavone

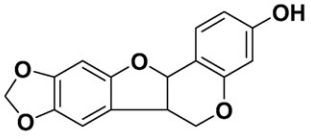




Sophoraisoflavanone A (20)

(F) Isoflavanone





Maachiain (21)

(G) Polycyclic Flavonoid

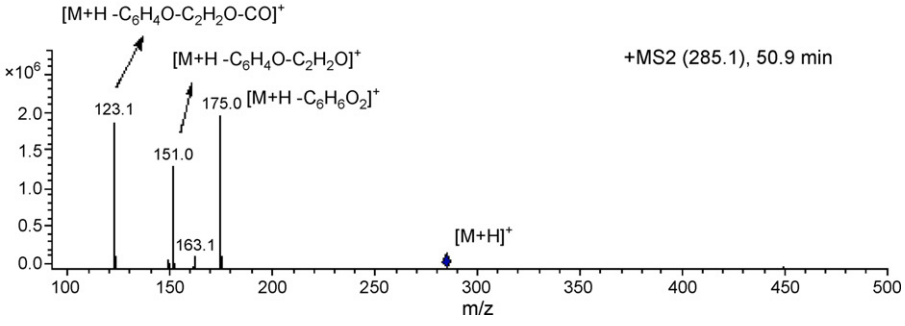
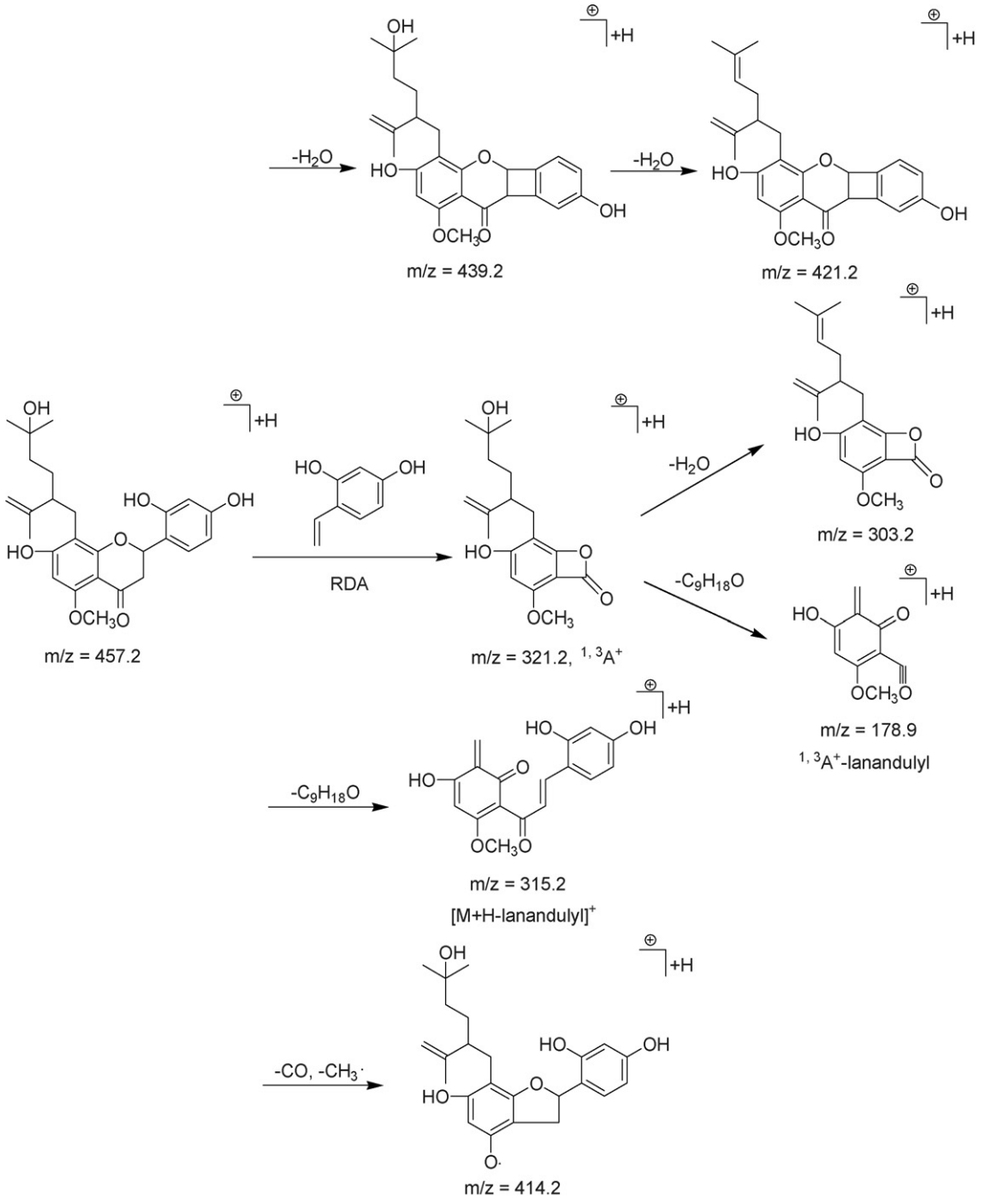
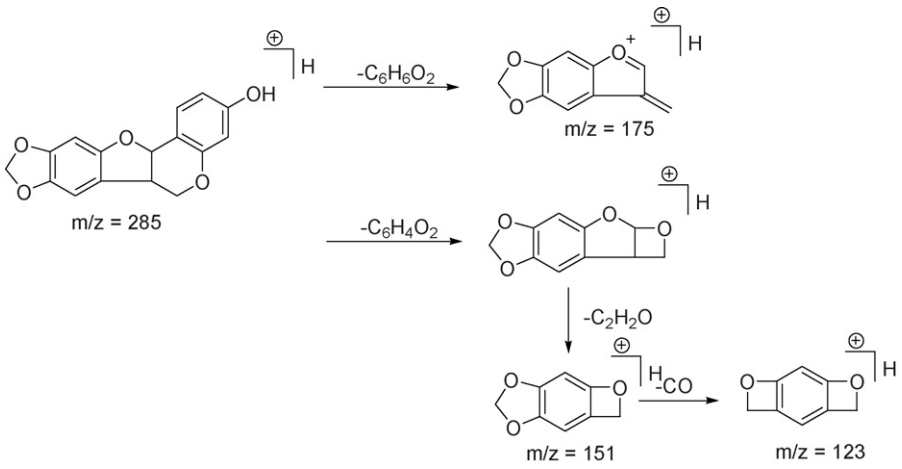


Fig. 3. (*Continued*).



Scheme 1. Proposed fragmentations of the flavonoid kurarinol in positive ions mode.

happening between C″-α and C″-β, and lanandulyl chain losses were observed undergoing through the rearrangement pathway of some protons. Most prenylated flavonoids researched here exhibited the fragments of *m/z* 179 or 165 in the positive ions mode, consistent with those of *m/z* 177 or 163 in the negative ions mode. The existence of ions 179 posi (177 nega)/165 posi (163 nega) suggested such groups as one –OH, one $–OCH_3$ and one lanandulyl chains/two –OH and one lanandulyl chains were of great possibility in ring-A. Thus these fragments could aid in deducing the structures of flavonoids. The fragmentation pathways presumed upon kurarinol (**18**) were shown in Scheme 1, and from its fragmentation behavior, other flavonoids of different types could be proposed owning to much comparability in their molecule structures. There is no literature concerning the fragmentation of pterocarpans except some common losses such as the losses of H_2O, CO and CO_2. Taking maackiain (**19**) as an example, its potential fragmentation pathways were tentatively summarized in Scheme 2. Owning to lack of the cases about this type of flavonoids, the fragmentation pathways are probably not universal.



Scheme 2. Proposed fragmentations of the flavonoid maackiain in positive ions mode.

4. Conclusion

This paper described a sensitive, specific and simple method for qualification of major constituents of *S. flavescens* extracts. Twenty-four flavonoids of the types of flavanone, flavanonol, flavonol, chalcone, isoflavone, isoflavanone and pterocarpine were identified or tentatively characterized in one LC–MS^n run. Results found in the study could significantly decrease the time required for the identification of some known flavonoids present in *S. flavescens* extracts as isolation and purification of authentic references were unnecessary. This study also provided the chemical support for the chromatographic fingerprint technology and facilitates to improve the quality control standard of *S. flavescens*.

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