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ORIGINAL ARTICLE

Application of Ultra-performance Liquid Chromatography with Time-of-Flight Mass Spectrometry for the Rapid Analysis of Constituents and Metabolites from the Extracts of *Acanthopanax senticosus* Harms Leaf

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ABSTRACT

Acanthopanax senticosus (Rupr and Maxim) Harms (AS), a member of Araliaceae family, is a typical folk medicinal herb, which is widely distributed in the Northeastern part of China. Due to lack of this resource caused by the extensive use of its root, this work studied the chemical constituents of leaves of this plant with the purpose of looking for an alternative resource. In this work, a fast and optimized ultra-performance liquid chromatography method with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) has been developed for the analysis of constituents in leaves extracts. A total of 131 compounds were identified or tentatively characterized including triterpenoid saponins, phenols, flavonoids, lignans, coumarins, polysaccharides, and other compounds based on their fragmentation behaviors. Besides, a total of 21 metabolites were identified in serum in rats after oral administration, among which 12 prototypes and 9 metabolites through the metabolic pathways of reduction, methylation, sulfate conjugation, sulfoxide to thioether and deglycosylation. The coupling of UPLC-QTOF-MS led to the in-depth characterization of the leaves extracts of AS both *in vitro* and *in vivo* on the basis of retention time, mass accuracy, and tandem MS/MS spectra. It concluded that this analytical tool was very valuable in the study of complex compounds in medicinal herb.

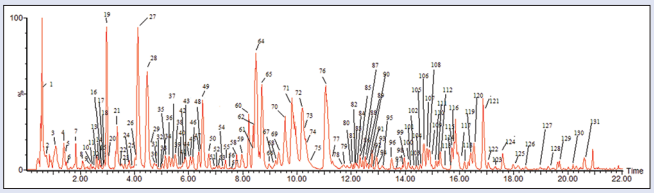
Key words: *Acanthopanax senticosus* (Rupr. and Maxim) Harms, chemical constituents, extracts, metabolites, Traditional Chinese herbal medicine, ultra-performance liquid chromatography method with quadrupole time-of-flight mass spectrometry

HIGHLIGHTS OF PAPER

- A fast UPLC-QTOF-MS has been developed for analysis of constituents in leaves extracts
- A total of 131 compounds were identified in leaves extracts
- A total of 21 metabolites including 12 prototypes and 9 metabolites were identified *in vivo*.

SUMMARY

- Constituent's analysis of *Acanthopanax senticosus* Harms leaf by ultra-performance liquid chromatography method with quadrupole time-of-flight mass spectrometry.



Abbreviations used: AS: *Acanthopanax senticosus* (Rupr and Maxim) Harms, TCHM: Traditional Chinese herbal medicine, UPLC-QTOF-MS: Ultra-performance liquid chromatography method with time-of-flight mass spectrometry, MS/MS: Tandem mass spectrometry, PCA: Principal component analysis, PLS-DA: Partial least squared discriminant analysis, OPLS-DA: Orthogonal projection to latent structure-discriminant analysis.

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INTRODUCTION

Acanthopanax senticosus (Rupr and Maxim.) Harms (AS) is a commonly used traditional Chinese herbal medicine (TCHM), which is widely distributed in the Northeastern part of China. The root and stem of AS are called "Ciwujia" in China, and it has long been widely used as tonifying and replenishing, heart-nourishing tranquilizing medicinal. In recent years, a great number of chemical, pharmacological, and clinical studies on AS has proved that it has the effect on immune regulation,[1] antistress,[2] antifatigue,[3] antitumors,[4] and treating cerebrovascular diseases.[5] However, because of the extensive use of root, the *Acanthopanax* plant resources are gradually exhausted and even cause the ecosystem crisis. Hopefully, the effect of leaves drew more and more attention over the years, not only because they may have the similar effects on the root but also the leaves have the ability of regeneration, which could be a substitutable resource.

Screening and identification of chemical constituents in TCHM are the first and indispensable steps of the development of TCHM.[6] However, for TCHM, the precise characterization of components can be considered as a challenge because of its complexity and variability.

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LC-mass spectrometry (MS)/MS has proved to be a very powerful tool in profiling of natural products with the advantages of its high resolution, high sensitivity, and accurate mass measurement.[7] Moreover, MS/MS offers unique structure identification capabilities that allow for the characterization of the components in mixtures directly and quickly without the reference standards. At the same time, this method also avoids the tedious and difficult ask of isolation, separation, and purification of substances whose structures are similar and even which are present in only trace amounts, which is obviously superior to other traditional methods.[8,9]

It is a major blockage to understanding and revealing the mystery of herbal medicines due to the lack of awareness in effective material basic and biological disposition. Information on identification of metabolites and metabolic fate of natural compounds *in vivo* is a key part of the equation in elucidating the effective constituents and understanding their potential effects.[10] Fortunately, serum pharmacochemistry has played an important role in explicating the effective constituents as well as their metabolites *in vivo*.[11] It has been recognized as an indispensable part in evaluating drug safety, efficacy, and drug-drug interactions in the whole pipeline of drug discovery and development.[12] Besides principal component analysis (PCA), partial least squared discriminant analysis (PLS-DA), and orthogonal projection to latent structure (OPLS-DA) are also developed to identify potential marker compounds.

Although comprehensively analyzing the chemical constituents having the medicinal and therapeutic potential of the natural plant is very crucial, the leaves of AS has not been studied in considerable details in this respect. Hence, the aim of our work here was to undertake a comprehensive characterization of leaves extracts of AS in order to get an in-depth knowledge of the active ingredients and identify compounds circulating in the blood stream by ultra-performance liquid chromatography method with quadrupole time-of-flight MS (UPLC-QTOF-MS).

EXPERIMENTAL

Chemicals and materials

Acetonitrile (ACN) (high-performance liquid chromatography [HPLC] grade) was purchased from Merck (Darmstadt, Germany). Distilled water was further purified by a Milli-Q system (Millipore, Bedford, MA, USA). Formic acid (HPLC grade) was purchased from Tianjin Kermel Reagent Company (Tianjin, China). The OASIS HLB SPE C_{18} columns (6cc, 200 mg) were purchased from Waters (Milford, MA, USA). Leucine enkephalin was purchased from Sigma-Aldrich (MO, USA). The dried leaves of *A. senticosus* (Rupr. and Maxim.) Harms were purchased from Qinghe Forestry Bureau (Heilongjiang, China), and authenticated by Prof. Xijun Wang, Department of Pharmacognosy of Heilongjiang University of Chinese Medicine.

Ultra-performance liquid chromatography-mass spectrometry conditions

Separation and detection of the components was performed on a Waters ACQUITY UPLC system (Waters Corp., Milford, MA, USA) coupled with a Waters Synapt™ High Definition TOF Mass system (Waters Corp., Milford, USA) equipped with the electrospray ionization. Chromatographic separations were achieved on an ACQUITY UPLC™ HSS T3 column (100 mm × 2.1 mm i.d., 1.8 um, Waters Corp.) at 40°C and the flow rate of the mobile phase was 0.50 mL/min. Mobile phase A consisted of 0.1% formic acid in ACN while mobile phase B consisted of 0.1% formic acid in water. The column was eluted with a linear gradient of 1–9% A over initial to 2.0 min, 9–20% A over 2.0–11.0 min, 20–45% A over 11.0–19.0 min, 45–100% A over 19.0–22.0 min.

The mass spectrometric full-scan data were acquired in the negative ion by V mode from 50 to 1500 Da with a 0.3 s scan time. Other conditions were as follows: Capillary voltage of 2.4 kV, sample cone voltage of 35 V, extraction cone voltage of 3.5 V, desolvation temperature of 300°C, source temperature of 110°C, cone gas flow of 50 L/h and desolvation gas flow of 650 L/h for negative ion mode. Data were centroided and mass was corrected during the acquisition using an external reference (Lock-Spray™) consisting of a 200 pg/mL solution of leucine enkephalin infused at a flow rate of 0.1 mL·min^{-1} via a lockspray interface, generating a reference ion for negative ion mode ($[M-H]^-$ = 554.2615 Da) to ensure accuracy during the MS analysis.

Preparation of sample solutions

The dried leaves of AS were crushed and was immersed 10 times with water for 2 h and then extracted by heating reflux for 2 h 2 times. The extract was merged and evaporated by rotary evaporation under vacuum. The residue was then freeze-dried. The dried powder of the leaves extracts (0.2 g) was accurately weighed and dissolved with 10 mL of 30% v/v methanol. After extracting in an ultrasonic bath for 30 min at room temperature, the solution was centrifuged at 13,000 rpm for 15 min, and the supernatant filtered through a 0.22 μm filter membrane before injecting 3 μl for UPLC-QTOF-MS analysis.

Preparation of drug administration and serum samples

Six-week-old male Sprague-Dawley rats were obtained from the Laboratory of Animal Center of the Heilongjiang University of Chinese Medicine. The animals were kept in a room maintained at 23–25°C and 50–60% humidity under a 12-h light/12-h dark cycle of artificial lighting starting at 7:00 h; food and water were available *ad libitum*. After an acclimation period of 1-week, all the rats were randomly divided into two groups of five rats each group: A Control group and dosed group. Prior to drug administration, the experimental animals were deprived of food for 16 h and were free to access the water. The freeze-dried powder of AS was dissolved in 0.5% CMC-Na to get a concentration equivalent to 0.6 g/ml. The dosed group was orally administered with AS extracts (1 mL/100 g body weight) while the control group received the same volume of 0.5% CMC-Na. The blood samples were collected from a hepatic portal vein at 60 min after the oral administration. and then the rat blood was immediately centrifuged at 4000 rpm for 15 min at 4°C. Forty microliters of phosphoric acid was added to 2.0 mL of the above supernatant and ultrasonicated for 1 min, and then vortexed for the 30s, and the serum samples were prepared using SPE column as follows: OASIS HLB SPE C_{18} columns previously activated using 3 mL of methanol and equilibrated with 3 mL water, successively. Then, 100% methanol was eluted, and the eluate was collected and evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was reconstituted in 100% methanol and vortex-mixed for 30 s, centrifuged at 13,000 rpm for 15 min at 4°C. A 5 μL aliquot of the solution was injected into the UPLC-QTOF-MS for analysis.

Data processing

All LC/MS data including retention time, accurate mass, and MS/MS spectra were acquired in the centroid mode by MarkerLynx software MassLynx™ V 4.1 software with QuanLynx™ program (Waters Corp., Milford, MA, USA). All mass spectra were aligned with mass tolerance of 0.02 Da and retention time window of 0.20 min. The noise elimination level was 6. Ion identification was based on the t_R, m/z, and MS/MS spectra. The three-dimensional were introduced into the EZinfo 2.0 software (Waters Corp, Milford, MA, USA) for PCA orthogonal partial

least-squares-discriminate analysis (OPLS-DA) with the purpose of visualizing discrimination between the dosed and control groups. The S-plot showing the combined covariance P (1) and correlation P (corr) from the PLS-DA model was used to visualize the metabolites contributing to the discrimination.

RESULTS AND DISCUSSION

To characterize the chemical constituents of leaves extracts, a UPLC-QTOF-MS method was established [Figure 1]. Structures of chemical compounds were characterized or tentatively characterized by comparing their chromatographic and spectrometric data with authentic standards or literature data. Ultimately, a total of 131 compounds [Table S1] were identified or tentatively characterized including triterpenoid saponins, phenols, flavonoids, lignans, coumarins, polysaccharides, and other compounds. Flavonoids are very important bioactive constituents widely found in leaves of AS. Among these, rutin (60), hyperin (63), and quercitrin (74) are the most cited in the literature.[13-15]

Compound 60 was characterized as rutin. For it, the $[M-H]^-$ precursor ion at m/z 609 gave one prominent fragment ion at m/z300 $[M-H-Rha-Glu]^-$ and subsequent fragmentation patterns with ions at m/z 271, 255, 243, and 150, whose fragmentation pathway was supported by comparing with its pure standard. For compound 63, the MS/MS spectrum also displayed a main peak at m/z 300, obviously by the elimination of a galactose unit (180-H_2O = 162 Da) to yield the $[M-H]^-$ ion of the aglycone quercetin. The presence of hyperoside in leaves of AS has been reported before, together with its mass spectrum of pure standard, the compound 59 was identified as being hyperoside. Compound 74 corresponded to quercitrin, the structure and fragmentation pathway of which are in complete agreement with that of the quercitrin standard sample. Flavonoids found in leaves extracts were characterized with typical anions of basic parent structures at m/z 300, 284 and 314 represented aglycone quercetin, kaempferol, isorhamnetin, respectively.

Eleutheroside E has been widely reported to be the key constituent of AS. For compound 58, the MS/MS spectra displayed exactly the same fragmentation patterns as those of the pure standard of Eleutheroside E. It had $[M-H]^-$ at m/z 741 which gave rise to an ion at m/z 417 by losing two galactose units (162 Da). Compound 80, giving the $[M-H]^-$ at m/z 417, exhibited the similar MS/MS behavior as compound 58, while its retention time was later than that of compound 58. Above those, compound 80 was proposed as syringaresinol. Triterpenoid compound are another kind of main ingredients in AS, which are mainly pentacyclic triterpene compounds such as oleanane-type and lupane-type. In the structure of triterpenoid saponins, sugar moieties are generally linked at C-3 or C-28 of their parent structures.[16]

Compound 131 gave an $[M-H]^-$ ion at m/z 733, which exhibited the fragment, typical of glycosyl derivatives (loss of terminal sugars) with ions at m/z 587 $[M-H-Rha]^-$ and m/z 455 $[M-H-Rha-Ara]^-$; this latter ion corresponded to the $[M-H]^-$ ion of the aglycone oleanane. Based on these, compound 130 was proposed as Eleutheroside I. Compound 109 showed an $[M-H]^-$ ion peak at m/z 1245, in accordance with an empirical molecular formula of $C_{60}H_{94}O_{27}$. The fragmentation patterns in the negative-ion MS/MS of it indicated loss of ester-linked sugar chain at C-28 (m/z 733 $[M-C_{20}H_{33}O_{15}]$). Combined with the previous literature, compound 109 was inferred as Acanthopanaxoside A. Compound 105 had an $[M-H]^-$ at m/z 1187, whose empirical molecular formula was $C_{58}H_{92}O_{25}$. Upon MS/MS fragmentation, it yielded ions at m/z 717 by losing a fragment of mass 469, which was another ester-linked sugar chain at C-28 and suggested the presence of one rhamnopyranosyl moiety and two glucopyranosyl moieties.

AS contains a large number of phenolic compounds, most of which exists as isomers. Compound 19, 27, and 28 had the same precursor ion at m/z 353 and the product ions of them were similar, among which the most significant difference is a change in base peak m/z 191 and m/z 179. After compared with pure standards, the three compounds were identified as neochlorogenic acid, chlorogenic acid, and cryptochlorogenic acid.

Compared with other kinds of compounds, only a few coumarin compounds have been reported from this plant. Isofraxidin is not only one of the main bioactive constituents but also regarded as the ideal marker compound for the quality assurance of this plant. As compound 38, its $[M-H]^-$ at m/z 221 produced MS/MS daughter ions at m/z 206, 191, which indicated loss two methyl moieties in sequence. Then, losing a 28 atomic mass unit, ion m/z 191 produced ion at m/z 163. From this information, we could conclude that compound 38 was isofraxidin and this assignment was supported by comparing its MS/MS spectra with those of a pure standard.

Global metabolite identification of complex compounds of herbal medicine in biological systems is a very challenging task. Using the optimal reversed-phase UPLC-MS conditions, all the data containing the retention time, peak intensity, and exact mass were imported in the Masslynx™ software for multiple statistical analyzes [Figure 2]. As an unsupervised pattern recognition method, PCA can effectively identify the differences between the control and dosed group, which indicated that these differences were caused by some exogenous constituents absorbed in serum after dosing [Figure 3]. Combining the results of the S-plot with VIP value from PLS-DA, significant difference points were selected [Figure 4]. However, these difference points were not all exogenous constituents, including some endogenous ones caused by the drug. For screening exogenous constituents absorbed in serum, the trend plot was used, displaying the ions only existed in the dosed group, taking the $[M-H]^-$ ion at m/z 609 ((t_R = 8.23) for example, shown in Figure 4c. By means of the above analysis, 12 prototype constituents were finally identified in rat serum after an oral administration leaves extracts.

The main chemical constituents of leaves extracts of AS are composed of glycosides, including flavonoid glycosides, triterpenoid glycosides, and so

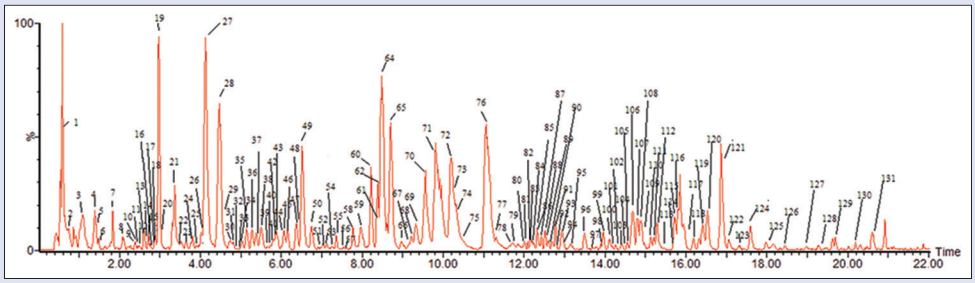

Figure 1: Liquid chromatography-mass spectrometry chromatograms for the leaves extracts of *Acanthopanax senticosus*

Table S1: Compounds identified in leaves extracts by UPLC-QTOF-MS in the negative ESI mode

Retention time (min)	Measured m/z	Formula	Theoretical m/z	Error (ppm)	Fragment	Proposed compound
0.619	341.107	$C_{12}H_{22}O_{11}$	341.108	−5.0	179, 161, 143, 113	Sucrose
0.894	503.161	$C_{18}H_{32}O_{16}$	503.161	−0.6	467, 367, 221, 161	Raffinose
1.262	233.066	$C_{9}H_{14}O_{7}$	233.066	−1.7	191, 173, 155, 111, 93	Allyl-glucopyranuronate
1.381	677.215	$C_{25}H_{42}O_{21}$	677.214	1.9	515, 425, 147, 115, 103	Xylopyranosyl-(1->4)-xylopyranosyl-(1->4)-xylopyranosyl-(1->4)-xylopyranosyl-(1->4)-xylopyranose
1.419	575.184	$C_{21}H_{36}O_{18}$	575.182	3.7	409, 367, 349, 221, 205, 101	Methyl-galactopyranosyl-(1->4)-galactopyranosyl-(1->4)-6-O-(carboxymethyl)-glucopyranoside
1.494	545.171	$C_{20}H_{34}O_{17}$	545.172	−0.9	509, 367, 221, 191, 131	Dimethyl-galactopyranosyl-(1->4)-glucopyranosyl-(1->4)-glucopyranosiduronate
1.84	515.192	$C_{27}H_{32}O_{10}$	515.192	0.6	369, 351, 207, 161, 113, 101	3,6-Di-O-acetyl-4-O-benzyl-2-O-[2-ethoxy-2-oxo-1-phenylethyl]-galactopyranose
2.134	371.098	$C_{16}H_{20}O_{10}$	371.098	0.5	353, 191, 135	3-{[3-(3,4-dihydroxyphenyl)-2-propenoyl]oxy}-1,4,5-trihydroxycyclohexanecarboxylic acid hydrate (1:1)
2.355	315.071	$C_{13}H_{16}O_{9}$	315.072	0.6	153, 109	Protocatechuic acid-glucoside
2.472	331.102	$C_{14}H_{20}O_{9}$	331.103	−1.5	168, 153, 137, 125, 110	3,5-dimethoxy-4-hydroxyphenyl-1-O-glucopyranoside
2.483	473.13	$C_{20}H_{26}O_{13}$	473.13	0	311, 267, 221, 189, 177, 161	6-O-[3,4-dihydroxy-4-(hydroxymethyl)tetrahydro-2-furanyl]-1-O-[3-(3,4-dihydroxyphenyl)-2-propenoyl]-glucopyranose
2.513	167.033	$C_{8}H_{8}O_{4}$	167.034	1.2	153, 139, 109	Protocatechuic acid methyl ester
2.6	153.016	$C_{7}H_{6}O_{4}$	153.019	1.5	109	Protocatechuic acid
2.724	557.204	$C_{22}H_{38}O_{16}$	557.208	−5.7	515, 369, 351, 207, 161, 113, 101	1,3,6-tri-O-acetyl-4-O-benzyl-2-O-[2-ethoxy-2-oxo-1-phenylethyl]-glucopyranose
2.776	515.14	$C_{22}H_{28}O_{14}$	515.14	−0.2	353, 323, 191, 179, 173, 161	Dicaffeoyl-quinic acid glucoside somer 1
2.787	219.046	$C_{9}H_{16}O_{6}$	219.045	6.4	173, 154, 129, 111	1-O-butyryl-xylopyranose
2.84	515.14	$C_{22}H_{28}O_{14}$	515.14	−0.2	353, 323, 191, 179, 173, 161	Dicaffeoyl-quinic acid glucoside isomer 2
2.913	447.113	$C_{18}H_{24}O_{13}$	447.114	−3.1	315, 153, 109	1-O-(3,4-dihydroxybenzoyl)-6-O-[-3,4-dihydroxy-4-(hydroxymethyl)tetrahydro-2-furanyl]-glucopyranose
2.934	353.087	$C_{16}H_{18}O_{9}$	353.087	−0.6	191, 179, 161, 135	Neochlorogenic acid
3.008	191.054	$C_{7}H_{12}O_{6}$	191.056	−8.9	173, 137, 127, 109	Quinic acid
3.46	137.02	$C_{8}H_{10}O_{2}$	137.021	−0.8	108, 93, 81	p-hydroxyphenylethyl alcohol
3.534	515.14	$C_{22}H_{28}O_{14}$	515.14	−1.2	353, 323, 191, 179, 173, 161	Dicaffeoyl-quinic acid glucoside isomer 3
3.87	341.088	$C_{15}H_{18}O_{9}$	341.087	1.8	179, 135, 109,	1-caffeoylglucose
3.923	311.113	$C_{15}H_{20}O_{7}$	311.131	−1.6	269, 242, 205, 187, 171, 162, 135	Sachaliside 1
3.965	451.218	$C_{20}H_{36}O_{11}$	451.218	0.2	407, 390, 363, 262, 218, 191, 135	Methyl-3-O-(2-O-acetyl-3,4-di-O-methyl-glucopyranosyl)-6-deoxy-3-C-methyl-2,4-di-O-methyl-mannopyranoside
4.038	271.095	$C_{16}H_{16}O_{4}$	271.097	−7.7	242, 213, 161, 149, 109	Trans-4,4'-dihydroxy-3,3'-dimethoxystilene
4.249	353.086	$C_{16}H_{18}O_{9}$	353.087	−4.0	191, 173, 161, 127, 85	Chlorogenic acid
4.544	625.141	$C_{27}H_{30}O_{17}$	625.141	1.3	463, 300, 271	Quercetin-3,4'-diglucoside
4.595	353.086	$C_{16}H_{18}O_{9}$	353.087	-4	191, 179, 173, 135, 85	Cryptochlorogenic acid
4.634	403.195	$C_{19}H_{32}O_{9}$	403.197	−1.7	243, 233, 205, 177, 165	1-hydroxy-4-[1-hydroxy-2,2,6-trimethyl-4-oxocyclohexyl]-3-buten-2-yl-glucopyranoside
4.785	379.159	$C_{16}H_{28}O_{10}$	379.16	−3.2	217, 191, 171, 113, 85	3-methyl-2-buten-1-yl6-O-[3,4-dihydroxy-4-(hydroxymethyl) tetrahydro-2-furanyl]-glucopyranoside
4.855	179.035	$C_{9}H_{8}O_{4}$	179.034	5.6	135, 117, 107	Caffeic acid
4.985	625.141	$C_{27}H_{30}O_{17}$	625.141	0.6	463, 300, 271	Quercetin-3,4'-diglucoside
5.016	221.045	$C_{11}H_{10}O_{5}$	221.045	−1.8	206, 191, 163, 135	Isofraxidin
5.027	369.081	$C_{16}H_{18}O_{10}$	369.082	−2.7	207, 191, 162, 135, 108	Pavlin
5.185	401.142	$C_{18}H_{26}O_{10}$	401.145	−6.7	269, 233, 161, 113, 101	Benzyl 2-O-xylopyranosyl-glucopyranoside
5.238	431.189	$C_{20}H_{32}O_{10}$	431.192	−6.0	315, 297, 191, 135	2-({6-O-[6-hydroxy-2,6-dimethyl-2,7-octadienoyl]-glucopyranosyl} oxy)-2-methylpropanoic acid
5.406	401.142	$C_{18}H_{26}O_{10}$	401.145	−6.0	269, 233, 161, 113, 101	Benzyl 6-O-(xylopyranosyl)-glucopyranoside
5.512	353.083	$C_{16}H_{18}O_{9}$	353.081	4	191, 173, 161	Caffeoylquinic acid isomer
5.61	677.1700	$C_{28}H_{38}O_{19}$	677.169	1.3	515, 353, 341, 191, 179, 161, 135	Caffeoylquinic acid glucoside
5.669	395.097	$C_{18}H_{20}O_{10}$	395.098	−1.8	335, 233, 191, 161,	Methyl [7-(galactopyranosyloxy)-2-oxo-2H-chromen-4-yl]acetate
5.806	385.186	$C_{17}H_{22}O_{10}$	385.186	0	223, 208, 192, 179, 164, 149, 135, 121	1-sinapoyl-glucose
5.812	335.075	$C_{16}H_{16}O_{8}$	335.077	−6.0	191, 179, 173, 161, 135	5-{[3-(3,4-dihydroxyphenyl)-2-propenoyl]oxy}-3,4-dihydroxy-1-cyclohexene-1-carboxylic acid
6.005	385.113	$C_{17}H_{22}O_{10}$	385.114	−0.3	223, 208, 192, 179, 164, 149, 135, 121	1-sinapoyl-glucose
6.069	415.16	$C_{19}H_{28}O_{10}$	415.16	−0.5	329, 285, 269, 191, 161, 101	Benzyl-6-O-(6-deoxy-α-L-mannopyranosyl)-glucopyranoside
6.195	433.207	$C_{27}H_{30}O_{5}$	433.207	−1.6	345, 234, 289, 161	2,3,4-tri-O-benzyl-fucopyranose
6.342	609.148	$C_{27}H_{30}O_{16}$	609.146	4.6	462, 447, 299	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 6-deoxy-2-O-glucopyranosyl-mannopyranoside
6.427	335.076	$C_{16}H_{16}O_{8}$	335.077	−2.7	191, 179, 173, 161, 135	3-{[3-(3,4-dihydroxyphenyl)-2-propenoyl]oxy}-4,5-dihydroxy-1-cyclohexene-1-carboxylic acid

Contd...

Table S1: Contd...

Retention time (min)	Measured m/z	Formula	Theoretical m/z	Error (ppm)	Fragment	Proposed compound
6.595	515.117	$C_{25}H_{24}O_{12}$	515.119	−3.1	353, 335, 191, 179, 173, 161, 135	Dicaffeoyl-quinic acid somer 1
6.805	367.102	$C_{17}H_{20}O_{9}$	367.103	−3.8	191, 173, 134, 93	3-O-Feruloylquinic Acid
7.057	337.091	$C_{16}H_{18}O_{8}$	337.092	6.2	191, 173, 135, 85	p-Coumaroyl quinic acid
7.088	625.137	$C_{27}H_{30}O_{17}$	625.141	−5.3	300, 271, 191, 151	Quercetin 3-O-sophoroside
7.11	415.16	$C_{19}H_{28}O_{10}$	415.16	−0.5	255, 240, 181, 165	Glucopyranoside, 2-phenylethyl 6-O-xylopyranosyl
7.278	365.181	$C_{16}H_{30}O_{9}$	365.181	−1.6	347, 203, 135.97	5-[1,2-dihydroxy-2-propyl]-2-hydroxy-2-methylcyclohexyl -glucopyranoside
7.384	393.176	$C_{17}H_{30}O_{10}$	393.176	−1.5	232, 191, 131, 89, 71,	3-methyl-3-buten-1-yl-4-O-(6-deoxy-mannopyranosyl)-glucopyranoside
7.405	335.077	$C_{16}H_{16}O_{8}$	335.077	0.3	191, 161, 135	5-hydroxy-1-naphthyl-glucopyranosiduronic acid
7.541	463.22	$C_{21}H_{36}O_{11}$	463.218	4.3	419, 331, 191, 99	2-hydroxy-5-isopropenyl-2-methylcyclohexyl 6-O-[3,4-dihydroxy-4-(hydroxymethyl)tetrahydro-2-furanyl]-glucopyranoside
7.784	741.267	$C_{34}H_{46}O_{18}$	741.261	8.5	417, 402, 191, 181, 166	Eleutheroside E
7.835	595.13	$C_{26}H_{28}O_{16}$	595.13	−0.2	300, 271, 255, 191, 179, 155	Quercetin-3-arabinoglucoside
8.277	609.145	$C_{27}H_{30}O_{16}$	609.146	−0.7	463, 300, 271, 255, 243, 178, 150	Rutin isomer
8.33	609.143	$C_{27}H_{30}O_{16}$	609.146	−4.1	463, 300, 271, 255, 243, 178, 150	Rutin
8.33	287.053	$C_{15}H_{12}O_{6}$	287.056	−10.1	157, 135, 111, 107	Eriodictyol
8.435	463.086	$C_{21}H_{20}O_{12}$	463.088	−3.2	300, 271, 255, 243, 178, 150	Hyperoside
8.571	477.064	$C_{21}H_{18}O_{13}$	477.067	−5.7	300, 283, 273, 255, 245, 178, 150	Quer-3-GlcA
8.814	463.088	$C_{21}H_{20}O_{12}$	463.088	0.6	300, 271, 255, 243, 178, 150	Isoquercitrin
9.045	593.152	$C_{27}H_{30}O_{15}$	593.151	2.4	284, 255, 227, 191, 151	Kaempferol-3-O-robinobioside
9.392	515.116	$C_{25}H_{24}O_{12}$	515.119	−5.2	353, 335, 191, 179, 173, 161, 135	Dicaffeoyl-quinic acid somer 2
9.466	447.09	$C_{21}H_{20}O_{11}$	447.093	−5.4	284, 255, 227, 191, 151	Kaempferol 3-O-glucoside
9.666	593.15	$C_{27}H_{30}O_{15}$	593.151	−1.5	284, 255, 227, 191, 151	Kaempfero-l3-O-rhamnopyranosy-l-(1–>2)-glucopyranoside
9.707	515.117	$C_{25}H_{24}O_{12}$	515.119	−3.9	353, 335, 191, 179, 173, 161, 135	Dicaffeoyl-quinic acid somer 3
9.728	515.118	$C_{25}H_{24}O_{12}$	515.119	−2.5	353, 335, 191, 179, 173, 161, 135	Dicaffeoyl-quinic acid somer 4
10.107	601.121	$C_{28}H_{32}O_{16}$	601.119	2.8	395, 335, 233, 191, 179, 173, 11, 135	1-methoxyoxalyl-3,5-dicaffeoylquinic acid
10.203	477.106	$C_{22}H_{22}O_{12}$	477.103	5.4	314, 285, 271, 243, 215, 151	Isorhamnetin 3-glucoside
10.318	447.09	$C_{21}H_{20}O_{11}$	447.093	−5.8	300, 271, 255, 243, 178, 150	Quercitrin
10.571	477.102	$C_{22}H_{22}O_{12}$	477.103	−2.3	314, 299, 285, 271, 257, 243, 215	Isorhamnetin 3-O-galactopyranoside
11.264	515.117	$C_{25}H_{24}O_{12}$	515.119	−4.3	353, 335, 191, 179, 173, 161, 135	Dicaffeoyl-quinic acid isomer
11.286	601.118	$C_{28}H_{26}O_{15}$	601.119	−1.8	395, 335, 233, 191, 179, 173, 11, 135	1-MO-3,5-DCQA
11.643	405.117	$C_{20}H_{22}O_{9}$	405.119	−5.2	344, 243, 153, 135, 109, 91	2,3,5,4'-tetrahydroxystilbene 2-O-glucopyranoside
11.707	557.128	$C_{27}H_{26}O_{13}$	577.13	−2.3	335, 233, 191, 179, 173, 161, 135	3-[2-(3,4-dihydroxyphenyl)vinyl]-5-hydroxyphenyl 6-O-(3,4,5-trihydroxybenzoyl)-glucopyranoside
12	431.095	$C_{21}H_{20}O_{10}$	431.098	−7.0	285, 255, 227, 207	Kaempferol 3-rhamnoside
12.022	417.246	$C_{22}H_{26}O_{8}$	417.246	−0.3	402, 191, 181, 166	Syringaresinol
12.138	497.103	$C_{25}H_{22}O_{11}$	497.103	1.8	335, 255, 211, 179, 161, 135	4,5-bis{[3-(3,4-dihydroxyphenyl)-2-propenoyl]oxy}-1-hydroxy-2-cyclohexene-1-carboxylic acid
12.161	461.106	$C_{22}H_{22}O_{11}$	461.108	−5.6	314, 299, 285, 271, 257, 243, 215	5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4-oxo-4H-chromen-3-yl 6-deoxy-allopyranoside
12.19	557.129	$C_{27}H_{26}O_{13}$	557.13	−1.3	395, 335, 255, 233, 191, 179, 173, 161, 135	3-[2-(3,4-dihydroxyphenyl)vinyl]-5-hydroxyphenyl 6-O-(3,4,5-trihydroxybenzoyl)-glucopyranoside
12.2	431.192	$C_{21}H_{20}O_{10}$	431.192	0.9	285, 255, 227, 207	Kaempferol-7-rhamnoside
12.317	1219.61	$C_{59}H_{96}O_{26}$	1219.61	−2.9	749, 469, 409, 367, 323, 161, 101	Ciwujianoside A3
12.379	515.119	$C_{25}H_{24}O_{12}$	515.119	0.6	353, 335, 191, 179, 173, 161, 135	Dicaffeoyl-quinic acid somer 5
12.442	1277.61	$C_{61}H_{98}O_{28}$	1277.62	−2.2	765, 603, 511, 469, 409, 367, 323, 161, 101	Ciwujianoside A4
12.485	497.108	$C_{25}H_{22}O_{11}$	497.108	−1.8	335, 255, 211, 179, 161, 135	1-hydroxy-7-oxo-6-oxabicyclo[3.2.1]octane-3,4-diyl bis[3-(3,4-dihydroxyphenyl)acrylate]
12.696	643.128	$C_{30}H_{28}O_{16}$	643.13	−2.3	567, 389, 275, 233, 215, 173, 135	6-hydroxy-2-oxo-3-[(2-oxo-2H-chromen-7-yl)oxy]-2H-chromen-7-yl 6-O-(4-carboxy-3-hydroxy-3-methylbutanoyl)-glucopyranoside
12.768	1261.62	$C_{61}H_{98}O_{27}$	1261.62	−3.2	749, 603, 511, 469, 409, 367, 323, 161, 101	Kizuta saponin K11
12.885	447.223	$C_{21}H_{20}O_{11}$	447.226	−2.4	315, 295, 213, 191, 161	Orientin
12.947	339.069	$C_{15}H_{16}O_{9}$	339.072	−6.8	309, 279, 153, 135, 109, 91	8-hydroxy-2-oxo-2H-chromen-7-yl-allopyranoside
12.969	207.061	$C_{8}H_{16}O_{6}$	207.063	−3.4	179, 161, 135	Eleutheroside C
13.147	1217.6	$C_{59}H_{94}O_{26}$	1217.6	−0.2	747, 469, 409, 367, 323, 161, 101	6-deoxy-α-L-mannopyranosyl-(1–>4)-glucopyranosyl-(1–>6)-1-O-[3-{[2-O-(6-deoxy-α-L-mannopyranosyl)-arabinopyranosyl] oxy}-23,28-dioxoolean-12-en-28-yl]-glucopyr anose
13.464	349.116	$C_{14}H_{22}O_{10}$	349.114	6	305, 229, 203, 181, 157, 145, 119	4,15-dioxo-5,8,11,14-tetraoxaoctadecane-1,18-dioic acid
13.683	1219.6	$C_{59}H_{96}O_{26}$	1219.61	−5.6	749, 469, 409, 367, 323, 161, 101	Kalopanax saponin A
13.873	765.446	$C_{41}H_{66}O_{13}$	765.443	4.8	619, 601, 487, 191, 179	3-{[2-O-(6-deoxy-mannopyranosyl)-arabinopyranosyl]oxy}-16,23-dihydroxyolean-12-en-28-oic acid
13.926	1203.58	$C_{58}H_{92}O_{26}$	1203.58	−1.4	733, 571, 409, 367, 323, 161, 101	Ciwujianoside A2

Contd...

Table S1: Contd...

Retention time (min)	Measured m/z	Formula	Theoretical m/z	Error (ppm)	Fragment	Proposed compound
14.094	357.132	$C_{20}H_{22}O_6$	357.134	−4.5	342, 313, 221, 161	Matairesinol
14.168	735.433	$C_{40}H_{64}O_{12}$	735.432	7	587, 571, 457, 441, 191, 161, 101	10-{[6-deoxy-4-O-(β-D-glucopyranosyl)-galactopyranosyl]oxy}-1,2,9,9,12a-pentamethyl-1,3,4,5,6,6b,7,8,8a,9,10,11,12,12a,12b,13,14,14b-octadecahydro-4a(2 H)-picenecarboxylic acid
14.262	1073.56	$C_{53}H_{86}O_{22}$	1073.55	6.4	749, 603, 469, 409, 367, 323, 161, 101	Hederacoside D
14.411	327.217	$C_{18}H_{32}O_5$	327.217	−1.8	309, 291, 229, 211, 183, 171	Malyngic acid
14.411	779.425	$C_{41}H_{64}O_{14}$	779.428	−3.5	735, 617, 573, 555, 455, 441, 191	10-[(2-O-glucopyranosyl-glucopyranosyl)oxy]-11-hydroxy-2,2,6a,6b,12a-pentamethyl-9-methylene-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-o ctadecahydro-4a(2H)-picenecarboxylic acid
14.461	1187.59	$C_{58}H_{92}O_{25}$	1187.58	1.9	717, 469, 409, 367, 323, 161, 101	Ciwujianoside B
14.557	765.44	$C_{41}H_{66}O_{13}$	765.443	−2.7	721, 603, 585, 471	3-[(4-O-glucopyranosyl-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid
14.757	1245.59	$C_{60}H_{94}O_{27}$	1245.59	−3.2	733, 571, 511, 469, 409, 367, 323, 161, 101	Acanthopanaxoside A
14.82	1087.57	$C_{54}H_{88}O_{22}$	1087.57	−0.1	617, 469, 409, 367, 323, 161, 101	Nipponoside B
14.83	1219.61	$C_{59}H_{96}O_{26}$	1219.61	−1.8	749, 587, 469, 409, 367, 323, 161, 101	Ciwujianoside A1
15.051	1041.53	$C_{52}H_{82}O_{21}$	1041.53	3.9	717, 469, 409, 367, 323, 161, 101	1-O-{[10-{[2-O-(6-deoxy-glycero-hexopyranosyl)-glycero-pentopyranosyl]oxy}-6a,6b,9,9,12a-pentamethyl-2-methylene-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydro-4a(2H)-picenyl]carbonyl}-6-O-β-D-threo-hexopyranosyl-β-D-threo-hexopyranose
15.115	1219.61	$C_{59}H_{96}O_{26}$	1219.61	−1.7	749, 587, 469, 409, 367, 323, 161, 101	Begoniifolide A
15.198	763.426	$C_{41}H_{64}O_{13}$	763.427	−1.8	719, 617, 599, 573, 555, 485, 441	(3β)-3-{[2-O-(6-deoxy-mannopyranosyl)-arabinopyranosyl]oxy} olean-12-ene-28,29-dioic acid
15.283	1041.53	$C_{52}H_{82}O_{21}$	1041.53	−1.3	571, 469, 409, 367, 323, 161, 101	Ciwujianoside C1
15.704	1229.59	$C_{60}H_{94}O_{26}$	1229.6	−3.7	717, 511, 469, 409, 367, 323, 161, 101	Ciwujianoside C2
15.763	749.449	$C_{41}H_{66}O_{12}$	749.448	1.5	603, 585, 471	Kalopanax saponin B
15.77	1203.61	$C_{59}H_{96}O_{25}$	1203.62	−2.7	733, 469, 409, 367, 323, 161, 101	Cussosaponin C
16.187	1261.62	$C_{61}H_{98}O_{27}$	1261.62	0.8	749, 587, 511, 469, 409, 367, 323, 161, 101	Acanthopanaxoside B
16.334	1057.56	$C_{53}H_{86}O_{21}$	1057.56	6	733, 587, 455	Anhuienside C
16.408	1083.55	$C_{54}H_{84}O_{22}$	1083.54	7.6	571, 511, 469, 409, 367, 323, 161, 101	Ciwujianoside D2
16.545	1057.57	$C_{53}H_{86}O_{21}$	1057.56	8.6	587, 469, 409, 367, 323, 161, 101	Ciwujianoside C3
16.819	1245.62	$C_{61}H_{98}O_{26}$	1245.63	−2.6	733, 511, 469, 409, 367, 323, 161, 101	Ciwujianoside C4
17.051	793.437	$C_{42}H_{66}O_{14}$	793.437	−0.8	749, 631, 569, 455	Silphioside G
17.579	1099.57	$C_{55}H_{88}O_{22}$	1099.57	1.6	733, 587, 511, 469, 409, 367, 323, 161, 101	Saniculoside N
17.987	749.446	$C_{41}H_{66}O_{12}$	749.448	−2.0	587, 471	a-hederin
18.155	747.43	$C_{41}H_{64}O_{12}$	747.432	−2.8	601, 469, 409, 367, 323, 161, 101	3-{[2-O-(6-deoxy-mannopyranosyl)-arabinopyranosyl]oxy}-23-oxoolean-12-en-28-oic acid
18.483	895.508	$C_{47}H_{76}O_{16}$	895.506	3.1	895, 733, 587, 455	1-O-[3-{[2-O-(6-deoxy-allopyranosyl)-lyxopyranosyl]oxy}-28-oxoolean-12-en-28-yl]-glucopyranose
18.996	603.39	$C_{35}H_{56}O_8$	603.39	0.3	471	3-(arabinopyranosyloxy)-19-hydroxyolean-12-en-28-oic acid
19.47	667.442	$C_{37}H_{64}O_{10}$	667.442	−0.4	621, 460, 309, 165	1-(decanoyloxy)-3-galactopyranosyloxy-2-propanyl 9,12,15-octadecatrienoate
19.701	1203.61	$C_{59}H_{96}O_{25}$	1203.62	−1.1	733, 587, 469, 455, 409, 367, 323, 161, 101	Hederasaponin B
20.195	717.422	$C_{40}H_{62}O_{11}$	717.421	0.6	571, 553, 439	Ciwujianoside E
20.595	733.453	$C_{41}H_{66}O_{11}$	733.453	0.1	587, 569, 455	Eleutheroside I

ESI: Electrospray ionization; UPLC-QTOF-MS: Ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry

on. However, these glycosides with the ability of water solubility are not easily absorbed in the intestines, and their biological availabilities are lower. Therefore, the deglycosylation process of plant glycosides is crucial for its pharmacological expression. These glycosides are generally hydrolyzed into active aglycones and then absorbed by intestinal flora.[17] In this study, we found flavonoid aglycones and triterpenoid aglycones, which were produced by these compounds with the same basic parent structure [Table S2].

CONCLUSION

A simple, reliable, and sensitive method was developed to separate and identify chemical compounds of leaves extracts of AS by UPLC-QTOF-MS. By using this method, 131 compounds have been characterized or tentatively characterized including triterpenoid saponins, phenols, flavonoids, lignans, coumarins, and polysaccharides. The experimental results, therefore, demonstrate that UPLC-QTOF-MS is a powerful analytical tool in the study of chemical compounds of herbal medicine. It also set a good example for the rapid identification of bioactive constituents in plant extracts and made it possible to fulfill the requirements for a modern drug with characters of safety, efficacy, and stability.

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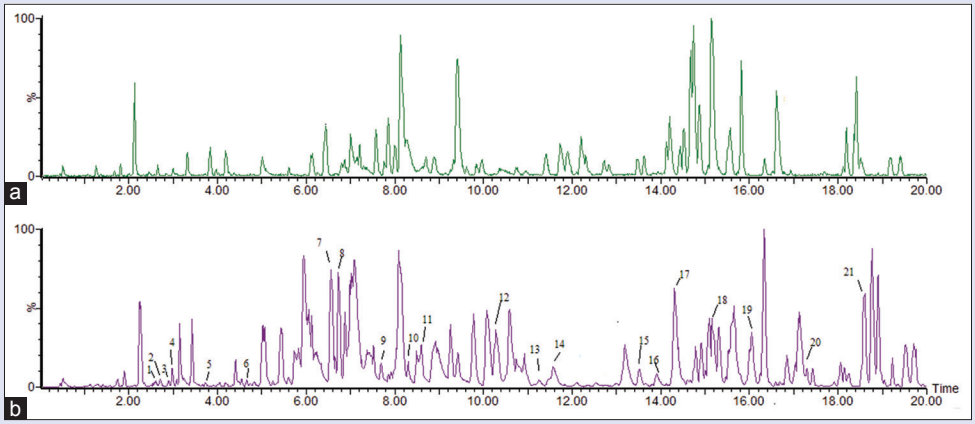

Figure 2: Liquid chromatography-mass spectrometry chromatograms of (a) control rat serum. (b) Dosed rat serum

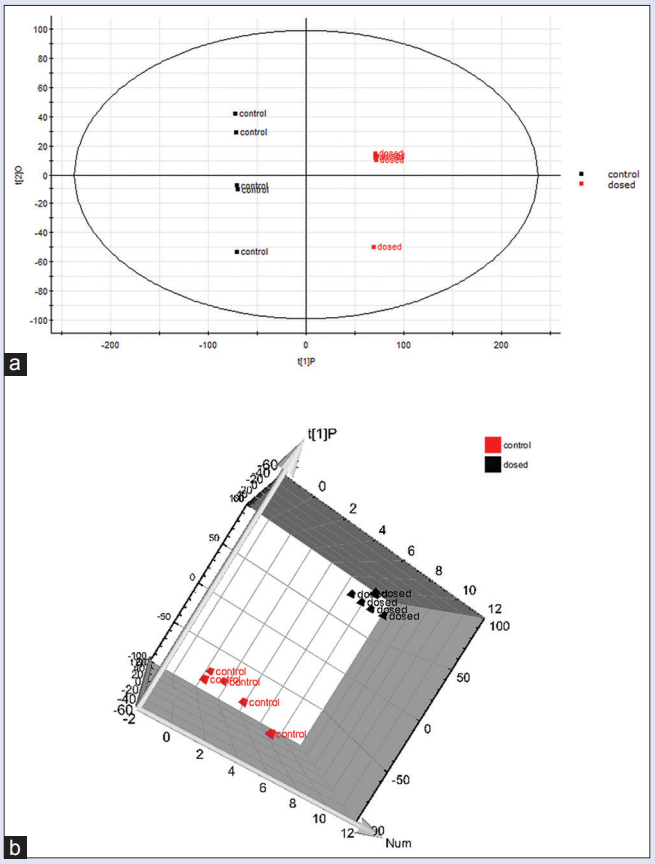

Figure 3: (a) Principal component analysis score plot for the control and dosed group. (b) Three-dimensional-principal component analysis plot for the control and dosed group

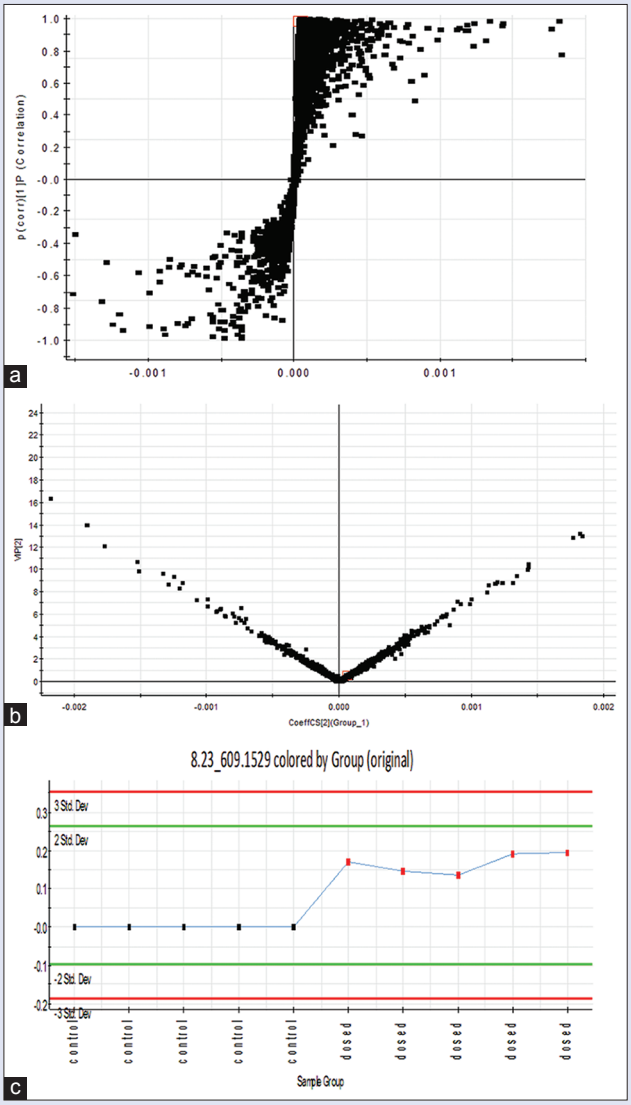

Figure 4: (a) S-plot of OPLS-discriminant analysis result for control and dosed group in negative mode. (b) VIP value result for control and dosed group in negative mode. (c) The trend plot of 8.23–609.1529

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Nil.

Conflicts of interest

There are no conflicts of interest.

Table S2: Identification of serum from leaves extracts-administrated-rats analyzed using UPLC-QTOF-MS

Retention time (min)	Metabolite name	Measured m/z	Formula	Fragment	Parent drug
2.66	Parent	153.052	$C_7H_6O_4$	109	Protocatechuic acid
2.78	Parent	219.0466	$C_9H_{16}O_6$	173, 154, 129, 111	1-O-butyryl-xylopyranose
2.95	Parent	191.0428	$C_7H_{12}O_6$	173, 137, 127, 109	Quinic acid
3.06	Reduction	181.0579	$C_9H_{10}O_4$	137, 119, 109	Caffeic acid
3.78	Sulfate conjugation	447.1034	$C_{17}H_{20}O_{12}S$	367, 191, 173, 134, 93	Feruloyl quinic acid
4.69	Parent	179.0341	$C_9H_8O_4$	135, 117, 107	Caffeic acid
6.74	Sulfoxide to thioether	163.0473	$C_9H_8O_3$	119	Caffeic acid
6.80	Parent	367.1021	$C_{17}H_{20}O_9$	191, 173, 134, 93	Feruloyl quinic acid
7.69	Metylation	193.0579	$C_{10}H_{10}O_4$	178, 149, 134	Caffeic acid
8.23	Parent	609.1529	$C_{27}H_{30}O_{16}$	463, 300, 271, 255, 243, 178, 150	Rutin
8.68	Parent	463.08	$C_{21}H_{20}O_{12}$	300, 271, 255, 243, 178, 150	Hyperoside
10.22	Parent	447.0857	$C_{21}H_{20}O_{11}$	300, 271, 255, 243, 178, 150	Quercitrin
11.24	Deglycosylation	300.0172	$C_{15}H_{10}O_7$	271, 255, 243, 178, 150	
11.61	Parent	405.1206	$C_{20}H_{22}O_9$	344, 243, 153, 135, 109, 91	2,3,5,4'-tetrahydroxystilbene 2-O-glucopyranoside
13.46	Parent	349.1147	$C_{14}H_{22}O_{10}$	305, 229, 203, 181, 157, 145, 119	4,15-dioxo-5,8,11,14-tetraoxaoctadecane-1,18-dioic acid
14.06	Deglycosylation	284.0210	$C_{15}H_{10}O_6$	255, 227, 150	
14.4	Parent	327.2154	$C_{18}H_{32}O_5$	309, 291, 229, 211, 183, 171	Malyngic acid
15.21	Parent	763.4344	$C_{41}H_{64}O_{13}$	719, 617, 599, 573, 555, 485, 441	3-{[2-O-(6-deoxy-mannopyranosyl)-arabinopyranosyl] oxy}olean-12-ene-28,29-dioic acid
16.12	Deglycosylation	314.041	$C_{16}H_{12}O_7$	300, 285, 271, 243, 151	
17.32	Deglycosylation	471.6897	$C_{30}H_{48}O_4$	248, 224, 203	
18.54	Deglycosylation	455.7021	$C_{30}H_{48}O_3$	248, 208, 203	

UPLC-QTOF-MS: Ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry

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