RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2020, **10**, 8396

Comprehensive metabolomics analysis based on UPLC-Q/TOF-MS^E and the anti-COPD effect of different parts of *Celastrus orbiculatus* Thunb.

Na Yang,[a] Han Wang,[a] Hongqiang Lin,[a] Junli Liu,[a] Baisong Zhou,[a] Xiaoling Chen,[a] Cuizhu Wang,[ab] Jinping Liu *[ab] and Pingya Li*[a]

The root, stem and leaf of *Celastrus orbiculatus* Thunb. (COT) have all been used as Chinese folk medicine. Aiming at revealing the secondary metabolites and screening the anti-COPD effect of COT, the comprehensive phytochemical and bioassay studies were performed. Based on the ultra-high performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF-MS^E), the screening analysis of components in COT was conducted with the UNIFI platform, the metabolomics of the three parts were analyzed with multivariate statistical analysis. Cigarette smoke extract (CSE)-stimulated inflammatory model in A549 cells was used to investigate the biological effect of the three parts. A total of 120 compounds were identified or tentatively characterized from COT. Metabolomics analysis showed that the three parts of COT were differentiated, and there were 13, 8 and 5 potential chemical markers discovered from root, stem and leaf, respectively. Five robust chemical markers with high responses could be used for further quality control in different parts of COT. The root, stem and leaf of COT could evidently reduce the levels of pro-inflammatory factors in a dose-dependent way within a certain concentration range. The stem part had a stronger anti-COPD effect than root and leaf parts. This study clarified the structural diversity of secondary metabolites and the various patterns in different parts of COT, and provided a theoretical basis for further utilization and development of COT.

Received 28th November 2019
Accepted 18th February 2020

DOI: 10.1039/c9ra09965d

rsc.li/rsc-advances

1. Introduction

Celastrus orbiculatus Thunb. (COT), belonging to the family Celastraceae and the genus *Celastrus* L., is widely distributed throughout China.[1] The parts including root, stem and leaf all could been used as Chinese folk medicine to treat rheumatoid arthritis, vomiting, abdominal pain, and snakebites.[2,3] The root of COT was reported to possess anti-tumor,[4] antiviral,[5] bacteriostatic[6] and lipid-lowering[1] activities, and about 50 compounds, including triterpenes, sesquiterpenoids, steroids and organic acids, were isolated from the root or root bark.[3,7–9] The stem was also reported to have anti-inflammation,[10] anti-cancer[11] and fatty liver amelioration[12] effects, and nearly 100 compounds including triterpenes, diterpenoids, steroids, flavonoids, phenolics and benzoquinone were isolated.[13–16] For the leaf part, previous studies have found that the extract of it had insecticidal effect and hypoglycemic effect,[17] while only a few flavonoids were isolated.[18] It was revealed that there were significant variation for the contents of celastrol or total alkaloids in different parts of COT.[19,20] Along with continuous expansion of the folk and clinical application of COT, an in-depth study on the chemical constituents in different parts of COT has attracted more and more attention. However, the comprehensive comparative study on the chemical composition between root, stem and leaf parts of COT has not been reported so far.

Recently, the UPLC-Q/TOF-MS method has been innovatively used for screening and identifying chemical components in herbal medicines and traditional Chinese medicine. And the global profiling of various metabolites were reported. As part of these research works, we reported this method to detect some natural products including *Platycodon grandiflorum* and Ginseng root.[21,22] Our research results showed that this method is high throughput, comprehensive, simple and efficient. As far as we know, the UPLC-Q/TOF-MS method has not been reported to identify the components in COT. So, the study in this paper comparatively analyzes the phytochemicals of root, stem and leaf parts of COT by using the UPLC-Q/TOF-MS method for the first time and finds out the similarities and differences between them.

Chronic Obstructive Pulmonary Disease (COPD), predicted to rank as the third leading cause of death in the world,[23] is mainly caused by significant exposure to harmful gases or

[a]*School of Pharmaceutical Sciences, Jilin University, Fujin Road 126, Changchun 130021, Jilin, China. E-mail: liujp@jlu.edu.cn; lipy@jlu.edu.cn; Tel: +86-431-85619803*

[b]*Research Center of Natural Drug, Jilin University, Changchun 130021, Jilin, China*

particles.[24] Cigarette smoking was the leading environmental risk factor for COPD around the world, and cigarette smokers were more likely to develop respiratory symptoms and had a higher COPD mortality rate. Along with the progressive lung inflammation, some pro-inflammatory mediators such as IL-1β, IL-6 and TNF-α participated in the occurrence and development of COPD.[25] Although the COT had been used in treating various inflammatory diseases, the effect on the cigarette smoke extract (CSE)-induced inflammatory reaction has not been reported so far.

In the present study, the main medicinal parts of COT (root, stem and leaf) were chosen as the test sample. On one hand, the similarities and differences of phytochemicals in three parts were analyzed by using UNIFI platform and untargeted metabolomics based on UPLC-Q/TOF-MS^E. The components and potential chemical markers to profile diverse classifications of metabolites of three parts were investigated. On the other hand, the effects on CSE-induced inflammatory reaction of these three parts were explored in A549 cells. The anti-COPD activity of different parts was preliminarily discussed. This comprehensive study could reveal the structural diversity of secondary metabolites and the different patterns of main medicinal parts of COT, and provide the data for further clinical application in anti-COPD. The study on the phytochemistry and the pharmacological activity of various parts were both significantly valuable to the research and development of COT.

2. Experiment

2.1. Materials and reagents

A total of 10 batches of fresh COT were collected from different growth areas in China (Table 1). All herbs were authenticated by the authors according to Hunan Province Local Standard for Traditional Chinese Medicine (2009 edition) for "*Celastrus orbiculatus* Thunb.". The corresponding specimens had been deposited in the Research Center of Natural Drug, Jilin University, China.

Methanol and acetonitrile were of LC/MS grade purchased from Fisher Chemical Company. Formic acid was bought from Sigma-Aldrich Company, St. Louis, MO, USA. Deionized water was purified by Millipore water purification system (Millipore, Billerica, MA, USA). All other chemicals were analytically pure. Cigarettes for bioassay analysis were Xiongshi cigarette (China Tobacco Zhejiang Industrial Co., Ltd, Hangzhou, China), each cigarette contained 11 mg of tar, 0.7 mg of nicotine, and 13 mg of carbon monoxide. Human lung carcinoma A549 cells were obtained from the Department of Pathogen Biology, Basic Medical College, Jilin University. ELISA kits were bought from Nanjing Jiancheng Bio-engineering Institute.

2.2. Sample preparation of three parts of COT

Took the whole fresh COT, and separated the root, stem and leaf part respectively to get 30 test samples including root part (R1–R10) samples, stem part (S1–S10) samples and leaf part (L1–L10) samples. The aforementioned parts were air-dried, grinded and sieved with Chinese National Standard Sieve No. 3, R40/3 series to obtain the homogeneous powder respectively. Each powder was weighted (2.0 g) accurately and extracted thrice (3 hours per time) with 100 mL of 80% methanol at 80 °C, cooled, filtered, collected and combined the filtrate of each sample, concentrated and evaporated to dryness.

For metabonomics analysis, each residue (all approximately 2.0 mg) was dissolved in 1.0 mL of 80% methanol respectively, after being filtered with a syringe filter (0.22 μm), 30 test solutions (R_{M1}–R_{M10}, S_{M1}–S_{M10} and L_{M1}–L_{M10}) were obtained, which was injected into the UPLC system directly. Furthermore, to ensure the suitability consistency and the stability of MS analysis, a sample for quality control (QC) was prepared by pooling 20 μL from every test solution, namely containing all of the constituents in this analysis.

For screening analysis, the test solutions of root part (R_S), stem part (S_S) and leaf part (L_S) were prepared by pooling 100 μL from R_{M1}–R_{M10}, S_{M1}–S_{M10} and L_{M1}–L_{M10} solutions, respectively.

For bioassay analysis, the test samples (R_{bio}, S_{bio} and L_{bio}) of root part, stem part and leaf part were prepared by combining each residue of R_1–R_{10}, S_1–S_{10} and L_1–L_{10}, respectively. Then, R_{bio}, S_{bio} and L_{bio} were dissolved in water at the concentration of 3.2 mg mL^{-1} to get the stock solutions stored in 4 °C.

2.3. Ultra-high performance liquid chromatography combined with quadrupole time-of-flight tandem mass spectrometry (UPLC-Q/TOF-MS^E)

The separation and detection of components were performed on the UPLC system combined with Xevo G2-XS Q/TOF mass

Table 1 The detail information of the collected COT samples

No.	Source	Collection time
COT 01	Yichun city, Heilongjiang province, China	12th August, 2017
COT 02	Changchun city, Jilin province, China	3rd August, 2017
COT 03	Huhhot city, Inner Mongolia autonomous region, China	10th August, 2017
COT 04	Lanzhou city, Gansu province, China	13th August, 2017
COT 05	Taian city, Shandong province, China	5th July, 2017
COT 06	Xi'an city, Shanxi province, China	14th July, 2017
COT 07	Zhengzhou city, Henan province, China	15th July, 2017
COT 08	Chengdu city, Sichuan province, China	7th July, 2017
COT 09	Changsha city, Hunan province, China	27th July, 2017
COT 10	Jinhua city, Zhejiang province, China	2nd July, 2017

Table 2 Compounds identified from the root, stem and leaf of COT by UPLC-QTOF-MS^{E}[a]

No.	t_R (min)	Formula	Calculated mass (Da)	Theoretical mass (Da)	Mass error (ppm)	MS^{E} fragmentation	Identification	Source	Ref.
1	0.59	$C_{29}H_{28}O_8$	504.1828	504.1843	−2.7	549.1810 $[M + H - COO]^-$, 382.1400 $[M - H - C_7H_5O_2]^-$, 311.0857 $[M - H - C_{11}H_{12}O_3]^-$	Interiotherin A	R	33
2#	1.23	$C_{19}H_{20}O_6$	344.1256	344.1260	−1.1	367.1486 $[M + Na]^+$, 327.1185 $[M + H - H_2O]^+$, 315.0707 $[M + H - 2 \times CH_3]^+$, 238.0591 $[M + H - C_7H_7O]^+$, 224.0341 $[M + H - C_8H_9O]^+$	5,7-Dihydroxy-6,8-dimethyl-3(*S*)-3-(3-methoxy-4′-hydroxybenzyl) chroman-4-one	S	—
3	2.43	$C_9H_{12}O_4$	184.0728	184.0736	−4.4	185.0791 $[M + H]^+$, 167.0584 $[M + H - H_2O]^+$, 136.0593 $[M + H - H_2O - CH_2OH]^+$	Eucommidiol	R, S	34
4	3.60	$C_{16}H_{18}N_2O_3$	286.1328	286.1317	3.5	309.1220 $[M + Na]^+$, 242.0841 $[M + H - 3 \times CH_3]^+$, 225.0989 $[M + H - 2 \times OCH_3]^+$	Picrasidine B	S, L	a
5	3.64	$C_{22}H_{28}O_{11}$	468.1616	468.1632	−3.4	469.1660 $[M + H]^+$, 290.1036 $[M + H\text{-Glu}]^+$, 232.0691 $[M + H - C_3H_7O\text{-Glu}]^+$	Cimicifuga glycoside	R	35
6	3.66	$C_{19}H_{30}O_8$	386.1925	386.1941	−4.2	409.1811 $[M + Na]^+$, 226.0124 $[M + H - C_6H_9O_5]^+$, 178.0837 $[M + H - 2 \times CH_3\text{-Glu}]^+$, 190.1255 $[M + H - H_2O\text{-Glu}]^+$	Roseoside	R, S, L	36
7	3.97	$C_{25}H_{28}O_6$	424.1901	424.1886	3.6	447.1812 $[M + Na]^+$, 407.1226 $[M + H - H_2O]^+$, 303.0539 $[M + H - C_9H_{14}]^+$, 176.0533 $[M + H - H_2O - C_9H_{14} - C_6H_5O_2]^+$	Norkurarinone	R	37
8	4.17	$C_{19}H_{20}O_6$	344.1244	344.1260	−4.7	345.1316 $[M + H]^+$, 222.0530 $[M + H - CH_3 - C_7H_7O]^+$, 153.0511 $[M + H - C_{11}H_{12}O_3]^+$	2,3-Dihydro-5,7-dihydroxy-8-methoxy-3-[(4-methoxyphenyl)methyl]-6-methyl-4*H*-1-benzopyran-4-one	S	38
9	4.44	$C_{15}H_{18}O_8$	326.0993	326.1002	−2.9	325.0870 $[M - H]^-$, 128.0426 $[M - H - H_2O\text{-Glu}]^-$, 100.0409 $[M - H - HCOOH\text{-Glu}]^-$, 75.0570 $[M - H - C_3H_3O_2\text{-Glu}]^-$	Glucosido-*p*-coumaric acid	R	s
10	4.60	$C_{20}H_{20}O_5$	340.1295	340.1311	−4.5	341.1355 $[M + H]^+$, 256.0641 $[M + H - C_5H_9O]^+$, 238.0692 $[M + H - H_2O - C_5H_9O]^+$, 193.0713 $[M + H - C_9H_7O_2]^+$	Psorachalcone A	S	—
11※	5.02	$C_{32}H_{34}O_{12}$	610.2048	610.2050	−0.4	609.1446 $[M - H]^-$, 541.2011 $[M - H - O - C_2H_3O]^-$, 371.1898 $[M - H - O - 2 \times C_5H_3O_3]^-$	Orbiculin I	L	—
12	5.28	$C_{27}H_{36}O_{13}$	568.2139	568.2156	−2.8	591.2031 $[M + Na]^+$, 359.1459 $[M + H - CH_2OH\text{-Glu}]^+$, 345.1251 $[M + H - CH_3 - C_{11}H_{13}O_4]^+$	Citrusin B	S, L	s
13	5.39	$C_{15}H_{10}O_7$	302.0415	302.0427	−4.1	303.0484 $[M + H]^+$, 178.0320 $[M + H - C_6H_5O_3]^+$, 108.0286 $[M + H - H_2O - C_9H_5O_4]^+$	Isoetin	S	s
14※	5.53	$C_{27}H_{30}O_{16}$	610.1539	610.1534	0.9	611.1613 $[M + H]^+$, 432.1040 $[M + H\text{-Glu}]^+$, 253.0388 $[M + H - 2 \times Glu]^+$	Luteolin 7,4′-diglucoside	L	39
15	5.65	$C_{24}H_{32}O_{10}$	480.2007	480.1996	2.3	503.1899 $[M + Na]^+$, 386.1517 $[M + H - 2 \times H_2O - COOCH_3]^+$, 360.1426 $[M + H - C_8H_9O]^+$, 191.0715 $[M + H - C_8H_9O - C_9H_{13}O_3]^+$	Ilexin L3	S	—
16	5.66	$C_{20}H_{24}O_7$	376.1505	376.1522	−4.5	399.1388 $[M + Na]^+$, 316.1217 $[M + H - 2 \times CH_3 - CH_2OH]^+$, 310.1320 $[M + H - 2 \times H_2O - CH_3O]^+$, 138.0597 $[M + H - C_{12}H_{15}O_5]^+$	Vladinol C	R	40
17	5.74	$C_{15}H_{10}O_7$	302.0418	302.0427	−2.9	303.0491 $[M + H]^+$, 285.0363 $[M + H - H_2O]^+$, 110.0281 $[M + H - C_9H_5O_5]^+$	Quercetin	S	b

Table 2 (*Contd.*)

No.	t_R (min)	Formula	Calculated mass (Da)	Theoretical mass (Da)	Mass error (ppm)	MS^E fragmentation	Identification	Source	Ref.
18	5.87	$C_{19}H_{18}O_4$	310.1193	310.1205	−3.8	309.1266 $[M-H]^-$, 279.0821 $[M-H-2\times CH_3]^-$, 278.0977 $[M-H-OCH_3]^-$, 173.0532 $[M-H-OCH_3-C_8H_9]^-$, 104.0703 $[M-H-C_{11}H_9O_4]^-$	6,7-Dimethoxy-2-phenethylchromone	S	s
19	5.96	$C_{10}H_{10}O_3$	178.0623	178.0630	−3.9	179.0687 $[M+H]^+$, 164.0374 $[M+H-CH_3]^+$, 120.0491 $[M+H-COOCH_3]^+$, 86.0307 $[M+H-C_6H_5O]^+$	Methyl-*p*-coumarate	R	41
20	6.75	$C_{17}H_{14}O_8$	346.0688	346.0689	−0.1	347.0761 $[M+H]^+$, 329.0629 $[M+H-H_2O]^+$, 298.0688 $[M+H-H_2O-OCH_3]^+$, 219.0460 $[M+H-H_2O-C_6H_5O_2]^+$	Aksilarin	R	s
21	7.22	$C_{14}H_{16}O_4$	248.1040	248.1049	−3.5	249.1105 $[M+H]^+$, 206.0877 $[M+H-CH_3CO]^+$, 193.1007 $[M+H-C_3H_4O]^+$	Evodinnol	R	42
22	7.58	$C_{21}H_{22}O_6$	370.1410	370.1416	−1.7	371.1483 $[M+H]^+$, 220 $[M+H-C_9H_{11}O_2]^+$, 152 $[M+H-C_{12}H_{11}O_4]^+$	(+)-7,8-Didehydroarctigenin	R	43
23	7.73	$C_{17}H_{20}O_4$	288.1360	288.1362	−0.5	311.1252 $[M+Na]^+$, 274.1090 $[M+H-CH_3]^+$, 238.0842 $[M+H-2\times H_2O-CH_3]^+$	(+)-Celaphanol A	R, S	s
24	8.47	$C_{34}H_{44}O_{14}$	676.2738	676.2731	1.0	677.2811 $[M+H]^+$, 585.2390 $[M+H-H_2O-CH_3-C_2H_3O_2]^+$, 556.2650 $[M+H-C_7H_5O_2]^+$, 441.1997 $[M+H-4\times C_2H_3O_2]^+$	Celangulin IV	S, L	—
25	8.57	$C_{22}H_{24}O_7$	400.1407	400.1522	−3.8	401.1559 $[M+H]^+$, 383.0623 $[M+H-H_2O]^+$, 365.2118 $[M+H-2\times H_2O]^+$, 234.0763 $[M+H-C_9H_{11}O_3]^+$	Aschantin	R, S	44
26	8.83	$C_{20}H_{20}O_5$	340.1295	340.1311	−4.5	341.1355 $[M+H]^+$, 326.0641 $[M+H-CH_3]^+$, 299.0562 $[M+H-C_3H_7]^+$, 150.0562 $[M+H-C_4H_7-C_8H_7O_2]^+$	Corylifol B	S	45
27	8.94	$C_{20}H_{18}O_5$	338.1143	338.1154	−3.3	339.1209 $[M+H]^+$, 218.0945 $[M+H-C_8H_7O]^+$, 176.0735 $[M+H-H_2O-C_9H_7O_2]^+$	Demethoxycurcumin	R	46
28	8.95	$C_{20}H_{20}O_6$	356.1247	356.1260	−3.6	357.1320 $[M+H]^+$, 245.0820 $[M+H-CH_3-C_6H_5O_2]^+$, 96.0346 $[M+H-H_2O-C_{14}H_{11}O_4]^+$	Leachianone G	R	s
29	8.96	$C_{18}H_{20}O_5$	316.1317	316.1311	1.8	339.1209 $[M+Na]^+$, 251.0624 $[M+H-2\times H_2O-2\times CH_3]^+$, 174.0900 $[M+H-2\times H_2O-C_7H_7O]^+$	(3*R-cis*)-3,4-Dihydro-3,4-diol-7-methoxy-3-[(4-methoxyphenyl)methyl]-2*H*-1-benzopyran	R, S	—
30#	8.98	$C_{22}H_{28}O_8$	420.1799	420.1784	3.4	443.1696 $[M+Na]^+$, 205.0843 $[M+H-2\times CH_2OH-C_8H_9O_3]^+$, 167.0664 $[M+H-C_{13}H_{18}O_5]^+$	(+)-Lyoniresinol	S	47
31	9.81	$C_{24}H_{26}O_8$	442.1611	442.1628	−3.8	443.1675 $[M+H]^+$, 384.1466 $[M+H-C_2H_3O_2]^+$, 381.1268 $[M+H-2\times CH_3O]^+$, 307.0731 $[M+H-CH_3-2\times CH_3O-C_2H_3O_2]^+$	Interiorin C	R, S	—
32	10.35	$C_{20}H_{18}O_4$	322.1218	322.1205	4.0	323.1291 $[M+H]^+$, 268.1004 $[M+H-C_4H_7]^+$, 254.0834 $[M+H-C_5H_9]^+$	Neobavaisoflavone	R	48
33	11.39	$C_{18}H_{34}O_5$	330.2392	330.2406	−4.0	353.2284 $[M+Na]^+$, 277.2129 $[M+H-3\times H_2O]^+$, 150.1100 $[M+H-2\times H_2O-C_8H_{17}O_2]^+$, 81.0569 $[M+H-H_2O-C_4H_7O_2-C_8H_{17}O_2]^+$	9-Octadecenoic acid	R, S	s
34	13.75	$C_{18}H_{20}O_4$	300.1348	300.1362	−4.3	323.1240 $[M+Na]^+$, 138.0556 $[M+H-C_{10}H_{11}O_2]^+$, 107.0465 $[M+H-CH_3O-C_{10}H_{11}O_2]^+$	*trans*-3,3′,5,5′-Tetramethoxystilbene	S	—
35*	14.06	$C_{30}H_{48}O_6$	504.3448	504.3451	−0.6		Virgaureagenin G	R	49

Table 2 (*Contd.*)

No.	t_R (min)	Formula	Calculated mass (Da)	Theoretical mass (Da)	Mass error (ppm)	MS^E fragmentation	Identification	Source	Ref.
						549.3417 $[M + HCOO]^-$, 263.3423 $[M - H - C_{14}H_{24}O_3]^-$, 239.3239 $[M - H - C_{16}H_{24}O_3]^-$			
36	14.33	$C_{28}H_{42}O_5$	458.3053	458.3032	4.6	457.2981 $[M - H]^-$, 344.3321 $[M - H - 2 \times H_2O - HCOOH - CH_2OH]^-$, 233.2957 $[M - H - C_{13}H_{20}O_3]^-$,	3β,4β,23-Trihydroxy-24,30-dinorolean-12,20(29)-dien-28-oic acid	R	50
37	15.19	$C_{28}H_{46}O_5$	462.3349	462.3345	0.8	461.3276 $[M - H]^-$, 425.3270 $[M - H - 2 \times H_2O]^-$, 193.2396 $[M - H - C_{17}H_{32}O_2]^-$	Polyporusterone F	R	51
38	15.26	$C_{39}H_{54}O_6$	618.3926	618.3920	1.0	641.3857 $[M + Na]^+$, 533.3344 $[M + H - C_3H_4 - HCOOH]^+$, 398.2486 $[M - 2 \times CH_3 - C_3H_4 - C_8H_7O_3]^+$	Lup-20(29)-en-28-oic-3β-yl caffeate	R	s
39*	15.47	$C_{30}H_{40}O_4$	464.2934	464.2927	1.5	509.2551 $[M + HCOO]^-$, 386.2693 $[M - H - H_2O - C_2H_3O_2]^-$, 199.2228 $[M - H - C_{17}H_{28}O_2]^-$	Pristimerin	R	52
40※	15.84	$C_{33}H_{38}O_9$	578.2535	578.2516	3.3	577.2683 $[M - H]^-$, 561.2680 $[M - H - O]^-$, 547.2187 $[M - H - 2 \times CH_3]^-$, 534.2176 $[M - H - C_2H_3O]^-$	Orbiculin A	R, L	s
41	16.28	$C_{30}H_{46}O_4$	470.3399	470.3396	0.6	469.3326 $[M - H]^-$, 454.3157 $[M - H - CH_3]^-$, 451.3303 $[M - H - H_2O]^-$, 342.3522 $[M - H - C_8H_{15}O]^-$	11-Oxokansenonol	R	53
42	16.52	$C_{30}H_{46}O_6$	502.3315	502.3294	4.1	547.3326 $[M + HCOO]^-$, 455.3236 $[M - H - HCOOH]^-$, 401.3505 $[M - H - 3 \times H_2O - HCOOH]^-$	Esculentic acid	R	s
43	16.66	$C_{31}H_{44}O_6$	512.3118	512.3138	−3.9	511.3045 $[M - H]^-$, 495.2929 $[M - H - O]^-$, 467.2412 $[M - H - CO_2]^-$, 233.2535 $[M - H - C_{17}H_{26}O_3]^-$	Paeonenolide G	R, L	54
44	16.79	$C_{32}H_{40}O_8$	552.2717	552.2723	−1.1	597.2699 $[M + HCOO]^-$, 487.2250 $[M - H - H_2O - CH_3 - CH_3O]^-$, 193.1065 $[M - H - C_{21}H_{26}O_5]^-$	Saucerneol A	R, S, L	55
45	16.80	$C_{27}H_{44}O_8$	496.3055	496.3036	3.8	541.3050 $[M + HCOO]^-$, 461.3026 $[M - H - 2 \times H_2O]^-$, 378.2876 $[M - H - C_6H_{13}O_2]^-$	Turkesterone	R, L	56
46	16.81	$C_{30}H_{50}O_2$	442.3907	442.3811	−0.9	441.3713 $[M - H]^-$, 423.3286 $[M - H - H_2O]^-$, 233.3456 $[M - H - C_{14}H_{24}O]^-$	Oleanolic alcohol	S, L	57
47	17.06	$C_{20}H_{34}O_4$	338.2461	338.2457	1.1	361.2353 $[M + Na]^+$, 267.2239 $[M + H - 4 \times H_2O]^+$, 262.1673 $[M + H - CH_3 - 2 \times CH_2OH]^+$, 278.0124 $[M + H - C_3H_5O_2]^+$	Hallol	R	a
48	17.22	$C_{18}H_{28}O_2$	276.2086	276.2089	−1.0	277.2141 $[M + H]^+$, 248.2197 $[M + H - C_2H_5]^+$, 222.1520 $[M + H - C_4H_7]^+$, 67.0579 $[M + H - C_2H_5 - C_{11}H_{17}O_2]^+$	6,9,12,15-Octadecatetraenoic acid	R	58
49	17.23	$C_{16}H_{30}O_2$	254.2249	254.2246	1.2	277.2141 $[M + Na]^+$, 125.1160 $[M + H - C_4H_9 - C_3H_5O_2]^+$, 112.0804 $[M + H - H_2O - C_9H_{17}]^+$	Oleopalmitic acid	R, S, L	59
50*	17.25	$C_{30}H_{44}O_4$	468.3225	468.3240	−3.2	467.2441 $[M - H]^-$, 434.2905 $[M - H - H_2O - CH_3]^-$, 423.3320 $[M - H - CO_2]^-$	3β-Hydroxy-2-oxoolean-12-ene-22,29-actone	R	—
51*	17.30	$C_{27}H_{40}O_6$	460.2833	460.2825	1.7	483.2751 $[M + Na]^+$, 361.2355 $[M + H - C_6H_{12}O]^+$, 417.2282 $[M + H - CO_2]^+$, 319.2480 $[M + H - C_8H_{14}O_2]^+$	Lucidenic acid N	R	60
52#	17.38	$C_{27}H_{44}O_4$	432.3223	432.3240	−3.8		Chlorogenin	R, S, L	a

Table 2 (*Contd.*)

No.	t_R (min)	Formula	Calculated mass (Da)	Theoretical mass (Da)	Mass error (ppm)	MS^E fragmentation	Identification	Source	Ref.
						431.2217 $[M - H]^-$, 397.3428 $[M - H - O - H_2O]^-$, 395.3258 $[M - H - 2 \times H_2O]^-$, 249.1019 $[M - H - C_{11}H_{18}O_2]^+$			
53	17.56	$C_{20}H_{22}O_4$	326.1515	326.1518	−0.8	327.1588 $[M + H]^+$, 312.1295 $[M + H - CH_3]^+$, 256.1067 $[M + H - 2 \times CH_3 - C_3H_5]^+$, 203.2237 $[M + H - C_7H_8O_2]^+$	Dehydrodiisoeugenol	R	61
54※	17.62	$C_{30}H_{48}O_5$	488.3523	488.3502	4.4	487.2917 $[M - H]^-$, 469.3240 $[M - H - H_2O]^-$, 433.3297 $[M - H - 3 \times H_2O]^-$, 411.1033 $[M - H - 2 \times CH_3 - HCOOH]^+$, 405.3029 $[M - H - 2 \times H_2O - HCOOH]^-$	Orthosphenic acid	R, S, L	a
55	17.80	$C_{35}H_{40}O_7$	572.2788	572.2774	2.4	571.2738 $[M - H]^-$, 555.2826 $[M - H - O]^-$, 331.2334 $[M - H - O - C_6H_5 - C_9H_7O_2]^-$	Celastrine B	R, S, L	—
56	17.84	$C_{30}H_{46}O_3$	454.3445	454.3447	−0.5	455.3492 $[M + H]^+$, 408.3435 $[M + H - H_2O - CHO]^+$, 325.2291 $[M + H - CH_3 - C_6H_{11}O]^+$	(13α,14β,17α,20*S*,24*Z*)-Lanosta-26-hydroxy-3-oxo-7,24-dien-21-al	R, S	a
57*	18.02	$C_{27}H_{44}O_7$	480.3106	480.3087	4.0	479.2439 $[M - H]^-$, 464.2903 $[M - H - CH_3]^-$, 461.2934 $[M - H - H_2O]^-$, 414.2779 $[M - H - H_2O - C_2H_7O]^-$, 202.0239 $[M - H - C_{11}H_{22}O_3]^+$	Viticosterone	R, S, L	62
58	18.11	$C_{30}H_{46}O_3$	454.3464	454.3447	3.8	453.3414 $[M - H]^-$, 435.3608 $[M - H - H_2O]^-$, 409.3319 $[M - H - CO_2]^-$, 376.3716 $[M - H - H_2O - CH_3 - CO_2]^-$, 223.2100 $[M - H - C_{15}H_{18}O_2]^-$	Wilforlide A	R	s
59	18.20	$C_{28}H_{48}O_2$	416.3636	416.3654	−4.1	461.3618 $[M + HCOO]^-$, 265.2591 $[M - H - C_9H_{10}O_2]^-$, 149.2309 $[M - H - C_{19}H_{38}]^-$	γ-Tocopherol	R	63
60	18.24	$C_{30}H_{38}O_{11}$	574.2423	574.2414	1.5	597.2315 $[M + Na]^+$, 395.1897 $[M + H - C_2H_3O_2 - C_7H_5O_2]^+$, 339.1724 $[M + H - 4 \times C_2H_3O_2]^+$	Ejap 3	R, L	a
61#	18.38	$C_{16}H_{32}O_2$	256.2393	256.2402	−3.7	279.2303 $[M + Na]^+$, 199.1787 $[M + H - C_4H_9]^+$, 69.0553 $[M + H - 2 \times CH_3 - C_9H_{17}O_2]^+$	Methyl (12*S*)-12-methyltetradecanoate	S	a
62	18.41	$C_{32}H_{44}O_8$	556.3016	556.3036	−3.6	555.2910 $[M - H]^-$, 463.2604 $[M - H - H_2O - CH_3 - C_2H_3O_2]^-$, 313.2285 $[M - H - H_2O - C_7H_{13}O_2 - C_5H_3O_2]^-$	14,15-Epoxy-14*H*-cyclopenta [*a*] phenanthrene, bufa-20,22-dienolide deriv.	R	—
63*	18.46	$C_{30}H_{42}O_7$	514.2931	514.2931	0.1	515.3017 $[M + H]^+$, 469.2859 $[M + H - HCOOH]^+$, 417.2608 $[M + H - C_6H_{10}O]^+$, 377.2297 $[M + H - C_9H_{14}O]^+$	Ganoderenic acid A	R	60
64#	18.67	$C_{30}H_{48}O_3$	456.3583	456.3604	−4.7	457.3675 $[M + H]^+$, 439.3520 $[M + H - H_2O]^+$, 424.2688 $[M + H - H_2O - CH_3]^+$, 330.2465 $[M + H - C_8H_{15}O]^+$	Kansenonol	S	64
65	18.69	$C_{16}H_{32}O_2$	256.2394	256.2402	−2.7	301.2376 $[M + HCOO]^-$, 97.0991 $[M - H - C_6H_{13} - C_3H_5O_2]^-$, 68.0554 $[M - H - CH_3 - C_8H_{17} - C_2H_3O_2]^-$	4,8,12-Trimethyltridecanoic acid	S	65
66	18.69	$C_{18}H_{30}O_2$	278.2233	278.2246	−4.5	279.2292 $[M + H]^+$, 261.2353 $[M + H - H_2O]^+$, 72.0554 $[M + H - C_{11}H_{19} - C_2H_3O_2]^+$	Gamolenic acid	R, S	66
67	18.74	$C_{30}H_{46}O_4$	470.3399	470.3396	0.6	469.3326 $[M - H]^-$, 454.3157 $[M - H - CH_3]^-$, 451.3303 $[M - H - H_2O]^-$, 407.3726 $[M - H - H_2O - CO_2]^-$, 261.4102 $[M - H - C_{14}H_{24}O]^-$	3β-Hydroxy-11α,12α-epoxy-13β,28-ursolide	R	a

Table 2 (*Contd.*)

No.	t_R (min)	Formula	Calculated mass (Da)	Theoretical mass (Da)	Mass error (ppm)	MS^E fragmentation	Identification	Source	Ref.
68	18.83	$C_{28}H_{36}O_7$	484.2458	484.2461	−0.6	469.2597 $[M - OH]^+$, 363.2350 $[M + H - C_7H_6O_2]^+$, 345.1852 $[M + H - H_2O - C_7H_6O_2]^+$, 218.1708 $[M + H - 2 \times H_2O - C_7H_6O_2 - C_6H_5O_2]^+$	Scutellone I	R	67
69	18.94	$C_{30}H_{46}O_4$	470.3399	470.3396	0.6	471.3326 $[M + H]^+$, 425.3157 $[M + H - HCOOH]^+$, 289.3241 $[M + H - C_{11}H_{18}O_2]^+$, 249.3383 $[M + H - C_{14}H_{22}O_2]^+$	Hederagonic acid	R	68
70	18.97	$C_{30}H_{48}O_3$	456.3606	456.3604	0.5	457.3678 $[M + H]^+$, 439.2231 $[M + H - H_2O]^+$, 249.3437 $[M + H - C_{14}H_{24}O]^+$, 203.1653 $[M + H - C_{14}H_{24}O - HCOOH]^+$	Ursolic acid	R, S, L	69
71	19.00	$C_{18}H_{30}O_3$	294.2184	294.2195	−3.6	317.2060 $[M + Na]^+$, 277.2115 $[M + H - H_2O]^+$, 172.0863 $[M + H - C_9H_{15}]^+$, 124.1081 $[M + H - C_9H_{15}O_3]^+$	9-Oxooctadeca-10,12-dienoic acid	R, S	70
72	19.13	$C_{30}H_{42}O_7$	514.2931	514.2931	0.1	515.3004 $[M + H]^+$, 454.2660 $[M + H - CH_3 - HCOOH]^+$, 415.2676 $[M + H - C_6H_{12}O]^+$, 321.2393 $[M + H - C_{11}H_{14}O_3]^+$	Ganoderenic acid B	R	60
73*	19.30	$C_{11}H_{14}O_2$	178.1001	178.0994	4.2	201.0886 $[M + Na]^+$,164.0739 $[M + H - CH_3]^+$, 107.0589 $[M + H - CH_3O - C_3H_5]^+$, 76.0507 $[M + H - 2 \times CH_3O - C_3H_5]^+$	Isohomogenol	R	71
74	19.35	$C_{29}H_{48}O_2$	428.3675	428.3654	4.7	451.3568 $[M + Na]^+$, 411.2449 $[M + H - H_2O]^+$, 274.2205 $[M + H - C_{10}H_{19}O]^+$, 256.2260 $[M + H - H_2O - C_{10}H_{19}O]^+$	Seringosterol	R, L	72
75	19.46	$C_{30}H_{44}O_7$	516.3080	516.3087	−1.3	515.3007 $[M - H]^-$, 500.2865 $[M - H - CH_3]^-$, 497.3383 $[M - H - H_2O]^-$, 322.1767 $[M - H - 2 \times H_2O - C_8H_{13}O_3]^-$	Ganoderic acid A	R	73
76	19.72	$C_{22}H_{30}O_4$	358.2132	358.2144	−3.3	381.2024 $[M + Na]^+$, 316.2063 $[M + H - C_2H_3O]^+$, 273.1648 $[M + H - C_3H_7 - C_2H_3O_2]^+$	(3*S*,4a*S*,10a*S*)-3-(Acetyloxy)-2,3,4,4*a*,10,10*a*-hexahydro-6-hydroxy-1,1,4*a*-trimethyl-7-(1-methylethyl)-9(1*H*)-phenanthrenone	R	s
77	19.73	$C_{30}H_{50}O_6$	506.3632	506.3607	4.9	505.3559 $[M - H]^-$, 43.3909 $[M - H - 4 \times H_2O]^-$, 431.2834 $[M - H - CH_3 - C_3H_7O]^-$	13β,17β-Epoxyalisol A	R	s
78	19.89	$C_{30}H_{46}O_3$	454.3438	454.3447	−2.0	455.3492 $[M + H]^+$, 409.3435 $[M + H - HCOOH]^+$, 391.1517 $[M + H - H_2O - HCOOH]^+$, 249.3317 $[M + H - C_{14}H_{22}O]^+$	Oleanonic acid	R, S	74
79	20.05	$C_{30}H_{46}O_5$	486.3334	486.3345	−2.4	487.3406 $[M + H]^+$, 441.3344 $[M + H - HCOOH]^+$, 413.3436 $[M + H - H_2O - CH_3 - HCOOH]^+$, 395.3278 $[M + H - 2 \times HCOOH]^+$, 249.5358 $[M + H - C_{14}H_{22}O_3]^+$	Ceanothic acid	R	a
80*	20.24	$C_{28}H_{42}O_6$	474.2986	474.2981	1.0	497.2908 $[M + Na]^+$, 457.2342 $[M + H - H_2O]^+$, 411.2324 $[M + H - H_2O - CH_3 - CH_3O]^+$, 285.0399 $[M + H - H_2O - C_9H_{16}O_3]^+$	Methyl lucidenate Q	R	75
81	20.36	$C_{29}H_{34}O_8$	510.2256	510.2254	0.3	533.2148 $[M + Na]^+$, 410.1760 $[M + H - C_2H_3O - C_3H_6O]^+$, 268.1455 $[M + H - C_2H_3O - C_7H_5O - C_5H_3O_2]^+$	Orbiculin B	R	—
82	20.36	$C_{30}H_{48}O_3$	456.3617	456.3604	3.0		Oleanolic acid	R, S, L	74

Table 2 (*Contd.*)

No.	t_R (min)	Formula	Calculated mass (Da)	Theoretical mass (Da)	Mass error (ppm)	MS^E fragmentation	Identification	Source	Ref.
						455.3544 $[M - H]^-$, 391.3440 $[M - H - H_2O - HCOOH]^-$, 149.3707 $[M - H - H_2O - C_{18}H_{27}O_2]^-$			
83	21.14	$C_{19}H_{38}O_4$	330.2770	330.2770	0.1	353.2632 $[M + Na]^+$, 313.2160 $[M + H - H_2O]^+$, 300.0486 $[M + H - CH_3O]^+$, 240.0679 $[M + H - C_3H_7O_3]^+$	β-Monopalmitin	S, L	76
84#	21.46	$C_{39}H_{54}O_6$	618.3949	618.3920	4.6	619.4002 $[M + H]^+$, 565.4126 $[M + H - 3 \times H_2O]^+$, 472.3557 $[M + H - C_9H_7O_2]^+$, 456.3443 $[M + H - C_9H_7O_3]^+$, 411.1356 $[M + H - C_{14}H_{24}O]^+$	27-*p*-*E*-Coumaroyloxyursolic acid	S	77
85#	21.49	$C_{30}H_{46}O_2$	438.3485	438.3498	−2.9	439.3559 $[M + H]^+$, 421.3432 $[M + H - H_2O]^+$, 390.1126 $[M + H - H_2O - CH_3O]^+$, 250.3443 $[M + H - C_{13}H_{17}O]^+$	5-Dehydrokarounidiol	S	—
86*	21.72	$C_{13}H_{12}O_2$	200.0834	200.0831	−3.3	201.0886 $[M + H]^+$, 186.0597 $[M + H - CH_3]^+$, 165.0996 $[M + H - 2 \times H_2O]^+$, 140.0533 $[M + H - C_2H_5O_2]^+$	Safynol	R	78
87*	22.03	$C_{12}H_{16}O_2$	192.1157	192.1150	3.7	215.1045 $[M + Na]^+$, 163.0490 $[M + H - 2 \times CH_3]^+$, 150.1739 $[M + H - C_3H_7]^+$, 135.2173 $[M + H - CH_3 - C_3H_7]^+$, 119.0695 $[M + H - CH_3 - C_2H_3O_2]^+$, 91.0556 $[M + H - C_3H_7 - C_2H_3O_2]^+$	Carvacryl acetate	R	a
88*	22.04	$C_{29}H_{38}O_4$	450.2766	450.2770	−0.9	451.2844 $[M + H]^+$, 433.2591 $[M + H - H_2O]^+$, 405.2771 $[M + H - HCOOH]^+$, 215.3621 $[M + H - C_{15}H_{24}O_2]^+$, 200.0917 $[M + H - CH_3 - C_{15}H_{24}O_2]^+$	Celastrol	R, S, L	79
89	22.05	$C_9H_{10}O$	134.0737	134.0732	3.4	157.0632 $[M + Na]^+$, 120.1173 $[M + H - CH_3]^+$, 106.0704 $[M + H - C_2H_5]^+$, 77.0477 $[M + H - C_2H_5 - CHO]^+$	4-Ethylbenzaldehyde	R	80
90*	22.22	$C_{29}H_{36}O_4$	448.2609	448.2614	−1.0	449.2679 $[M + H]^+$, 434.2548 $[M + H - CH_3]^+$, 403.2606 $[M + H - HCOOH]^+$, 267.3315 $[M + H - C_{12}H_6O_2]^+$	25-(9→8)Abeo-24-nor-friedelan-2,3-dioxo-1(10),4,6,9(11)-tetraen-29-oic acid	R	—
91	22.46	$C_{38}H_{44}N_2O_6$	624.3200	624.3220	3.2	647.3113 $[M + Na]^+$, 503 $[M + H - C_8H_9O]^+$, 314.1395 $[M + H - CH_3 - C_{18}H_{16}NO_2]^+$, 297 $[M + H - C_7H_7O - C_{11}H_{17}O_2N]^+$, 266.0790 $[M + H - CH_3 - C_8H_9O - C_{13}H_{18}NO_2]^+$	Neferin	R	81
92	22.78	$C_{32}H_{48}O_5$	512.3486	512.3502	−3.1	511.3413 $[M - H]^-$, 493.2436 $[M - H - H_2O]^-$, 465.1023 $[M - H - HCOOH]^+$, 434.2379 $[M - H - H_2O - C_2H_3O_2]^-$, 370.2491 $[M - H - C_8H_{13}O_2]^-$	Ganoderic acid X	R	82
93	22.80	$C_{12}H_{16}O_2$	192.1159	192.1150	4.2	215.1052 $[M + Na]^+$, 178.0893 $[M + H - CH_3]^+$, 107.5147 $[M - C_5H_{11}O]^+$, 78.0571 $[M + H - C_6H_{11}O_2]^+$	Amyl benzoate	R	s
94	22.81	$C_{30}H_{52}O_3$	460.3920	460.3917	0.8	483.3813 $[M + Na]^+$, 407.3593 $[M + H - H_2O]^+$, 368.3012 $[M + H - H_2O - C_3H_7O_2]^+$, 253.0154 $[M + H - C_{14}H_{24}O]^+$	Olibanumol H	S, L	83
95	22.82	$C_{30}H_{48}O_4$	472.3543	472.3553	−2.1	473.3615 $[M + H]^+$, 455.3483 $[M + H - H_2O]^+$, 427.3561 $[M + H - HCOOH]^+$, 333.1527 $[M + H - C_9H_{16}O]^+$, 290.0146 $[M + H - CH_3 - C_{11}H_{20}O]^+$	Echinocystic acid	R, S	84
96	22.83	$C_{16}H_{32}O_2$	256.2390	256.2402	−4.5		Methyl pentadecanoate	R, S, L	s

Table 2 (*Contd.*)

No.	t_R (min)	Formula	Calculated mass (Da)	Theoretical mass (Da)	Mass error (ppm)	MS^E fragmentation	Identification	Source	Ref.
						279.2276 $[M + Na]^+$, 155.1230 $[M + H - C_5H_{11} - CH_3O]^+$, 85.0488 $[M + H - C_{10}H_{21} - OCH_3]^+$			
97	23.02	$C_{22}H_{36}O_4$	364.2601	364.2614	−3.7	365.2665 $[M + H]^+$, 266.2190 $[M + H - C_6H_{11}O]^+$, 219.1749 $[M + H - H_2O - C_2H_3O_2 - C_5H_9O]^+$	Vitetrifolin E	S	85
98	24.29	$C_{12}H_{16}O_2$	192.1159	192.1150	4.2	215.1052 $[M + Na]^+$, 164.0893 $[M + H - CH_3]^+$, 136.0587 $[M + H - C_4H_9]^+$, 78.0571 $[M + H - C_6H_{11}O_2]^+$	Isopentyl benzoate	R	s
99	24.31	$C_{11}H_{14}O_2$	178.1003	178.0994	4.7	201.0895 $[M + Na]^+$, 147.0441 $[M + H - CH_3]^+$, 136.0491 $[M + H - C_2H_3O]^+$, 122.0477 $[M + H - C_3H_5O]^+$, 77.0146 $[M + H - CH_3O - C_4H_7O]^+$	Anisylacetone	R	86
100#	24.51	$C_{30}H_{52}O_3$	460.3898	460.3917	−4.1	483.3682 $[M + Na]^+$, 425.3325 $[M + H - 2 \times H_2O]^+$, 336.2443 $[M + H - C_9H_{17}]^+$, 295.0357 $[M + H - C_{12}H_{22}]^+$	Neopanaxadiol	S	87
101	24.71	$C_{30}H_{48}O_2$	440.3656	440.3654	0.3	441.3729 $[M + H]^+$, 423.3611 $[M + H - H_2O]^+$, 259.1687 $[M + H - C_{11}H_{18}O_2]^+$, 219.0312 $[M + H - C_{14}H_{22}O_2]^+$	Daturanolone	R, S	88
102	24.94	$C_{30}H_{48}O$	424.3701	424.3705	−1.0	425.3774 $[M + H]^+$, 407.3633 $[M + H - H_2O]^+$, 392.3459 $[M + H - H_2O - CH_3]^+$, 218.1926 $[M + H - C_{14}H_{23}O]^+$	Olean-9(11),12-dien-3β-ol	R, S	s
103*	25.48	$C_{21}H_{24}O_4$	340.1672	340.1675	−0.8	363.1573 $[M + Na]^+$, 189.0711 $[M + H - CH_3 - C_8H_9O_2]^+$, 173.0939 $[M + H - CH_3O - C_8H_9O_2]^+$, 107.0494 $[M + H - CH_3O - C_{13}H_{15}O_2]^+$	Dehydrodiisoeugenol methyl ether	R	89
104	25.67	$C_{30}H_{46}O_4$	470.3372	470.3396	−4.1	471.3445 $[M + H]^+$, 456.2016 $[M + H - CH_3]^+$, 398.5107 $[M + H - C_3H_5O_2]^+$, 379.0317 $[M + H - 2 \times HCOOH]^+$, 312.2370 $[M + H - H_2O - C_8H_{13}O_2]^+$, 234.4581 $[M + H - CH_3 - C_3H_5O_2 - C_8H_{13}O_2]^+$	Nigranoic acid	R	a
105	25.70	$C_{37}H_{42}O_{13}$	694.2801	694.2778	3.6	695.2873 $[M + H]^+$, 515.2201 $[M + H - 3 \times C_2H_4O_2]^+$, 393.2228 $[M + H - 3 \times C_2H_4O_2 - C_7H_6O_2]^+$, 331.0167 $[M + H - 2 \times C_2H_4O_2 - C_7H_6O_2 - C_7H_5O_2]^+$	Celahin D	S, L	s
106	25.91	$C_{28}H_{48}O$	400.3698	400.3705	−1.6	423.3591 $[M + Na]^+$, 386.2638 $[M + H - CH_3]^+$, 326.2823 $[M + H - H_2O - CH_3 - C_3H_7]^+$, 254.2266 $[M + H - H_2O - C_8H_{17}O]^+$, 213.2252 $[M + H - H_2O - C_{11}H_{22}O]^+$	Fungisterol	R, S	a
107	25.93	$C_{27}H_{44}O$	384.3390	384.3392	−0.6	385.3463 $[M + H]^+$, 367.0417 $[M + H - H_2O]^+$, 256.2547 $[M + H - H_2O - C_8H_{15}]^+$, 215.2415 $[M + H - H_2O - C_{11}H_{20}]^+$	(*E*)-22-Dehydrocholesterol	R	90
108	25.96	$C_{29}H_{48}O$	412.3686	412.3705	−4.6	413.3746 $[M + H]^+$, 260.2204 $[M + H - CH_3 - C_{10}H_{19}]^+$, 257.2151 $[M + H - H_2O - C_{10}H_{19}]^+$	28-Isofucosterol	R, S	a
109	26.22	$C_{30}H_{48}O_3$	456.3588	456.3604	−3.5	457.3651 $[M + H]^+$, 439.2142 $[M + H - H_2O]^+$, 411.3584 $[M + H - HCOOH]^+$, 265.1812 $[M + H - C_{13}H_{20}O]^+$	Maytenoic acid	R	s
110	26.28	$C_{29}H_{48}O$	412.3702	412.3705	−0.8	413.3775 $[M + H]^+$, 395.3676 $[M + H - H_2O]^+$, 256.2275 $[M + H - H_2O - C_{10}H_{19}]^+$, 218.7218 $[M + H - CH_3 - C_{13}H_{24}]^+$	Chondryllasterol	R	91

Table 2 (*Contd.*)

No.	t_R (min)	Formula	Calculated mass (Da)	Theoretical mass (Da)	Mass error (ppm)	MS^E fragmentation	Identification	Source	Ref.
111	26.31	$C_{18}H_{36}O_2$	284.2724	284.2715	2.7	307.2616 $[M + Na]^+$, 140.1312 $[M + H - 2 \times CH_3 - C_6H_{11}O_2]^+$, 70.0583 $[M + H - 2 \times CH_3 - C_{11}H_{21}O_2]^+$	Hexadecanoic acid	R	s
112	26.62	$C_{30}H_{48}O$	424.3686	424.3705	−4.4	425.3747 $[M + H]^+$, 410.3584 $[M + H - CH_3]^+$, 367.3099 $[M + H - CH_3 - C_3H_7]^+$, 273.3120 $[M + H - C_{11}H_{20}]^+$	Fernenone	R, L	92
113	26.83	$C_{30}H_{48}O_2$	440.3656	440.3654	0.3	441.3729 $[M + H]^+$, 423.3611 $[M + H - H_2O]^+$, 412.0147 $[M + H - CHO]^+$, 233.1890 $[M + H - C_{14}H_{24}O]^+$, 204.3167 $[M + H - CHO - C_{14}H_{24}O]^+$	Oleanolic aldehyde	R, S, L	a
114	27.08	$C_{28}H_{48}O_2$	416.3667	416.3654	2.9	439.3559 $[M + Na]^+$, 192.1212 $[M + H - C_{16}H_{33}]^+$, 151.0731 $[M + H - C_{19}H_{38}]^+$	Cumotocopherol	R	93
115	27.25	$C_{24}H_{38}O_4$	390.2752	390.2770	−4.3	413.2644 $[M + Na]^+$, 179.0809 $[M + H - C_2H_5 - C_4H_9 - C_8H_{17}O]^+$, 149.1469 $[M + H - C_8H_{17} - C_8H_{17}O]^+$, 77.0216 $[M + H - 2 \times C_9H_{17}O_2]^+$	Fleximel	R, S, L	b
116	27.52	$C_{30}H_{48}O$	424.3709	424.3705	0.9	425.3782 $[M + H]^+$, 410.3572 $[M + H - CH_3]^+$, 259.2141 $[M + H - C_{11}H_{18}O]^+$, 221.1939 $[M + H - C_{14}H_{20}O]^+$	β-Amyron	R, S, L	94
117※	27.84	$C_{36}H_{44}O_9$	620.3003	620.2985	2.9	621.3083 $[M + H]^+$, 562.2851 $[M + H - C_2H_3O_2]^+$, 461.2303 $[M + H - C_2H_3O_2 - C_5H_9O_2]^+$, 291.2088 $[M + H - C_2H_5 - C_2H_3O_2 - 2 \times C_7H_5O_2]^+$	Celafolin D-3	S, L	s
118	27.88	$C_{30}H_{48}O_2$	440.3656	440.3654	0.3	441.3729 $[M + H]^+$, 423.3611 $[M + H - H_2O]^+$, 398.5359 $[M + H - C_3H_7]^+$, 288.2288 $[M + H - C_{11}H_{21}]^+$, 247.0117 $[M + H - C_{14}H_{26}]^+$	Polasterol A	R, S, L	a
119	28.10	$C_{29}H_{46}O$	410.2535	410.2549	−3.4	411.3599 $[M + H]^+$, 393.3696 $[M + H - H_2O]^+$, 272.2261 $[M + H - C_{10}H_{19}]^+$, 231.2084 $[M + H - C_{13}H_{24}]^+$	Corbisterin	R, L	95
120	28.49	$C_{30}H_{50}O_2$	442.3813	442.3811	0.5	465.3705 $[M + Na]^+$, 425.3647 $[M + H - H_2O]^+$, 291.3580 $[M + H - C_{10}H_{16}O]^+$, 251.5133 $[M + H - C_{13}H_{20}O]^+$	3-Oxo-11β-hydroxyfriedelane	S	96

[a] * Characteristic component in root; # characteristic component in stem; ※ characteristic component in leaf; a: compared with spectral data obtained from Wiley Subscription Services, Inc. (USA); b: compared with NIST Chemistry WebBook; s: compared with the reference compounds; R: the root of *Celastrus orbiculatus* Thunb.; S: the stem of *Celastrus orbiculatus* Thunb.; L: the leaf of *Celastrus orbiculatus* Thunb.

spectrometer (Waters Co., Milford, MA, USA) with an electrospray ionization (ESI) interface.

The ACQUITY UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm) was bought from Waters Corporation (Milford, MA, USA). The moving phrase was consisted of eluent A (0.1% methanoic acid in water, v/v) and eluent B (0.1% methanoic acid in acetonitrile, v/v) in a liner gradient program (0–2 min, 10% B; 2–26 min, 10 → 90% B; 26–28 min, 90% B; 28–28.1 min, 90 → 10% B; 28.1–40 min, 10% B) with a flow rate of 0.4 mL min^{-1}. Set the temperature of column and the sample manager at 30 °C and 15 °C, respectively. 10% and 90% acetonitrile in aqueous solution were used as weak and strong wash solvents respectively.

The optimized MS parameters were as follows: source temperature (150 °C), desolvation temperature (400 °C), cone voltage (40 V), capillary voltage at 2.6 kV (ESI^+) and 2.2 kV (ESI^-), cone gas flow (50 L h^{-1}) and desolvation gas flow (800 L h^{-1}). MS^E mode was chosen with low energy of 6 V and high energy of 20–40 V.[26,27] The mass spectrometer was calibrated with sodium formate in the range of 100 to 1200 Da in order to ensure the mass reproducibility and accuracy. Leucine enkephalin (*m*/*z* 556.2771 in ESI^+ and 554.2615 in ESI^-) was used as external reference for Lock Spray™ injected at a constant flow of 10 μL min^{-1}. The QC sample was injected randomly 4 times throughout the whole work list. All of the volume injection of the samples and QC was 5 μL per run. During data acquisition, the data for screening analysis was performed in MS^E continuum mode, the data for metabolomics analysis was performed in MS^E centroid mode. Data recording was performed on MassLynx V4.1 workstation (Waters, Manchester, UK).

2.4. Screening analysis of components in three parts of COT by UNIFI platform

UNIFI 1.7.0 software (Waters, Manchester, UK) was used for data analysis.[28,29]

Firstly, in addition to the internal Traditional Medicine Library on UNIFI platform, the chemical constituent investigation was conducted. As the result, a self-built database of chemical compounds isolated from the genus of *Celastrus* L. was established by searching the online databases including Web of Science, Medline, PubMed, ChemSpider and China National Knowledge Infrastructure (CNKI). The compound name, molecular formula and chemical structure of components were obtained in the database.

Secondly, the raw data obtained from Masslynx workstation were compressed by Waters Compression and Archival Tool v1.10, then were imported into the UNIFI software.

Thirdly, the compressed data were processed by the streamlined work flow of UNIFI software in order to quickly identify the chemical compounds which were matched the criteria with Traditional Medicine Library and self-built database. The main parameters of processed method were as follow: 2D peak detection was set to 200 as the minimum peak area. In the 3D peak detection, the peak intensity of high energy and low energy was taken more than 200 and 1000 times as the parameter respectively. Selected +H and +Na as positive adducts and +COOH and –H as negative adducts. Leucine enkephalin was used as reference compound in order to get exact mass accuracy, with $[M + H]^+$ 556.2766 for positive ion and $[M - H]^-$ 554.2620 for negative ion. As a result, the comprehensive chemical constituents screening list was accomplished.

Finally, a filter was set to refine the results, with the mass error between −5 and 5 ppm and response value over 5000. Each compound was verified by compared with the characteristic MS fragmentation patterns reported in literature or the retention time of the reference substances.

2.5. Metabolomics analysis of three parts of COT

The raw data acquired by Masslynx workstation was processed on MakerLynx XS V4.1 software (Waters, Milford, CT, USA). Firstly, the raw data were processed with alignment, deconvolution, and data reduction, *etc*. The main parameters of the process method were as follows: retention time (0–28 min), retention time window (0.20), mass (100–1200 Da), mass tolerance (0.10), mass window (0.10), minimum intensity (5%), marker intensity threshold (2000 counts) and noise elimination (level 6). As a result, the list with mass and retention time corresponded to the responses based on all the detected peaks from each data file were shown in Extended Statistics (XS) Viewer. Secondly, multivariate statistical analysis, both principle component analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA), were performed on the MakerLynx software to analyze the resulting data.

PCA, a classical unsupervised low dimensional pattern recognition model, was used to show pattern recognition and maximum variation, and the overview and classification were obtained. OPLS-DA was used to obtain the maximum separation between two different groups. *S*-plots, which could provide visualization of the OPLS-DA predictive results, were created to explore the potential chemical markers which contributed to the differences.

Meanwhile, metabolites with VIP value > 4.0 and *p*-value < 0.001 were considered as potential chemical markers.[30,31] Futhermore, permutation test was also performed to provide a reference distribution with the R^2/Q^2 values to indicate statistical significance. Finally, the analysis results were shown in Simca 15.0 software (Umetrics, Malmö, Sweden).

2.6. Bioassay analysis of three parts of COT

2.6.1 Preparation of cigarette smoke extract. The preparation of the cigarette smoke extract (CSE) was basically the same as previous reports.[32] Put the smoke from one cigarette into 20 mL culture medium (300 s per cigarette). The CSE solution was incubated at 37 °C for 30 min after being filtered with a 0.22 μm sterile filter. The CSE solution was prepared freshly and was used within 30 min. This prepared CSE solution was considered to have the highest concentration (100%).

2.6.2 Cell viability assay. The final concentrations (20.0, 40.0, 80.0, 160.0, 320.0 μg mL^{-1}) of each test samples (R_{bio}, S_{bio} and L_{bio}) were acquired by diluting the stock solutions with Dulbecco's Modified Eagle Medium (DMEM). A549 cells were

cultured in 96-well plates at a density of 5×10^5 cells per well treated with CSE (0%, 5%, 10%, 20%, 30% and 40%) for 18 h, or treated with R_{bio}, S_{bio} and L_{bio} solutions (0.0, 20.0, 40.0, 80.0, 160.0, 320.0 μg mL^{-1}) for 24 h. The growth-inhibition effect of CSE and the effect of the drugs on viability of A549 cells were evaluated by MTT assay.

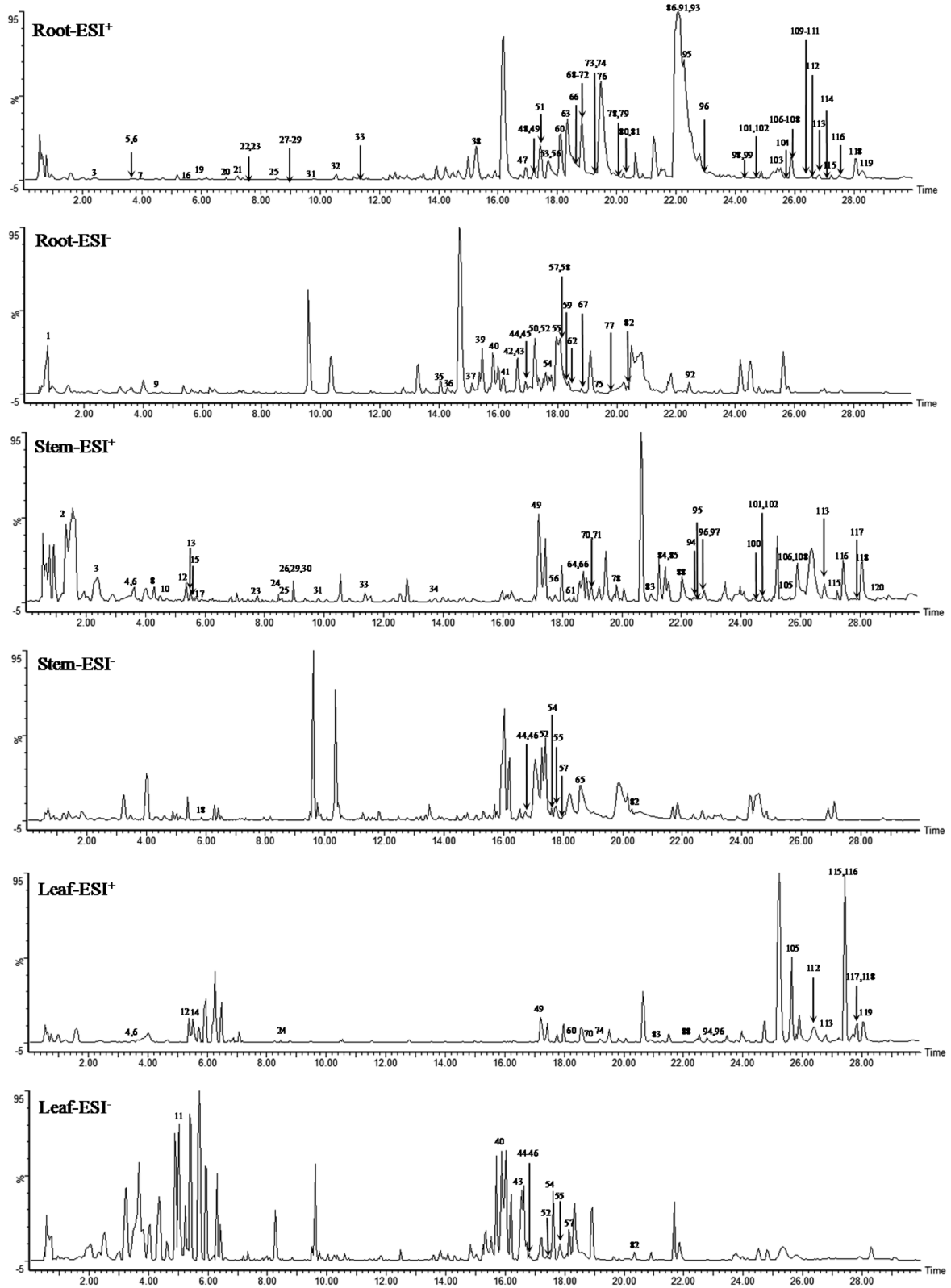


Fig. 1 The base peak intensity (BPI) chromatograms in root, stem and leaf of COT in ESI^+ and ESI^-.

2.6.3 Drug treatment. For all groups, A549 cells were cultured in 96-well plates at a density of 5×10^5 cells per mL for 18 h. In CSE group, the cells were treated with a certain dose of CSE without drug intervened. In drug groups, the cells were treated with both CSE and drugs (R_{bio}, S_{bio} or L_{bio}). In control group, A549 cells were cultured normally without the CSE or

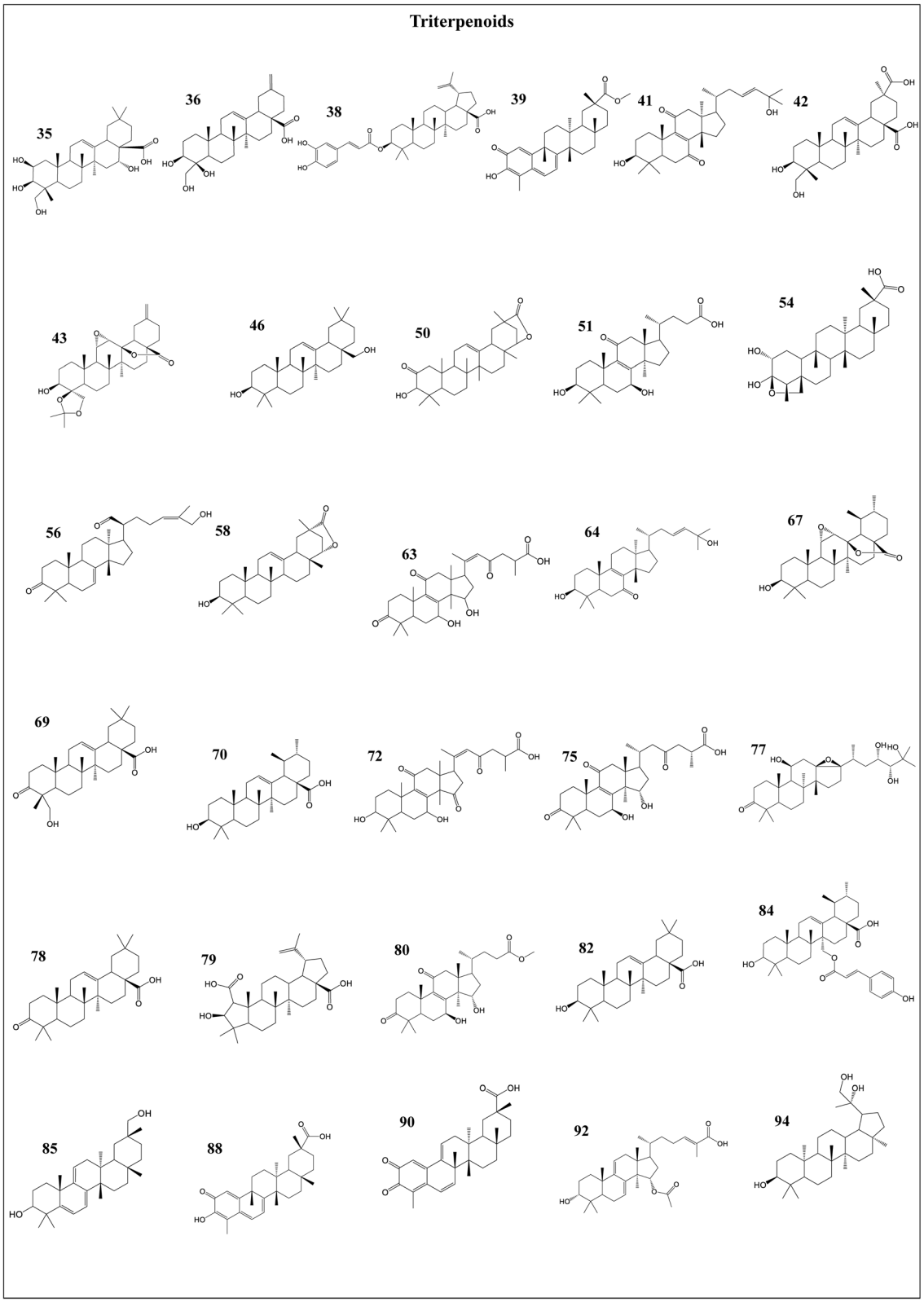


Fig. 2 Chemical structures of compounds identified in the root, stem and leaf of COT.

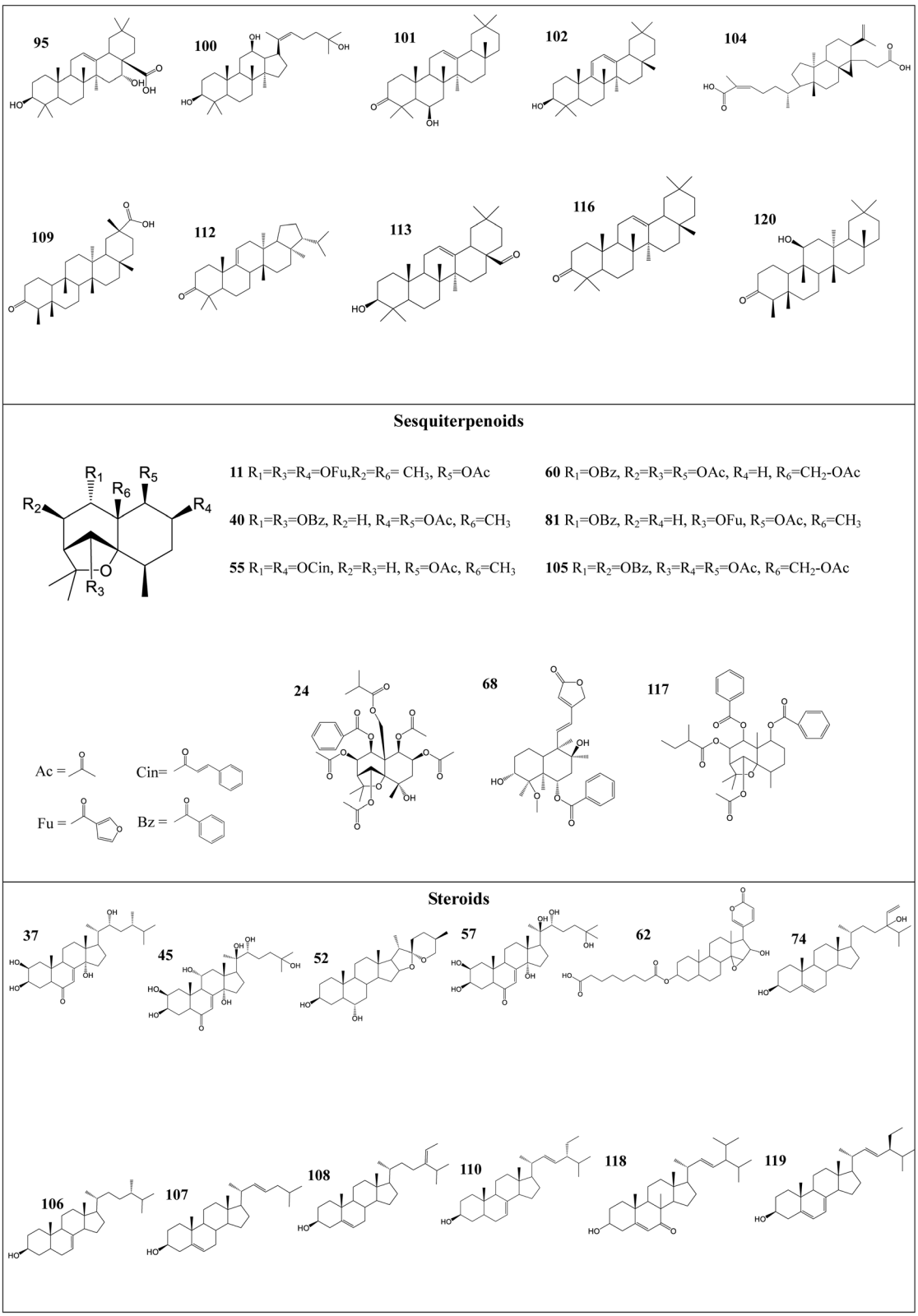


Fig. 2 (*contd.*)

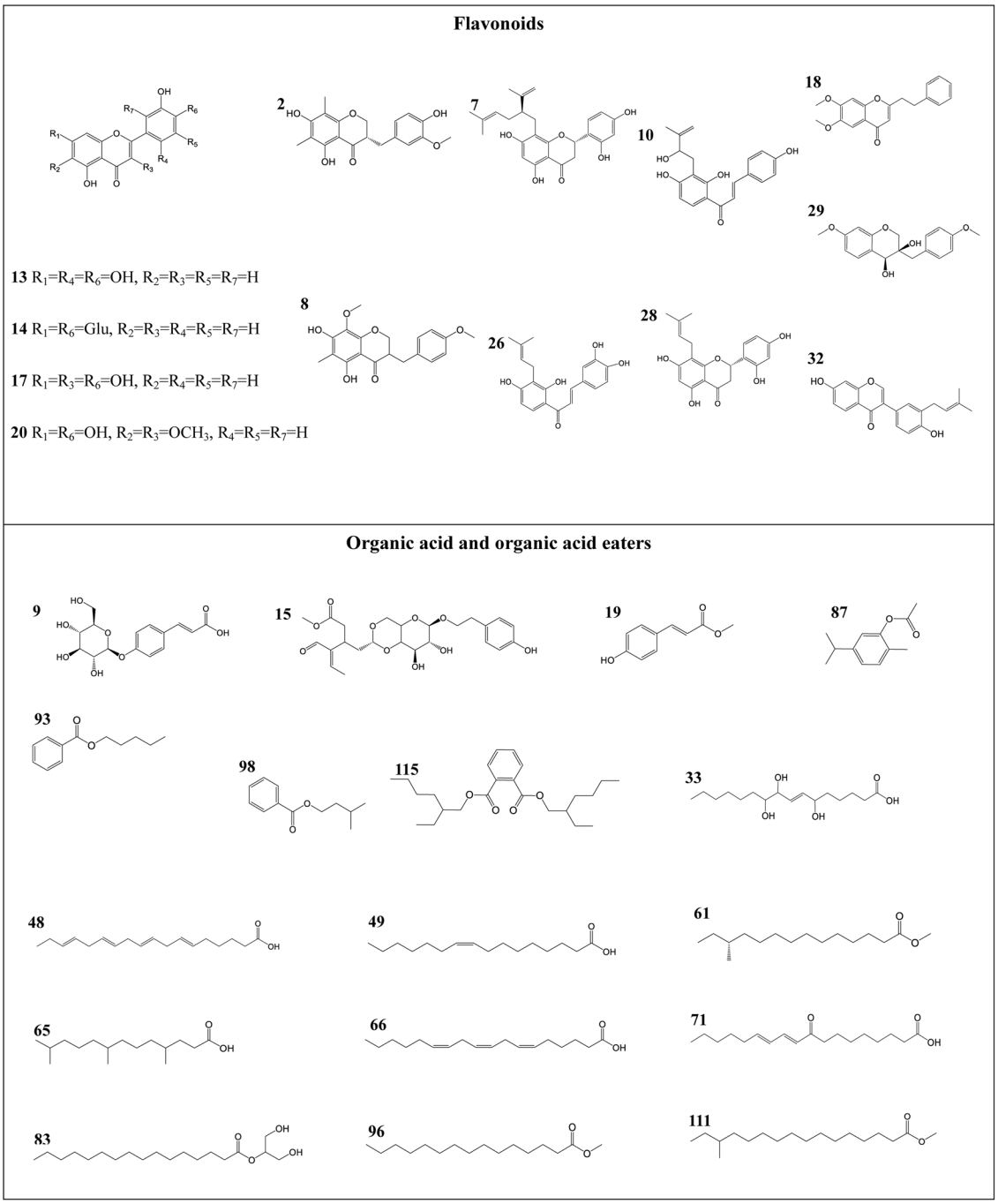


Fig. 2 (*contd.*)

drugs. In positive group, the cells were treated with both CSE and dexamethasone (5 μg mL^{-1}).

2.6.4 Enzyme-linked immunosorbent assay. The contents of IL-1β, IL-6 and TNF-α in the cell culture supernatant were determined with ELISA kits. All procedures were performed according to the manufacturer's instructions.

2.6.5 Statistical analysis. Statistical analysis was performed on Graphpad Prism 6.0 software (CA, USA). The results were expressed as mean ± SD. Two tailed test or a one-way analysis of variance (ANOVA) was used to calculate statistical significant difference ($p < 0.05$).

3. Results and discussion

3.1. Screening analysis of components of three parts of COT

A total of 120 compounds, including 91 in ESI^+ mode and 29 in ESI^- mode, were identified or tentatively characterized from three parts of COT (Table 2). The base peak intensity (BPI) chromatograms were shown in Fig. 1. The chemical structures were shown in Fig. 2, the results showed that COT was rich in natural components with various structural patterns. On one hand, according to the reference, there were nearly 50, 100, 10 compounds were reported from the root, stem, leaf parts of COT,

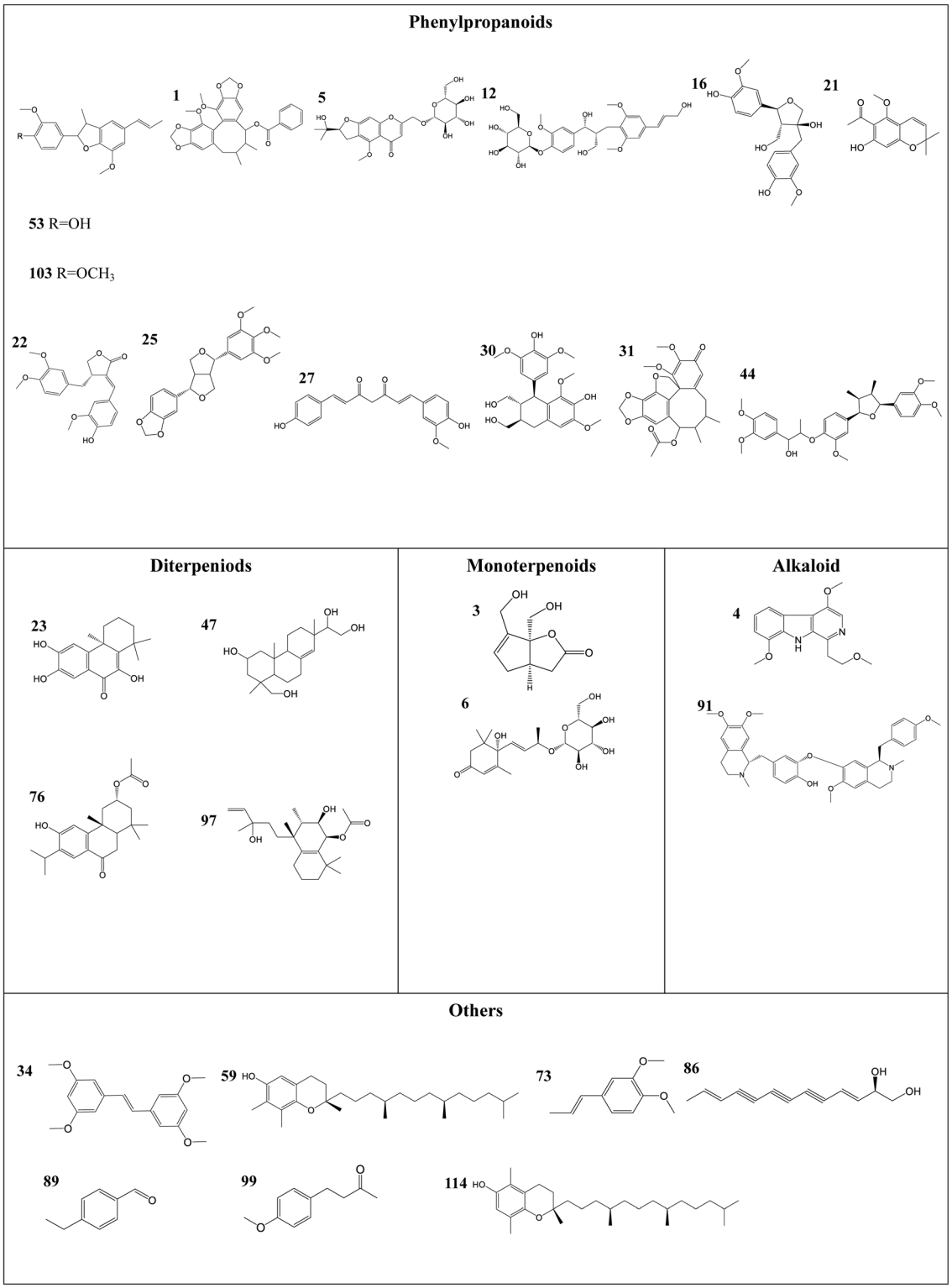
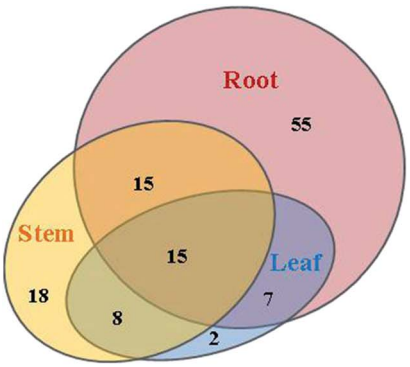


Fig. 2 (*contd.*)

respectively. While in this study, there were 92, 56 and 32 components were identified or tentatively characterized from root part, stem part and leaf part of COT, respectively. And most of the components were identified from COT for the first time. Various kinds of structures, including triterpeniods, sesquiterpenoids, steroids, flavonoids, organic acid and organic acid esters, phenylpropanoids, diterpeniods, monoterpenoids, alkaloid and others, were contained in each part of COT. The numbers (% of the total identified components in each part) and structural types of compounds identified from root, stem and leaf



Type of compounds	R	S	L	R∩S	R∩L	S∩L	R∩S∩L
Triterpenoids	34 (37%)	18 (32%)	10 (31%)	5 (33%)	2 (29%)	2 (25%)	6 (40%)
Organic acid and organic acid esters	13 (14%)	10 (18%)	4 (13%)	3 (20%)	-	1 (13%)	3 (20%)
Steroids	12 (13%)	5 (9%)	6 (19%)	2 (13%)	3 (43%)	-	3 (20%)
Flavonoids	5 (5%)	8 (14%)	1 (3%)	1 (7%)	-	-	-
Phenylpropanoids	11 (12%)	5 (9%)	2 (6%)	2 (13%)	-	1 (13%)	1 (7%)
Sesquiterpenoids	5 (5%)	4 (7%)	7 (22%)	-	2 (29%)	3 (38%)	1 (7%)
Diterpenoids	3 (3%)	2 (4%)	-	1 (7%)	-	-	-
Monoterpenoids	2 (2%)	2 (4%)	1 (3%)	1 (7%)	-	-	1 (7%)
Alkaloid	1 (1%)	1 (2%)	1 (3%)	-	-	1 (13%)	-
Others	6 (7%)	1 (2%)	-	-	-	-	-
Total	92	56	32	15	7	8	15

Fig. 3 The numbers (% of the total identified components in each part) and structural types of compounds identified from root, stem and leaf of COT. R: the root of *Celastrus orbiculatus* Thunb.; S: the stem of *Celastrus orbiculatus* Thunb.; L: the leaf of *Celastrus orbiculatus* Thunb.

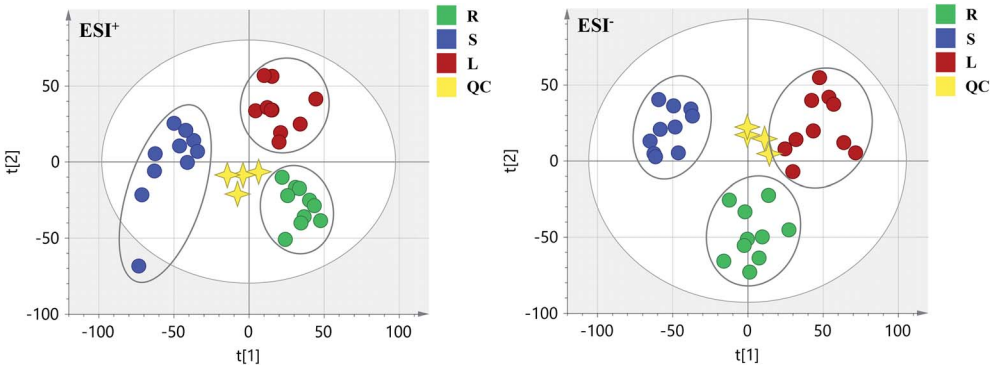


Fig. 4 The PCA of root (R), stem (S), leaf (L) groups in ESI^+ and ESI^-. QC: quality control.

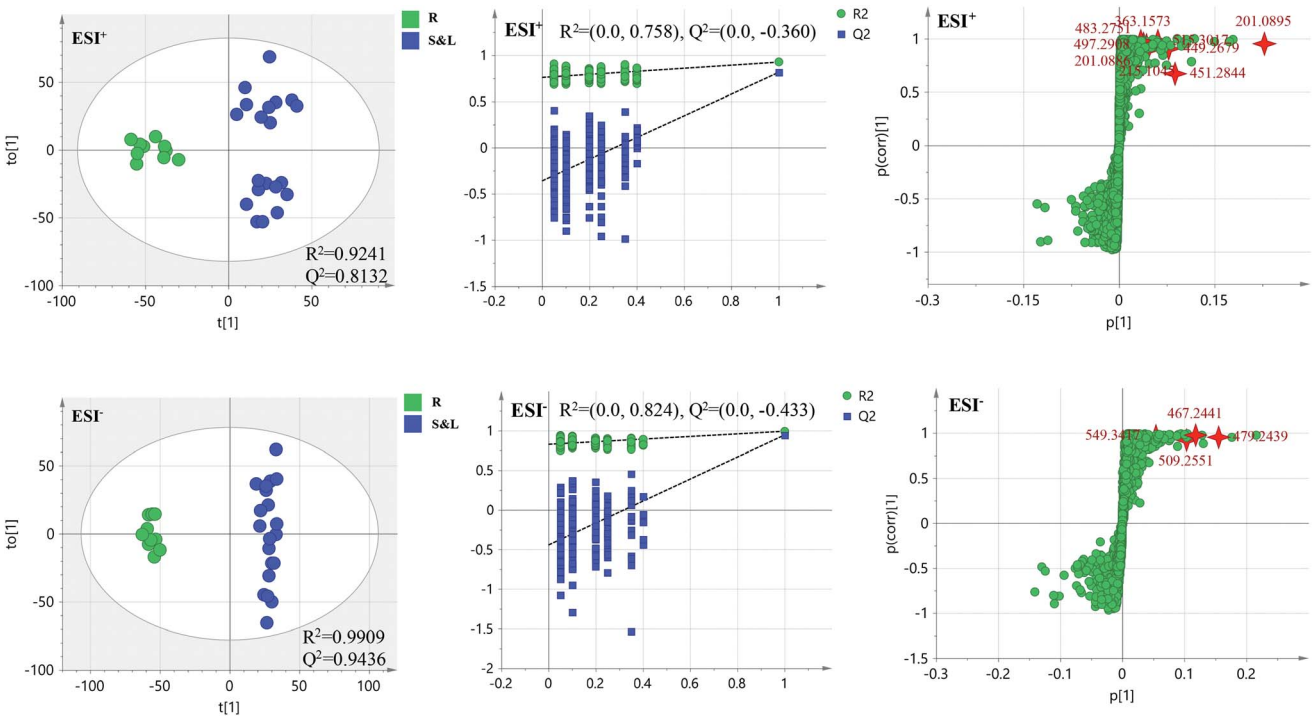


Fig. 5 OPLS-DA plots/permutation tests/*S*-plots between R and S&L. R: the root of *Celastrus orbiculatus* Thunb.; S: the stem of *Celastrus orbiculatus* Thunb.; L: the leaf of *Celastrus orbiculatus* Thunb.

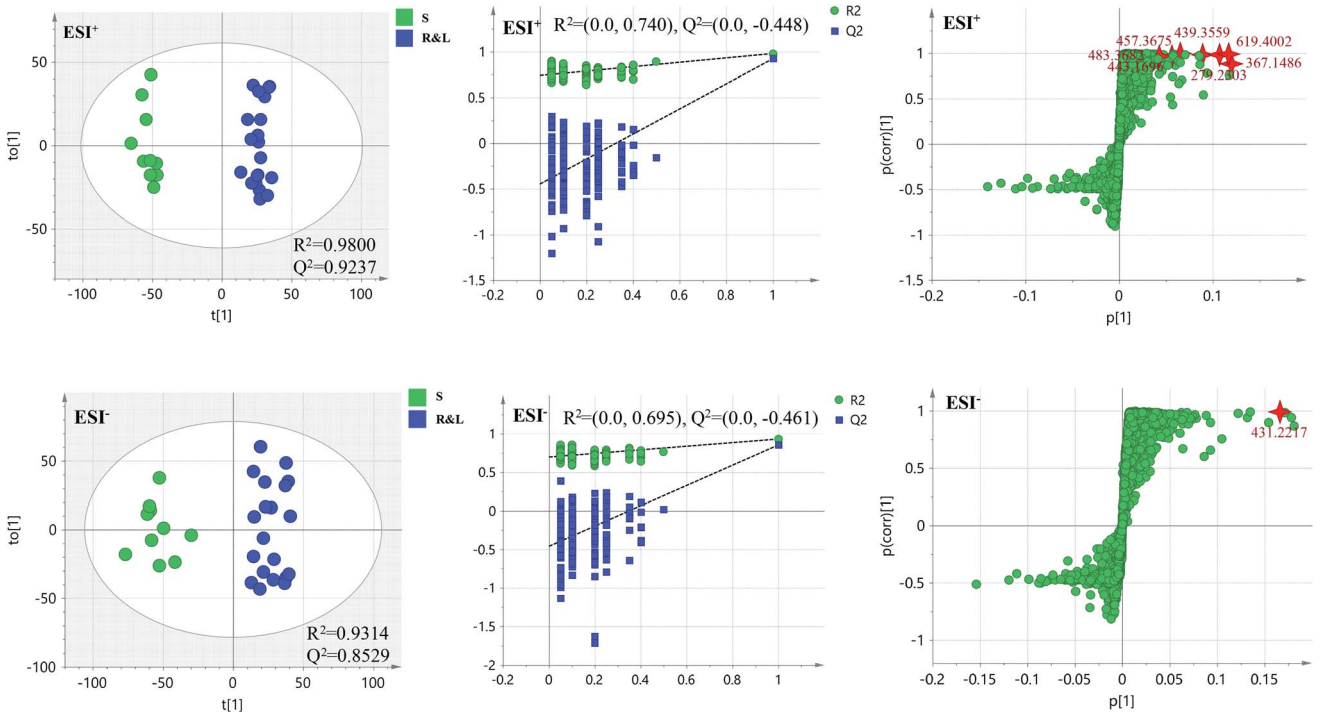


Fig. 6 OPLS-DA plots/permutation tests/*S*-plots between S and R&L. R: the root of *Celastrus orbiculatus* Thunb.; S: the stem of *Celastrus orbiculatus* Thunb.; L: the leaf of *Celastrus orbiculatus* Thunb.

of COT were shown in Fig. 3. There were 34, 18 and 10 triterpeniods identified from root, stem and leaf, respectively, accounted for 37%, 32% and 31% of the total components in each part. So, it was concluded that triterpeniods were the major constituents in three parts of COT. Moreover, according to each percentage, the root part of COT was also rich in organic acid and organic

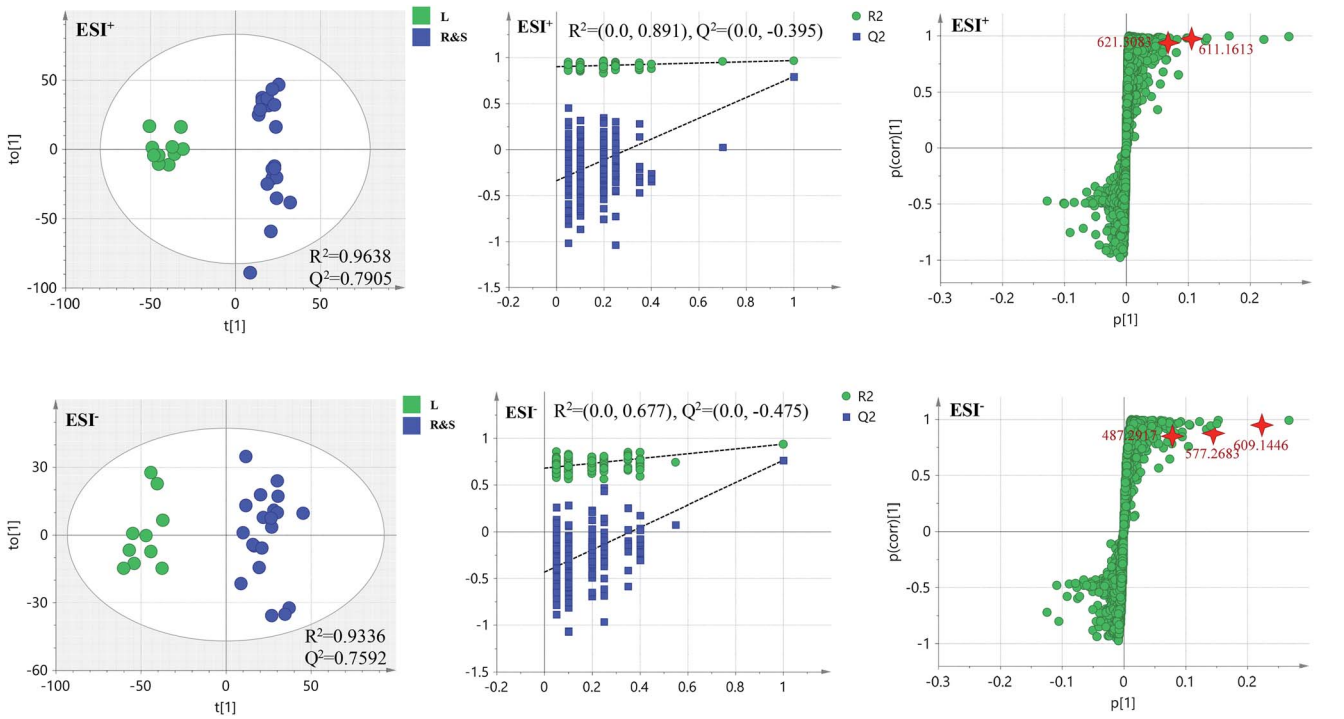


Fig. 7 OPLS-DA plots/permutation tests/*S*-plots between L and R&S. R: the root of *Celastrus orbiculatus* Thunb.; S: the stem of *Celastrus orbiculatus* Thunb.; L: the leaf of *Celastrus orbiculatus* Thunb.

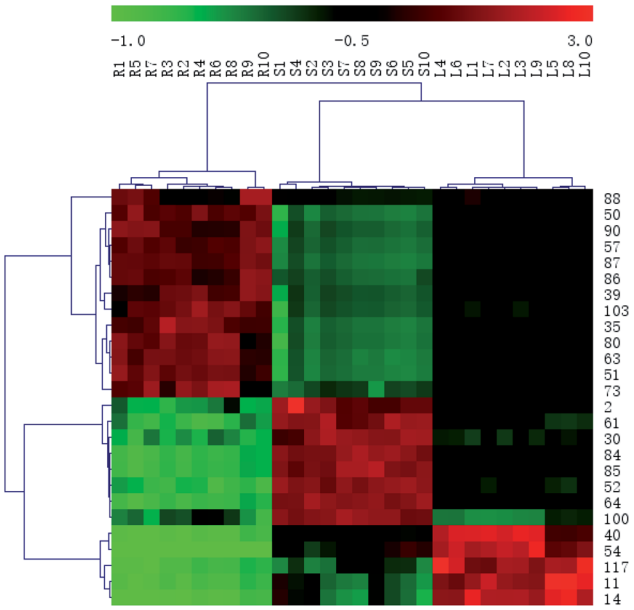


Fig. 8 Heat-map visualizing the intensities of the potential chemical markers.

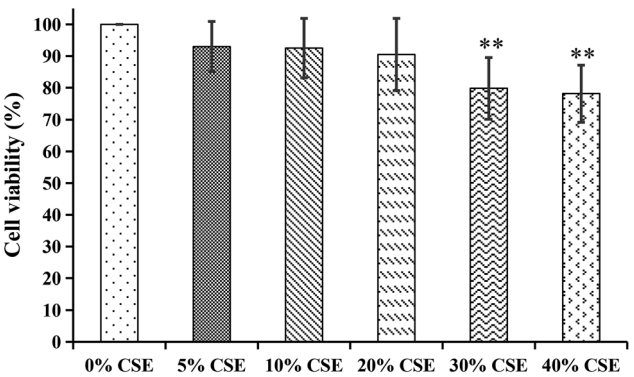


Fig. 9 The cytotoxicity effects of different concentrations of cigarette smoke extract (CSE) on A549 cells. $^{**}p < 0.01$, compared with 0% CSE group.

acid esters, steroids and phenylpropanoids. The stem part was also rich in organic acid and organic acid esters, and flavonoids. The leaf part was also rich in steroids, and sesquiterpenoids. The percentage of flavonoids in the total identified components in stem was higher than the percentages in root part or in leaf part. The percentages of sesquiterpeniods and steroids in the total identified components in leaf was higher than the percentages in root part or in stem part. On the other hand, the shared components (30 for root and stem, 22 for root and leaf, 23 for stem and leaf, 15 for root, stem and leaf) were also found in our study. As shown in Fig. 3, the structures of shared components were various, while triterpeniods held the majority. Celastrol, one of the triterpeniods, was shown to distribute in root, stem and leaf, which was consistent with the ref. 19. So our research work could provide the scientific data to clarify the chemical composition of COT, particularly for the root and the leaf parts.

Although the study provided evidences to elucidate the chemical composition of COT, there were still some unresolved issues. For example, as shown in BPI chromatograms, there were some unidentified components. Further research should be carried on the identification of these unknown compounds.

3.2. Metabolomics analysis of three parts of COT

PCA score 2D plots in both ESI^+ and ESI^- were established as shown in Fig. 4. The QC samples were clustered tightly and were in the middle of the three groups in PCA, which indicated the system had satisfactory stability. The samples from root of COT were clearly gathered together, which indicated there was a good similarity among them, and this phenomenon was also observed in stem and leaf of COT. Meanwhile, the root, stem and leaf groups were easily divided into three clusters, indicating that these three parts of COT could be differentiated in both ESI^+ and ESI^-.

In order to further distinguish one part from the other two parts, OPLS-DA plots, *S*-plots, permutation tests, and VIP values were obtained to see which variables were responsible for sample separation[97] (Fig. 5–7). In OPLS-DA plots, each spot represented a sample. From the perspective of OPLS-DA, one part was clearly separated from the other two parts. The parameters such as R^2 and Q^2 indicated the model had good

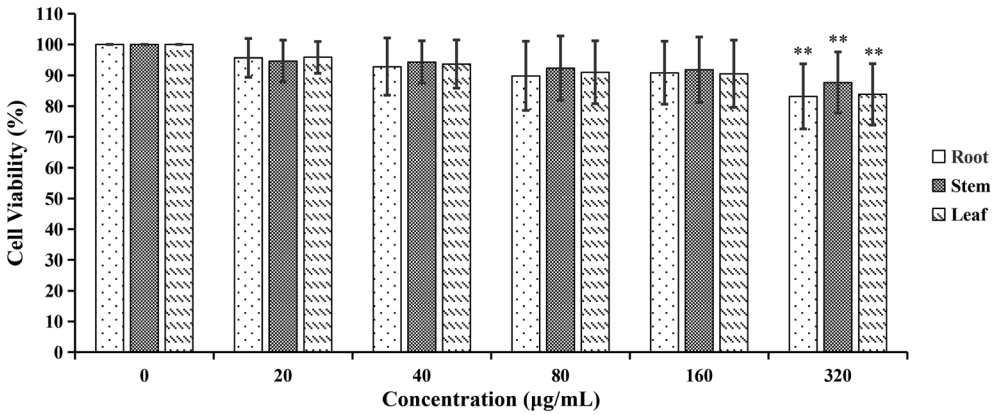


Fig. 10 The cell viability effects of different concentrations of R_{bio}, S_{bio} and L_{bio} solution on A549 cells. $^{**}p < 0.01$, compared with 0 μg mL^{-1} group.

ability of prediction and reliability in both ESI^+ and ESI^- modes. The permutation plots showed the original point on the right was clearly higher than all Q^2-values (blue) on the left, which indicated the original models were valid. To identify the metabolites contributing to the discrimination, *S*-plots were generated under OPLS-DA model. Each spot in *S*-plots represented a variable. The variables with VIP > 4 and $p < 0.001$ were considered as potential chemical markers. The possible molecular formula of the markers were calculated by high-accuracy quasi-molecular ion with mass error between ±5 ppm. A total of 26 robust known chemical markers (marked in Table 2) enabling the differentiation between one part with the other two parts were identified and marked in *S*-plots.

According to the reference, it was revealed that there was significant variation for the contents of celastrol or total alkaloids in different parts of COT. While in this study, there were 13, 8 and 5 potential chemical markers including celastrol discovered from root, stem and leaf, respectively. The markers in root including 8 triterpenoids (**35**, **39**, **50**, **51**, **63**, **80**, **88**, **90**), 1 steroids (**57**), 1 organic acid esters (**87**), 1 phenylpropanoids (**103**) and 2 other compounds (**73**, **86**). The markers in stem including 4 triterpenoids (**64**, **84**, **85**, **100**), 1 flavonoids (**2**), 1 phenylpropanoids (**30**), 1 steroids (**52**) and 1 organic acid esters (**61**). The markers in leaf including 3 sesquiterpenoids (**11**, **40**, **117**), 1 flavonoids (**14**) and 1 triterpenoids (**54**). Additionally, among these potential chemical markers, the contents of **57** and **88** in root, **52** in stem, **40**, **54** and **117** in leaf were much higher than in the other two parts ($p <$

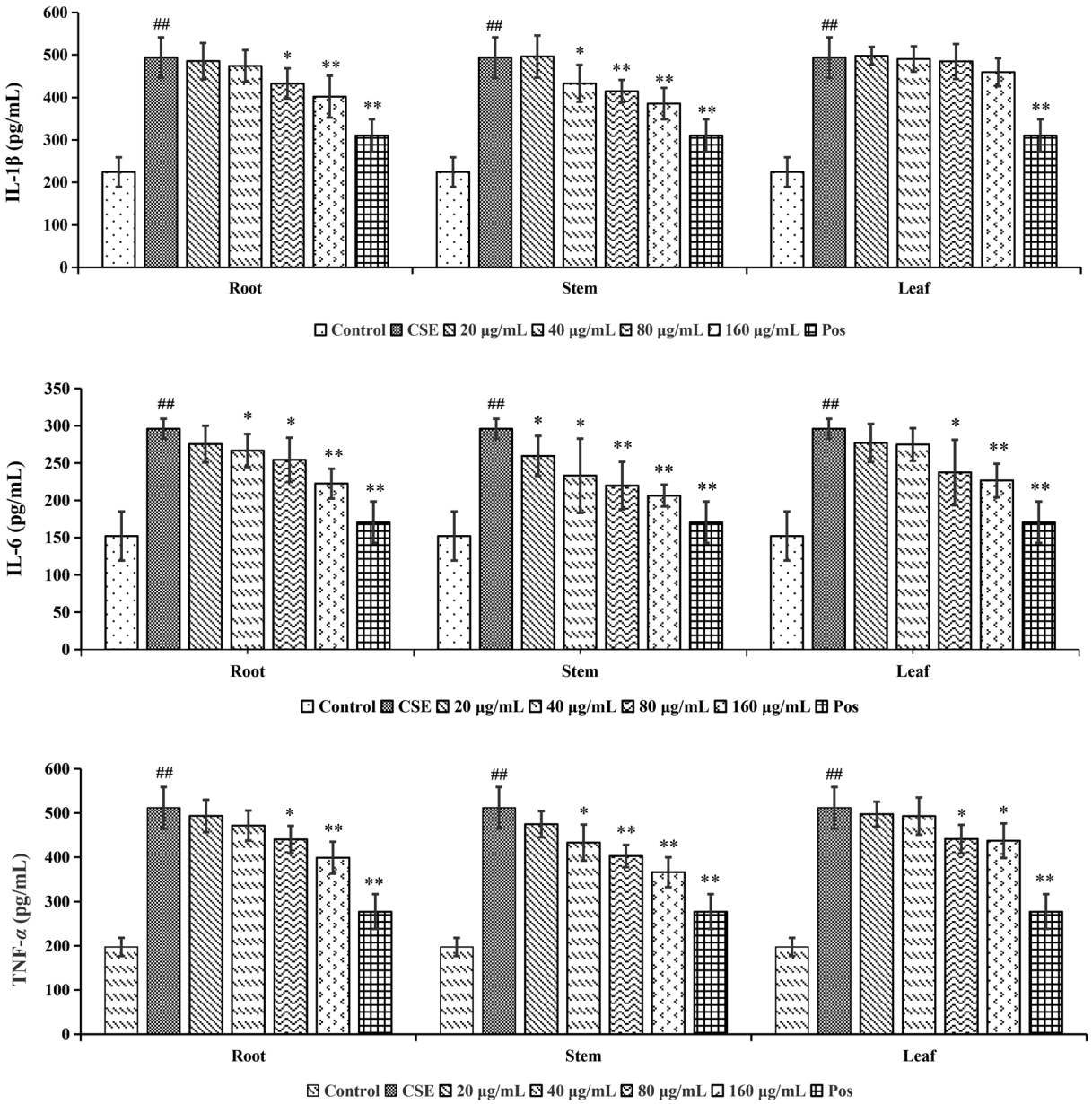


Fig. 11 The effects of inflammatory cytokine IL-1β, IL-6 and TNF-α on root, stem and leaf (20–160 μg mL^{-1}) of COT in CSE-stimulated A549 cells. $^{\#\#}p < 0.01$, compared with control group; $^{*}p < 0.05$, compared with CSE group; $^{**}p < 0.01$, compared with CSE group.

0.001). While components **35**, **39**, **50**, **51**, **63**, **73**, **80**, **86**, **87**, **90** and **103** were detected only in root, components **2**, **30**, **61**, **64**, **84**, **85** and **100** were detected only in stem, components **11** and **14** were detected only in leaf part under the detect condition.

The detected result of **88** (celastrol) in our study, with much higher contents in root than in stem and leaf, was consistent with the ref. 19. While there were a few differences between our results and the references. In the present study, compound **39** (pristimerin) was only detected in root, and **11** (orbiculin I) was only detected in leaf. According to the reports, **39** was once isolated from stem[14] though mainly from root,[98,99] and **11** isolated from root.[3] The reason was the concentrations of them were lower than the lowest detection limits. It was worth mentioning that some chemical markers with high responses in UPLC-MS, two triterpenoids (**39** and **88**) in root, one flavonoids (**2**) in stem and two sesquiterpenoids (**11** and **40**) in leaf, could be used for further quality control of three parts of COT respectively.

In order to systematically evaluate the chemical markers, a heat-map was generated. The hierarchical clustering heat map, intuitively visualizing the difference level of potential chemical markers in different parts, was shown in Fig. 8. The higher values were indicated by red squares, the lower values were indicated by green squares.

3.3. Bioactivity evaluation

3.3.1 Cytotoxicity of CSE and the three parts of COT on the viability of A549 cells. The results of MTT showed that the viability of A549 cells was obviously affected ($p < 0.01$) by 30% or 40% CSE (Fig. 9). Therefore, 20% CSE was chosen as stimulus in the following experiments. Additionally, as shown in Fig. 10, the viability of A549 cells were not significantly affected by the R_{bio}, S_{bio} and L_{bio} solutions at 20–160 μg mL^{-1}. So we evaluated the effects of R_{bio}, S_{bio} and L_{bio} solutions at 20–160 μg mL^{-1} on CSE-stimulated A549 cells.

3.3.2 Effect of root, stem and leaf of COT on CSE-stimulated pro-inflammatory cytokine levels in A549 cells. The inflammatory development was characterized by the release of pro-inflammatory mediators such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Whether the root, stem and leaf of COT could inhibit the release of IL-1β, IL-6 and TNF-α in CSE-stimulated lung epithelial cells was investigated in this paper. As shown in Fig. 11, the production of IL-1β, IL-6 and TNF-α in A549 cells was obviously increased after treated with CSE ($p < 0.01$). However, treated with the R_{bio}, S_{bio} and L_{bio} solutions could evidently decrease the levels of pro-inflammatory factors in a good dose-dependent way with a certain range of 20–160 μg mL^{-1} in CSE-stimulated cells. The R_{bio} solution could significantly decrease the levels of IL-1β, TNF-α (80 μg mL^{-1}, $p < 0.05$; 160 μg mL^{-1}, $p < 0.01$) and IL-6 (40 and 80 μg mL^{-1}, $p < 0.05$; 160 μg mL^{-1}, $p < 0.01$). The S_{bio} solution could significantly decrease the levels of IL-1β, TNF-α (40 μg mL^{-1}, $p < 0.05$; 80 and 160 μg mL^{-1}, $p < 0.01$) and IL-6 (20 and 40 μg mL^{-1}, $p < 0.05$; 80 and 160 μg mL^{-1}, $p < 0.01$). The L_{bio} solution could significantly decrease the levels of IL-6 (80 μg mL^{-1}, $p < 0.05$; 160 μg mL^{-1}, $p < 0.01$) and TNF-α (80 and 160 μg mL^{-1}, $p < 0.05$), but showed no significantly effect on IL-1β. The above results showed that the S_{bio} solution had a stronger anti-inflammation effect than R_{bio} and L_{bio}. It is suggested that to explore the anti-COPD effect of the COT stem *in vivo* is meaningful in further research. The different activities of root, stem and leaf of COT might be caused by the various phytochemicals in these three parts of COT. The phytochemical study showed that three parts of COT were differentiated. The bioassay study showed that three parts of COT could reduce the levels of pro-inflammatory factors to varying degrees. And the stem part had a stronger anti-COPD effect than root and leaf parts. As we all know, the material basis of different pharmacological activities is the different chemical composition. The results of the two parts of our study showed that the different activities of root, stem and leaf of COT might be caused by the various phytochemicals in these three parts of COT.

4. Conclusions

In conclusion, for screening analysis, a total of 120 compounds (15 shared components), including 92 from root, 56 from stem and 32 from leaf, were identified or tentatively characterized from COT. Each part of COT was rich in various kinds of structures, especially triterpeniods held the majority. For metabolomic analysis, the root, stem and leaf of COT were differentiated in both ESI^+ and ESI^- modes. There were 13, 8 and 5 potential chemical markers identified from root, stem and leaf, respectively. Among the above robust markers, 5 robust chemical markers with high responses in UPLC-MS, 2 triterpenoids (pristimerin and celastrol) in root, 1 flavonoids {5,7-dihydroxy-6,8-dimethyl-3(*S*)-3-(3-methoxy-4′-hydroxybenzyl) chroman-4-one} in stem and 2 sesquiterpenoids (orbiculin I and orbiculin A) in leaf, could be used for further quality control in three parts of COT respectively. For bioassay analysis, the root, stem and leaf of COT could evidently reduce the levels of pro-inflammatory factors in a dose-dependent way within a certain range of 20–160 μg mL^{-1} in CSE-induced A549 cells. The results showed that the stem part had a stronger anti-COPD effect than root and leaf parts. The different activities might be caused by the various phytochemicals in these three parts of COT. This comprehensive phytochemical study revealed both the structural diversity of secondary metabolites and the different distributions in different parts of COT. It could provide a theoretical basis for further utilization and development of COT. And the identification of anti-COPD components from COT will be explored deeply in the future based on the current results of this study.

Conflicts of interest

The authors declare no conflicts of interests.

References

1 Y. Zhang, Y. H. Si, L. Zhai, S. D. Guo, J. L. Zhao, H. Sang, X. F. Pang, X. Zhang, A. B. Chen and S. C. Qin, Celas*trus orbiculatus* Thunb. reduces lipid accumulation by

promoting reverse cholesterol transport in hyperlipidemic mice, *Lipids*, 2016, **51**, 677–692.
2 M. R. Wang, Research progress of a Chinese herb *Celastrus* on anti-tumor effect, *J. Chin. Med.*, 2010, **6**, 1055–1057.
3 H. Z. Jin, B. Y. Hwang, H. S. Kim, J. H. Lee, Y. H. Kim and J. J. Lee, Antiinflammatory constituents of *Celastrus orbiculatus* inhibit the NF-kappaB activation and NO production, *J. Nat. Prod.*, 2002, **65**, 89–91.
4 D. Q. Hou, N. Bai, Z. C Wei, B. Li, G. X. Tang and Y. F. Gao, *Celastrus orbiculatus Celastraceae Thunb* extracts inhibit proliferation and migration of oral squamous cell carcinoma cells by blocking NF-κB pathway, *Trop. J. Pharm. Res.*, 2019, **18**, 1259–1264.
5 Z. Q. Liu, R. X. Zhou and M. Q. Wu, Study on antiviral components of *Celastrus orbiculatus* Thunb., *Acta Acad. Med. Jiangxi*, 1983, 1–5.
6 X. G. Tao, *Bacteriostatic effect of Celastrus root skin extraction fluid on Staphylococcus aureus*, Hunan Agric. Univ., 2012.
7 H. Y. Ni, Z. H. Zhang, W. J. Song and L. F. Guo, Chemical constituents of root bark of *Celastrus orbiculatus* Thunb., *Chin. Pharmaceut. J.*, 2014, **49**, 21.
8 Y. Jiang, H. Li and S. Q. Luo, Study on the chemical constituents of *Celastrus orbiculatus* Thunb., *Chin. Tradit. Herb. Drugs*, 1996, **27**, 73–89.
9 J. Wu, 1. Preparation and biological activity of cucurbitacin derivatives. 2. Study on the chemical constituents and biological activities of the root bark of *Celastrus orbiculatus* Thunb., Dissertation, University of Chinese Academy of Sciences, 2011.
10 Y. D. Zhu, L. Liu, L. Hu, W. Q. Dong, M. Zhang, Y. Q. Liu and P. Li, Effect of *Celastrus orbiculatus* in inhibiting Helicobacter pylori induced inflammatory response by regulating epithelial mesenchymal transition and targeting miR-21/PDCD4 signaling pathway in gastric epithelial cells, *BMC Complementary Altern. Med.*, 2019, **19**, 91.
11 H. B. Wang, L. D. Tao, T. Y. Ni, H. Gu, F. Jin, X. J. Dai, J. Feng, Y. B. Ding, W. M. Xiao, S. Y. Guo, T. D. S. Hisamitsud, Y. Y. Qian and Y. Q. Liu, Anticancer efficacy of the ethyl acetate extract from the traditional Chinese medicine herb *Celastrus orbiculatus* against human gastric cancer, *J. Ethnopharmacol.*, 2017, **205**, 147–157.
12 Y. Zhang, Y. H. Si, L. Zhai, N. N. Yang, S. T. Yao, H. Sang, D. D. Zu, X. Xu, S. C. Qin and J. H. Wang, *Celastrus orbiculatus Thunb*. ameliorates high-fat diet-induced non-alcoholic fatty liver disease in guinea pigs, *Pharmazie*, 2013, **68**, 850–854.
13 B. Y. Hwang, H. S. Kim, J. H. Lee, Y. S. Hong, J. S. Ro, K. S. Lee and J. J. Lee, Antioxidant benzoylated flavan-3-ol glycoside from *Celastrus orbiculatus*, *J. Nat. Prod.*, 2001, **64**, 82–84.
14 J. Li, Discovery of natural products with lipid-lowering activity and their mechanism of action, Dissertation, University of Chinese Academy of Sciences, 2018.
15 J. J. Li, J. Yang, F. Lu, Y. T. Qi, Y. Q. Liu, Y. Sun and Q. Wang, Chemical constituents from the stems of *Celastrus orbiculatus*, *Chin. J. Nat. Med.*, 2012, **10**, 279–283.
16 X. Q. Chen, K. Zan and Q. Wang, Olean-type triterpenes of *Celastrus orbiculatus*, *Biochem. Syst. Ecol.*, 2012, **44**, 338–340.
17 Y. W. Liu, Efficacy test of crude ethanol extracts of *Celastrus orbiculatus* Thunb. and *Ginkgo biloba* leaves on *Agrolimax agrestis* Linnaeus, *J. Anhui Agric. Sci.*, 2011, **39**, 19176–19185.
18 X. X. Yu, T. T. Zhang and D. Y. Wang, Chemical constituents in hypoglycemic active fraction of *Celastrus orbiculatus* leaf, *J. Chin. Med. Mater.*, 2014, **6**, 998–1000.
19 Y. Zhang, Y. J. Cui, S. D. Guo, N. N. Yang, Y. H. Si and S. C. Qin, Using response surface methodology for optimization of extraction process followed by capillary zone electrophoresis for determination of celastrol in *Celastrus orbiculatus* Thunb, *Chin. J. Hosp. Pharm.*, 2014, **34**, 704–709.
20 Y. H. Zhang, L. H. Yang and C. M. Li, Determination of general alkaloids from *Celastrus orbiculatus* Thunb. in Guilin area, *Acad. J. Second Mil. Med. Univ.*, 2004, **25**, 1012.
21 C. Z. Wang, N. Q. Zhang, Z. Z. Wang, Z. Qi, B. Z. Zheng, P. Y. Li and J. P. Liu, Rapid characterization of chemical constituents of *Platycodon grandiflorum* and its adulterant Adenophora stricta by UPLC-QTOF-MS/MS, *J. Mass Spectrom.*, 2017, **52**, 643–656.
22 H. Q. Lin, H. L. Zhu, J. Tan, C. Z. Wang, Q. H. Dong, F. L. Wu, H. Wang, J. L. Liu, P. Y. Li and J. P. Liu, Comprehensive investigation on metabolites of wild-simulated american Ginseng root based on ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry, *J. Agric. Food Chem.*, 2019, **67**, 5801–5819.
23 W. S. Tang, H. Y. Peh, W. P. Liao, C. H. Pang, T. K. Chan, S. H. Lau, V. T. Chow and W. S. Wong, Cigarette smoke-induced lung disease predisposes to more severe infection with nontypeable haemophilus influenzae: protective effects of andrographolide, *J. Nat. Prod.*, 2016, **79**, 1308–1315.
24 L. M. Fabbri, F. Luppi, B. Beghe and K. F. Rabe, Update in chronic obstructive pulmonary disease 2005, *Am. J. Respir. Cell Mol. Biol.*, 2006, **175**, 1056–1065.
25 X. Liang, J. Wang, R. J. Guan, L. Zhao, D. F. Li, Z. Long, Q. Yang, J. Y. Xu, Z. Y. Wang, J. K. Xie and W. J. Lu, Limax extract ameliorates cigarette smoke-induced chronic obstructive pulmonary disease in mice, *Int. J. Immunopharmacol.*, 2018, **54**, 210–220.
26 H. Q. Lin, H. L. Zhu, J. Tan, H. Wang, Q. H. Dong, F. L. Wu, Y. H. Liu, P. Y. Li and J. P. Liu, Non-targeted metabolomic analysis of methanolic extracts of wild-simulated and field-grown American Ginseng, *Molecules*, 2019, **24**, 1053.
27 Y. R. Wang, C. Z. Wang, H. Q. Lin, Y. H. Liu, Y. M. Li, Y. Zhao, P. Y. Li and J. P. Liu, Discovery of the potential biomarkers for discrimination between *Hedyotis diffusa* and *Hedyotis corymbosa* by UPLC-QTOF/MS metabolome analysis, *Molecules*, 2018, **23**, 1525.
28 H. L. Zhu, H. Q. Lin, J. Tan, C. Z. Wang, H. Wang, F. L. Wu, Q. H. Dong, Y. H. Liu, P. Y. Li and J. P. Liu, UPLC-QTOF/MS-based nontargeted metabolomic analysis of mountain- and garden-cultivated Ginseng of different ages in northeast China, *Molecules*, 2019, **24**, 33.
29 H. Q. Lin, H. L. Zhu, J. Tan, H. Wang, Z. Y. Wang, P. Y. Li, C. F. Zhao and J. P. Liu, Comparative analysis of chemical constituents of *Moringa oleifera* leaves from China and

India by ultra-performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry, *Molecules*, 2019, **24**, 942.

30 C. Z. Wang, N. Q. Zhang, Z. Z. Wang, Z. Qi, H. L. Zhu, B. Z. Zheng, P. Y. Li and J. P. Liu, Nontargeted metabolomic analysis of four different parts of *Platycodon grandiflorum* grown in northeast China, *Molecules*, 2017, **22**, 1280.

31 Z. J. Zou, Z. H. Liu, M. J. Gong, B. Han, S. M. Wang and S. W. Liang, Intervention effects of puerarin on blood stasis in rats revealed by a 1H-NMR-based metabonomic approach, *Phytomedicine*, 2015, **22**, 333–343.

32 L. Chen, Q. Ge, G. Tjin, H. Alkhouri, L. H. Deng, C. A. Brandsma, I. Adcock, W. Timens, D. Postma, J. K. Burgess, J. L. Black and B. G. G. Oliver, Effects of cigarette smoke extract on human airway smooth muscle cells in COPD, *Eur. Respir. J.*, 2014, **44**, 634–646.

33 R. S. Coleman, S. R. Gurrala, S. Mitra and A. Raao, Asymmetric total synthesis of dibenzocyclooctadiene lignan natural products, *J. Org. Chem.*, 2005, **70**, 8932.

34 H. M. Hua, H. Q. Yin, B. Q. Li and Y. H. Pei, Study on the constituents of the bark of *Eucommia ulmoides*, *Mol. Plant Breed.*, 2003, **1**, 801–803.

35 Z. G. Zheng, R. S. Wang, H. Q. Cheng, T. T. Duan, B. He, D. Tang, F. Gu and Q. Zhu, Isolated perfused lung extraction and HPLC-ESI-MS(n) analysis for predicting bioactive components of *Saposhnikoviae Radix*, *J. Pharm. Biomed. Anal.*, 2011, **54**, 614–618.

36 C. Masuoka, M. Ono, Y. Ito and T. Nohara, Antioxidative, antihyaluronidase and antityrosinase activities of some constituents from the aerial part of *Piper elongatum* VAHL, *Food Sci. Technol. Res.*, 2003, **9**, 197–201.

37 S. Sato, J. Takeo, C. Aoyama and H. Kawahara, Na^+-glucose cotransporter (SGLT) inhibitory flavonoids from the roots of *Sophora flavescens*, *Bioorg. Med. Chem.*, 2007, **15**, 3445–3449.

38 Y. N. Lin, D. N. Zhu, J. Qi, M. J. Qin and B. Y. Yu, Characterization of homoisoflavonoids in different cultivation regions of *Ophiopogon japonicus* and related antioxidant activity, *J. Pharm. Biomed. Anal.*, 2010, **52**, 757–762.

39 S. Schwaiger, C. Seger, B. Wiesbauer, P. Schneider, E. P. Ellmerer, S. Sturm and H. Stuppner, Development of an HPLC-PAD-MS assay for the identification and quantification of major phenolic edelweiss (*Leontopodium alpium* Cass.) constituents, *Phytochem. Anal.*, 2006, **17**, 291–298.

40 M. J. Chang, T. M. Hung, B. S. Min, J. C. Kim, M. H. Woo, J. S. Choi, H. K. Lee and K. Bae, Lignans from the fruits of *Forsythia suspensa* (Thunb.) Vahl protect high-density lipoprotein during oxidative stress, *J. Agric. Chem. Soc. Jpn.*, 2008, **72**, 2750–2755.

41 S. I. Falcao, M. Vilas-Boas, L. M. Estevinho, C. Barros, M. Domingues and S. M. Cardoso, Phenolic characterization of northeast Portuguese propolis: usual and unusual compounds, *Anal. Bioanal. Chem.*, 2010, **396**, 887–897.

42 V. Kumar, V. Karunaratne, M. R. Meegalle and K. Sanath, 1-[2′,4′-dihydroxy-3′,5′-di-(3″-methylbut-2″-enyl)-6′-methoxy] phenylethanone from *Acronychia pedunculata*, root bark, *Phytochemistry*, 1989, **28**, 1278–1279.

43 T. Matsumoto, K. Hosono-Nishiyama and H. Yamada, Antiproliferative and apoptotic effects of butyrolactone lignans from *Arctium lappa* on leukemic cells, *Planta Med.*, 2005, **72**, 276–278.

44 T. J. Schmidt, S. Hemmati, E. Fuss and A. W. Alfermann, A combined HPLC-UV and HPLC-MS method for the identification of lignans and its application to the lignans of *Linum usitatissimum* L. and L. bienne Mill, *Phytochem. Anal.*, 2006, **17**, 299–311.

45 S. Yin, C. Q. Fan, Y. Wang, L. Dong and J. M. Yue, Antibacterial prenylflavone derivatives from *Psoralea corylifolia*, and their structure-activity relationship study, *Bioorg. Med. Chem.*, 2004, **12**, 4387–4392.

46 H. L. Jiang, A. Somogyi, N. E. Jacobsen, B. N. Timmermann and D. R. Gang, Analysis of curcuminoids by positive and negative electrospray ionization and tandem mass spectrometry, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 1001–1012.

47 Q. H. Ye, W. M. Zhao and G. W. Qin, Lignans from *Dendrobium chrysanthum*, *J. Asian Nat. Prod. Res.*, 2004, **6**, 39–43.

48 X. Y. Guan, H. F. Li, W. Z. Yang, C. H. Lin, C. Sun, B. R. Wang, D. A. Guo and M. Ye, HPLC-DAD-MSn analysis and HPLC quantitation of chemical constituents in Xian-ling-gu-bao capsules, *J. Pharm. Biomed. Anal.*, 2011, **55**, 923.

49 H. C. Beatriz, C. P. Miriam, V. C. Claudia, L. O. Jesus F, A. G. Agustin and J. N. Pedro, New saponins from Sechium mexicanum, *Magn. Reson. Chem.*, 2009, **11**, 994–1003.

50 S. J. Xu, L. Yang, X. Zeng, M. Zhang and Z. T. Wang, Characterization of compounds in the Chinese herbal drug Mu-Dan-Pi by liquid chromatography coupled to electrospray ionization mass spectrometry, *Rapid Commun. Mass Spectrom.*, 2010, **20**, 3275–3288.

51 Y. Y. Zhao, X. L. Cheng, Y. M. Zhang, Y. Zhao, R. C. Lin and W. J. Sun, Simultaneous determination of eight major steroids from *Polyporus umbellatus* by high-performance liquid chromatography coupled with mass spectrometry detections, *Biomed. Chromatogr.*, 2010, **24**, 222–230.

52 C. Niampoka, R. Suttisri, R. Bavovada, H. Takayama and N. Aimi, Potentially cytotoxic triterpenoids from the root bark of *Siphonodon celastrineus* Griff, *Arch. Pharmacal Res.*, 2005, **28**, 546–549.

53 L. Y. Wang, N. L. Wang, X. S. Yao, S. Miyata and S. Kitanaka, Euphane and tirucallane triterpenes from the roots of *Euphorbia kansui* and their in vitro effects on the cell division of Xenopus, *J. Nat. Prod.*, 2003, **66**, 630–633.

54 X. Li, S. H. Li, J. X. Pu, S. X. Huang and H. D. Sun, Chemical constituents from *Paeonia anomala subsp*.veitchii (Paeoniaceae), *Acta Bot. Yunnanica*, 2007, **29**, 259–262.

55 C. F. Hossain, Y. P. Kim, S. R. Baerson, L. Zhang, R. K. Bruick, K. A. Mohammed, A. K. Agarwal, D. G. Nagle and Y. D Zhou, Saururus cernuus lignans-potent small

molecule inhibitors of hypoxia-inducible factor-1, *Biochem. Biophys. Res. Commun.*, 2005, **333**, 1026–1033.

56 F. M. Darwish and M. G. Reinecke, Ecdysteroids and other constituents from *Sida spinosa* L., *Phytochemistry*, 2003, **62**, 1179–1184.

57 N. Sanchez-Avila, F. Priego-Capote, J. Ruiz-Jimenez and M. D. Luque de Castro, Fast and selective determination of triterpenic compounds in olive leaves by liquid chromatography-tandem mass spectrometry with multiple reaction monitoring after microwave-assisted extraction, *Talanta*, 2009, **78**, 0–48.

58 M. A. Alamsjah, S. Hirao, F. Ishibashi and Y. Fujita, Isolation and structure determination of algicidal compounds from *Ulva fasciata*, *J. Agric. Chem. Soc. Jpn.*, 2005, **69**, 2186–2192.

59 T. Sugiura, S. Nakane, S. Kishimoto, K. Waku, Y. Yoshioka and A. Tokumura, Lysophosphatidic acid, a growth factor-like lipid, in the saliva, *J. Lipid Res.*, 2002, **43**, 2049–2055.

60 C. R. Cheng, M. Yang, Z. Y. Wu, Y. Wang, F. Zeng, W. Y. Wu, S. H. Guan and D. A. Guo, Fragmentation pathways of oxygenated tetracyclic triterpenoids and their application in the qualitative analysis of *Ganoderma lucidum* by multistage tandem mass spectrometry, *Rapid Commun. Mass Spectrom.*, 2011, **25**, 1323–1335.

61 R. Bortolomeazzi, G. Verardo, A. Liessi and A. Callea, Formation of dehydrodiisoeugenol and dehydrodieugenol from the reaction of isoeugenol and eugenol with DPPH radical and their role in the radical scavenging activity, *Food Chem.*, 2009, **118**, 256–265.

62 M. B. Gallo, W. C. Rocha, U. S. da Cunha, F. A. Diogo, F. C. da Silva, P. C. Vieira, J. D. Vendramim, J. B. Fernandes, M. F. da Silva and L. G. Batista-Pereira, Bioactivity of extracts and isolated compounds from *Vitex polygama* (Verbenaceae) and *Siphoneugena densiflora* (Myrtaceae) against *Spodoptera frugiperda* (Lepidoptera: Noctuidae), *Pest Manage. Sci.*, 2006, **62**, 1072–1081.

63 K. Nagy, C. Courtet, C. Marie, B. Holst and M. Kussmann, Comprehensive analysis of vitamin E constituents in human plasma by liquid chromatography-mass spectrometry, *Anal. Chem.*, 2007, **79**, 7087–7096.

64 L. Y. Wang, N. L. Wang, X. S. Yao, S. Miyata and S. Kitanaka, Euphane and Tirucallane Triterpenes from the Roots of *Euphorbia kansui* and their in vitro Effects on the Cell Division of Xenopus, *J. Nat. Prod.*, 2003, **66**, 630–633.

65 H. Simone and V. Walter, Exploring the fatty acids of vernix caseosa in form of their methyl esters by off-line coupling of non-aqueous reversed phase high performance liquid chromatography and gas chromatography coupled to mass spectrometry, *J. Chromatogr. A*, 2010, **52**, 8270–8278.

66 C. Pettinella, S. H. Lee, F. Cipollone and I. A. Blair, Targeted quantitative analysis of fatty acids in atherosclerotic plaques by high sensitivity liquid chromatography/tandem mass spectrometry, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2007, **850**, 168–176.

67 Y. L. Lin and Y. H. Kuo, Four new neoclerodane-type diterpenoids, scutellones B, G, H, and I, from aerial parts of *Scutellaria rivularis*, *Chem. Pharm. Bull.*, 1989, **37**, 582–585.

68 X. A. Wen, J. Liu, L. Y. Zhang, P. Z. Ni and H. B. Sun, Synthesis and biological evaluation of arjunolic Acid, bayogenin, hederagonic acid and 4-epi-hederagonic acid as glycogen phosphorylase inhibitors, *Chin. J. Nat. Med.*, 2010, **8**, 441–448.

69 R. Tundis, B. Deguin, F. Menichini and F. Tillequin, Iridoids from *Putoria calabrica*, *Biochem. Syst. Ecol.*, 2002, **30**, 689–691.

70 X. Li, P. Gao, B. Gjetvaj, N. Westcott and M. Y. Gruber, Analysis of the metabolome and transcriptome of *Brassica carinata* seedlings after lithium chloride exposure, *Plant Sci.*, 2009, **177**, 68–80.

71 K. Kuroda and A. Nakagawaizumi, Analytical pyrolysis of lignin: products stemming from β-5 substructures, *Org. Geochem.*, 2006, **37**, 665–673.

72 S. E. Ayyad, S. Z. Sowellim, M. S. El-Hosini and A. Abo-Atia, The structural determination of a new steroidal metabolite from the brown alga *Sargassum asperifolium*, *Z. Naturforsch., C: J. Biosci.*, 2003, **58**, 333–336.

73 H. W. Seo, T. M. Hung, M. K. Na, H. J. Jung, J. C. Kim, J. S. Choi, J. H. Kim, H. K. Lee, I. S. Lee, K. H. Bae, M. S. Hattori and B. S. Min, Steroids and triterpenes from the fruit bodies of Ganoderma lucidum and their anti-complement activity, *Arch. Pharmacal Res.*, 2009, **32**, 1573–1579.

74 T. H. Lee, J. L. Chiou, C. K. Lee and Y. H. Kuo, Separation and determination of chemical constituents in the roots of *Rhus Javanica* L. Var. Roxburghiana, *J. Chin. Chem. Soc.*, 2005, **52**, 833–841.

75 K. Iwatsuki, T. Akihisa, H. Tokuda, M. Ukiya, M. Oshikubo, Y. Kimura, T. Asano, A. Nomura and H. Nishino, Lucidenic acids P and Q, methyl lucidenate P, and other triterpenoids from the fungus *Ganoderma lucidum* and their inhibitory effects on Epstein-Barr virus activation, *J. Nat. Prod.*, 2003, **66**, 1582.

76 C. A. Luo, W. N. Zhang, C. Q. Sheng, C. J. Zheng, J. Z. Yao and Z. Y. Miao, Chemical composition and antidiabetic activity of *Opuntia Milpa Alta* extracts, *Chem. Biodiversity*, 2010, **7**, 2869–2879.

77 B. S. Siddiqui, U. Ghani, S. T. Ali, S. B. Usmani and S. Begum, Triterpenoidal constituents of the leaves of *Carissa carandas*, *Nat. Prod. Lett.*, 2003, **17**, 153.

78 L. Liu, Y. Cheng and H. Zhang, Phytochemical Analysis of Anti-atherogenic Constituents of Xue-Fu-Zhu-Yu-Tang using HPLC-DAD-ESI-MS, *Chem. Pharm. Bull.*, 2004, **52**, 1295–1301.

79 D. Q. Luo, H. Wang, X. Tian, H. J. Shao and J. K. Liu, Antifungal properties of pristimerin and celastrol isolated from *Celastrus hypoleucus*, *Pest Manage. Sci.*, 2005, **61**, 85–90.

80 M. Skoumal, R. M. Rodriguez, P. L. Cabot, F. Centellas, J. A. Garrido, C. Arias and E. Brillas, Electro-Fenton, UVA photoelectro-Fenton and solar photoelectro-Fenton degradation of the drug ibuprofen in acid aqueous medium using platinum and boron-doped diamond anodes, *Electrochim. Acta*, 2009, **54**, 2077–2085.

81 J. Yang and K. L. Zhou, NMR spectroscopic analysis of neferine and isoliensinine, *Magn. Reson. Chem.*, 2004, **42**, 994–997.

82 C. H. Li, P. Y. Chen, U. M. Chang, L. S. Kan, W. H. Fang, K. S. Tsai and S. B. Lin, Ganoderic acid X, a lanostanoid triterpene, inhibits topoisomerases and induces apoptosis of cancer cells, *Life Sci.*, 2005, **77**, 0–265.

83 M. Yoshikawa, T. Morikawa, H. Oominami and H. Matsuda, Absolute Stereostructures of olibanumols A, B, C, H, I, and J from olibanum, gum-resin of *Boswellia carterii*, and inhibitors of nitric oxide production in lipopolysaccharide-activated mouse peritoneal macrophages, *Cheminform*, 2009, **57**, 957.

84 E. H. Joh and D. H. Kim, A sensitive liquid chromatography-electrospray tandem mass spectrometric method for lancemaside A and its metabolites in plasma and a pharmacokinetic study in mice, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2010, **878**, 1875–1880.

85 G. Alam, I. G. Gandjar, L. Hakim, H. Timmerman, R. Verpoorte and S. Wahyuono, Tracheospasmolytic activity of vitetrifolin-E isolated from the leaves of *Vitex trifolia* L, *Maj. Farm. Indones.*, 2003, **14**, 188–194.

86 M. Meier, B. Kohlenberg and N. Braun, Isolation of anisyl acetone from Agarwood Oil, *J. Essent. Oil Res.*, 2003, **15**, 54–56.

87 L. N. Tao, Q. Y. Meng, Y. Jian, R. Xing and H. R. Guo, A new panaxadiol from the acid hydrolysate of Panax ginseng, *Chin. Chem. Lett.*, 2009, **20**, 687–689.

88 K. Wang, H. Sun, B. Wu and Y. Pan, Two Novel Olean triterpenoids from *Celastrus hypoleucus*, *Helv. Chim. Acta*, 2010, **88**, 990–995.

89 G. Li, C. S. Lee, M. H. Woo, S. H. Lee, H. W. Chang and J. K. Son, Lignans from the bark of *Machilus thunbergii* and their DNA topoisomerases I and II inhibition and cytotoxicity, *Biol. Pharm. Bull.*, 2004, **27**, 1147–1150.

90 L. P. Ponomarenko, I. V. Stonik, N. A. Aizdaicher, T. Y. Orlova, G. I. Popovskaya, G. V. Pomazkina and V. A. Stonik, Sterols of marine microalgae Pyramimonas cf.cordata (Prasinophyta), Attheya ussurensis sp. nov. (Bacillariophyta) and a spring diatom bloom from Lake Baikal, *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.*, 2004, **138**, 65–70.

91 J. Wandji, F. Tillequin, D. A. Mulholland, J. C. Shirri, N. Tsabang, E. Seguin, P. Verite, F. Libot and Z. T. Fomum, Pentacyclic triterpenoid and saponins from Gambeya boukokoensis, *Phytochemistry*, 2003, **64**, 845–849.

92 F. Wu, K. Koike, T. Nikaido, K. Ishii, T. Ohmoto and K. Ikeda, Terpenoids and flavonoids from *Arenaria kansuensis*, *Chem. Pharm. Bull.*, 1990, **38**, 2281–2282.

93 C. R. Chen, L. H. Chao, M. H. Pan, Y. W. Liao and C. I. Chang, Tocopherols and Triterpenoids from *Sida acuta*, *J. Chin. Chem. Soc.*, 2007, **54**, 41–45.

94 C. M. Sandison, R. Alexander, R. I. Kagi and C. J. Boreham, Early diagenetic transformation of organic matter in a marine-influenced lignite, *Org. Geochem.*, 2003, **34**, 1081–1102.

95 C. Johannes and R. L. Lorenz, Preparation and mass spectrometry of 14 pure and 18O2-labeled oxidation products from the phytosterols b-sitosterol and stigmasterol, *Anal. Biochem.*, 2004, **325**, 107–116.

96 M. X. Chen, D. Y. Wang and J. Guo, ChemInform abstract: 3-Oxo-11β-hydroxyfriedelane from the roots of *Celastrus monospermus*, *Cheminform*, 2010, **34**, 114–117.

97 X. W. Chang, B. R. Wang, T. Wang, D. K. Li, Y. Zhao, Y. L. Zhang, D. Z. Zhou and Z. L. Ye, Plant metabolomics approach for age discrimination of mountain cultivated ginseng using UPLC-Q-TOF/MS, *China J. Chin. Mater. Med.*, 2016, **41**, 3609–3614.

98 J. Wu, Y. Zhou, L. Y. Wang, J. P. Zuo and W. M. Zhao, Terpenoids from root bark of *Celastrus orbiculatus*, *Phytochemistry*, 2012, **7**, 159–168.

99 L. Zhang, Z. J. Xu, Y. J. Feng and D. Y. Wang, Chemical constituents of roots of *Celastrus orbiculatus*, *J. Chin. Med. Mater.*, 2013, **36**, 569.

