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Analysis of Chemical Constituents of *Melastoma dodecandrum* Lour. by UPLC-ESI-Q-Exactive Focus-MS/MS

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Abstract: The ethnic drug *Melastoma dodecandrum* Lour. (MDL) is widely distributed throughout South China, and is the major component of Gong Yan Ping Tablets/Capsules and Zi Di Ning Xue San. Although the pharmacological effects of MDL have been well documented, its chemical profile has not been fully determined. In this study, we have developed a rapid and sensitive UPLC-ESI-Q-Exactive Focus-MS/MS method to characterize the chemical constituents of MDL in the positive and negative ionization modes. A comparison of the chromatographic and spectrometric data obtained using this method with data from databases, the literature and reference standards allowed us to identify or tentatively characterize 109 compounds, including 26 fatty acids, 26 organic acids, 33 flavonoids, six tannins, 10 triterpenoids, two steroids and six other compounds. Notably, 55 of the compounds characterized in this study have never been detected before in this plant. The information obtained in this study therefore enriches our understanding of the chemical composition of MDL and could be used in quality control, pharmacological research and the development of drugs based on MDL. In addition, this study represents the first reported comprehensive analysis of the chemical constituents of MDL.

Keywords: *Melastoma dodecandrum* Lour.; ultra performance liquid chromatography; mass spectrometry; chemical constituents; identification

1. Introduction

Traditional Chinese medicine (TCM) has been used for thousands of years to treat a variety of different diseases. Based on its theoretical therapeutic efficacy and wide range of clinical applications, TCM has received considerable interest from healthcare professionals, as well as those working towards the identification of new therapeutic agents for commercialization. In contrast to the pharmacological characteristics of single agent drugs, multicomponent drugs can exhibit synergistic pharmacological effects, through a "network" approach, where multiple compounds interact with multiple targets, pharmacokinetic or physicochemical synergisms in vivo with interdependent activities to achieve an improved optimal effect [1–3]. It is therefore essential to evaluate the chemical composition

of each TCM, so that this information can be used to support further studies, such as drug effect, toxicity and metabolism studies.

Melastoma dodecandrum Lour. (MDL) is extensively distributed throughout the southern provinces of China, including Guizhou, Fujian, Zhejiang, Jiangxi and Yunnan. This plant is widely used for its medicinal properties by the Yao, Miao and She people, as well as several other minority groups. Modern pharmacological studies have shown that MDL exhibits several biological effects, including antihypoglycemic, hemostatic, analgesic, anti-inflammatory, blood lipid reducing, antioxidant and liver protection properties [4]. MDL has been used to treat a variety of different ailments, including dysmenorrhea, postpartum abdominal pain, metrorrhagia, leucorrhea, hematochezia, dysentery, carbuncle swollen and boils. To date, only 76 compounds have been isolated from MDL, including organic acids, flavonoids, triterpenoids and steroids [5–13]. However, much of the chemical composition of MDL remains unknown, making it difficult to rationalize its bioactivity or evaluate the safety of this material as a therapeutic agent. There is therefore an urgent need to develop an analytical method capable of determining the chemical composition of MDL. With this in mind, the aim of the current study was to establish a rapid and sensitive method for identifying the constituents of MDL. In this study, we used a Q-Exactive Focus MS/MS method to obtain high-resolution mass spectra of the different components. This method was proven to be an advanced, accurate and reliable tool for the comprehensive identification of compounds belonging to a wide range of structural classes [14–21]. Using this method, we tentatively identified a total of 109 compounds, highlighting the efficiency and accuracy of this new technique.

2. Results and Discussion

Melastoma dodecandrum Lour. was analyzed in the positive and negative ionization modes using a Q Exactive Focus mass spectrometer, and the base peak chromatogram (BPC) chromatograms for both of these ESI modes are shown in Figure 1. Some of the compounds found in this study were identified based on a comparison of their analytical data (i.e., retention times and high-resolution mass spectra) with those of several reference standards. Thus compounds **10**, **34**, **35**, **46**, **68**, **79**, and **84** were unambiguously identified as gallic acid, luteolin, kaempferide, quercetin, oleanic acid, asiatic acid, and rutin, repectively. Moreover, the fragmentation patterns and pathways of the standards helped further confirm the structures of the derivatives of the reference compounds. Compounds without reference standards were identified by determining the elemental compositions of the precursor and product ions. The molecular formula and rational fragmentation patterns and pathways of these compounds were then identified based on a comparison of these data with chemical databases and the literature as described below in Section 3.5. In this way, we used a UPLC-ESI-Q-Exactive Focus-MS/MS method in combination with available standards, databases and literature data to characterize 109 compounds from MDL. Seven of these compounds were unambiguously identified based on a comparison with the corresponding reference standards. Data for all of these compounds are summarized in Table 1.

Table 1. Tentative identification of the chemical constituents of *Melastoma dodecandrum* Lour. by UPLC-ESI-Q-Exactive Focus-MS/MS in negative and positive modes.

No.	Tentative Compound	t_R (min)	Molecular Formula	Measured *m*/*z*	*m*/*z* Error in ppm	MS/MS (*m*/*z*)	Type of Compounds
1	Malic acid	0.99	$C_4H_6O_5$	133.01422 $[M–H]^-$	−0.19	115.00355 $[M – H – H_2O]^-$ 71.0138 $[C_3H_3O_2]^-$ 89.02435 $[M – H – CO_2]^-$	A
2	Salicylic acid	9.34	$C_7H_6O_3$	137.02438 $[M – H]^-$	−0.25	93.03443 $[M – H – CO_2]^-$	B
3	*m*-Salicylic acid	10.00	$C_7H_6O_3$	137.02437 $[M – H]^-$	−0.36	93.03445 $[M – H – CO_2]^-$	B
4	Citramalic acid	1.41	$C_5H_8O_5$	147.02991 $[M – H]^-$	0.06	129.01924 $[M – H – H_2O]^-$ 101.02427 $[M – H – HCOOH]^-$ 85.02937 $[C_4H_5O_2]^-$	A
5	Protocatechuic acid	5.55	$C_7H_6O_4$	153.01932 $[M – H]^-$	−0.09	109.02937 $[M – H – CO_2]^-$	B
6	Gentisic acid	9.51	$C_7H_6O_4$	153.01932 $[M – H]^-$	−0.09	109.02943 $[M – H – CO_2]^-$ 108.02159 $[C_6H_4O_2]^-$	B
7	Pimelic acid	13.87	$C_7H_{12}O_4$	159.0663 $[M – H]^-$	0.11	115.07629 $[M – H – CO_2]^-$ 97.06577 $[C_6H_9O]^-$ 141.0556 $[M – H – H_2O]^-$	A
8	Coumaric acid	13.94	$C_9H_8O_3$	163.04005 $[M – H]^-$	−0.08	119.05009 $[M – H – CO_2]^-$	B
9 [#]	Vanillic acid	11.72	$C_8H_8O_4$	167.03494 $[M – H]^-$	−0.23	152.0114 $[M – H – CH_3]^-$ 123.045 $[M – H – CO_2]^-$ 108.02161 $[C_6H_4O_2]^-$	B
10 [*,#]	Gallic acid	2.23	$C_7H_6O_5$	169.01416 $[M – H]^-$	−0.51	125.02429 $[M – H – CO_2]^-$ 97.0294 $[C_5H_5O_2]^-$ 81.03452 69.03456 $[C_4H_5O]^-$	B
11	Shikimic acid	1.15	$C_7H_{10}O_5$	173.04547 $[M – H]^-$	−0.44	155.03479 $[M – H – H_2O]^-$ 137.02423 $[M – H – 2H_2O]^-$ 111.04502 $[M – H – H2O – CO_2]^-$ 93.03445 $[C_6H_5O]^-$ 73.02941 $[C_3H_5O_2]^-$	B
12	2-Isopropylmalic acid	11	$C_7H_{12}O_5$	175.06120 $[M – H]^-$	0.03	157.05048 $[M – H – H_2O]^-$ 115.03992 $[C_5H_7O_3]^-$ 113.06075 $[C_6H_9O_2]^-$ 85.0658 $[C_5H_9O]^-$	B

Table 1. *Cont.*

No.	Tentative Compound	t_R (min)	Molecular Formula	Measured *m*/*z*	*m*/*z* Error in ppm	MS/MS (*m*/*z*)	Type of Compounds
13	Glucose	0.89	$C_6H_{12}O_6$	179.05605 $[M-H]^-$	−0.37	59.01379 $[C_2H_3O_2]^-$ 71.01382 $[C_3H_3O_2]^-$ 89.02422 $[C_3H_5O_3]^-$ 101.02422 $[C_4H_5O_3]^-$	F
14	2-Hydroxy-3-(2-hydroxyphenyl) propanoic acid	9.78	$C_9H_{10}O_4$	181.05064 $[M-H]^-$	0.06	163.03993 $[M-H-H_2O]^-$ 135.04512 $[M-H-HCOOH]^-$ 119.0501 $[M-H-H_2O-CO_2]^-$	B
15 #	Methyl gallate	10.88	$C_8H_8O_5$	183.02988 $[M-H]^-$	−0.11	168.00624 $[M-H-CH_3]^-$ 140.0114 $[M-H-CH_3-CO]^-$ 124.01643 $[C_6H_4O_3]^-$	B
16	Citric acid	0.99	$C_6H_8O_7$	191.01971 $[M-H]^-$	−0.06	111.00864 $[M-H-H_2O-COOH-OH]^-$ 87.00864 $[C_3H_3O_3]^-$ 129.01915 $[M-H-H_2O-CO_2]^-$ 85.02939	A
17 #	Ferulic acid	18.04	$C_{10}H_{10}O_4$	193.05052 $[M-H]^-$	−0.57	178.02702 $[M-H-CH_3]^-$ 149.06059 $[M-H-CO_2]^-$ 134.03719 $[M-H-CH_3-CO_2]^-$	B
18 #	Vanillylmandelic acid	14.47	$C_9H_{10}O_5$	197.04573 $[M-H]^-$	0.93	153.05563 $[M-H-CH_3]^-$ 138.03203 $[M-H-CH_3\text{-}CO_2]^-$ 121.0294 $[M-H-CH_3-CO_2-OH]^-$	B
19	Sebacic acid	20.14	$C_{10}H_{18}O_4$	201.11327 $[M-H]^-$	0.17	183.10249 $[M-H-H_2O]^-$ 139.11273 $[M-H-H_2O-CO_2]^-$	A
20	1-Oxo-1,2,4-butanetricarboxylic acid	1.48	$C_7H_8O_7$	203.01970 $[M-H]^-$	−0.13	141.01923 $[M-H-H_2O-CO_2]^-$ 97.02934 $[M-H-H_2O-2CO_2]^-$ 69.03453 $[C_4H_5O]^-$	A
21	Undecanedioic acid	22.12	$C_{11}H_{20}O_4$	215.12874 $[M-H]^-$	−0.67	197.11826 $[M-H-H_2O]^-$ 153.12842 $[M-H-H_2O-CO_2]^-$	A
22	2-Hydroxysebacic acid	16.5	$C_{10}H_{18}O_5$	217.10825 $[M-H]^-$	0.46	199.09734 $[M-H-H_2O]^-$ 171.10257 $[M-H-HCOOH]^-$ 155.10768 $[M-H-H_2O-CO_2]^-$	A
23	Glucoheptonic acid	0.82	$C_7H_{14}O_8$	225.06168 $[M-H]^-$	0.38	179.05602 $[C_6H_{11}1O_6]^-$ 161.04546 $[C_6H_9O_5]^-$ 87.00864 $[C_3H_3O_3]^-$	A
24	Traumatic Acid	22.97	$C_{12}H_{20}O_4$	227.12894 $[M-H]^-$	0.24	183.13876 $[M-H-CO_2]^-$ 165.12823 $[M-H-H_2O-CO_2]^-$	A
25	1-*O*-galloyl-glycerol	8.17	$C_{10}H_{12}O_7$	243.05095 $[M-H]^-$	−0.32	169.01408 $[M-H-CO_2-2CH_3]^-$ 125.02431 $[M-H-2CO_2-2CH_3]^-$	B

Table 1. *Cont.*

No.	Tentative Compound	t_R (min)	Molecular Formula	Measured *m*/*z*	*m*/*z* Error in ppm	MS/MS (*m*/*z*)	Type of Compounds
26	Oxododecanedioic acid	18.35	$C_{12}H_{20}O_5$	243.12381 $[M-H]^-$	0.05	225.1131 $[M-H-H_2O]^-$ 207.10254 $[M-H-H_2O]^-$ 181.1234 $[C_{11}H_{17}O_2]^-$	A
27	Palmitic acid	39.09	$C_{16}H_{32}O_2$	255.23297 $[M-H]^-$	0.07	237.06160 $[M-H-H_2O]^-$	A
28	Abscisic acid	19.75	$C_{15}H_{20}O_4$	263.12885 $[M-H]^-$	−0.12	219.13887 $[M-H-CO_2]^-$ 204.11528 $[M-H-CO_2-CH_3]^-$ 151.07634 $[C_9H_{11}O_2]^-$	B
29 [#]	Apigenin	19.79	$C_{15}H_{10}O_5$	269.04559 $[M-H]^-$	0.17	117.03447 $[C_8H_5O]^-$ 151.00354 $[C_7H_3O_4]^-$ 107.01373 $[C_6H_3O_2]^-$	C
30 [#]	Naringenin	21.35	$C_{15}H_{12}O_5$	271.06122 $[M-H]^-$	0.08	177.01917 $[C_9H_5O_4]^-$ 151.00352 $[C_7H_3O_4]^-$ 119.05009 $[C_8H_7O]^-$	C
31	Hydroxyhexadecanoic acid	36.77	$C_{16}H_{32}O_3$	271.22797 $[M-H]^-$	0.36	225.22221 $[M-H-HCOOH]^-$	A
32	Oleic acid	39.49	$C_{18}H_{34}O_2$	281.24860 $[M-H]^-$	−0.01	237.06163 $[M-H-CO_2]^-$	A
33	Stearic acid	41.08	$C_{18}H_{36}O_2$	283.26425 $[M-H]^-$	−0.01	265.14810 $[M-H-H_2O]^-$ 237.06181 $[M-H-HCOOH]^-$	A
34 [*,#]	Kaempferol	19.95	$C_{15}H_{10}O_6$	285.04047 $[M-H]^-$	0.02	257.04535 $[M-H-CO]^-$ 241.05112 $[M-H-CO_2]^-$ 151.00352 $[C_7H_3O_4]^-$ 133.02942 $[C_8H_5O_2]^-$	C
35 [*,#]	Luteolin	21.79	$C_{15}H_{10}O_6$	285.04062 $[M-H]^-$	0.56	239.03464 $[M-H-CO-H_2O]^-$ 185.06078 $[C_{12}H_9O_2]^-$ 159.04491 $[C_{10}H_7O_2]^-$ 93.03454 $[C_6H_5O]^-$	C
36	Hexadecanedioic acid	30.38	$C_{16}H_{30}O_4$	285.20709 $[M-H]^-$	−0.14	267.19635 $[M-H-H_2O]^-$ 223.20653 $[M-H-H_2O-CO_2]^-$	A
37	3,5-Dihydroxy-hexadecanoic acid	23.84	$C_{16}H_{32}O_4$	287.22278 $[M-H]^-$	−0.01	269.21246 $[M-H-H_2O]^-$ 241.21735 $[M-H-H_2O-CO_2]^-$	A
38	Epicatechin	11.49	$C_{15}H_{14}O_6$	289.07193 $[M-H]^-$	0.58	245.0816 $[M-H-CO_2]^-$ 203.07123 $[C_{12}H_{11}O_3]^-$ 137.02423 $[C_7H_5O_3]^-$ 109.02934 $[C_6H_5O_2]^-$	C

Table 1. *Cont.*

No.	Tentative Compound	t_R (min)	Molecular Formula	Measured *m*/*z*	*m*/*z* Error in ppm	MS/MS (*m*/*z*)	Type of Compounds
39	Catechin	13.27	$C_{15}H_{14}O_6$	289.07184 $[M-H]^-$	0.27	245.0089 $[M-H-C_3H_8]^-$ 217.01398 $[M-H-C_3H_8-CO]^-$ 189.01923 $[M-H-C_3H_8-2CO]^-$ 173.0242 $[C_{10}H_5O_3]^-$ 145.0294 $[M-H-C_3H_8-2CO-CO_2]^-$	C
40	4,9-Dihydroxy-6,7-dimethoxynaphtho (2,3-*d*)-1,3-dioxole-5,8-dione	14.56	$C_{13}H_{10}O_8$	293.03040 $[M-H]^-$	0.36	249.04028 $[M-H-CO_2]^-$ 225.11308 $[C_{12}H_{17}O_4]^-$ 162.0321 $[M-H-CO_2-CH_3-CO]^-$	F
41	9-Hode	32.28	$C_{18}H_{32}O_3$	295.22791 $[M-H]^-$	0.12	277.21716 $[M-H-H_2O]^-$ 171.1026 $[C_9H_{15}O_3]^-$	A
42	Ricinoleic acid	32.63	$C_{18}H_{34}O_3$	297.24353 $[M-H]^-$	0.0	183.13885 $[C_{11}H_{19}O_2]^-$	A
43	2-Glucopyranosyloxybenzoic acid	9.95	$C_{13}H_{16}O_8$	299.07730 $[M-H]^-$	0.2	137.02425 $[M-H-Glc]^-$ 93.03445 $[M-H-glc-CO_2]^-$	B
44	Hydroxystearic acid	39.09	$C_{18}H_{36}O_3$	299.25919 $[M-H]^-$	0.06	253.25348 $[M-H-HCOOH]^-$ 225.22246 $[C_{15}H_{29}O]^-$	A
45 [#]	Ellagic acid	15.56	$C_{14}H_6O_8$	300.99899 $[M-H]^-$	0	283.99619 $[M-H-OH]^-$ 245.009 $[C_{12}H_5O_6]^-$ 229.01402 $[M-H-CO_2-CO]^-$ 201.01927 $[M-H-CO_2-2CO]^-$ 185.02431 $[C_{11}H_5O_3]^-$	D
46 [*,#]	Quercetin	19.95	$C_{15}H_{10}O_7$	301.03534 $[M-H]^-$	−0.12	178.99843 $[C_8H_3O_5]^-$ 151.00351 $[M-H-C_6H_6-CO_2-CO]^-$ 107.01373 $[M-H-C_6H_6-2CO_2-CO]^-$	C
47	Gallocatechin	11.12	$C_{15}H_{14}O_7$	305.06683 $[M-H]^-$	0.52	261.0766 $[M-H-CO_2]^-$ 219.06612 $[C_{12}H_{11}O_4]^-$ 167.03488 $[C_8H_7O_4]^-$ 137.02432 $[C_7H_5O_3]^-$ 125.02433 $[C_6H_5O_3]^-$	C
48	Eicosanoic acid	42.84	$C_{20}H_{40}O_2$	311.29562 $[M-H]^-$	0.22	293.17941 $[M-H_2O]^-$	A
49	Glucovanillin	10.72	$C_{14}H_{18}O_8$	313.09308 $[M-H]^-$	0.6	161.04539 $[C_6H_9O_5]^-$ 113.02431 $[C_5H_5O_3]^-$ 101.02427 $[C_4H_5O_3]^-$ 71.01381 $[C_3H_3O_2]^-$	F
50	Octadecanedioic acid	29.15	$C_{18}H_{34}O_4$	313.23849 $[M-H]^-$	0.2	295.2272 $[M-H-H_2O]^-$	A

Table 1. *Cont.*

No.	Tentative Compound	t_R (min)	Molecular Formula	Measured *m*/*z*	*m*/*z* Error in ppm	MS/MS (*m*/*z*)	Type of Compounds
51 #	2,3,8-Trihydroxy-7-methoxychromeno [5,4,3-cde]chromene-5,10-dione	21.54	$C_{15}H_8O_8$	315.01489 $[M-H]^-$	0.80	300.99841 $[C_{14}H_5O_8]^-$ 269.10269 161.04578 $[C_6H_9O_5]^-$ 71.01388 $[C_3H_3O_2]^-$	D
52	Dihydroxystearic acid	30.79	$C_{18}H_{36}O_4$	315.25403 $[M-H]^-$	−0.17	297.24344 $[M-H-H_2O]^-$ 201.11363 $[M-H-C_8H_{18}]^-$	A
53	Digallate	11.39	$C_{14}H_{10}O_9$	321.02530 $[M-H]^-$	0.29	169.01404 $[C_7H_5O_5]^-$ 125.02428 $[C_6H_5O_3]^-$	B
54	2,3-Di-*O*-methylellagic acid	20.25	$C_{16}H_{10}O_8$	329.03040 $[M-H]^-$	0.32	314.00681 $[M-H-CH_3]^-$ 298.98325 $[C_{14}H_3O_8]^-$ 270.98834 $[M-H-2CH_3-CO]^-$	D
55	Woodorien	9.46	$C_{14}H_{18}O_9$	329.08795 $[M-H]^-$	0.44	167.03482 $[M-H\text{-}Glc]^-$ 152.01135 $[M-H\text{-}Glc-CH_3]^-$ 121.0294 $[M-H-Glc-HCOOH]^-$ 108.02159 $[M-H\text{-}Glc-CH_3-CO_2]^-$	B
56	Galloylglucose	3.25	$C_{13}H_{16}O_{10}$	331.06729 $[M-H]^-$	0.67	271.04575 $[C_{11}H_{11}O_8]^-$ 211.02464 $[C_9H_7O_6]^-$ 169.01405 $[M-H\text{-}Glc]^-$ 125.02432 $[C_6H_5O_3]^-$	B
57	Caffeic acid-3-glucoside	11.08	$C_{15}H_{18}O_9$	341.08798 $[M-H]^-$	0.52	305.06638 $[M-H-2H_2O]^-$ 281.06647 $[C_{13}H_{13}O_7]^-$ 251.05588 $[C_{12}H_{11}O_6]^-$ 221.04532 $[C_{11}H_9O_5]^-$ 179.03485 $[M-H-Glc]^-$ 135.04509 $[M-H-Glc-CO_2]^-$	B
58	2-Hydroxy-3,7,8-trimethoxychromeno [5,4,3-cde]chromene-5,10-dione	23.33	$C_{17}H_{12}O_8$	343.04590 $[M-H]^-$	−0.13	328.0224 $[M-H-CH3]^-$ 312.99899 $[M-H-2CH_3]^-$ 297.97522 $[M-H-3CH_3]^-$ 269.98053 $[M-H-3CH_3-CO]^-$	D
59	Theogallin	6.68	$C_{14}H_{16}O_{10}$	343.06720 $[M-H]^-$	0.38	169.01408 $[C_7H_5O_5]^-$ 125.02427 $[C_6H_5O_3]^-$	B
60	Chlorogenic acid	9.88	$C_{16}H_{18}O_9$	353.08813 $[M-H]^-$	0.93	191.05602 $[C_7H_{11}O_6]^-$ 179.0349 $[C_9H_7O_4]^-$ 135.04512 $[C_8H_7O_2]^-$	B
61 #	Vitexin	15.74	$C_{21}H_{20}O_{10}$	431.09833 $[M-H]^-$	−0.1	341.06631 $[C_{18}H_{13}O_7]^-$ 311.05603 $[C_{17}H_{11}O_6]^-$ 283.06088 $[C_{16}H_{11}O_5]^-$	C

Table 1. *Cont.*

No.	Tentative Compound	t_R (min)	Molecular Formula	Measured *m*/*z*	*m*/*z* Error in ppm	MS/MS (*m*/*z*)	Type of Compounds
62 #	9,10-Dihydro-10-(4-hydroxyphenyl)-pyrano[2,3-*h*]epicatechin-8-one	19.9	$C_{24}H_{20}O_8$	435.10876 $[M-H]^-$	0.51	341.06641 $[M-H-Phenol]^-$ 217.01392 $[C_{11}H_5O_5]^-$ 189.01918 $[C_{10}H_5O_4]^-$ 177.01915 $[C_9H_5O_4]^-$	C
63	Epicatechin monogallate	15.92	$C_{22}H_{18}O_{10}$	441.08286 $[M-H]^-$	0.31	289.07166 $[C_{15}H_{13}O_6]^-$ 169.0141 $[C_7H_5O_5]^-$ 125.02431 $[C_6H_5O_3]^-$	C
64	Astragalin	15.06	$C_{21}H_{20}O_{11}$	447.09344 $[M-H]^-$	0.36	357.06146 $[C_{18}H_{13}O_8]^-$ 327.05087 $[C_{17}H_{11}O_7]^-$ 299.05569 $[C_{16}H_{11}O_6]^-$	C
65 #	Kaempferol-3-glucoside	16.47	$C_{21}H_{20}O_{11}$	447.09354 $[M-H]^-$	0.56	285.03983 $[M-H-Glc]^-$	C
66 #	Luteolin-7-glucoside	17.4	$C_{21}H_{20}O_{11}$	447.09357 $[M-H]^-$	0.63	284.0325 $[M-H-Glc]^-$ 255.02986 $[C_{14}H_7O_5]^-$ 227.03485 $[C_{13}H_7O_4]^-$	C
67 #	Ursolic acid	35.96	$C_{30}H_{48}O_3$	455.35315 $[M-H]^-$	0.18	407.33292 $[C_{29}H_{43}O]^-$	E
68 *,#	Oleanic acid	36.51	$C_{30}H_{48}O_3$	455.35333 $[M-H]^-$	0.57	407.33292 $[C_{29}H_{43}O]^-$	E
69 #	Quercetin-3-alloside	16.21	$C_{21}H_{20}O_{12}$	463.08856 $[M-H]^-$	0.78	300.02731 $[M-H-Gal]^-$ 271.02469 $[C_{14}H_7O_6]^-$ 255.02979 $[C_{14}H_7O_5]^-$	C
70	Nigranoic acid	32.15	$C_{30}H_{46}O_4$	469.33249 $[M-H]^-$	0.33	423.327 $[C_{29}H_{43}O_2]^-$	B
71 #	4-*O*-(6″-*O*-*p*-Coumaroyl-glucopyranosyl)-*p*-coumaric acid	18.64	$C_{24}H_{24}O_{10}$	471.12994 $[M-H]^-$	0.58	307.08206 $[C_{15}H_{15}O_7]^-$ 163.03993 $[C_9H_7O_3]^-$ 119.05008 $[C_8H_7O]^-$	B
72	Corosolic acid	31.81	$C_{30}H_{48}O_4$	471.34802 $[M-H]^-$	0.08	453.33932 $[C_{30}H_{45}O_3]^-$	E
73 #	7-Hydroxy-3,8-dimethoxy-5,10-dioxo-5,10-dihydro-chromeno[5,4,3-cde]chromen-2-yl 6-deoxymannopyranoside	18.83	$C_{22}H_{20}O_{12}$	475.08853 $[M-H]^-$	0.7	460.06451 $[M-H-CH_3]^-$ 328.02231 $[M-H-Rha]^-$ 312.99887 $[M-H-Rha-CH_3]^-$ 269.98038 $[C_{13}H_2O_7]^-$	D
74	Isorhamnetin-7-*O*-glucopyranoside	17.62	$C_{22}H_{22}O_{12}$	477.10416 $[M-H]^-$	0.64	314.04306 $[M-H-Glc]^-$ 271.02448 $[M-H-Glc-CH_3-CO]^-$ 243.02959	C

Table 1. *Cont.*

No.	Tentative Compound	t_R (min)	Molecular Formula	Measured *m*/*z*	*m*/*z* Error in ppm	MS/MS (*m*/*z*)	Type of Compounds
75	Isomyricitrin	14.78	$C_{21}H_{20}O_{13}$	479.08301 $[M-H]^-$	−0.22	316.02216 $[M-H-Glc]^-$ 271.02454 $[C_{14}H_7O_6]^-$	C
76	1,6-Bis-*O*-galloyl-glucose	11.52	$C_{20}H_{20}O_{14}$	483.07819 $[M-H]^-$	0.33	465.10410 $[M-H-H_2O]^-$ 439.08768 $[M-H-CO_2]^-$	B
77	Quillaic acid	26.36	$C_{30}H_{46}O_5$	485.32706 $[M-H]^-$	−0.39	407.29538 $[M-H-CH_3-H_2O]^-$ 241.10255	E
78 #	2-*O*-(*E*)-caffeoyl-1-*O*-*p*-(*E*)-coumaroylglucopyrannose	12.86	$C_{24}H_{24}O_{11}$	487.12473 $[M-H]^-$	0.29	323.07706 $[C_{15}H_{15}O_8]^-$ 161.02429 $[C_9H_5O_3]^-$ 119.05011 $[C_8H_7O]^-$	B
79 *,#	Asiatic acid	26.85	$C_{30}H_{48}O_5$	487.34271 $[M-H]^-$	−0.38	469.33224 $[M-H-H_2O]^-$	E
80	Oenin	18.16	$C_{23}H_{24}O_{12}$	491.11981 $[M-H]^-$	0.64	313.03531 $[M-H-Glc-CH]^-_3$ 299.01956 $[C_{15}H_7O_7]^-$ 285.04028 $[M-H-Glc-CH_3-CO]^-$	C
81	Medicagenic acid	24.38	$C_{30}H_{46}O_6$	501.32227 $[M-H]^-$	0.21	455.31689 $[M-H-HCOOH]^-$	E
82	2″-*O*-Galloylisovitexin	17.72	$C_{28}H_{24}O_{14}$	583.10974 $[M-H]^-$	0.71	431.09842 $[C_{21}H_{19}O_{10}]^-$ 341.06635 $[C_{18}H_{13}O_7]^-$ 311.056 $[C_{17}H_{11}O_6]^-$ 283.061 $[C_{16}H_{11}O_5]^-$	C
83	Nicotiflorin	17.02	$C_{27}H_{30}O_{15}$	593.15167 $[M-H]^-$	0.81	285.03989 $[C_{15}H_9O_6]^-$ 255.02983 $[C_{14}H_7O_5]^-$ 227.03491 $[C_{13}H_7O_4]^-$	C
84 *	Rutin	15.9	$C_{27}H_{30}O_{16}$	609.14630 $[M-H]^-$	0.32	300.02737 $[C_{15}H_8O_7]^-$ 271.02469 $[C_{14}H_7O_6]^-$ 255.02977 $[C_{14}H_7O_5]^-$	C
85	2′-*O*-Galloylhyperin	15.56	$C_{28}H_{24}O_{16}$	615.09943 $[M-H]^-$	0.44	463.08804 $[C_{21}H_{19}O_{12}]^-$ 300.02734 $[C_{15}H_8O_7]^-$ 271.02454 $[C_{14}H_7O_6]^-$ 255.02972 $[C_{14}H_7O_5]^-$	C
86	3-*O*-*trans*-*p*-Coumaroylmaslinic acid	34.83	$C_{39}H_{54}O_6$	617.38501 $[M-H]^-$	0.4	145.02936 $[C_9H_5O_2]^-$	E
87	Delphinidin-3-caffeoylglucoside	18.25	$C_{30}H_{26}O_{15}$	625.12048 $[M-H]^-$	0.94	463.08832 $[C_{21}H_{19}O_{12}]^-$ 300.02731 $[C_{15}H_8O_7]^-$ 271.02472 $[C_{14}H_7O_6]^-$ 255.02985 $[C_{14}H_7O_5]^-$	C
88	3-*O*-*trans*-*p*-Coumaroyltormentic acid	31.21	$C_{39}H_{54}O_7$	633.37982 $[M-H]^-$	0.23	163.04001 $[C_9H_7O_3]^-$ 145.02939 $[C_9H_5O_2]^-$	E

Table 1. *Cont.*

No.	Tentative Compound	t_R (min)	Molecular Formula	Measured *m*/*z*	*m*/*z* Error in ppm	MS/MS (*m*/*z*)	Type of Compounds
89	1,3,6-Tri-*O*-galloylglucose	14.4	$C_{27}H_{24}O_{18}$	635.08948 $[M-H]^-$	0.77	465.06714 $[C_{20}H_{17}O_{13}]^-$ 211.02463 $[C_9H_7O_6]^-$ 169.01404 $[C_7H_5O_5]^-$ 125.02427 $[C_6H_5O_3]^-$	B
90	(3,5,9)-3-Hydroxy-27-{[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoyl]oxy}olean-12-en-28-oic acid	35.86	$C_{40}H_{56}O_7$	647.39587 $[M-H]^-$	0.84	632.37152 $[C_{37}H_{52}O_7]^-$ 453.33835 $[C_{30}H_{45}O_3]^-$ 175.03993 $[C_{10}H_7O_3]^-$ 133.02946 $[C_8H_5O_2]^-$	E
91	3-*O*-*trans*-Feruloyleuscaphic acid	32.02	$C_{40}H_{56}O_8$	663.39069 $[M-H]^-$	0.67	648.36658 $[C_{39}H_{52}O_8]^-$ 175.03989 $[C_{10}H_7O_3]^-$ 160.01643 $[C_9H_4O_3]^-$ 132.02156 $[C_8H_4O_2]^-$	E
92 #	4′-Hydroxyacetophenone	12.32	C8H8O2	137.05969 $[M+H]^+$	−0.01	122.03635 $[M+H-CH_3]^+$ 109.06508 $[M+H-CO]^+$	F
93	Dihydroxyacetophenone	13.7	$C_8H_8O_3$	153.05461 $[M+H]^+$	−0.06	125.05981 $[M+H-CO]^+$ 111.04433 $[C_6H_7O_2]^+$	F
94	*N*-Lauryldiethanolamine	37.04	$C_{16}H_{35}O_2N$	256.26334 $[M+H]^+$	−0.6	144.1382 $[C_8H_{18}ON]^+$ 116.1072 $[C_6H_{14}ON]^+$	F
95	Licanic acid	26.99	$C_{18}H_{28}O_3$	293.21085 $[M+H]^+$	−0.94	275.20044 $[M+H-H_2O]^+$ 257.19003 $[M+H-2H_2O]^+$	A
96	Kamlolenic acid	33.51	$C_{18}H_{30}O_3$	295.22678 $[M+H]^+$	0.06	277.21609 $[M+H-H_2O]^+$ 259.20532 $[M+H-2H_2O]^+$ 231.21051 $[M+H-3H_2O]^+$	A
97	Diosmetin	21.99	$C_{16}H_{12}O_6$	301.0705 $[M+H]^+$	−0.56	286.04681 $[M+H-CO_3]^+$ 258.05197 $[M+H-CH_3-CO]^+$	C
98 #	Sitosterol	39.21	$C_{29}H_{50}O$	397.38269 $[M+H-H_2O]^+$	−0.47	243.21089 $[C_{18}H_{27}]^+$ 175.14799 $[C_{13}H_{19}]^+$ 147.11684 $[C_{11}H_{15}]^+$	G
99 #	Stigmasterol	38.08	$C_{29}H_{48}O$	395.36725 $[M+H-H_2O]^+$	0.05	241.1945 $[C_{18}H_{25}]^+$ 199.14772 $[C_{15}H_{19}]^+$ 173.13248 $[C_{13}H_{17}]^+$	G
100	Apigenin-7-*O*-glucoside	17.83	$C_{21}H_{20}O_{10}$	433.1127 $[M+H]^+$	−0.51	271.05975 $[C_{15}H_{11}O_5]^+$ 153.01814 $[C_7H_5O_4]^+$	C
101 #	Quercetin-3-arabinoside	16.62	$C_{20}H_{18}O_{11}$	435.09235 $[M+H]^+$	0.36	303.04959 $[C_{15}H_{11}O_7]^+$ 153.01819 $[C_7H_5O_4]^+$	C
102	Luteolin-7-galactoside	12.52	$C_{21}H_{20}O_{11}$	449.10745 $[M+H]^+$	−0.86	287.05453 $[M-H-Glc]^+$ 269.044 $[M+H-Glc]^+$	C

Table 1. *Cont.*

No.	Tentative Compound	t_R (min)	Molecular Formula	Measured *m*/*z*	*m*/*z* Error in ppm	MS/MS (*m*/*z*)	Type of Compounds
103	Pelargonidin-3-*O*-(6-caffeoyl-glucoside)	19.91	$C_{30}H_{26}O_{13}$	595.14459 $[M + H]^+$	−0.04	433.11255 $[M - H - C_6H_6 - 3CO]^+$ 313.07028 $[C_{17}H_{13}O_6]^+$ 163.03888 $[C_9H_7O_3]^+$	C
104	Tiliroside	20.42	$C_{30}H_{26}O_{13}$	595.14435 $[M + H]^+$	−0.45	287.0546 $[C_{15}H_{11}O_6]^+$ 147.04393 $[C_9H_7O_2]^+$ 119.04929 $[C_8H_7O]^+$	C
105	Kaempferol-3-(6″-galloylgalactoside)	16.8	$C_{28}H_{24}O_{15}$	601.11914 $[M + H]^+$	0.57	287.05457 $[C_{15}H_{11}O_6]^+$ 153.01813 $[C_7H_5O_4]^+$ 137.0233 $[C_7H_5O_3]^+$	C
106	Quercetin-3-*O*-(6″-*O*-p-coumaroyl)-glucopyranoside	19.41	$C_{30}H_{26}O_{14}$	611.13922 $[M + H]^+$	−0.51	147.04388 $[C_9H_7O_2]^+$ 303.04953 $[C_{15}H_{11}O_7]^+$	C
107	2′-*O*-Galloylhyperin	15.51	$C_{28}H_{24}O_{16}$	617.11322 $[M + H]^+$	−0.89	153.01813 $[C_7H_5O_4]^+$ 303.0495 $[C_{15}H_{11}O_7]^+$	C
108	Quercetin-3-(6″-caffeoylgalactoside)	18.29	$C_{30}H_{26}O_{15}$	627.13409 $[M + H]^+$	−0.56	163.03873 $[C_9H_7O_3]^+$ 303.04941 $[C_{15}H_{11}O_7]^+$	C
109 #	Casuarinin	12.61	$C_{41}H_{28}O_{26}$	937.09332 $[M + H]^+$	−0.89	153.01817 $[C_7H_5O_4]^+$ 171.04417 $[C_{11}H_7O_2]^+$ 277.03397 $[C_{13}H_9O_7]^+$ 345.02377 $[C_{16}H_9O_9]^+$	D

* These compound were unambiguously identified by the use of authentic reference compounds. # These compound were isolated from *Melastoma dodecandrum* Lour. according to the literature [5–13]. Glc, glucopyranosyl, Rha, rhamnopyranosyl. A, fatty acid; B, organic acid; C, flavonoid; D, tannin; E, pentacyclic triterpene; F, others; G, steroid.

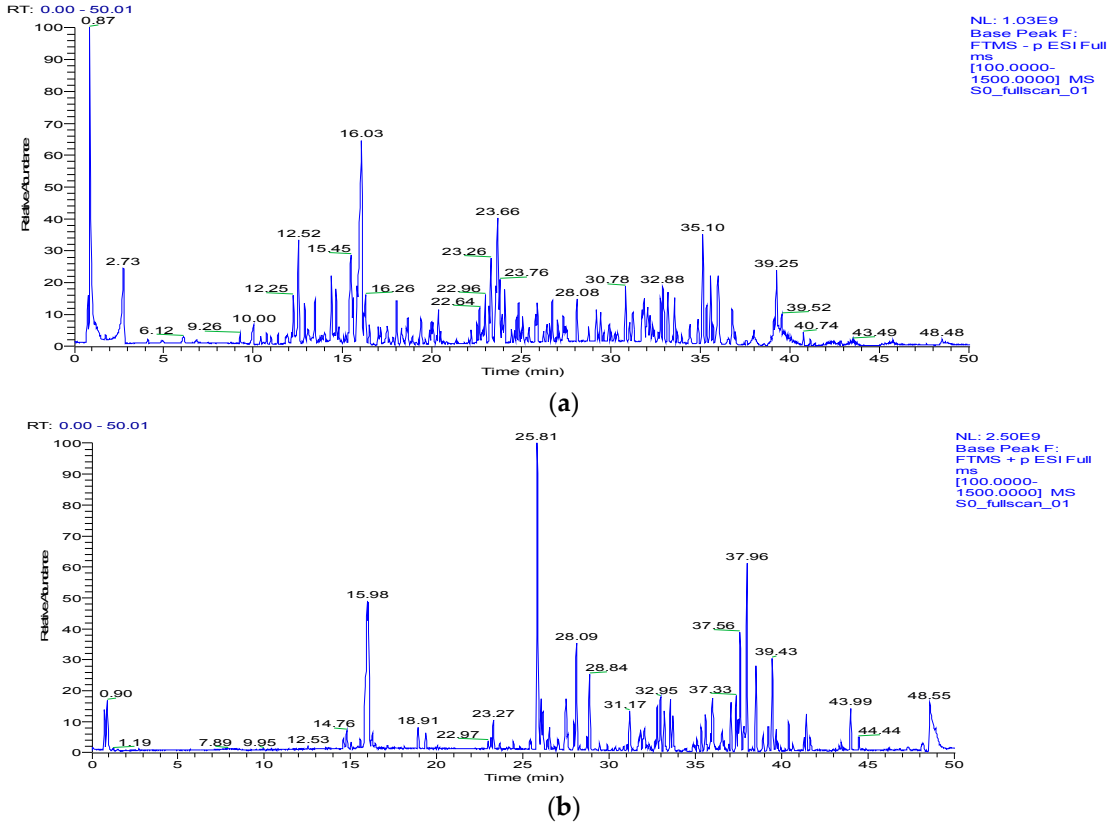


Figure 1. Base peak chromatogram of *Melastoma dodecandrum* Lour. in negative ion mode (**a**) and positive ion mode (**b**) using UPLC-ESI-Q-Exactive Focus-MS/MS.

2.1. Fragmentation Pattern of Main Compounds

2.1.1. Flavonoids

Flavonoids are 2-phenylchromone systems that consist of two benzene rings (A and B) connected by a pyran ring (ring C), which is fused to the A ring. Flavonoids can be classified into several subclasses, including flavones, flavonols, flavanones, flavanonols, anthocyanidins, chalcones, isoflavonoids and flavan-3-ols, depending on the nature of the substituents attached to the different rings. Based on the results of accurate molecular mass measurements and the MS^2 fragmentation pathways of the different materials [16,22,23], we characterized a total of 33 different flavonoids (aglycones) in MDL, including eight flavones, one flavanone, five flavan-3-ols, two anthocyanidins and 17 flavonols. The structures of the 33 flavonoids are shown below (Figure 2).

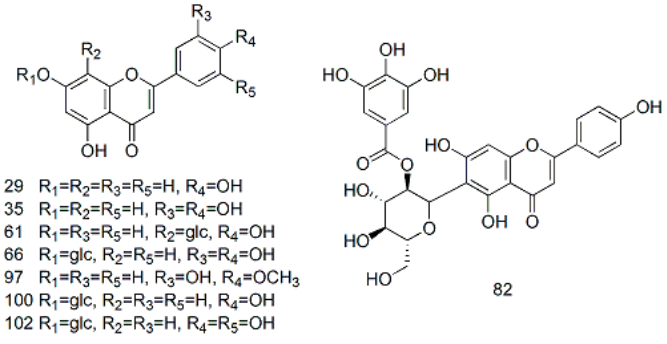


Figure 2. *Cont.*

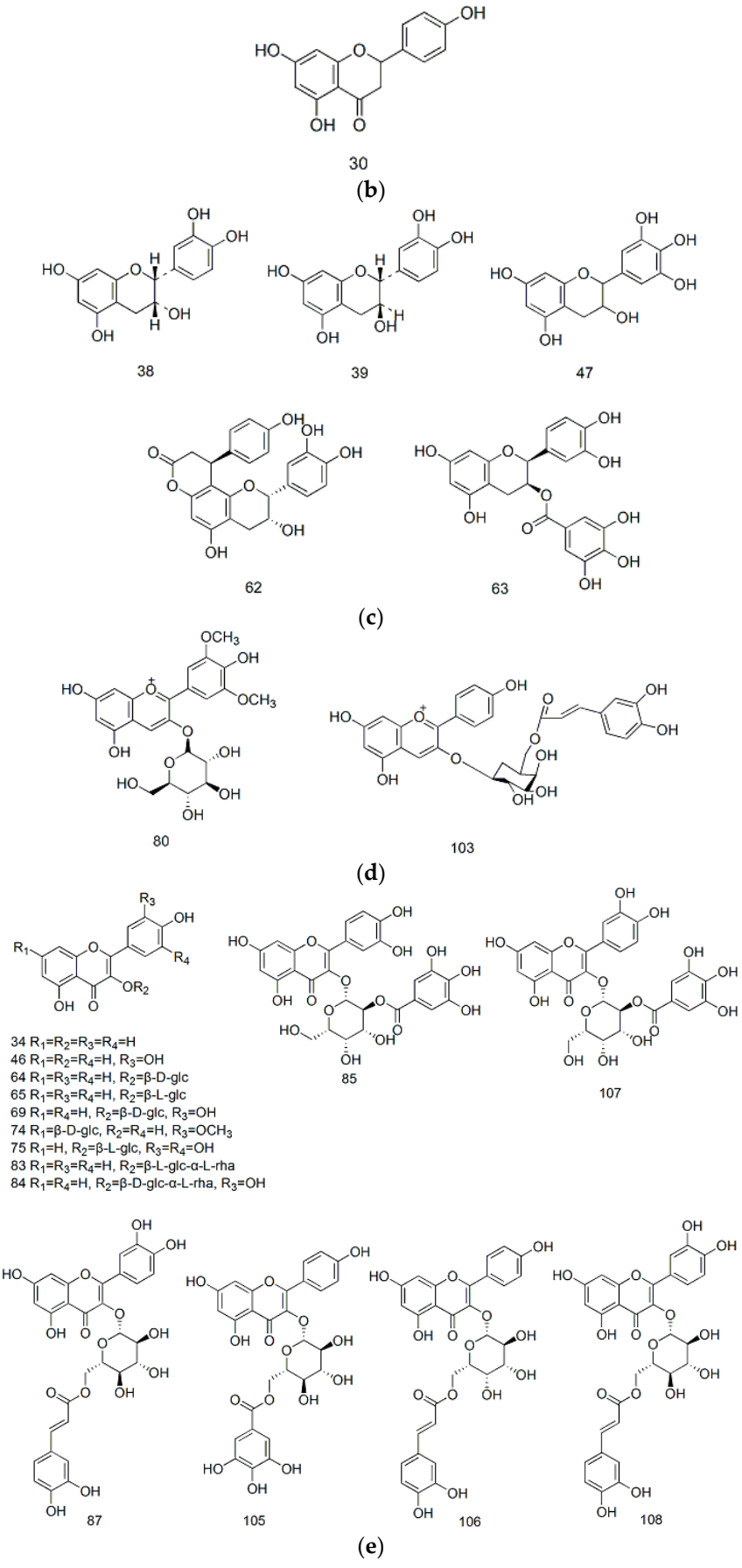


Figure 2. Structure of flavones (**a**); flavanones (**b**); flavan-3-ols (**c**); anthocyanidins (**d**); and flavonols (**e**).

Two different fragmentation patterns and pathways were observed for the flavonoids (I and II, shown in Figure 3). The retro-Diels–Alder (RDA) fragmentation (I) would result in the formation of A 1, 3 and B 1, 3 as the main fragment ions of the flavonoid moiety (aglycones) because of the X 1, 3 cleavage of the C ring. The substituent groups on the parent compounds were determined based on the compositions of the A 1, 3 and B 1, 3 fragments. The fragmentation of the parent compound according to Pattern I resulted in high-intensity fragment ions, whereas the fragmentation according to pattern II result in low-intensity fragment ions. Furthermore, the main fragmentation patterns of flavonoids (glycosides) typically consist of fragments associated with deglycosylation, demethylation and decarboxylation yielding $[M - H - Glc]^-$, $[M - H - CH_3]^-$ and $[M - H - CO_2]^-$ ions, respectively. The structures of the fragments resulting from the RDA fragmentation are shown in Table 2.

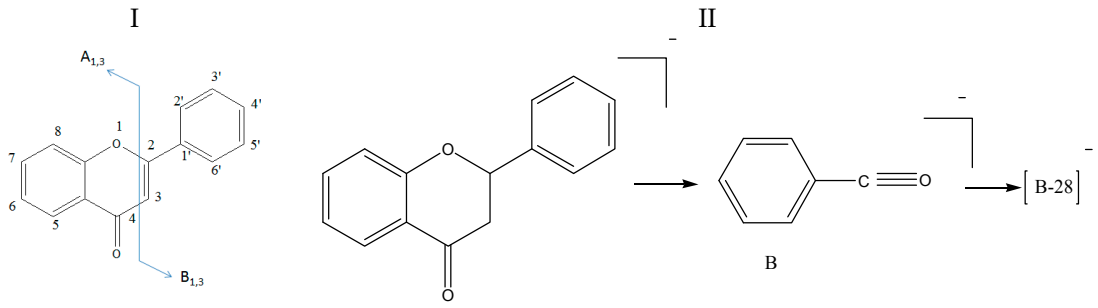


Figure 3. Fragmentation pattern I and II.

Table 2. RDA fragmentation pathways of compound **29**, **30**, **46**, and **63**.

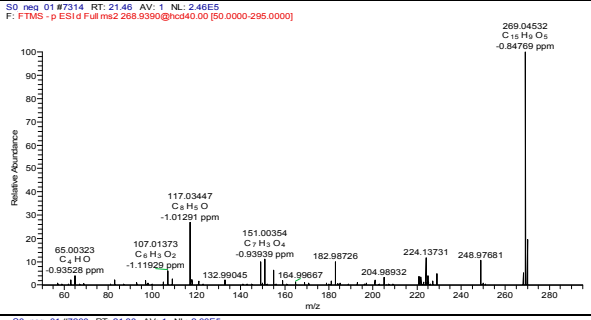
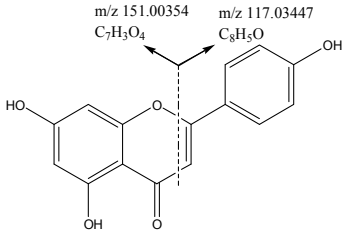
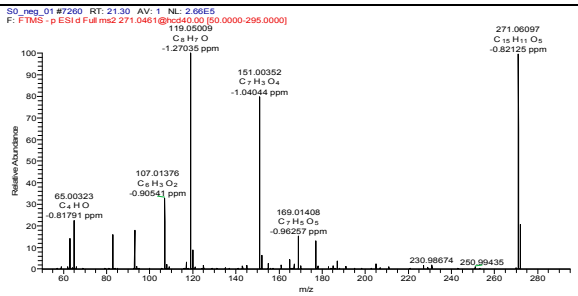
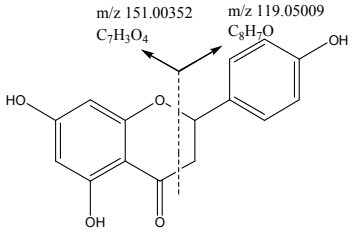
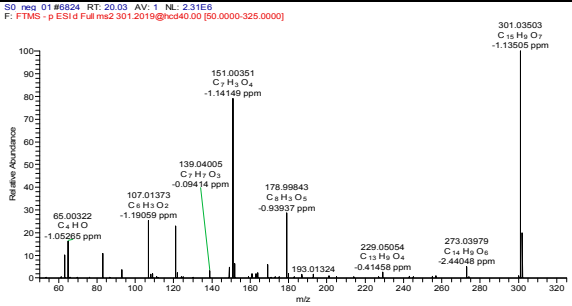
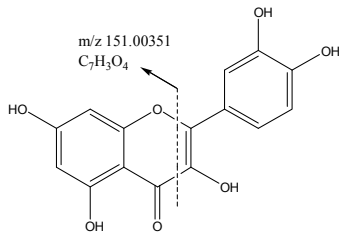
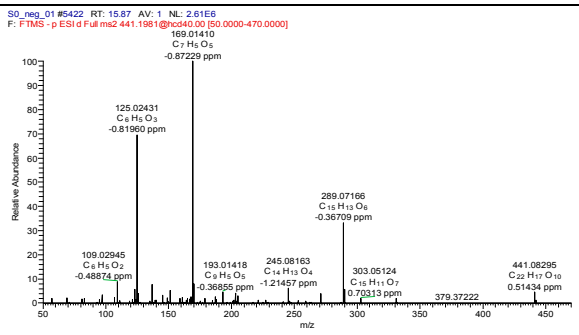
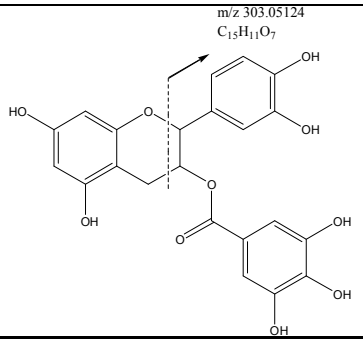
Compound No.	Mass Spectra of MS/MS	RDA Fragmentation Pathway
29		
30		
46		

Table 2. *Cont.*

Compound No.	Mass Spectra of MS/MS	RDA Fragmentation Pathway
63		

2.1.2. Pentacyclic Triterpenes

Pentacyclic triterpenes are wildly distributed in Nature and consist of five rings, which are typically referred to as the A, B, C, D and E rings. Pentacyclic triterpenes can be divided into several different categories, depending on the nature of their E ring. In this study, we characterized two different types of pentacyclic triterpenes, including ursane- and oleanane-type pentacyclic triterpenes. The *endo*-double bond in pentacyclic triterpenes can readily undergo a RDA reaction during MS analysis (shown in Figure 4). Dehydration and decarboxylation are also observed as common fragmentation pathways in these systems during MS analysis [21–23]. Depending on the different substituents attached to their endo-double bonds and their accurate mass measurements, we were able to fully characterize all of the pentacyclic triterpenes found in MDL. As shown in Figure 5, we characterized a total of nine pentacyclic triterpenes using high-resolution MS^2 mass spectrometry, including four ursane-type and five oleanane-type compounds pentacyclic triterpenes.

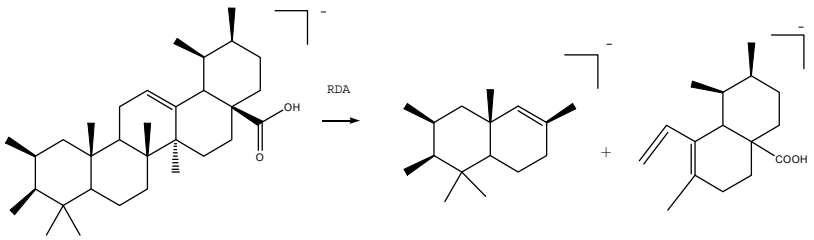


Figure 4. RDA fragmentation pathway of pentacyclic triterpene with endo-double bond.

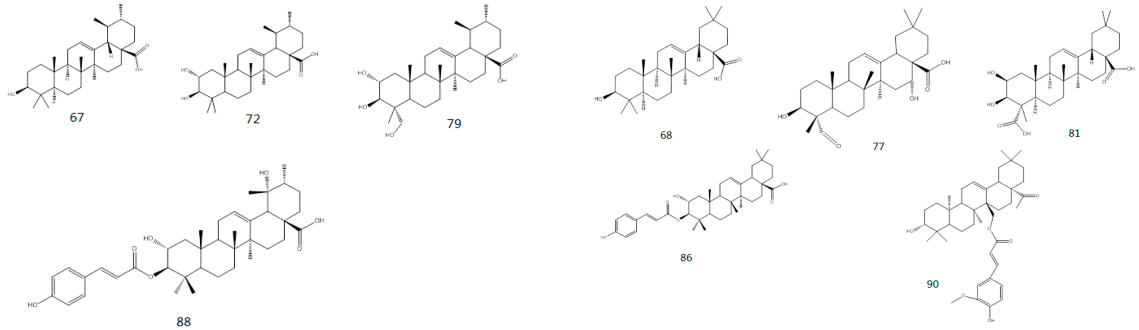


Figure 5. Structure of ursane-type (**67**, **72**, **79**, **88**) and oleanane-type (**68**, **77**, **81**, **86**, **90**) pentacyclic triterpene. This figure is fuzzy, please replace it with sharper figure.

2.1.3. Tannins

The main structural features of tannins include their gallic acid ester moieties (or polymer) and glucose core (or other polyols). The MS^2 spectra of the tannins revealed fragment ions with *m*/*z* values of 1091, 939, 769, 617, 599, 447 and 277. The *m*/*z* differences between these fragment ions were 152 and 170 atomic mass units (amu), which indicated that the fragments ions were produced by the successive removal of *O*-galloylhyperin and gallic acid anions. The fragment ion observed with an *m*/*z* value of 169 was characteristic of the fragment ions derived from *O*-galloylhyperin, whereas the fragment ion observed with an *m*/*z* value of 125 was attributed to the loss of CO_2 from gallic acid [17]. A total of six other tannins were characterized in this way using high-resolution MS^2 mass spectrometry. The structure of tannins characterized were shown in Figure 6.

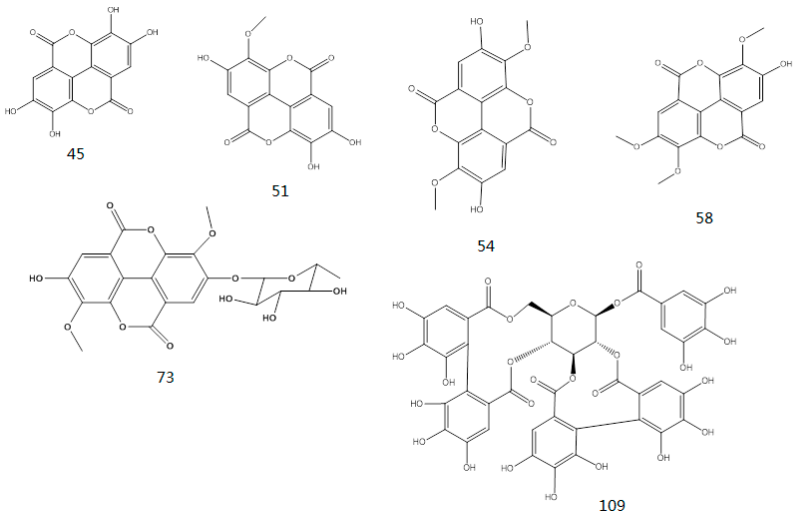


Figure 6. Structure of tannins.

Taking compound **109** as a representative example, the analysis of this compound in the positive ionization mode (t_R = 12.61 min) gave an $[M + H]^+$ ion with an *m*/*z* value of 937.09332, indicating a molecular formula of $C_{41}H_{28}O_{26}$ (Δ = −0.89 ppm) (Table 2). The MS^2 spectrum of compound **109** contained five fragment ions with *m*/*z* values of 345.02377, 277.03397, 231.02875, 171.04417 and 153.01817. The fragment pathway for this compound is shown in Figure 7. Based on these results, compound **109** was characterized as casuarinin.

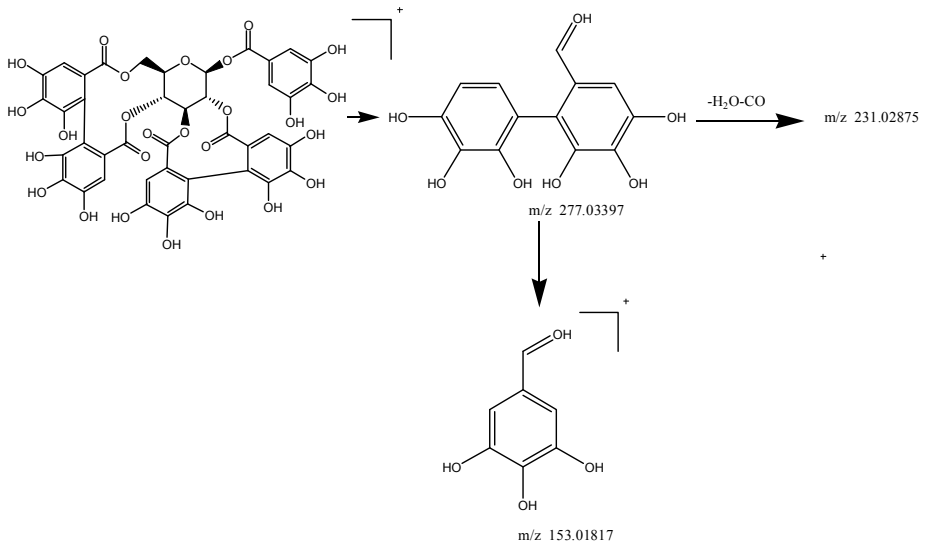


Figure 7. Fragmentation pathway of compound **109**.

2.1.4. Organic Acids

Compound **4** (t_R = 1.41 min) was analyzed in the negative ionization mode and gave an $[M - H]^-$ ion with an *m*/*z* value of 147.02991, which indicated a molecular formula of $C_5H_8O_5$ (Δ = 0.06 ppm) (Table 1). The MS^2 spectrum of compound **4** gave three fragment ions with *m*/*z* values of 129.01924 $[M - H - H_2O]^-$, 101.02427 $[M - H - HCOOH]^-$ and 85.02937 $[M - H - H_2O - CO_2]^-$. This fragmentation process was used to confirm the identities of the other organic acids, resulting in the characterization of 26 compounds as organic acids.

3. Materials and Methods

3.1. Chemicals and Reagents

Acetonitrile and methanol (HPLC grade) were purchased from Fisher Scientific (Waltham, MA, USA). Distilled water was purchased from Watson's Food & Beverage Co., Ltd. (Guangzhou, China). Formic acid (MS grade) was purchased from Fisher Scientific (Waltham, MA, USA). Reference standards of oleanolic acid (94.9%, batch No. 110709-201206), kaempferol (93.2%, batch No. 110861-201209), luteolin (100%, batch No. 111520-200504) and quercetin (97.4%, batch No. 100081-200907) were purchased from the National Institutes for Food and Drug Control (Beijing, China). A reference standard of asiatic acid (99%, batch No. 20150901) was purchased from CRM/RM center of China (Beijing, China). Reference standards of rutin (98%, batch No. 153-18-4) and gallic acid (98.5%, batch No. 149-91-7) were purchased from Chengdu-PUSH Bio-Technology Co., Ltd. (Chengdou, Sichuan, China).

3.2. Plant Materials and Sample Preparation

The whole plants of MDL were collected from Yunnan Province in China and authenticated by Professor Yaojun Yang (Beijing University of Chinese Medicine, Beijing, China). Voucher specimens (DN001) of the plant were deposited at the authors' laboratory. The samples were dried and powdered, before being sieved through a 40-mesh sieve. A sample of the powder (approximately 2.0 g) was suspended in 25 mL of methanol, and the resulting mixture was subjected to ultrasonic treatment for 30 min before being cooled to room temperature. Methanol was added to compensate for the lost weight and the resulting mixture was filtered through a 0.22-μm PTFE syringe filter. The filtrate was collected and subjected to centrifugation (13,000 rpm, 10 min). The supernatant was then transferred to an autosampler vial for analysis by UPLC-ESI-Q-Exactive Focus-MS/MS.

3.3. UPLC-ESI-Q-Exactive Focus-MS/MS Analysis

UPLC analysis was performed on a Thermo Scientific Ultimate 3000 system (Sunnyvale, CA, USA) equipped with a binary solvent delivery manager and a sample manger. Chromatographic separations were performed on a Thermo Scientific Hypersil GOLD C18 column (100 mm × 2.1 mm, 1.9 μm). The column temperature was maintained at 40 °C. A mobile phase consisting of 0.1% formic acid in water (A) and acetonitrile (B) was used to elute the column according to the optimized gradient program, as follows: 98% A from 0 to 5 min; 98%–80% A from 5 to 15 min; 80%–40% A from 15 to 30 min; 40%–2% A from 30 to 40 min; 2% A from 40 to 47 min; 2%–98% A from 47 to 47.1 min; 98% A from 47.1 to 50 min. The flow rate was set at 0.3 mL/min. An injection volume of 5 μL was used for the reference compounds and then analytical samples. For MS detection, the operating parameters were as follows: spray voltage, +3500 V/–3200 V; atomization temp, 350 °C; sheath gas pressure, 35 arb; aux gas pressure, 10 arb; capillary temperature, 320 °C; S-lens RF, 60 V; resolution, MS full scan 70,000 FWHM, MS/MS full scan 15,000 FWHM; scan range, *m*/*z* 100–1500 for MS; *m*/*z* 30–1500 for MS/MS; scanning mode, fullscan-ddms2.

3.4. Optimization of Analytical Conditions

To obtain better chromatographic separation and mass spectrometric detection, we evaluated three different mobile phase systems, including aqueous methanol, aqueous acetonitrile and aqueous acetonitrile-formic acid solutions. The aqueous acetonitrile solution resulted in the best separation of

the major components of MDL. Furthermore, the addition of 0.1% formic acid to this mobile phase resulted in a considerable improvement in the symmetry properties of most of the chromatographic peaks. We also varied the flow rate (0.25, 0.3, and 0.35 mL/min), column temperature (30, 35, and 40 °C) and injection volume (2, 3, and 5 μL) during method development. The results of these optimization experiments established the following conditions for the chromatographic separation of the different components of MDL: mobile phase, aqueous acetonitrile containing 0.1% formic; flow rate, 0.3 mL/min; column temperature, 40 °C; and injection volume, 5 μL.

3.5. Structure Analysis Procedure

In the positive and negative scan mode, based on the high-accuracy precursor ions and product ions obtained from Q-Exactive Focus-MS/MS, the elemental compositions were calculated when the maximum tolerance of mass error for all the precursor ions and product ions was set at 1.5 ppm, which can satisfy the requirements for positive identification. Based on the elemental compositions of the precursors, the most rational molecular formula was sought in different chemical databases such as the Spectral Database for Organic Compounds SDBS (http://sdbs.db.aist.go.jp), *m*/*z* cloud (https://www.mzcloud.org) and ChemSpider (http://www.chemspider.com). Meanwhile by searching literature sources, such as PubMed of the U.S. National Library Medicine and the National Institutes of Health, Scifinder Scholar of the American Chemical Society, Science Direct of Elsevier and Chinese National Knowledge Infrastructure (CNKI) of Tsinghua University, all components reported in the literatures on MDL and plants of the same family were summarized in a Microsoft Office Excel table to establish an in-house library [5–13] for searching the most rational molecular formula. When several matching compounds with the same formula were found, the fragmentation patterns and pathways of the compounds were analyzed and then validated by Mass Frontier 7.0 (Thermo Scientific) for positive identification.

4. Conclusions

A new UPLC-ESI-Q-Exactive Focus-MS/MS method was developed to analyze the chemical constituents of MDL based on their mass spectral fragmentation patterns. This new method resulted in the characterization of 109 compounds. The results of this study therefore provide an important reference to improve our understanding of the composition of MDL. We found that flavonoids are the main components of MDL, especially the flavonols, which possess a wide range of interesting pharmacological activities, such as anticancer, antibacterial, and antiviral activities. In terms of their structural characteristics, the triterpenoids found in MDL were ursane- and oleanane-type systems. Several tannins and steroids were also found in MDL. In addition to the fatty acids found in MDL, we found 55 other compounds that have never been reported in MDL besides fatty acids. Further studies pertaining to the chemical constituents in *Melastoma dodecandrum* Lour. are currently underway in our laboratory. Moreover, The study shows that, with the application of the UPLC-ESI-Q-Exactive Focus-MS/MS to characterizing the constituents of MDL, this method offers a rapid, sensitive and high throughput methodology for the identification of constituents of TCM prescriptions and herbal medicines.

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Sample Availability: Samples of the compound **10**, **34**, **35**, **46**, **68**, **79**, and **84** are available from the authors.