Journal of Chemical Biology & Therapeutics

Karar and Kuhnert, J Chem Biol Ther 2015, 1:1
DOI: 10.4172/2572-0406.1000102

Research Article Open Access

UPLC-ESI-Q-TOF-MS/MS Characterization of Phenolics from *Crataegus monogyna* and *Crataegus laevigata* (Hawthorn) Leaves, Fruits and their Herbal Derived Drops (Crataegutt Tropfen)

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Abstract

Crataegus species are medicinal plants naturally growing in Europe, Asia and the north of Africa. The plant extracts have been used for a long time in traditional medicine for the treatment of cardiovascular diseases. Many natural health product, including tablets, teas, and aqueous extracts are made from Crataegus species. These products are currently marketed as an alternative therapy for New York Heart Association (NYHA I-III) heart failure. But further studies suggested the use of the plant extracts for various other cardiovascular diseases including hypertension, hyperlipidemia, arrhythmia and angina. Thus, due to the important role that hawthorn plays in medicine and human health, we have investigated qualitatively the phytoconstituents of *C. monogyna* and *C. laevigata*, leaves, fruits and their herbal derived drops (Crataegutt Tropfen) using UPLC-ESI-Q-TOF-MS/MS and HPLC-ESI-MS^n. A total of 113 compounds were identified, characterized or tentatively assigned on the basis of their accurate mass data generated by Q-TOF-MS, MS/MS, MS^n fragmentation patterns, retention behaviors, or by comparison with commercial reference standards and literature data. The identified constituents belonged to chlorogenic acids, phenolic acids, proanthocyanidins, flavonoid glycosides, flavonoid aglycones and derivatives, and other compounds. To our knowledge 63 of the identified phytoconstituents were not reported previously in Crataegus species and two of them for the first time in nature. Additionally, it is important to highlight that this is the first comprehensive study of the bioactive phenolic compounds of *C. monogyna* and *C. laevigata*, leaves, fruits and the herbal derived drops (Crataegutt Tropfen).

Keywords: Herbal drugs; natural products; *C. monogyna*; *C. laevigata*; polyphenols; flavonoid glycosides; mass spectrometry

Introduction

Crataegus species also known as hawthorn (Rosaceae) are small trees and shrubs naturally growing in Europe, Asia and the north of Africa. Many natural health products, including tablets, teas, and aqueous extracts are made from Crataegus species. Crataegus is described in various pharmacopoeia including German Pharmacopoeia and Chinese Pharmacopoeia. Several reports suggested that hawthorn and the herbal derived drops (Crataegutt Tropfen) could be used as an alternative therapy for various cardiovascular diseases, such as hyperlipidemia, hypertension, arrhythmia, angina and New York Heart Association (NYHA) class I-III heart failure [1,2]. Thus, confirmed the use of hawthorn flowers, leaves and berries, alone or in combination traditionally in Europe for the treatment of a variety of ailments including high blood pressure and heart disorders [3]. However, the Complete German Commission E Monographs declares that *Crataegus monogyna* and *Crataegus laevigata* can be used to treat cases of cardiac failure [4].

Polyphenols are natural compounds characterized by a high structural diversity, and many of them are essential components in our diets. They are found only in plants and certain fungal species and are not synthesized by humans or animals [5]. Currently, polyphenols are seen as secondary metabolites characterized by a high range of physiological functions [5]. Several studies have shown that the high consumption of polyphenols have protective effects against cancer and inflammatory diseases [6]. The anti-inflammatory effects of phenolic compounds have been attributed mostly to their antioxidant activity presumably scavengers the reactive oxygen and nitrogen species in vivo [7,8]. However, the antioxidant hypothesis is under intense scrutiny [9]. It is also reported that the phenolics have anti-tumor, antidiabetic, anti-mutagenic and anti-HIV properties [10,11]. Moreover, there are several studies suggesting benefits of polyphenols intake for reducing the risks of cardiovascular problems, skin diseases, asthma, wound healing, protect from drug toxicity and UV radiations [10,12]. Although dietary plants polyphenols have been associated with several beneficial health effects for humans, their bioavailability is still under discussion [13,14].

A number of analytical techniques had been used such as liquid chromatography with diode array detection (HPLC-DAD) and liquid chromatography coupled to electrospray ionization multi-stage mass spectrometry (LC-ESI-MS^n) to identify different classes of phytoconstituents in hawthorn species including proanthocyanidins, phenolic acids, flavonoid glycosides, flavonoid aglycones, triterpene acids, sterols and chlorogenic acids [3,15-18]. Herein, the aim of this study was to improve the knowledge of the methanol: water (2:1) mixture extract of the fruits and leaves of *C. monogyna* and *C. laevigata* and the traditionally derived drops (Crataegutt Tropfen) by a comprehensive identification and characterization of their bioactive phenolic compounds by using ultra-performance liquid chromatography coupled to electrospray ionization quadropole-time-of-fight mass spectrometry (UPLC-ESI-Q-TOF-MS) in addition to the

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Received September 17, 2015; **Accepted** November 03, 2015; **Published** November 10, 2015

Citation: Karar MGE, Kuhnert N (2015) UPLC-ESI-Q-TOF-MS/MS Characterization of Phenolics from *Crataegus monogyna* and *Crataegus laevigata* (Hawthorn) Leaves, Fruits and their Herbal Derived Drops (Crataegutt Tropfen). J Chem Biol Ther 1: 102. doi: 10.4172/2572-0406.1000102

LC-ESI-MS^n as a powerful analytical techniques. In this contribution, we have tested the methanolic extract of the plant materials in particular, because it known to be more effective in extraction of low molecular weight phenolic compounds. The obtained results may contribute to a better understanding of influence of hawthorn phenolics on biological, nutritional and medicinal prosperities.

Recently, the improvement of ultra-high-pressure pump systems and small size filling substances leads to improve resolution, greater separation, high peak efficiency and reduced solvent consumption and running time compared with usual HPLC. In this regard, the combination of UPLC with MS allows a better separation, identification and characterisation of bioactive compounds in medicinal plants and complex mixtures. Consequently, the mass analyser Q-TOF-MS combines the high efficiency of TOF analysis in both MS and tandem MS (MS/MS) manners, providing high mass accuracy and better sensitivity for both precursor and fragment ions [19,20].

On numerous occasions we have shown that use of modern analytical instrumentation frequently merits reinvestigation of well characterized medicinal or dietary plant material. Using improved resolution and sensitivity on occasions large number of previously overlooked secondary metabolites can be readily identified. For this reasons we decided to re-investigate Crataegus as one of the best and most intensity studied medicinal plants.

Experimental

Chemicals and standards

All the chemicals (analytical grade) and authentic standards of polyphenols were purchased from Sigma-Aldrich, Applichem, HWI analytic, and Phytolab (Germany). 3,4-di-O-caffeoylquinic acid 69, 3,5-di-O-caffeoylquinic acid 70, 4,5-di-O-caffeoylquinic acid 71, epicatechin 30, quercetin 86, quercetin 3-O-(6-O-rhamnosyl-glucoside) (rutin) 65, quercetin 3-O-glucoside 68, kaempferol 7-O-glucoside 63, kaempferol 3-O-rutinoside 59, luteolin 7-O-glucoside 62, luteolin 8-C-glucoside 48, quercetin 3-O-arabinoside, quercetin 3-O-rhamnoside (quercitrin) 77, apigenin 7-O-glucoside 76, phloretin 2'-O-glucoside (phlorizin) 81, p-coumaric acid 44, malic acid 106, ascorbic acid 108 and quinic acid 1 were used as authentic standards.

Plant materials and the herbal drops

Leaves and fruits of *C. monogyna* and *C. laevigata* were freshly collected from a garden in Jacobs University, Bremen, Germany. The apple (*Malus domestica*) fruits were purchased from a local market in Bremen, Germany. The herbal drops (Crataegutt Tropfen, Schwabe, Karlsruhe) was granted from a pharmacy in Bremen, Germany.

Sample preparation

The sample preparation was achieved as previously described [21]. Fresh hawthorn samples (10 g of each) were freeze dried with the liquid nitrogen and crushed by a mortar. Then, the samples were extracted with methanol:water (2:1) mixture by sonication for 30 min and filtered through a Whatman no. 1 filter paper. The solvents were removed by evaporation in vacuo and the extracts were stored at −20°C until required, thawed at room temperature, dissolved in methanol (50 mg/10 mL of methanol), filtered through a membrane filter (0.45 µm) and used directly for UPLC-ESI-Q-TOF-MS/MS and HPLC-ESI-MS^n. The herbal drops (Crataegutt Tropfen, 94 mg/mL, extracting agent: Ethanol 45%) was diluted 10 times in methanol and directly analyzed.

UPLC- Q-TOF-MS/MS

The UPLC system (Agilent infinity 1260 series, Germany) was incorporated a binary pump, an auto sampler (G1367E), degasser (G1322A) and a DAD detector (1315D) with a light-pipe flow cell (recording at 280 and 320 nm). This was coupled to Ultra-High-resolution-Quadropole-Time-of-Flight (UHR-Q-TOF) (Bruker Impact HD, Bruker DaltoniK GmbH, Bremen Germany) equipped with an ESI source operating on Auto-MS/MS mode. The analysis was achieved in the negative ion mode in a mass range from m/z 50-1200. The ESI source parameters were: capillary voltage 4.5 KV; nebulising gas pressure 1.8 Bar; drying gas temperature 200.0°C, drying gas flow 9.0 L/min; Funnel 1RF 250.0 Vpp; transfer time 50.0 µs; and pre-pulse storage 2.0 µs. The MS data were analyzed through Data Analysis 4.2 software (Bruker Daltonics, Bremen, Germany). Internal calibration was achieved with 10 mL of 0.1 M sodium formate solution injected through a six port valve prior to each chromatographic run. Calibration was done using the High Precision Calibration (HPC).

UPLC and HPLC

The UPLC separation was achieved on a Polaris reverse phase C18 amide (RF-C18-A), 150 length x 2 mm inner-diameter, particle size 3 µm column (Agilent, Germany). Solvent A was water : formic acid (1000 : 0.05 v/v) and solvent B was methanol. Solvents were delivered at a total flow rate of 0.2 mL/min. The gradient profile was from 10% B to 80% B linearly in 70 min followed by 10 min isocratic and a return to 10% B at 90 min and 10min isocratic to re-equilibrate. The injection volume was 2 µL.

The HPLC separation was achieved on a 250 length x 3 mm-inner-diameter column containing 5 µm C18 amide, with a 5 mm x 3 mm-inner-diameter guard column (Varian, Darmstadt, Germany). Solvent A was water : formic acid (1000 : 0.05 v/v), and solvent B was acetonitrile. Solvents were delivered at a total flow rate of 0.5 mL/min. The gradient profile was from 6% B to 80% B linearly in 70 min followed by 10 min isocratic and a return to 10% B at 90 min and 10 min isocratic to re-equilibrate. The injection volume was 5 µL [22,23].

HPLC-MS^n

The HPLC-MS^n analysis was achieved as previously described [21-23].

UV Irradiation

UV irradiation experiments were performed as previously reported [24].

Results and Discussion

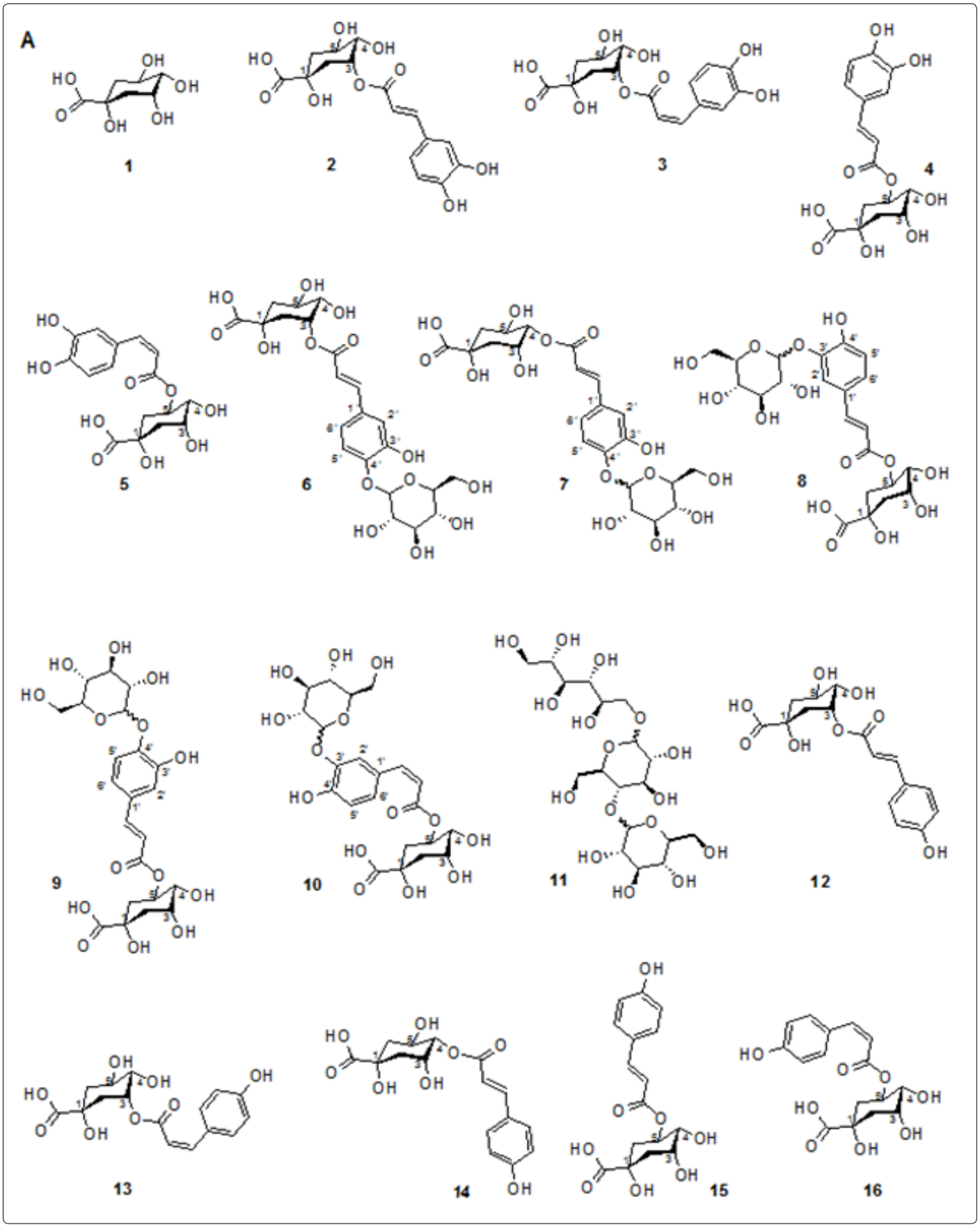
A reversed phase UHPLC-MS method was developed, allowing separation of 113 peaks in methanolic Crataegus extracts. Compound assignment was carried out for the identified phytoconstituents along with their m/z experimental and calculated, error (ppm), MS/MS fragments and molecular formula is presented in Table 1. Chemical structures of the identified phytoconstituents are shown in Figure 1. All identified compounds displaying a mass error of below 2 ppm thus confirming their elemental composition. Only three compounds errors were higher than 2 and below 5 ppm but their MS/MS and tandem MS data were with agreement with molecular formulas and suggested structures. Moreover, in this study not all identified compounds were fragmented in the Q-TOF. Consequently, the tandem MS fragmentation obtained from the LC-MS^n was considered using a quadruple ion trap MS detector (Table 2). The UV chromatograms at 280 nm are shown

No.	Compound Identity	Mol. Formula	The. m/z [M−H]	Exp. m/z [M−H]	Err. [ppm]	MS/MS fragments
1	Quinic acid	$C_7H_{12}O_6$	191.0561	191.0561	0.1	173.0453, 127.0400, 111.0451, 85.0297
2	3-O-Caffeoylquinic acid	$C_{16}H_{18}O_9$	353.0878	353.0875	1.0	191.0562, 179.0350, 173.0455, 133.0296
3	cis-3-O-Caffeoylquinic acid	$C_{16}H_{18}O_9$	353.0878	353.0875	0.9	191.0561, 179.0350, 177.0811, 173.0446, 135.0447
4	5-O-Caffeoylquinic acid	$C_{16}H_{18}O_9$	353.0878	353.0872	1.8	191.0559, 179.0342, 173.0449
5	cis- 5-O-Caffeoylquinic acid	$C_{16}H_{18}O_9$	353.0878	353.0873	1.6	191.0549, 179.0347, 133.0301
6	3-O-(4'-O-Caffeoyl glucosyl)quinic acid	$C_{22}H_{28}O_{14}$	515.1406	515.1403	0.6	Not fragmented
7	4-O-(4'-O-Caffeoyl glucosyl)quinic acid	$C_{22}H_{28}O_{14}$	515.1406	515.1406	0.5	Not fragmented
8	5-O-(3'-O-Caffeoyl glucosyl)quinic acid	$C_{22}H_{28}O_{14}$	515.1406	515.1403	0.6	179.0350, 191.0541
9	5-O-(4'-O-Caffeoyl glucosyl)quinic acid	$C_{22}H_{28}O_{14}$	515.1406	515.1399	1.4	323.0811, 191.0563, 96.9699
10	cis 5-O-(3'-O-Caffeoyl glucosyl)quinic acid	$C_{22}H_{28}O_{14}$	515.1406	515.1407	0.0	Not fragmented
11	Di-O-glycosyl-glucitol	$C_{18}H_{34}O_{16}$	505.1774	505.1772	0.5	Not fragmented
12	3-O-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0929	337.0928	0.3	163.0401, 119.0501
13	cis-3-O-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0929	337.0927	0.6	191.0558, 173.0449, 163.0398, 93.0346
14	4-O-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0929	337.0925	1.1	173.0455, 163.0395, 93.0364
15	5-O-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0929	337.0925	1.0	191.0542, 163.0397, 119.0501
16	cis-5-O-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0929	337.0923	1.8	191.0551, 173.0452, 163.0398, 119.0494
17	(Epi)catechin-4,8′-(epi)catechin C-hexoside	$C_{36}H_{36}O_{17}$	739.1880	739.1885	-0.7	Not fragmented
18	(Epi)catechin-4,8′-(epi)catechin C-hexoside	$C_{36}H_{36}O_{17}$	739.1880	739.1880	0.4	Not fragmented
19	p-Coumaric acid O-hexoside	$C_{15}H_{18}O_8$	325.0929	325.0927	0.7	163.0401, 119.0503
20	(Epi)catechin C-hexoside	$C_{21}H_{24}O_{11}$	451.1246	451.1241	1.0	331.0820, 290.0922, 272.0797
21	Cinchonain Ia (isomer)	$C_{24}H_{20}O_9$	451.1035	451.1030	1.0	341.0658, 315.0866, 289.0709, 287.0553, 217.0133
22	Cinchonain Ia (isomer)	$C_{24}H_{20}O_9$	451.1035	451.1035	-0.1	341.0659, 287.0562, 217.0142, 161.0245, 112.9856
23	Benzyl alcohol-hexose-pentose	$C_{18}H_{26}O_{10}$	401.1453	401.1455	-0.3	269.1041
24	Quercetin 3,7-di-O-hexoside	$C_{27}H_{30}O_{17}$	625.1410	625.1411	-0.1	Not fragmented
25	(Epi)catechin-4,8′-(epi)catechin (isomer)	$C_{30}H_{26}O_{12}$	577.1351	577.1348	0.6	425.0883, 289.0704, 331.0803, 245.0461, 125.0239
26	(Epi)catechin-4,8′-(epi)catechin (isomer)	$C_{30}H_{26}O_{12}$	577.1351	577.1347	0.7	425.0865, 407.0762, 451.1021, 289.0711, 245,0450
27	(Epi)catechin-4,8′-(epi)catechin (isomer)	$C_{30}H_{26}O_{12}$	577.1351	577.1350	0.3	289.0729, 287.0585, 161.0260, 125.0249
28	Luteolin 6,8-di-C-hexoside (isomer)	$C_{27}H_{30}O_{16}$	609.1461	609.1459	0.3	Not fragmented
29	Luteolin 6,8-di-C-hexoside (isomer)	$C_{27}H_{30}O_{16}$	609.1461	609.1462	-0.2	Not fragmented
30	Epicatechin	$C_{15}H_{14}O_6$	289.0718	289.0716	0.6	245.0825, 200.0570, 128.0356
31	Cyanidin 7-O-glucoside	$C_{21}H_{21}O_{11}^+$	449.1089	449.1082	1.6	287.0554, 259.0613, 125.0230
32	Cyanidin 3-O-galctoside	$C_{21}H_{21}O_{11}^+$	449.1089	449.1085	0.9	287.0558, 151.0037
33	Cyanidin 3-O-glucoside	$C_{21}H_{21}O_{11}^+$	449.1089	449.1082	1.7	287.0575, 341.0648, 112.9842
34	Apigenin 6,8-di-C-galactoside (vicenin-4)	$C_{27}H_{30}O_{15}$	593.1512	593.1507	0.8	133.0143, 96.9706
35	Apigenin 6,8-di-C-glucoside (vicenin-3)	$C_{27}H_{30}O_{15}$	593.1512	593.1503	1.5	293.0435, 159.0 297
36	5-O-Feruloylquinic acid	$C_{17}H_{20}O_9$	367.1035	367.1034	0.1	191.0559, 173.0450, 111.0474, 93.0339
37	Apigenin 6-C-pentosyl-8-C-hexoside (isomer)	$C_{26}H_{28}O_{14}$	563.1406	563.1405	0.3	133.0143, 96.9695

38	Apigenin 6-C-pentosyl-8-C-hexoside (isomer)	$C_{26}H_{28}O_{14}$	563.1406	563.1403	0.5	357.1313, 164.9578, 133.0138, 96.9649
39	Apigenin 6-C-hexosyl-8-C-pentoside (isomer)	$C_{26}H_{28}O_{14}$	563.1406	563.1403	0.5	443.0980, 413.0861, 293.0446
40	Apigenin 6-C-pentosyl-8-C-hexoside (isomer)	$C_{26}H_{28}O_{14}$	563.1406	563.1407	-0.1	443.0974, 413.0872, 293.0447, 133.0143
41	3-O-Caffeoylshikimic acid	$C_{16}H_{16}O_{8}$	335.0772	335.0773	-0.1	133.0301, 93.0318
42	(Epi)catechin-4,8′-(epi)catechin-4′,8″-(epi) catechin (isomer)	$C_{45}H_{38}O_{18}$	865.1985	865.1947	4.5	Not fragmented
43	(Epi)catechin-4,8′-(epi)catechin-4′,8″-(epi) catechin (isomer)	$C_{45}H_{38}O_{18}$	865.1985	865.1976	1.1	Not fragmented
44	p-Coumaric acid	$C_{9}H_{8}O_{3}$	163.0401	163.0401	-0.4	119.0502, 96.9615
45	Eriodictyol-di-C-hexoside	$C_{27}H_{32}O_{16}$	611.1618	611.1615	0.3	Not fragmented
46	(Epi)afzelechin-(epi)catechin	$C_{30}H_{26}O_{11}$	561.1402	561.1402	0.1	289.0715, 271.0604, 125.0237, 96.9695
47	Luteolin 8-C-galactoside	$C_{21}H_{20}O_{11}$	447.0933	447.0929	0.9	327.0505, 357.0611, 297.0392, 163.0760
48	Luteolin 8-C-glucoside (orientin)	$C_{21}H_{20}O_{11}$	447.0933	447.0932	0.1	357.0609, 327.0506, 297.0398, 285.0368
49	Naringenin C-hexoside (isomer)	$C_{21}H_{22}O_{10}$	433.1140	433.1136	1.0	313.0708, 343.0835, 161.0458, 96.9694
50	Naringenin C-hexoside (isomer)	$C_{21}H_{22}O_{10}$	433.1140	433.1138	0.6	343.0830, 313.0720, 96.9688, 78.9593
51	Quercetin 3-O-(2, 6-di-O-rhamnosyl-glucoside)	$C_{33}H_{40}O_{20}$	755.2040	755.2037	0.4	Not Fragmented
52	Apigenin 8-C-glucoside (vitexin)	$C_{21}H_{20}O_{10}$	431.0984	431.0982	0.3	311.0557, 341.0659, 284.0621
53	Apigenin 6-C-glucoside (isovitexin)	$C_{21}H_{20}O_{10}$	431.0984	431.0985	0.3	311.0560, 341.0663, 413.0868, 191.0560, 283.0607, 296.0456
54	Isorhamnetin 7-O-(6-O-rhamnosyl-glucoside)	$C_{28}H_{32}O_{16}$	623.1618	623.1619	-0.2	150.0310, 112.9861, 96.9681, 78.9583, 293. 0461
55	Isorhamnetin 3-O-(6-O-rhamnosyl-galactoside)	$C_{28}H_{32}O_{16}$	623.1618	623.1601	2.7	112.9858
56	Isorhamnetin 3-O-(6-O-rhamnosyl-glucoside)	$C_{28}H_{32}O_{16}$	623.1618	623.1616	0.2	Not fragmented
57	Kaempferol 7-O-(6-O-rhamnosyl-glucoside)	$C_{27}H_{30}O_{15}$	593.1512	593.1517	-0.9	159.0296, 112.9848
58	Kaempferol 3-O-(6-O-rhamnosyl-galactoside)	$C_{27}H_{30}O_{15}$	593.1512	593.1511	0.1	151.0411, 112.9856
59	Kaempferol 3-O-(6-O-rhamnosyl-glucoside)	$C_{27}H_{30}O_{15}$	593.1512	593.1512	0.2	Not fragmented
60	Diosmetin 7-O-rutinoside (diosmin)	$C_{28}H_{32}O_{15}$	607.1668	607.1665	0.5	Not fragmented
61	Kaempferol 3-O-glucuronide	$C_{21}H_{18}O_{12}$	461.0725	461.0725	0.2	Not fragmented
62	Luteolin 7-O-glucoside	$C_{21}H_{20}O_{11}$	447.0933	447.933	0.0	285.0406, 249.0532, 174.09551
63	Kaempferol 7-O-glucoside	$C_{21}H_{20}O_{11}$	447.0933	447.0930	0.7	284.0323, 174.9585, 242.9443
64	Kaempferol 3-O-glucoside	$C_{21}H_{20}O_{11}$	477.0933	447.0927	1.4	285.0393, 256.0357, 174.9558
65	Quercetin 3-O-(6-O-rhamnosyl-glucoside) (rutin)	$C_{27}H_{30}O_{16}$	609.1461	609.1458	0.6	301.0351, 187.0974, 111.0060
66	Myricetin 3-O-rhamnoside (myricitrin)	$C_{21}H_{20}O_{12}$	463.0882	463.0880	0.4	316.0216, 178.9982, 151.0037
67	Quercetin 3-O-galactoside	$C_{21}H_{20}O_{12}$	463.0982	463.0878	0.8	301.0342, 300.0270, 273.0054, 178.9968, 151.0038
68	Quercetin 3-O-glucoside	$C_{21}H_{20}O_{12}$	463.0882	463.0876	1.2	301.0347
69	3,4-di-O-Caffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1195	515.1193	0.4	Not fragmented
70	3,5-di-O-Caffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1195	515.1194	0.3	Not fragmented
71	4,5-di-O-Caffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1195	515.1192	0.5	353.0898, 179.0377, 143.1068, 126.9994
72	Isorhamnetin O-hexoside	$C_{22}H_{22}O_{12}$	477.1038	477.1036	0.6	315.0502, 300.0264
73	Naringenin 7-O-glucoside	$C_{21}H_{22}O_{10}$	433.1140	433.1139	0.3	271.0611, 151.0032, 227.0715
74	Apigenin 7-O- rutinoside	$C_{27}H_{30}O_{14}$	577.1563	577.1557	1.0	296.0466, 112.9857, 96.9693
75	Quercetin 3-O-xyloside	$C_{20}H_{18}O_{11}$	433.0776	433.0772	1.0	300.0273, 301.0334, 167.0326, 149.0235
76	Apigenin 7-O-glucoside	$C_{21}H_{20}O_{10}$	431.0984	431.0979	1.0	269.0435, 173.0443, 149.0222, 96.9676

77	Quercetin 3-O-rhamnoside (quercitrin)	$C_{21}H_{20}O_{11}$	447.0933	447.0928	0.4	300.0268, 301.0330, 271.0244, 255.0290, 151.0031
78	Pectolinarin	$C_{29}H_{34}O_{15}$	621.1825	621.1827	-0.2	313.0754, 295.0595, 175.0040
79	Quercetin O-acetyl hexoside (isomer)	$C_{23}H_{22}O_{13}$	505.0988	505.0984	0.3	300.0248, 301.0283, 271.0262, 255.0239, 151.0027
80	Quercetin O-acetyl hexoside (isomer)	$C_{23}H_{22}O_{13}$	505.0988	505.0982	0.5	300.0274, 271.0260, 255.0307, 151.0014
81	Phloretin 2'-O-glucoside (phlorizin)	$C_{21}H_{24}O_{10}$	435.1297	435.1294	0.7	273.0781
82	(−)-11-hydroxy-9,10-dihydrojasmonic acid 11-β-D-glucoside	$C_{18}H_{30}O_{9}$	389.1817	389.1812	0.7	183.1376, 134.0263, 101.0216, 227.1339
83	Kaempferol 3-O-rhamnoside	$C_{21}H_{20}O_{10}$	431.0984	431.0980	0.9	Not fragmented
84	Cyanidin	$C_{15}H_{11}O_{6}^{+}$	287.0561	287.0561	0.0	151.0017, 135.0446, 107.0153
85	3-O-Methylellagic acid 4'-(2'',3''-di-O-acetyl)-rhamnoside	$C_{25}H_{22}O_{14}$	545.0937	545.0920	1.7	299.0190, 314.0443
86	Quercetin	$C_{15}H_{10}O_{7}$	301.0354	301.0349	0.5	151.0021, 130.9922
87	(Epi)catechin-(4,8′)-(epi)catechin-(4′,8″/2′,7″)-(epi)catechin (isomer)	$C_{45}H_{36}O_{18}$	863.1829	863.1843	-1.6	Not fragmented
88	(Epi)catechin-(4,8′)-(epi)catechin-(4′,8″/2′,7″)-(epi)catechin (isomer)	$C_{45}H_{36}O_{18}$	863.1829	863.1839	-1.2	Not fragmented
89	(Epi)catechin-(4,8′)-(epi)catechin-(4′,8″/2′,7″)-(epi)catechin (isomer)	$C_{45}H_{36}O_{18}$	863.1829	863.1855	-3.1	Not fragmented
90	(Epi)catechin-(4,8′)-(epi)catechin-(4′,8″/2′,7″)-(epi)catechin (isomer)	$C_{45}H_{36}O_{18}$	863.1829	863.1847	-2.1	Not fragmented
91	Protocatechuic acid O-hexoside	$C_{13}H_{16}O_{9}$	315.0772	315.0770	0.5	153.0184, 152.0113, 109.0288, 108.0213
92	Citric acid	$C_{6}H_{8}O_{7}$	191.0197	191.0198	-0.2	102.9491, 85.0296
93	Syringic acid O- hexoside	$C_{15}H_{20}O_{10}$	359.0984	359.0988	-0.6	197.0440, 175.0063, 153.0556, 149.0230
94	Salicylic acid O-galactoside	$C_{13}H_{16}O_{8}$	299.0772	299.0774	-0.5	137.0245, 93.0347, 239.0560, 179.0353
95	Salicylic acid O-glucoside	$C_{13}H_{16}O_{8}$	299.0772	299.0773	-0.1	137.0237, 93.0346
96	Homovanillic acid O-hexoside	$C_{15}H_{20}O_{9}$	343.1035	343.1034	0.1	181.0510, 163.0396, 119.0494
97	Sinapic acid O-hexoside	$C_{17}H_{22}O_{10}$	385.1140	385.1137	0.9	223.0618, 179.0698
98	Salicylic acid	$C_{7}H_{6}O_{3}$	137.0244	137.0244	-0.2	93.0347
99	Phloretin	$C_{15}H_{14}O_{5}$	273.0768	273.0767	0.1	Not fragmented
100	(Epi)catechin-(4,8′/2,7′)-(epi)catechin (isomer)	$C_{30}H_{24}O_{12}$	575.1195	575.1196	-0.2	423.0742, 289.0718, 285.0403, 125.0242
101	(Epi)catechin-(4,8′/2,7′)-(epi)catechin (isomer)	$C_{30}H_{24}O_{12}$	575.1195	575.1191	0.8	449.0821, 423.0782, 289.0720, 285.0400, 245.0462, 152.0249
102	(Epi)catechin-(4,8′/2,7′)-(epi)catechin (isomer)	$C_{30}H_{24}O_{12}$	575.1195	575.1192	0.6	449.0864, 407.0818, 327.0480, 289.0706, 285.0401, 125.0245
103	(Epi)catechin-(4,8′/2,7′)-(epi)catechin (isomer)	$C_{30}H_{24}O_{12}$	575.1195	575.1192	0.5	287.0546, 125.0242
104	Vanillic acid O-hexoside	$C_{14}H_{18}O_{9}$	329.0878	329.0873	0.9	167.0345, 152.0140, 123.0451, 108.0230
105	Syringic acid	$C_{9}H_{10}O_{5}$	197.0455	197.0457	-0.7	Not fragmented
106	Malic acid	$C_{4}H_{6}O_{5}$	133.0142	133.0142	0.2	115.0034
107	Pyruvic acid	$C_{3}H_{4}O_{3}$	87.0088	87.00	0.2	Not fragmented
108	Ascorbic acid	$C_{6}H_{8}O_{6}$	175.0248	175.0248	-0.0	87.0090
109	Cratenacin (isomer)	$C_{29}H_{32}O_{15}$	619.1668	619.1668	0.1	413.0874, 293.0458
110	Cratenacin (isomer)	$C_{29}H_{32}O_{15}$	619.1668	619.1667	0.2	413.0872, 293.0447, 499.1215, 353.0709, 577.1545
111	Cratenacin (isomer)	$C_{29}H_{32}O_{15}$	619.1668	619.1668	-0.0	413.0871, 293.0452, 499.1244
112	Vitexin 2″-O-rhamnoside	$C_{27}H_{30}O_{14}$	577.1563	577.1561	0.3	413.0872, 457. 1133, 293, 0457
113	Isovitexin 2″-O-rhamnoside	$C_{27}H_{30}O_{14}$	577.1563	577.0561	0.2	413.0870, 293.0456, 457.1128

Table 1: UPLC-QTOf-MS/MS mass spectral data in negative ion mode of *C. laevigata, C. monogyna* and the herbal drops (Crataegutt Tropfen) phenolics.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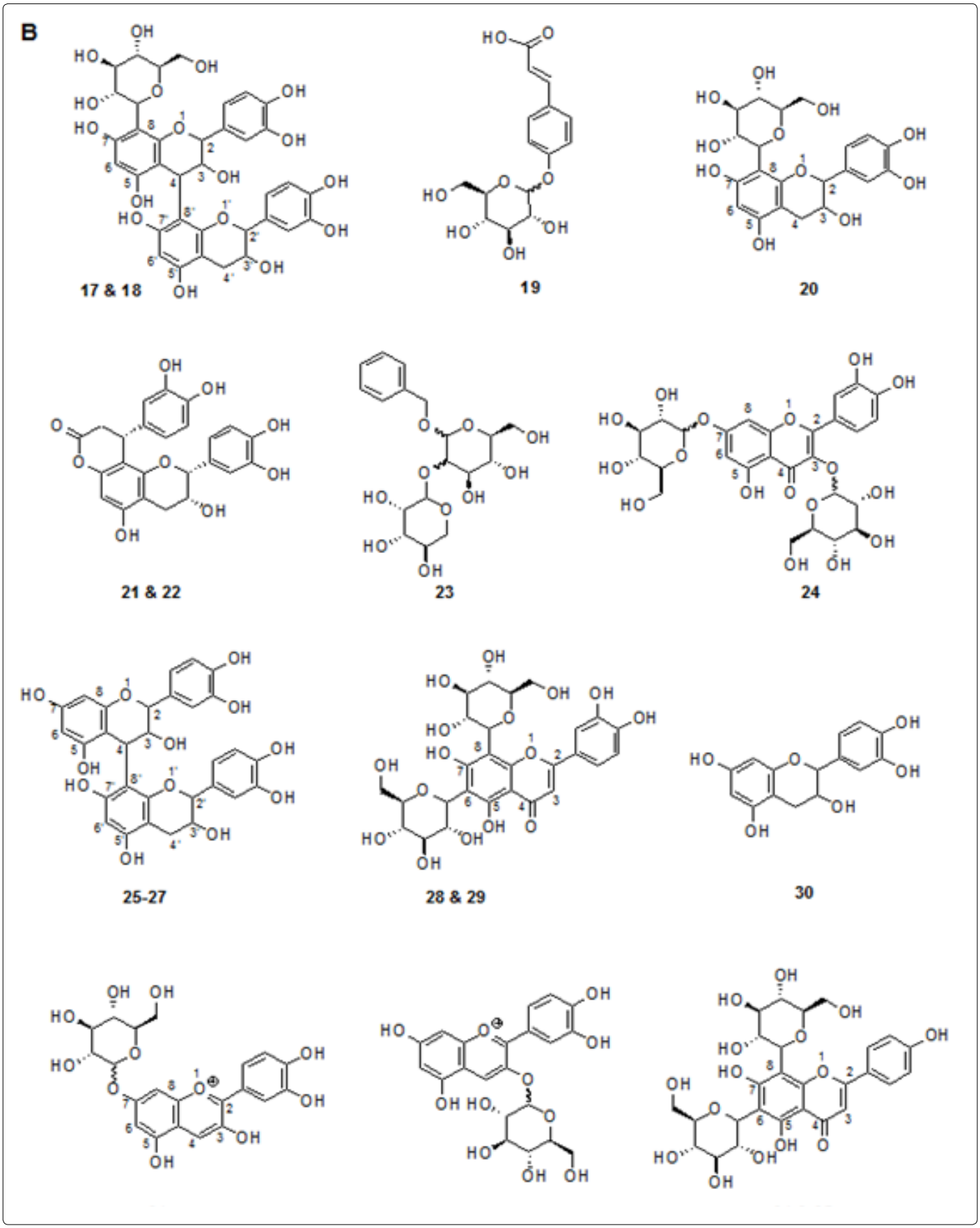
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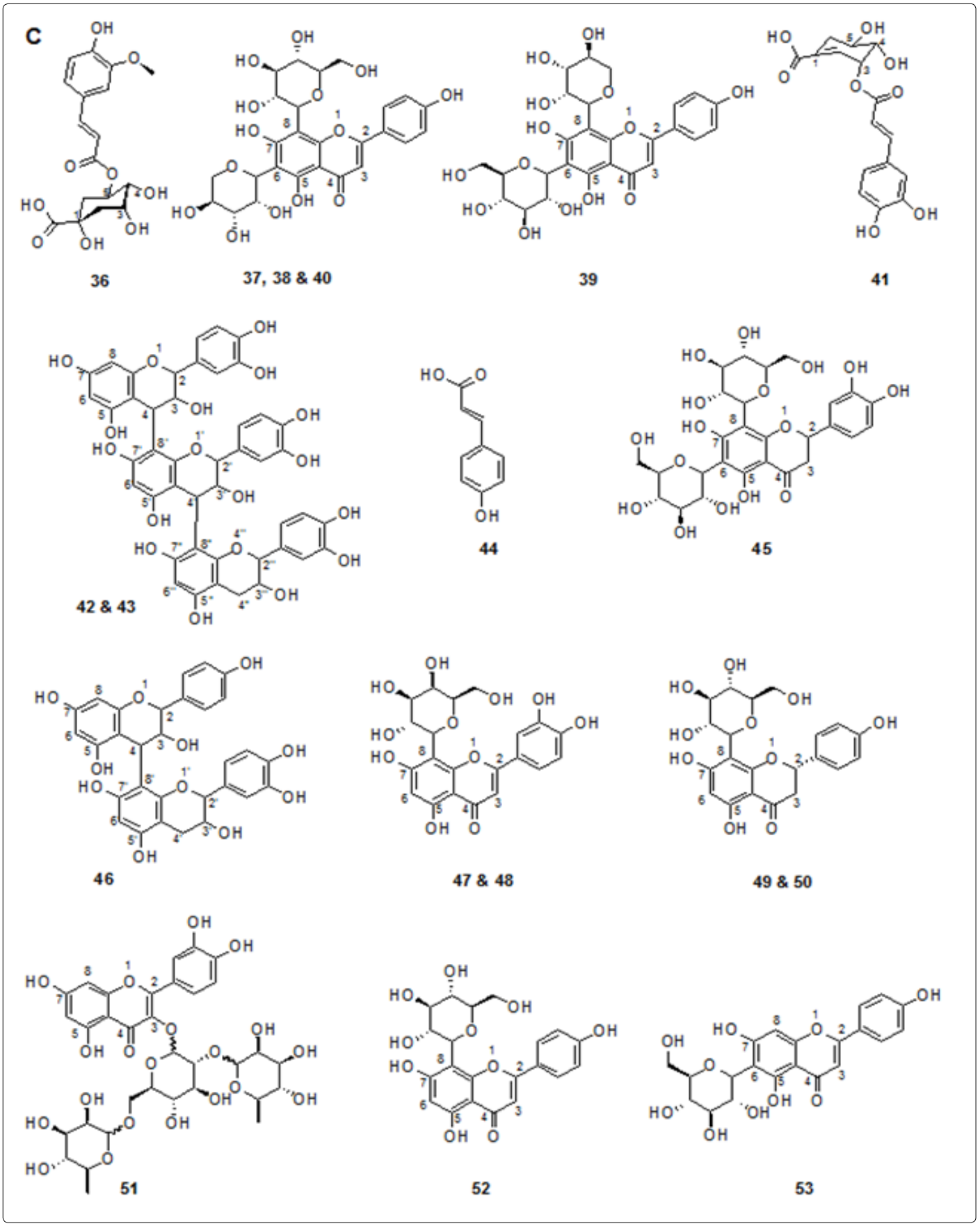
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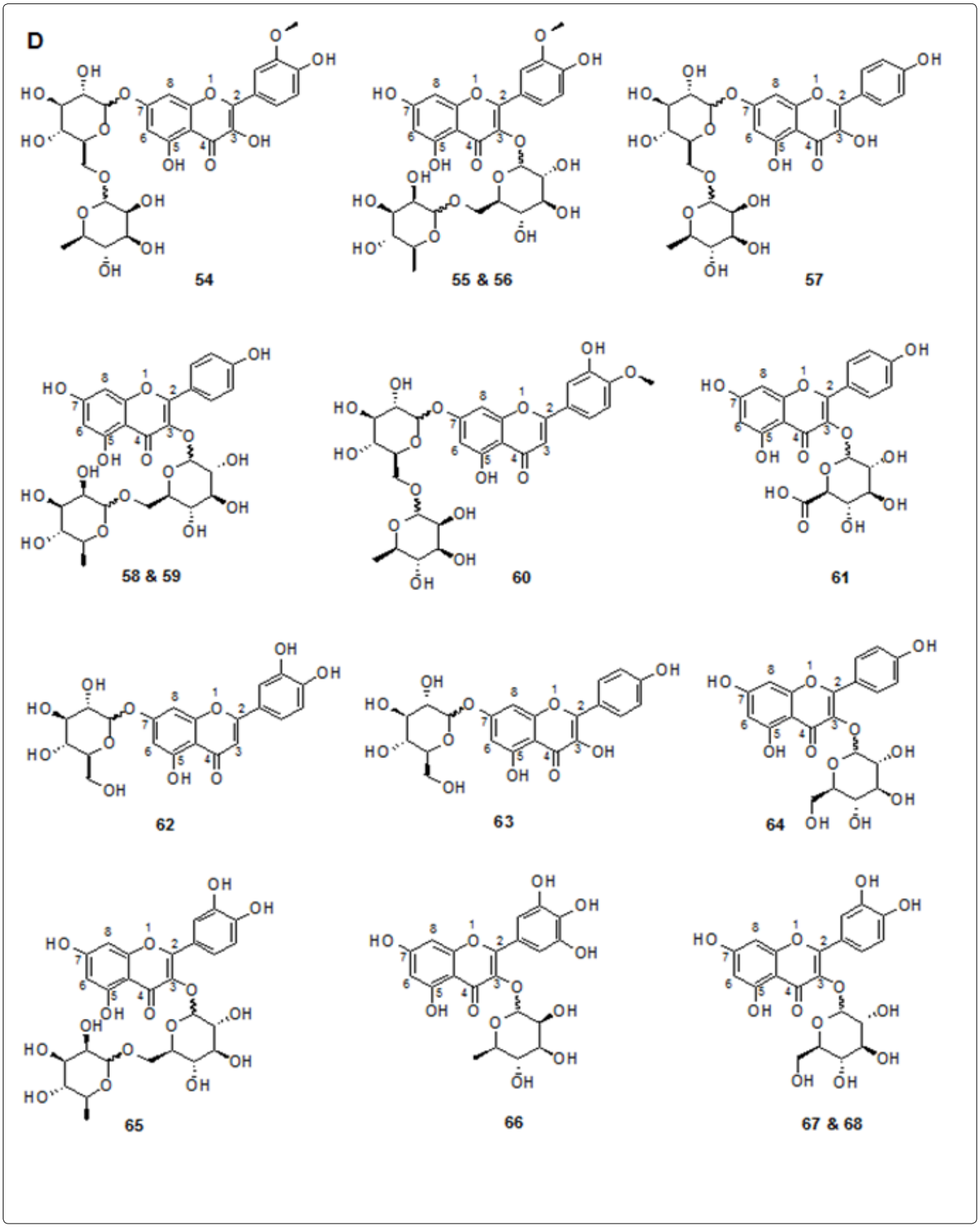
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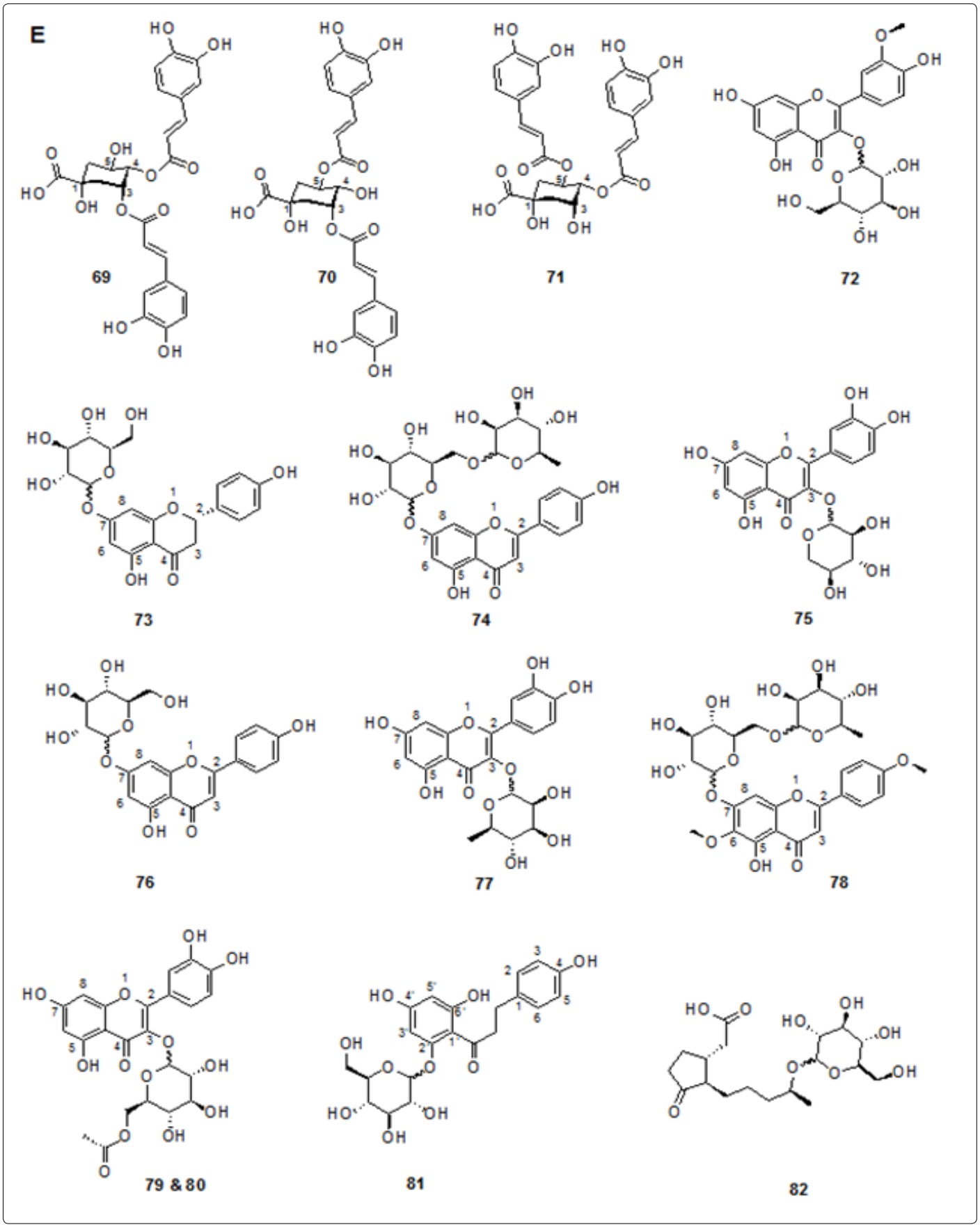
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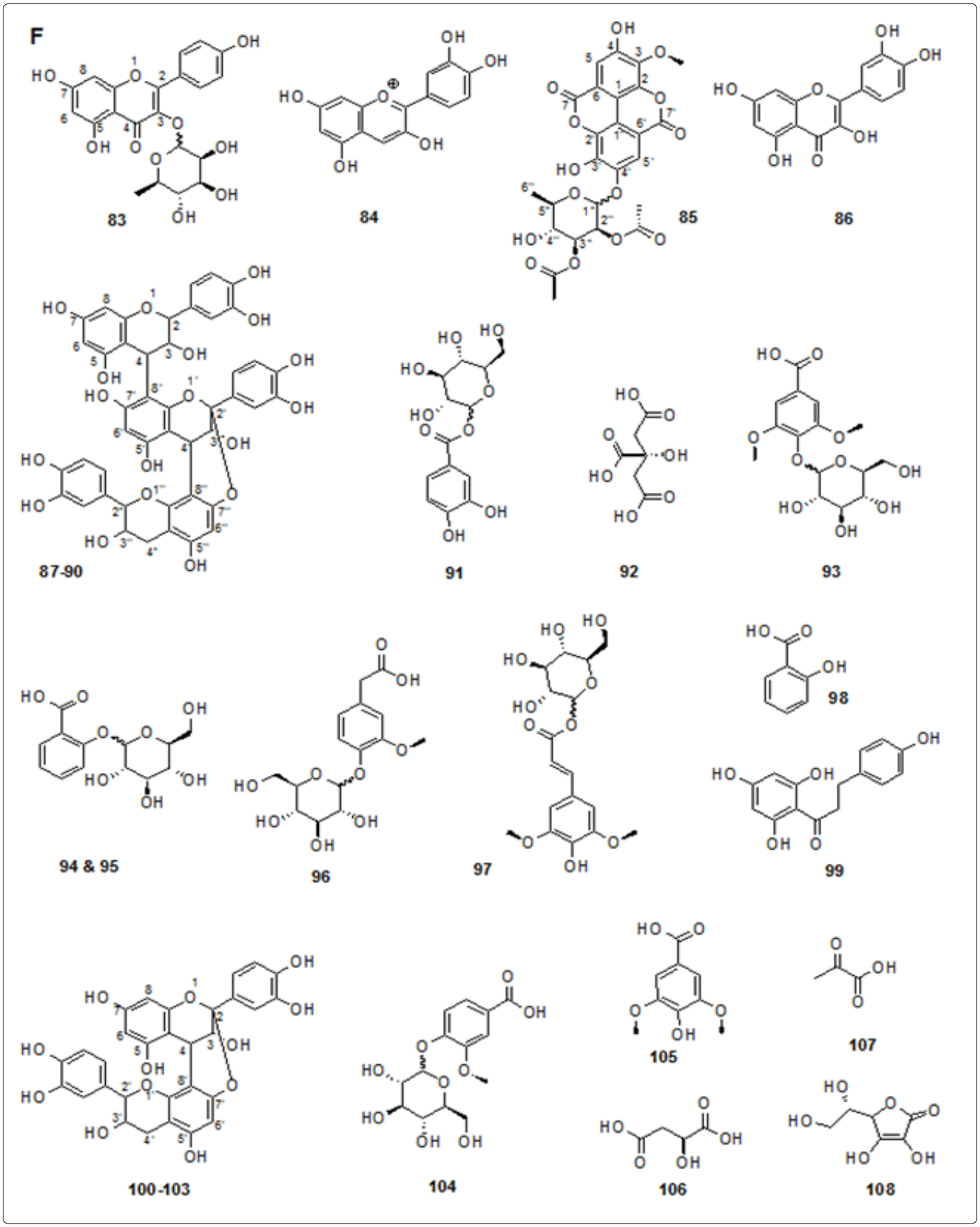
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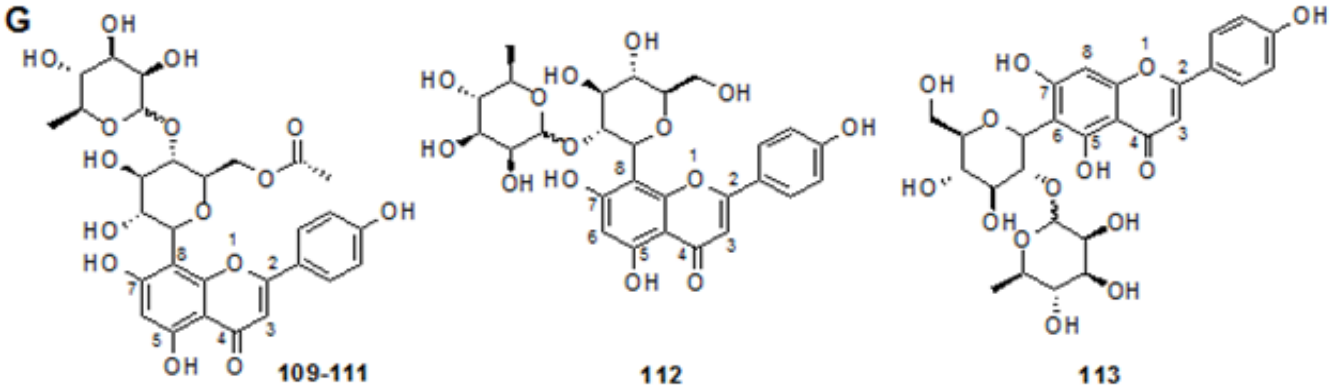

Figure 1: Representative structures of *C. laevigata, C. monogyna* and their herbal drops (Crataegutt Tropfen) phenolics.

in Figure S_1 in the **Supporting Information**. Furthermore, compounds were also compared with authentic reference standards, structured under the same experimental conditions. Taking into account the data previously reported in literature, the flavonol glycosides were allowed further structure assignment and differentiated by using suggested rules and identification criteria previously reported [25].

In the following section structure assignment of selected compounds is illustrated. Full assignment arguments are provided in the **Supporting Information**.

Characterization of phenolic acids and phenolic acid glycosides

Eight phenolic acids and seven phenolic acid glycosides were identified in the plant extracts and the herbal drug.

Characterization of phenolic acids: Compounds 1, 44, 106 and 108 with retention times (t_R) of 2.9, 32.1, 3.4, 3.9 min and m/z of 191, 163, 133, and 175 were identified as quinic acid, p-coumaric acid, malic acid and ascorbic acid, respectively (Table 1), by comparing their retention times and fragmentation behaviour to the MS/MS spectral data of the corresponding phenolic acids authentic standards. Peaks 92, 98, 105 and 107 (t_R 4.2, 7.6, 11.6 and 3.9 min) were assigned as citric acid, salicylic acid, syringic acid and pyruvic acid, respectively. The structural identification of these compounds was based on a comparison of their MS/MS and MS^n data (Table 1 and 2) with those reported in literature [26-28]. Phenolic acids have previously been identified and quantified in Crataegus species [29-31].

Characterization of phenolic acids glycosides

Compound 91 (t_R 9.0 min) was identified as protocatechuic acid O-hexoside with a pseudomolecular ion $[M–H]^-$ of 315.0772 (Table 1). It produced daughter ions at *m/z* 153.0148 corresponding to protocatechuic acid after the neutral loss of the hexoside group and at m/z 109.0288 by the neutral loss of a hexose moiety followed by the neutral loss of CO_2 (44 Da).

Similarly, compounds 93-97 and 104 with t_R 11.8, 7.7, 11.3, 20.8 and 9.6 min (Table 2) were assigned as syringic acid O-hexoside, salicylic acid O-galactoside, salicylic acid O-glucoside, homovanillic acid O-hexoside, sinapic acid O-hexoside and vanillic acid O-hexoside, respectively as previously reported [32-36]. Although aromatic acids and phenolic acids have been previously identified and quantified in Crataegus species [29-31], their hexosides derivatives have been reported here for the first time.

Characterization of benzyl alcohol-hexose-pentose

One benzoic acid derivative was detected at t_R of 21.6 min and m/z of 401.1455 ($C_{18}H_{25}O_{10}$) in the plant extracts and regarded as benzyl alcohol-hexose-pentose 23 based on the MS/MS data, which provided a daughter ion at m/z 269.1041 ($C_{13}H_{17}O_6$) resulted from the neutral loss of pentose unit. Moreover, the compound showed a mass error below 1 ppm thus confirming its elemental composition. This compound was already mentioned in the literature [33,37].

Characterization of 3-O-methylellagic acid 4'-(2",3"-di-O-acetyl)-rhamnoside

With retention time 64.8 min, one ellagic acid derivative was detected at m/z 545.0920 ($C_{25}H_{21}O_{14}$) and was tentatively assign ed as 3-O-methylellagic acid 4'-(2",3"-di-O-acetyl)-rhamnoside 85. Compound 85 produced the MS^2 base peak at m/z 315 $[M–H–230]^-$ by the neutral loss of a rhamnosyl unit and the two acetyl groups connected and a secondary peak at m/z 300 $[M–2H–230–15]^-$ (**Table 2**) due to the subsequence neutral loss of the methyl group. It produced the MS^3 base peak at m/z 300 $[\text{ellagic acid}–2H]^-$. Similar ellagic acid derivatives were already mentioned in the literature [38]. To the best of our knowledge, compound 85 was not previously reported in nature.

Characterization of (–)-11-hydroxy-9,10-dihydrojasmonic acid 11-β-D-glucoside

One fatty acid derivative (t_R 58.5 min) exhibited a deprotonated molecule at m/z 389.1817 was suggested as (–)-11-hydroxy-9,10-dihydrojasmonic acid 11-β-D-glucoside 82. This compound showed MS^2 fragment ion at m/z 227, which indicate the neutral loss of glucose moiety $[M–H–162]^-$ (Table 2). It also represented MS/MS fragment at m/z 183.1376 due to the neutral loss of sugar unit followed by the neutral [38] loss of CO_2 $[M–H–162–44]^-$ (Table 1). The precursor ion has already been reported in the literature in *Nicotiana tabacum* [39]. To our knowledge this compound has been reported in Crataegus derived herbal medicine for the first time.

Characterization of the trisaccharide di-O-glucosyl-glucitol

Compound 11 (t_R 12.1 min), which showed an m/z of 505.1772, was assigned as di-O-glucosyl-glucitol based on the presence of two main fragments at m/z 343, which resulted from a neutral loss of glucosyl molecule $[M–H–162]^-$, and m/z 181 $[\text{glucitol}–H]^-$ due to the neutral loss of the second glucosyl unit $[M–H–324]^-$ (Table 1 and 2). It is worth noting that this compound has been reported in Crataegus for the first time.

No.	Compound Identity	t_R (min)	[M−H]	Characteristic m/z of ions in negative ion mode	Herbal drug	*C. laevigata*		*C. monogyna*	
						F	L	F	L
1	Quinic acid	2.9	191	$MS^2 \rightarrow$ 173 (100), 127 (97), 111 (37), 85 (68); $MS^3 \rightarrow$ 127 (96), 111 (100)	P	P	P	P	P
2	3-O-Caffeoylquinic acid	12.6	353	$MS^2 \rightarrow$ 191 (100), 179 (49); $MS^3 \rightarrow$ 127 (100), 173 (81), 85 (78)	P	P	P	P	-
3	cis-3-O-Caffeoylquinic acid	13.4	353	$MS^2 \rightarrow$ 191 (100), 179 (42); $MS^3 \rightarrow$ 127 (100), 173 (35), 85 (31)	P	P	-	-	-
4	5-O-Caffeoylquinic acid	20.0	353	$MS^2 \rightarrow$ 191 (100); $MS^3 \rightarrow$ 173 (100), 127 (75), 111 (53), 85 (98); $MS^4 \rightarrow$ 93 (100), 109 (37)	P	P	P	P	P
5	cis- 5-O-Caffeoylquinic acid	23.2	353	$MS^2 \rightarrow$ 191 (100); $MS^3 \rightarrow$ 127 (100), 173 (32), 85 (63)	P	P	-	P	-
6	3-O-(4'-O-Caffeoyl glucosyl)quinic acid	11.8	515	$MS^2 \rightarrow$ 353 (100), 191 (30), 341 (28), 323 (04), 335 (07), 179 (11); $MS^3 \rightarrow$ 191 (100), 179 (09); $MS^4 \rightarrow$ 127 (100), 173 (85), 157 (39), 85 (39), 111 (72)	P	-	P	P	P
7	4-O-(4'-O-Caffeoyl glucosyl)quinic acid	13.5	515	$MS^2 \rightarrow$ 341 (100), 353 (94), 173 (67), 179 (83), 323 (01); $MS^3 \rightarrow$ 173 (100), 179 (52), 191 (22)	P	-	-	-	-
8	5-O-(3'-O-Caffeoyl glucosyl)quinic acid	16.5	515	$MS^2 \rightarrow$ 323 (100), 353 (30), 341 (13), 191 (34), 161 (10); $MS^3 \rightarrow$ 161 (100), 133 (10)	P	-	P	P	P
9	5-O-(4'-O-Caffeoyl glucosyl)quinic acid	18.1	515	$MS^2 \rightarrow$ 353 (100), 341 (94), 395 (25), 323 (15), 191 (79), 179 (36); $MS^3 \rightarrow$ 191 (100), 179 (09)	-	-	-	-	P
10	cis 5-O-(3'-O-Caffeoyl glucosyl)quinic acid	22.2	515	$MS^2 \rightarrow$ 323 (100), 353 (38), 341 (10), 191 (155); $MS^3 \rightarrow$ 161 (100), 133 (04), 323 (99); $MS^4 \rightarrow$ 161 (100), 117(99)	-	-	-	-	P
11	Di-O-glycosyl-glucitol	12.1	505	$MS^2 \rightarrow$ 343 (100), 181 (25); $MS^3 \rightarrow$ 181 (100)	P	-	P	-	-
12	3-O-p-Coumaroylquinic acid	17.1	337	$MS^2 \rightarrow$ 163 (100), 191 (12); $MS^3 \rightarrow$ 119 (100)	P	P	-	P	-
13	cis-3-O-p-Coumaroylquinic acid	17.8	337	$MS^2 \rightarrow$ 163 (100), 191 (07); $MS^3 \rightarrow$ 119 (100)	P	P	-	P	P
14	4-O-p-Coumaroylquinic acid	26.1	337	$MS^2 \rightarrow$ 173 (100), 163 (10); $MS^3 \rightarrow$ 93 (100), 155 (29), 111 (65), 71 (51)	P	P	-	P	-
15	5-O-p-Coumaroylquinic acid	27.8	337	$MS^2 \rightarrow$ 191 (100), 163 (10); $MS^3 \rightarrow$ 127 (100), 173 (58), 85 (74), 109 (23); $MS^4 \rightarrow$ 109 (100), 81 (15)	P	P	P	-	-
16	cis-5-O-p-Coumaroylquinic acid	29.7	337	$MS^2 \rightarrow$ 191 (100), 163 (06); $MS^3 \rightarrow$ 127 (100), 173 (38), 85 (29), 109 (27), 93 (13)	P	p	P	P	P
17	(Epi)catechin-4,8′-(epi)catechin C-hexoside	17.3	739	$MS^2 \rightarrow$ 449 (100), 587 (57), 331 (56), 288 (19); $MS^3 \rightarrow$ 327 (100), 289 (71), 245 (28), 167 (18)	P	-	-	-	-
18	(Epi)catechin-4,8′-(epi)catechin C-hexoside	21.8	739	$MS^2 \rightarrow$ 449 (100), 289 (15), 467 (46), 649 (62), 619 (68), 587 (40), 329 (52), 299 (12); $MS^3 \rightarrow$ 329 (100), 289 (26), 245 (10), 287 (26); $MS^4 \rightarrow$ 167 (100), 299 (49)	P	-	-	-	-
19	p-Coumaric acid O-hexoside	17.6	325	$MS^2 \rightarrow$ 163 (100), 119 (15); $MS^3 \rightarrow$ 119 (100)	P	-	P	P	P
20	(Epi)catechin C-hexoside	18.3	451	$MS^2 \rightarrow$ 331 (100), 361 (21), 289 (12); $MS^3 \rightarrow$ 313 (100), 287 (41), 269 (29), 245 (20); $MS^4 \rightarrow$ 231 (100), 245 (15), 203 (46)	P	P	-	-	-
21	Cinchonain Ia (isomer)	65.2	451	$MS^2 \rightarrow$ 313 (100), 289 (52), 331 (79), 169 (10), 137 (11); $MS^3 \rightarrow$ 134 (100), 168 941), 269 (31), 295 (14); $MS^4 \rightarrow$ 123 (100), 150 (89)	P	-	P	P	-
22	Cinchonain Ia (isomer)	66.7	451	$MS^2 \rightarrow$ 289 (100), 331 (18); $MS^3 \rightarrow$ 215 (100), 245 (79), 267 (43), 93 (17); $MS^4 \rightarrow$ 106 (100), 146 (43), 172 (83), 197 (19)	P	-	P	P	-
23	Benzyl alcohol-hexose-pentose	21.6	401	$MS^2 \rightarrow$ 269 (100)	-	P	P	P	P
24	Quercetin 3,7-di-O-hexoside	21.9	625	$MS^2 \rightarrow$ 463 (100), 301 (42); $MS^3 \rightarrow$ 301 (100), 300 (27), 271 (09); $MS^4 \rightarrow$ 271 (98), 299 (100), 179 (18), 151 (19)	P	-	-	-	-
25	(Epi)catechin-4,8′-(epi)catechin (isomer)	22.3	577	$MS^2 \rightarrow$ 407 (100), 425 (79), 451 (20), 289 (18),; $MS^3 \rightarrow$ 285 (100), 389 (24), 297 (30), 255 (30), 255 (32), 243 (15)	P	P	-	-	-
26	(Epi)catechin-4,8′-(epi)catechin (isomer)	25.4	577	$MS^2 \rightarrow$ 407 (100), 425 (68), 451 (22), 289 (22); $MS^3 \rightarrow$ 285 (100), 281 (37), 389 (36), 255 (23), 423 (18); $MS^4 \rightarrow$ 257 (100), 283 (84)	P	P	P	-	P
27	(Epi)catechin-4,8′-(epi)catechin (isomer)	41.7	577	$MS^2 \rightarrow$ 407 (100), 425 (93), 451 (24), 287 (26); $MS^3 \rightarrow$ 285 (100), 281 (60); $MS^4 \rightarrow$ 255 (100)	-	P	P	-	-
28	Luteolin 6,8-di-C-hexoside (isomer)	23.9	609	$MS^2 \rightarrow$ 489 (100), 519 (21), 591 (8), 429 (5), 399 (15), 369 (26);$MS^3 \rightarrow$ 369 (100), 399 (52); $MS^4 \rightarrow$ 341 (100), 351 (20), 313 (28), 298 (10)	P	-	-	-	-
29	Luteolin 6,8-di-C-hexoside (isomer)	30.9	609	$MS^2 \rightarrow$ 429 (100), 489 (57), 339 (31), 357 (29), 327 (15), 309 (35); $MS^3 \rightarrow$ 309 (100), 339 (26), 285 (03), 351 (55); $MS^4 \rightarrow$ 309 (100), 267 (61), 238 (10), 176 (23), 172 (17)	P	-	-	-	-

30	Epicatechin	25.8	289	MS^2 → 245 (100), 205 (33), 179 (11); MS^3 → 203 (100), 227 (25), 187 (23), 161 (20)	P	P	P	P	-
31	Cyanidin 7-O-glucoside	27.0	449	MS^2 → 287 (100), 269 (51), 259 (42); MS^3 → 259 (100), 243 (16); MS^4 → 725 (100), 215 (76), 173 (51), 241 (20)	P	P	-	P	-
32	Cyanidin 3-O-galctoside	38.5	449	MS^2 → 287 (100), 269 (12), 151 (09); MS^3 → 151 (100); MS^4 → 107 (100)	P	P	P	P	-
33	Cyanidin 3-O-glucoside	56.8	449	MS^2 → 287 (100), 407 (05), 341 (53), 243 (58); MS^3 → 243 (100), 228 (16); MS^4 → 228 (100), 122 (82)	P	-	-	-	P
34	Apigenin 6,8-di-C-galactoside (vicenin-4)	27.1	593	MS^2 → 473 (100), 503 (24), 383 (35), 353 (69); MS^3 → 353 (100), 383 (14); MS^4 → 325 (100), 297 (56)	P	-	P	-	P
35	Apigenin 6,8-di-C-glucoside (vicenin-3)	36.0	593	MS^2 → 413 (100), 473 (05), 293 (35); MS^3 → 293 (100); MS^4 → 293 (100), 249 (19), 174 (21)	P	-	P	-	P
36	5-O-Feruloylquinic acid	28.6	367	MS^2 → 191 (100); MS^3 → 127 (100), 173 (32), 85 (55), 109 (20)	P	P	P	P	P
37	Apigenin 6-C-pentosyl-8-C-hexoside (isomer)	28.7	563	MS^2 → 353 (100), 543 (34), 503 (33), 473 (66), 443 (43), 383 (88), 289 (26); MS^3 → 325 (100), 297 (59); MS^4 → 297 (100)	P	P	-	-	-
38	Apigenin 6-C-pentosyl-8-C-hexoside (isomer)	30.6	563	MS^2 → 353 (100), 503 (43), 473 (99), 443 (78), 383 (72); MS^3 → 325 (100), 353 (17), 297 (64); MS^4 → 297 (100)	P	P	-	-	-
39	Apigenin 6-C-hexosyl-8-C-pentoside (isomer)	32.2	563	MS^2 → 443 (100), 383 (52), 353 (66), 473 (54); MS^3 → 325 (100), 353 (10), 297 (57); MS^4 → 297 (100), 325 (09)	P	P	P		P
40	Apigenin 6-C-pentosyl-8-C-hexoside (isomer)	35.0	563	MS^2 → 353 (100), 473 (68), 443 (38), 413 (43), 383, (56), 503 (15); MS^3 → 293 (100); MS^4 → 293 (100), 249 (36), 221 (11), 173 (26)	P	-	P	-	-
41	3-O-Caffeoylshikimic acid	29.7	335	MS^2 → 191 (100), 179 (70), 135 (19), 173 (9); MS^3 → 133 (100)	P	P	-	-	-
42	(Epi)catechin-4,8′-(epi)catechin-4′,8″-(epi)catechin (isomer)	26.5	865	MS^2 → 695 (100), 577 (70), 739 (30), 713 (39), 449 (31), 425 (30), 407 (05), 287 (47); MS^3 → 525 (100), 677 (49), 543 (31), 451 (64), 407 (83), 243 (53)	-	-	P	-	P
43	(Epi)catechin-4,8′-(epi)catechin-4′,8″-(epi)catechin (isomer)	31.2	865	MS^2 → 695 (100), 577 (56), 575 (23), 739 (32), 711 (32), 559 (14), 543 (31), 451 (30), 425 (46), 407 (64), 289 (22), 287 (31); MS^3 → 525 (100), 543 (95), 677 (53), 651 (12), 451 (33), 407 (44), 363 (25), 289 (13), 243 (53); MS^4 → 525 (100), 391 (10)	P	P	P	-	P
44	p-Coumaric acid	32.1	163	MS^2 → 119 (100)	P	-	-	-	-
45	Eriodictyol-di-C-hexoside	32.4	611	MS^2 → 431 (100), 449 (04), 327 (06), 251 (38); MS^3 → 251 (100), 309 (39), 207 (16); MS^4 → 207 (100), 189 (56)	P	P	P	P	P
46	(Epi)afzelechin-(epi) catechin	32.7	561	MS^2 → 289 (100), 407 (20), 425 (19), 435 (41), 543 (22), 329 (17), 271 (16); MS^3 → 245 (100), 205 (36), 179 (17); MS^4 → 203 (100), 226 (20), 188 (12)	P	P	-	-	-
47	Luteolin 8-C-galactoside	33.4	447	MS^2 → 327 (100), 357 (77); MS^3 → 299 (100), 327 (21), 284 (11); MS^4 → 299 (100), 282 (33), 271 (26), 255 (100), 213 (40)	P	P	P	P	-
48	Luteolin 8-C-glucoside (orientin)	34.5	447	MS^2 → 327 (100), 357 (81), 429 (17); MS^3 → 299 (100), 284 (16), 285 (05); MS^4 → 213 (100), 271 (15), 175 (28)	P	P	-	-	-
49	Naringenin C-hexoside (isomer)	34.1	433	MS^2 → 313 (100), 343 (18); MS^3 → 193 (100), 206 (10), 167 (17); MS^4 → 165 (100), 149 (57), 125 (44),	P	P	P	-	P
50	Naringenin C-hexoside (isomer)	35.2	433	MS^2 → 313 (100), 343 (28); MS^3 → 193 (100), 207 (11), 167 (15); MS^4 → 165 (100), 149 (99), 137 (29), 125 (31)	P	-	P	-	P
51	Quercetin 3-O-(2, 6-di-O-rhamnosyl-glucoside)	36.5	755	MS^2 → 300 (100), 301 (64), 609 (22), 591 (22), 489 (36), 343 (23), 737 (09), 271 (23), 255 (13); MS^3 → 271 (100), 255 (56), 179 (16), 151 (15); MS^4 → 271 (100), 255 (19), 243 (23), 267 (15), 151 (12)	P	-	-	-	-
52	Apigenin 8-C-glucoside (vitexin)	37.9	431	MS^2 → 311 (100), 283 (10), 341 (06); MS^3 → 283 (100); MS^4 → 163 (100), 283 (20), 239 (37), 197 (17), 211 (12)	P	P	P	-	P
53	Apigenin 6-C-glucoside (isovitexin)	41.4	431	MS^2 → 311 (100), 341 (45); MS^3 → 283 (100); MS^4 → 283 (100), 239 (46), 224 (36), 183 (28), 162 (34)	P	P	P	-	P
54	Isorhamnetin 7-O-(6-O-rhamnosyl-glucoside)	40.2	623	MS^2 → 299 (100), 300 (30), 314 (79), 315 (47), 477 (11), 271 (26), 177 (25); MS^3 → 271 (100), 255 (33), 243 (10)	P	-	-	-	-
55	Isorhamnetin 3-O-(6-O-rhamnosyl-galactoside)	47.1	623	MS^2 → 315 (100), 300 (28), 271 (13); MS^3 → 300 (100); MS^4 → 271 (100), 255 (79)	P	-	-	-	-
56	Isorhamnetin 3-O-(6-O-rhamnosyl-glucoside)	48.6	623	MS^2 → 315 (100), 300 (25), 271 (14); MS^3 → 300 (100); MS^4 → 271 (100), 255 (56), 221 (51)	P	-	-	-	-

57	Kaempferol 7-O-(6-O-rhamnosyl-glucoside)	40.3	593	MS^2 → 285 (100); MS^3 → 175 (100), 285 (76), 241 (86), 217 (56), 199 (91)	P	-	-	-	-
58	Kaempferol 3-O-(6-O-rhamnosyl-galactoside)	45.3	593	MS^2 → 285 (100), 284 (28), 257 (11); MS^3 → 255 (100), 239 (11), 151 (16)	P	-	-	-	-
59	Kaempferol 3-O-(6-O-rhamnosyl-glucoside)	48.3	593	MS^2 → 284 (100), 463 (05); MS^3 → 257 (100), 267 (54), 239 (41), 150 (30); MS^4 → 109 (100), 163 (80), 212 (36)	P	-	P	-	P
60	Diosmetin 7-O-rutinoside (diosmin)	40.6	607	MS^2 → 299 (100), 443 (27); MS^3 → 271 (100), 255 (79), 179 (11), 151 (16)	P	-	-	-	-
61	Kaempferol 3-O-glucuronide	40.9	461	MS^2 → 285 (100); MS^3 → 199 (100), 285 (52), 241 (45), 226 (35), 175 (35), 155 (11); MS^4 → 196 (100), 154 (64)	P	-	-	-	-
62	Luteolin 7-O-glucoside	41.5	447	MS^2 → 285 (100); MS^3 → 241 (100), 285 (72), 217 (55), 199 (77), 175 (85), 151 (52)	P	-	-	-	-
63	Kaempferol 7-O-glucoside	48.3	447	MS^2 → 284 (100), 285 (95), 255 (22); MS^3 → 255 (100); MS^4 → 255 (100), 227 (85), 211 (27)	P	P	P	P	-
64	Kaempferol 3-O-glucoside	50.5	447	MS^2 → 284 (100), 285 (20), 255 (20); MS^3 → 255 (100), 227 (17); MS^4 → 255 (100), 227 (68), 211 (36)	P	-	P	P	P
65	Quercetin 3-O-(6-O-rhamnosyl-glucoside) (rutin)	41.9	609	MS^2 → 301 (100), 300 (32), 271 (15); MS^3 → 151 (100), 179 (76), 255 (61), 300 (45), 179 (62), 107 (10)	P	P	P	P	P
66	Myricetin 3-O-rhamnoside (myricitrin)	42.2	463	MS^2 → 316 (100), 317 (49); MS^3 → 271 (100), 287 (32), 179 (32), 151 (13); MS^4 → (100)	P	-	-	-	-
67	Quercetin 3-O-galactoside	44.0	463	MS^2 → 301 (100), 300 (25); MS^3 → 179 (100), 151 (90), 271 (50), 255 (35); MS^4 → 151 (100)	P	P	P	P	P
68	Quercetin 3-O-glucoside	45.0	463	MS^2 → 301 (100); MS^3 → 179 (100), 151 (87), 255 (25), 271 (48); MS^3 → 151 (100), 107 (15)	P	P	P	P	P
69	3,4-di-O-Caffeoylquinic acid	45.6	515	MS^2 → 353 (100), 173 (23), 179 (15); MS^3 → 173 (100), 178 (56), 191 (51), 135 (10); MS^4 → 111 (100), 93 (74), 71 (21), 155 (53)	P	-	-	-	-
70	3,5-di-O-Caffeoylquinic acid	47.0	515	MS^2 → 353 (100), 191 (08); MS^3 → 191 (100), 179 (18), 135 (10); MS^4 → 93 (100), 173 (90), 127 (86), 111 (47), 85 (87)	P	P	-	-	-
71	4,5-di-O-Caffeoylquinic acid	51.0	515	MS^2 → 353 (100); MS^3 → 173 (100), 191 (28), 179 (62); MS^4 → 93 (100), 111 (67), 155 (22)	P	-	-	P	-
72	Isorhamnetin O-hexoside	45.9	477	MS^2 → 315 (100), 300 (73); MS^3 → 300 (100); MS^4 → 271 (100), 255 (99)	P	-	-	-	-
73	Naringenin 7-O-glucoside	46.5	433	MS^2 → 271 (100); MS^3 → 151 (100), 177 (26); MS^4 → 107 (100)	P	-	P	-	P
74	Apigenin 7-O-rutinoside	47.0	577	MS^2 → 269 (100); MS^3 → 269 (100), 227 (17), 200 (17),158 (18), 117 (12); MS^4 → 223 (100), (90)	P	-	-	-	-
75	Quercetin 3-O-xyloside	47.6	433	MS^2 → 301 (100), 300 (25); MS^3 → 151 (100), 271 (41), 179 (93); MS^4 → 151 (100)	P	-	-	-	-
76	Apigenin 7-O-glucoside	49.1	431	MS^2 → 269 (100), 301 (87); MS^3 → 268 (100), 269 (64), 225 (17), 197 (16); MS^4 → 197 (100)	P	-	-	-	-
77	Quercetin 3-O-rhamnoside (quercitrin)	51.0	447	MS^2 → 301 (100), 300 (23); MS^3 → 178 (100), 151 (91), 271 (42), 255 (28); MS^4 → 107 (100), 169 (84)	P	-	-	-	-
78	Pectolinarin	51.8	621	MS^2 → 313 (100), 343 (14), 413 (21), 501 (99); MS^3 → 193 (100), 269 (04), 285 (01), 167 (17); MS^4 → 165 (100), 149 (69), 137 (35), 81 (22)	P	-	P	-	-
79	Quercetin O-acetyl hexoside (isomer)	52.2	505	MS^2 → 301 (100), 300 (37), 271 (08); MS^3 → 271 (100), 255 (54), 179 (93), 151 (90), 107 (14); MS^4 → 151 (100)	P	P	-	-	-
80	Quercetin O-acetyl hexoside (isomer)	55.7	505	MS^2 → 300 (100), 301 (68); MS^3 → 271 (100), 300 (49), 255 (52), 179 (58), 151 (53); MS^4 → 271 (100), 255 (41), 243 (26)	P	-	-	-	-
81	Phloretin 2'-O-glucoside (phlorizin)	53.9	435	MS^2 → 273 (100), 229 (21); MS^3 → 229 (100), 107 (14); MS^4 → 107 (100), 121 (43)	P	-	-	-	P
82	(−)-11-hydroxy-9,10-dihydrojasmonic acid 11-β-D-glucoside	58.5	389	MS^2 → 227 (100), 183 (12); MS^3 → 183 (100), 165 (91); MS^4 → 112 (76), 163 (26)	P	-	-	-	-
83	Kaempferol 3-O-rhamnoside	58.8	431	MS^2 → 284 (100), 285 (43); MS^3 → 255 (100), 227 (23), 229 (13)	P	-	-	-	-
84	Cyanidin	62.6	287	MS^2 → 151 (100), 135 (10); MS^3 → 107 (100), 169 (10), 65 (20); MS^4 → 65 (100)	P	-	-	-	-
85	3-O-Methylellagic acid 4'-(2",3"-di-O-acetyl)-rhamnoside	64.8	545	MS^2 → 315 (100), 300 (68), 301 (12), 271 (21), 255 (27); MS^3 → 300 (100), 301 (10); MS^4 → 255 (100), 271 (67)	P	-	-	-	-

86	Quercetin	69.9	301	$MS^2 \rightarrow 179$ (100), 151 (83); $MS^3 \rightarrow 151$ (100), 169 (14); $MS^4 \rightarrow 107$ (100), 168 (14)	P	-	-	-	-
87	(Epi)catechin-(4,8′)-(epi)catechin-(4′,8″/2′,7″)-(epi) catechin (isomer)	31.4	863	$MS^2 \rightarrow 711$ (100), 695 (70), 577 (36), 451 (61), 407 (53), 287 (20), 575 (13), 425 (30)	-	P	-	-	-
88	(Epi)catechin-(4,8′)-(epi)catechin-(4′,8″/2′,7″)-(epi) catechin (isomer)	32.0	863	$MS^2 \rightarrow 711$ (100), 725 (41), 599 (39), 425 (23), 449 (15), 411(33), 287 (14); $MS^3 \rightarrow 559$ (100), 693 (98), 541 (59), 407 (42), 381 (25); $MS^4 \rightarrow 567$ (100), 658 (35), 407 (13)	-	P	-	-	-
89	(Epi)catechin-(4,8′)-(epi)catechin-(4′,8″/2′,7″)-(epi) catechin (isomer)	33.8	863	$MS^2 \rightarrow 573$ (100), 711 (50), 694 (25), 451 (51), 411) (82); $MS^3 \rightarrow 411$ (100), 273 (11); $MS^4 \rightarrow 227$ (100), 283 (24), 349 (45), 125 (14)	-	P	-	-	-
90	(Epi)catechin-(4,8′)-(epi)catechin-(4′,8″/2′,7″)-(epi) catechin (isomer)	38.2	863	$MS^2 \rightarrow 711$ (100), 693 (50), 599 (72), 407 (05); $MS^3 \rightarrow 559$ (100), 693 (82), 425 (10); $MS^4 \rightarrow 567$ (100), 483 (32), 405 (33), 282 (25)	-	P	-	-	-
91	Protocatechuic acid O-hexoside	9.0	315	$MS^2 \rightarrow 153$ (100), 109 (13); $MS^3 \rightarrow 109$ (100)	-	P	P	-	P
92	Citric acid	4.2	191	$MS^2 \rightarrow 111$ (100), 173 (22)	P	-	P	P	P
93	Syringic acid O-hexoside	11.8	359	$MS^2 \rightarrow 197$ (100), 182 (13); $MS^3 \rightarrow 182$ (100), 153 (23); $MS^4 \rightarrow 167$ (100), 123 (11)	P	P	P	P	P
94	Salicylic acid O-galactoside	7.7	299	$MS^2 \rightarrow 137$ (100); $MS^3 \rightarrow 93$ (100)	-	P	-	P	P
95	Salicylic acid O-glucoside	11.3	299	$MS^2 \rightarrow 137$ (100), 239 (70), 209 (19), 179 (73); $MS^3 \rightarrow 93$ (100)	P	-	-	P	P
96	Homovanillic acid O-hexoside	17.6	343	$MS^2 \rightarrow 181$ (100), 137 (11); $MS^3 \rightarrow 137$ (100); $MS^4 \rightarrow 95$ (100)	P	-	P	-	P
97	Sinapic acid O-hexoside	20.8	385	$MS^2 \rightarrow 223$ (100); $MS^3 \rightarrow 208$ (100), 179 (45), 165 (32); $MS^4 \rightarrow 164$ (100), 149 (22)	P	-	-	P	P
98	Salicylic acid	7.6	137	$MS^2 \rightarrow 93$ (100)	P	-	-	-	-
99	Phloretin	44.1	273	$MS^2 \rightarrow 167$ (100); $MS^3 \rightarrow 123$ (100); $MS^4 \rightarrow 95$ (100)	-	P	-	-	-
100	(Epi)catechin-(4,8′/2,7′)-(epi) catechin (isomer)	43.0	575	$MS^2 \rightarrow 423$ (100), 449 (43), 539 (10), 407 (11), 327 (10), 285 (27) 289 (15); $MS^3 \rightarrow 285$ (100); $MS^4 \rightarrow 241$ (100), 217 (71), 125 (40)	-	P	-	P	-
101	(Epi)catechin-(4,8′/2,7′)-(epi) catechin (isomer)	48.0	575	$MS^2 \rightarrow 423$ (100), 449 (77), 539 (52), 407 (26), 327 (10), 285 (34) 289 (29); $MS^3 \rightarrow 285$ (100); $MS^4 \rightarrow 241$ (100), 125 (53)	-	P	-	P	-
102	(Epi)catechin-(4,8′/2,7′)-(epi) catechin (isomer)	49.8	575	$MS^2 \rightarrow 447$ (56), 423 (50), 449 (30), 539 (61), 407 (23), 327 (18), 285 (33) 289 (27); $MS^3 \rightarrow 287$ (100), 243 (14); $MS^4 \rightarrow 285$ (100), 243 (58), 125 (71)	-	P	-	-	-
103	(Epi)catechin-(4,8′/2,7′)-(epi) catechin (isomer)	59.9	575	$MS^2 \rightarrow 287$ (97), 449 (100), 325 (14), 431 (47); $MS^3 \rightarrow 285$ (96), 125 (100)	-	P	-	P	-
104	Vanillic acid O-hexoside	9.6	329	$MS^2 \rightarrow 167$ (100); $MS^3 \rightarrow 152$ (100), 123 (54), 108 (23)	-	P	P	P	P
105	Syringic acid	11.6	197	$MS^2 \rightarrow 182$ (100), 153 (18); $MS^3 \rightarrow 167$ (100); $MS^4 \rightarrow 123$ (100)	-	P	-	-	-
106	Malic acid	3.4	133	$MS^2 \rightarrow 115$ (100); $MS^3 \rightarrow 71$ (100)	P	P	P	P	P
107	Pyruvic acid	3.8	87	Not fragmented	-	P	-	-	-
108	Ascorbic acid	3.9	175	$MS^2 \rightarrow 87$ (100), 157 (34)	-	P	-	-	-
109	Cratenacin (isomer)	41.1	619	$MS^2 \rightarrow 413$ (100), 293 (59); $MS^3 \rightarrow 293$ (100); $MS^4 \rightarrow 293$ (100), 249 (38), 173 (24)	P	-	P	-	-
110	Cratenacin (isomer)	42.2	619	$MS^2 \rightarrow 413$ (100), 293 (51); $MS^3 \rightarrow 293$ (100); $MS^4 \rightarrow 293$ (100), 249 (14), 175 (18)	P	-	P	-	-
111	Cratenacin (isomer)	49.8	619	$MS^2 \rightarrow 413$ (100), 293 (50); $MS^3 \rightarrow 293$ (100); $MS^4 \rightarrow 293$ (100), 249 (17), 173 (19)	P	P	P	P	-
112	Vitexin 2″-O-rhamnoside	37.2	577	$MS^2 \rightarrow 413$ (100), 293 (41); $MS^3 \rightarrow 293$ (100); $MS^4 \rightarrow 293$ (100), 249 (23), 221 (13), 174 (12), 117 (10)	P	P	P	P	P
113	Isovitexin 2″-O-rhamnoside	39.9	577	$MS^2 \rightarrow 413$ (100), 293 (41); $MS^3 \rightarrow 293$ (100); $MS^4 \rightarrow 293$ (100), 175 (24)	P	-	-	-	-

Table 2: HPLC retention times (t_R) and MS^n fragmentation in negative ion mode of *C. laevigata, C. monogyna* and the herbal drug phenols (where P=present, F=Fruits and L= Leaves).

Characterisation of flavonoids and flavonoids glycosides

Flavonoids are derived from the shikimate pathway in plant kingdom. They have a basic structure consisting of two aromatic benzene rings separated by an oxygenated heterocyclic ring. Several compounds from different flavonoid classes, such as flavonols, flavanones, flavones and others have been characterized and identified in hawthorn and the herbal drops samples. Crataegus species analyzed in this study showed C-glycosides and O-glycosides flavonoids isomers as represented in Table 1 and 2. All flavonoids O-glycosides showed a neutral loss of the glycan part. In contrast, flavonoid C-glycosides showed a series of characteristic fragments of C-glycosides flavonoids at m/z [M–H–18], [M–H–18]$^-$, [M–H–60]$^-$, [M–H–90]$^-$, [M–H–120]$^-$, [M–H–180]$^-$ and [M–H–210]$^-$ due to a cross-ring fragmentation of glucosides molecules [40].

Characterization of luteolin derivatives: Compounds 62, 65, 68, 77 and 86 (t_R 41.5, 41.9, 45.0, 51.1 and 69.9 min) with m/z of 447.0933, 609.1461, 463.0882, 447.0928 and 301.0349 were assigned as luteolin 7-O-glucoside, quercetin 3-O-(6-O-rhamnosyl-glucoside) (rutin), quercetin 3-O-glucoside, quercetin 3-O-rhamnoside (quercitrin) and quercetin, respectively, after comparison their retention times, UV spectra and MS/MS fragmentation patterns with authentic standards (Table 1).

Peaks 28 and 29 with retention times of 23.9 and 30.9 min and m/z of 609.1459 and 609.1462 were tentatively identified as luteolin 6,8-di-C-hexoside isomers. The HPLC-ESI-MS spectra of these peaks showed MS^2 fragment ions at [M–H–18]$^-$, [M–H–90]$^-$, [M–H–120]$^-$, [M–H–180]$^-$ and [M–H–210]$^-$ (Table 2) in addition the fragment ions at m/z 369 (aglycone + 83) and 399 (aglycone + 113), which characterize the luteolin with the residues of the hexoses that remained connected to it (Figure 2) [41].

Compounds 47 and 48, with precursor ions at m/z 447.0929 and 447.0932 and retention times of 33.4 and 34.5 min, respectively, were identified as luteolin C-hexoside isomers (Table 1 and 2). Compound 48 was characterized as luteolin 8-C-glucoside based on the comparison of the retention time and the fragmentation patterns with the authentic standard. From the literature and from our experiments, we have found that the flavonoids glycosylated with galactoside units are less polar than the flavonoids glycosylated with glucoside units [34, 35]. Based on the above argument, the earlier eluted isomer (t_R 33.4 min) was assigned as luteolin 8-C-galactoside 47. Luteolin and luteolin glycoside derivatives were already reported in the literature in Crataegus species [31,42].

Characterization of naringenin derivatives: Two naringenin *C*-hexosides 49 (m/z 433.1136) and 50 (m/z 433.1138) with retention times of 34.1 and 35.2 min were detected in the TOF-MS mode. The MS/MS product ion scan of these compounds showed characteristic neutral losses of 90 and 120 Da from the parent ion (m/z 433) corresponding to cross-ring cleavages in the sugar unit (Table 1). Although naringenin and naringenin 5,7-diglucoside were already mentioned in the literature in Crataegus species [43,44] these compounds have been reported here for the first time.

Peak 73 (m/z 433.1139) with a retention time of 46.5 min and molecular formula ($C_{21}H_{22}O_{10}$) was suggested as naringenin 7-O-glucoside (Table 1 and Figure 3A) based on the presence of fragment ion at m/z 271.0611 [naringenin–H]$^-$ and the neutral loss of hexose moiety, resulting in an ion at [M–H–162]$^-$ [45]. As far as we know this compound has been reported in Crataegus for the first time.

Characterization of apigenin derivatives: Apigenin 8-*C*-glucoside (vitexin) 52 and apigenin 6-C-glucoside (isovitexin) 53, vitexin 2″-O-rhamnoside 112, isovitexin 2″-O-rhamnoside 113 and cratenacin (4‴-acetylvitexin-2″-O-rhamnoside) isomers 109-111 were assigned on the bases of the product ion spectrum (Table 1) and comparison with previously reported data [15,45]. Vitexin derivatives are well-known compounds in Crataegus species [15,46].

Peaks 37, 38, 39 and 40 (t_R 28.7, 30.6, 32.2, 35.0 min) exhibited similar molecular formula ($C_{26}H_{28}O_{14}$). They showed MS/MS fragmentation characteristic to flavone di-C-glycosides with [M–H–18]$^-$, [M–H–60]$^-$, [M–H–90]$^-$, [M–H–120]$^-$, [M–H–180]$^-$ and [M–H–210]. The higher intensity of ion at m/z 473 [M–H–90]$^-$ relative to the ion at m/z 443 [M–H–120]$^-$ indicating 6-C-pentosyl-8-C-hexosyl linkage (Table 2) [47]. Thus, peaks 37, 38 and 40 were characterized as apigenin 6-C-pentosyl-8-C-hexoside isomers. In contrast, peak 39 was tentatively regarded as apigenin 6-C-hexosyl-8-C-pentoside hence it showed the highest intensity (100%) for the ion at m/z 443 [M–H–120]$^-$. From the leaves of *C. monogyna* and *C. pentagyna* apigenin di-C-glycosides were isolated previously [48,49].

Peaks 34 and 35 with m/z 593.1507 ($C_{27}H_{30}O_{15}$) were detected at retention times 27.1 and 36.0 min, respectively (Table 2). These compounds have shown the C-glycoside flavonoids fragmentation patterns similar to the apigenin derivatives mentioned above. Thus, these peaks were proposed as apigenin 6,8-di-C-hexoside isomers. The later eluted isomer 35 was identified as apigenin 6,8-di-C-glucoside (vicenin-3). This compound has been already reported in Crataegus species [46,48,49]. Tentatively, we assigned the earlier eluted isomer (34) as apigenin 6,8-di-C-galactoside (vicenin-4) [34,35]. As far as we

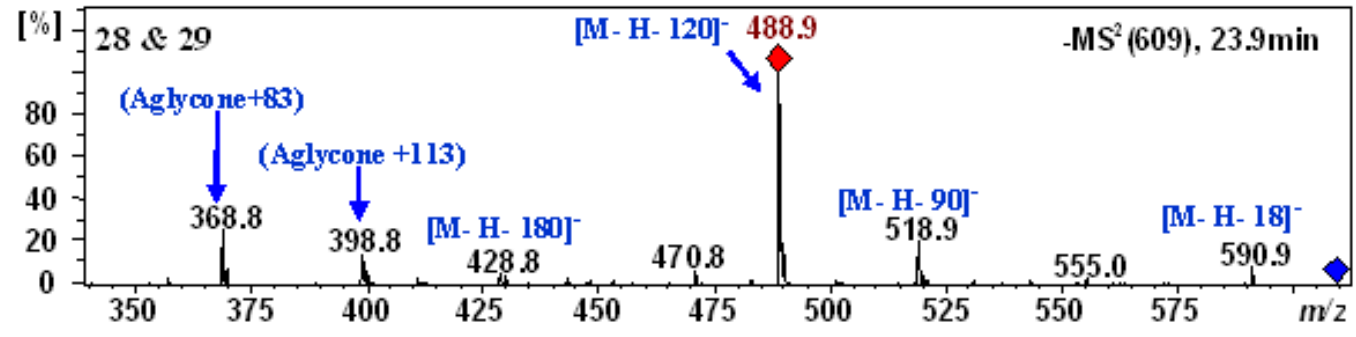

Figure 2: MS^2 spectra of luteolin 6,8-di-C-hexoside isomers 28 and 29 with precursor ion at m/z 609 and in negative ion mode

know, vicenin-4 has not been reported previously in nature.

In addition to the apigenin C-glycosides, we also identified two apigenin O-glycoside derivatives, namely, apigenin 7-O-rutinoside 74 and apigenin 7-O-glucoside 76 (Table 1). Compound 74 (t_R 47.0 min) showed a precursor ion at m/z 577.1557. The neutral loss of rutinoside $[M–H–308]^-$ and the appearance of the ion at m/z 269.0466 in the MS/MS spectra indicated the aglycone apigenin. The acceptable data of MS with the daughter fragment ions of MS/MS have supported compound 74 be apigenin 7-O-rutinoside. This compound was already mentioned in the literature [50]. On another hand, compound 76 (t_R 49.1 min) was identified by comparing its retention time and characteristic MS spectral data with apigenin 7-O-glucoside authentic standard.

Characterization of eriodictyol di-C-hexoside: Peak 45 (t_R 32.4 min), showed fragment ions of a di-C-hexoside flavonoid (Table 2). These fragments, together with the precursor ion at m/z 611.1615 and the molecular formula $C_{27}H_{31}O_{16}$ pointed to an eriodictyol di-C-hexoside. Eriodictyol has been previously isolated from C. microphylla [44]. Compound 45 was present in all investigated samples.

Characterization of quercetin derivatives: Compounds 24, 51, 67, 75, 79 and 80 with retention times of 21.9, 36.5, 44.0, 47.6, 52.2, 55.7 min and m/z of 625.1411, 755.2037, 463.0878, 433.0772, 505.0984 and 505.0982 were assigned as quercetin 3,7-di-O-hexoside, quercetin 3-O-(2,6-di-O-rhamnosyl-glucoside), quercetin 3-O-galactoside (Figure 3B), quercetin 3-O-xyloside and quercetin O-acetyl hexoside isomers, respectively (Tables 1 and 2) based on the previous studies [25,35,51,52].

Characterization of kaempferol derivatives: Kaempferol derivatives were also identified in *C. laevigata, C. monogyna* and the herbal drug. The detected ions at m/z 447.0930 and 447.0927, which correspond to peaks 63 and 64, showed a neutral loss of hexosyl moiety resulting in fragments at m/z 285.0393 and 284.0323 (Table 1), corresponded to the aglycone kaempferol. Thus, they were suggested as kaempferol-O-hexoside isomers. Peak 63 (t_R 48.3 min) was as assigned as kaempferol 7-O-glucoside after the comparison with the retention time and fragmentation pattern of kaempferol 7-O-glucoside authentic standard. Peak 64 (t_R 50.5 min) has been proposed as kaempferol 3-O-glucoside (Figure 3C).

One peak was readily detected with t_R of 40.9 min and of m/z 461.0725 in the EIC and was tentatively assigned as kaempferol 3-O-glucuronide 61. It produced the MS^2 base peak at m/z 285 $[kaempferol–H]^-$ by the neutral loss of a glucuronide residue (176 Da) and the characteristic MS fragments of kaempferol in MS^3 (Table 2). This compound has been recently reported by De Rosso et al. in hybrid grapes [53]. It is worth mentioning that this compound has been detected in Crataegus for the first time.

Compound 83 exhibited a deprotonated molecule at m/z 431.0980 ($C_{21}H_{19}O_{10}$) and MS^2 fragment ions at m/z 284 $[kaempferol–2H]^-$ (base peak) and m/z 285 $[kaempferol–H]^-$ due to the neutral loss of a rhamnoside moiety $[M–H–146]^-$. Based on our previous finding [25] and the acceptable MS data (Table 1 and 2) this compound has been assigned to kaempferol 3-O-rhamnoside.

With m/z 593.1512 ($C_{27}H_{29}O_{15}$), three compounds (57-59) were detected at retention times 40.3, 45.3 and 48.3 min (Figure 4). These compounds showed the same fragmentation patterns. In their MS^n they showed ions corresponding to the aglycone kaempferol (Table 2) [54] by the neutral loss of (rhamnosyl-hexoside) $[M–H–308]^-$. Therefore they were suggested to be kaempferol -O-(6-O-rhamnosyl-hexoside) isomers. We have found that flavonol-7-O-glycosides elute first followed by flavonol-3-O-glycosides [25]. Based on the order of elution and the similarity of the fragmentation behaviors, isomers 57 and 59 were assigned as kaempferol 7-O-(6-O-rhamnosyl-glucoside) and kaempferol 3-O-(6-O-rhamnosyl-glucoside), respectively. For further evidence, the UV spectrum, the MS fragmentation and the retention time of compound 59 were compared with the kaempferol 3-O-(6-O-rhamnosyl-glucoside) authentic standard. This compound has been previously recorded in quince (*Cydonia oblonga*) fruits [21]. Considering the elution order in the RF-C18-A column, isomer 58 was regarded as a glycosylated isomer of compound 59 and hence assigned as kaempferol 3-O-(6-O-rhamnosyl-galactoside) [34,35]. Recently, we have detected this compound in *I. coccinea* [25]. Although kaempferol glycosides derivatives were already mentioned in the literature in Crataegus species [31,55], these compounds have been reported here for the first time.

Characterization of isorhamnetin derivatives: Following the same strategies discussed above, peaks 54, 55 and 56 (t_R 40.2, 47.1 and 48.6 min) were assigned as isorhamnetin 7-O-(6-O-rhamnosyl-glucoside), isorhamnetin 3-O-(6-O-rhamnosyl-galactoside) and isorhamnetin 3-O-(6-O-rhamnosyl-glucoside), respectively (Table 1 and 2). Although an isorhamnetin glycoside derivative has been previously reported by Rodrigues et al. in *C. monogyna* [56], these compounds have been reported here for the first time.

Peak 72 (t_R 45.9 min) with m/z of 477.1036 ($C_{22}H_{21}O_{16}$), was identified as isorhamnetin O-hexoside according to the MS, MS/MS data (Table 1) and data from the literature [57]. The presence of the fragment ion at m/z 315.0502 ($C_{16}H_{11}O_7$) indicates the neutral loss of a hexosyl moiety and the presence of the aglycone isorhamnetin (Figure 3D).

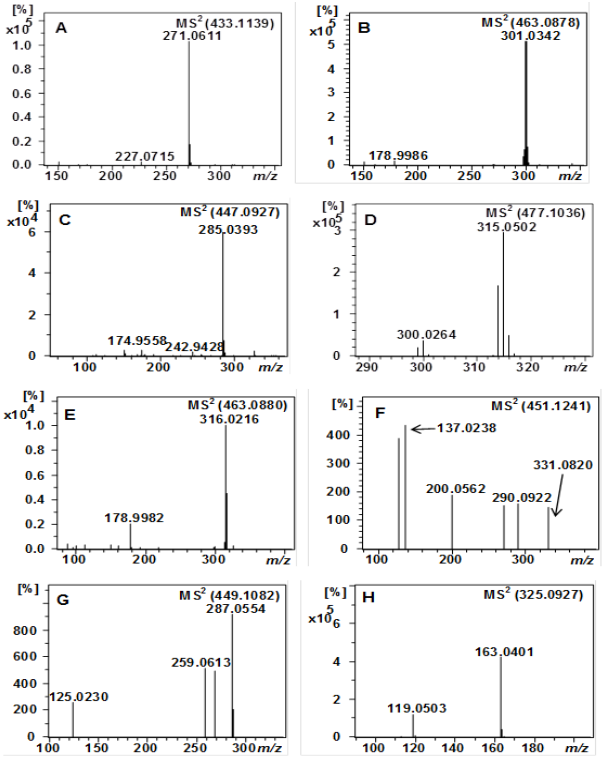

Figure 3: MS/MS spectra of some phenolic compounds detected in *C. laevigata*, *C. monogyna* extracts and the herbal drops (Crataegutt Tropfen)

Characterization of diosmin: Peak 60 (t_R 40.6 min) at m/z 577 was tentatively identified as diosmetin 7-O-rutinoside (diosmin), illustrated by the neutral loss of rutinoside unit [M–H –308]$^-$ and the presence of fragment ions at m/z 299 (base peak) (Table 2), which can be referred to [diosmetin–H]$^-$ [58]. This fragmentation is consistent with that reported by Lin L [59], who also found many isomers of O-glycosylated diosmetin in flower extract of chrysanthemum (*Chrysanthemum morifolium* Ramat).

Characterization of myricitrin: Compound 66 with retention time of 42.2 min and m/z of 463.0880 was suggested to myricetin 3-O-rhamnoside (myricitrin), relying on the clear neutral loss of rhamnoside moiety (146 Da) and the characteristic fragment ions at m/z 317, 316, 271, 179 and 151, which represented myricetin (Figure 3E, Table 1 and 2) [60]. In addition, the aglycone myricetin was already reported in a recent study in *C. cuneata* [61]. To our knowledge this compound has been reported in hawthorn for the first time.

Characterization of pectolinarin: The compound at the retention time 51.8 min and a pseudomolecular ion [M–H]$^-$ of 621.1825 was tentatively assigned as pectolinarin (78) based on the acceptable data of MS (Table 1) and the information obtained from the literature [62].

Characterisation of phloretin derivatives

The analysis in TOF-MS mode also showed the presence of phloretin derivatives in Crataegus species and the herbal drug (Crataegutt Tropfen). Compound 81 (t_R 53.9 min; m/z 435.1297; $C_{21}H_{24}O_{10}$) was positively identified as phloretin 2'-O-glucoside (phlorizin) based on the comparison of the retention time and the fragmentation patterns with the authentic standard (Table 1).

Compound 99 ($C_{15}H_{14}O_5$) with retention time of 41.1 min and pseudomolecular ion [M–H]$^-$ of m/z 273.0768 was assigned to phloretin relying on the acceptable data of MS (Table 1), fragmentation pattern of MS^2/MS^3 (Table 2) and the data obtained from literature [63]. As far as we know, phlorizin and phloretin have never been reported in Crataegus species before.

Characterisation of anthocyanidins, proanthocyanidins and their derivatives

Anthocyanidins, proanthocyanidins and their derivatives are well-known phytoconstituents in Crataegus species. Our findings boosted and were in line with previous studies [15,64,65].

Characterization of epicatechin derivatives: Peak 30 (t_R 25.8 min) at m/z 289.0716 was identified as epicatechin by comparison of UV spectrum and retention time with a commercial standard.

Compound 20 at m/z 451.1241 (t_R 18.3 min) with molecular formula $C_{21}H_{23}O_{11}$ was considered as (epi)catechin C-hexoside relying on its MS and MS/MS fragmentation pattern (Figure 3F) and the data obtained from literature [66].

Characterization of B-type proanthocyanidins and their derivatives: B-type proanthocyanidin oligomers were also detected in the herbal medicine and Crataegus samples. They showed UV spectra with λ_{max} 280 nm, characteristic of proanthocyanidins. Thus, peaks 25-27 ($C_{30}H_{26}O_{12}$), eluted between 22 and 42 min, showed a pseudomolecular ions [M–H]$^-$ at m/z 577.1348, 577.1347 and 577.1350 corresponding to dimeric B-type proanthocyanidins [21].

Peaks 42 (t_R 26.5 min) and 43 (t_R 31.2 min) were detected at m/z 865.1947 and 865.1976 in the EIC. These compounds were assigned as trimeric B-type proanthocyanidin with (epi)catechin monomeric units [21].

On the other hand, compound 46 (t_R 32.7 min) had pseudomolecular ion [M–H]$^-$ at m/z 561.1402 and an MS/MS fragment ion at m/z 289.0715 (epicatechin), which corresponds to the neutral loss of (epi) afzelechin (272 Da) (Table 1). Thus, this dimer was suggested to (epi) afzelechin-(epi)catechin. This compound has been previously reported in literature and its fragmentation pathway was described [67]. It is worth noting that peak 46 has been reported in Crataegus for the first time.

At m/z 739.1880 two compounds (17, 18) were detected at retention times of 17.3 and 21.8 min and were proposed as proanthocyanidin dimers having hexose groups attached to them, showed by the acceptable data of MS (Table 1), fragmentation pattern of MS^2/MS^3 (Table 2) and the data obtained from literature [68]. Moreover, these peaks represented the characteristic flavan-3-ols-C-glycosides ions at m/z 649 [M–H–90]$^-$, 619 [M–H–120]$^-$, and 589 [M–H–150]$^-$ in their MS^n (Figure 5). Consequently, compounds 17 and 18 were assigned as (epi)catechin-4,8′-(epi)catechin C-hexoside isomers. Nevertheless, assignment of positions and identity of the hexose units was not possible. According to our knowledge, these isomers have never been reported in Crataegus species before.

Characterization of A-type proanthocyanidins: With m/z 575 ($C_{30}H_{23}O_{12}$) four peaks were detected at retention times of 48.0, 49.8 and 59.9 min and assigned as dimeric A-type proanthocyanidin, tentatively (epi)catechin-(4,8′/2,7′)-(epi)catechin isomers (100-103) (Table 1 and 2).

We have calculated identical molecular formula ($C_{45}H_{36}O_{18}$) for peaks 87-90 at m/z 863 (Table 1). They eluted between 30 and 40 min in the developed reverse phase chromatographic method. These compounds were tentatively assigned as trimeric A-type proanthocyanidins with (epi)catechin monomeric units. The suggested structures and fragmentation pathways of A-type proanthocyanidin oligomers have been discussed in previous studies [21,23,25,67].

Characterization of cyanidin derivatives: The detected precursor ion at m/z 287.0561 ($C_{21}H_{21}O_{11}^+$), which correspond to peaks 84 (t_R 62.6 min) was assigned as cyanidin based on the acceptable data of MS with the daughter fragment ions of MS/MS (Table 1) in addition to the information previously reported in the literature [69].

With m/z 449.1082 three peaks with identical molecular formula $C_{21}H_{21}O_{11}^+$ were detected at retention times of 27.0, 38.5 and 56.8 min. These compounds showed a neutral loss of hexose moiety resulting in a fragment ion at m/z 287.0554, which corresponds to cyanidin (Table 1). Therefore, they were suggested as cyanidin O-hexoside isomers (31-33). Based on the order of elution these peaks were tentatively identified as cyanidin 7-O-glucoside 31, cyanidin 3-O-galactoside 32 and cyanidin 3-O-glucoside 33 [25,34,35,70]. Although compounds 32 and 33 were already mentioned in the literature in hawthorn [70], compound 31 has been reported here for the first time (Figure 3G).

Characterization of cinchonains Ia: The analysis in TOF-MS mode also showed the presence of flavalignan isomers (21 and 22) in the analyzed samples. These isomers (t_R 65.2 and 66.7 min) showed the same fragmentation patterns (Table 1). The acceptable data of MS with the produced fragment ions of MS/MS have directed to suggest the parent ions at m/z 451.1030 and 451.1035 to cinchonains Ia isomers. These data are in accordance with previous data cited in literature [71]. Cinchonains and their higher oligomeric have already been detected in Crataegus species [72].

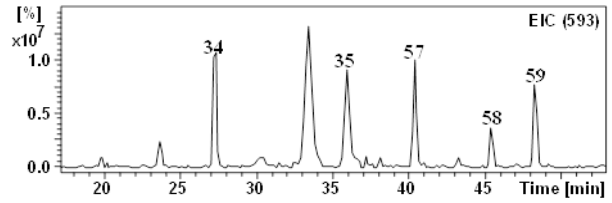

Figure 4: Extracted ion chromatogram (EIC) at m/z 593 in negative ion mode.

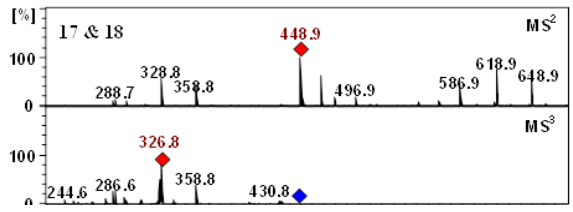

Figure 5: MS^3 spectra of (epi)catechin-4,8′-(epi)catechin C-hexoside isomers 17 and 18 with precursor ion at m/z 739 in negative ion mode

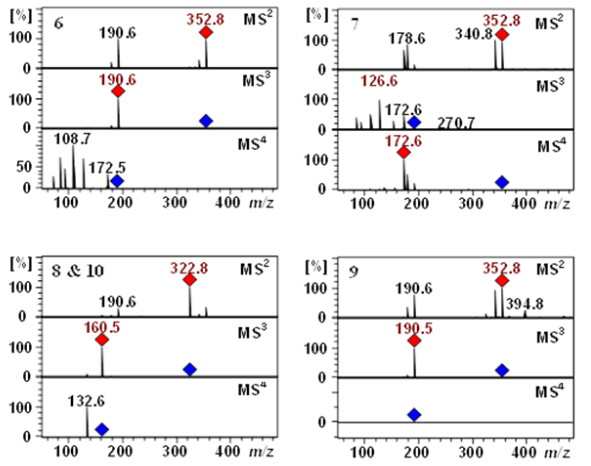

Figure 6: MS^4 spectra of caffeoylquinic acid glucosides 6-10 with precursor ion at m/z 515 in negative ion mode

Characterisation of acylated quinic acid derivatives

Several mono and di-acylated quinic acid derivatives including chlorogenic acids and chlorogenic acids glycosides were also detected in fruits and leaves of *C. monogyna* and *C. laevigata* and the traditionally derived drops (Crataegutt Tropfen).

Characterization of chlorogenic acids: Peaks 69 (t_R 45.6 min), 70 (t_R 47.0 min), and 71 (t_R 51.0 min) with identical molecular formula ($C_{25}H_{24}O_{12}$) were identified as 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid and 4,5-di-O-caffeoylquinic acid (Table 1) by comparison with their commercial standards. 3,5-di-O-caffeoylquinic acid has been previously reported in *C. monogyna* [56], while 3,4-di-O-caffeoylquinic acid and 4,5-di-O-caffeoylquinic acid have been reported here for the first time.

Four caffeoylquinic acids (2-5), four p-coumaroylquinic acids (12-16) and one feruloylquinic acid were identified in TOF-MS mode using accurate mass measurements and MS/MS fragmentations (Table 1) in addition to identification keys previously cited as 3-O-caffeoylquinic acid 2, cis-3-O-caffeoylquinic acid 3, 5-O-caffeoylquinic acid 4, cis-5-O-caffeoylquinic acid 5, 3-p-coumaroylquinic acid 12, cis-3-p-coumaroylquinic acid 13, 4-p-coumaroylquinic acid 14, 5-p-coumaroylquinic acid 15, cis-5-p-coumaroylquinic acid 16 and 5-O-feruloylquinic acid 36 [73-77]. Furthermore, compounds 2 and 4 were confirmed by chromatographic comparisons with authentic standards. Although several chlorogenic acid derivatives were already mentioned in the literature in *C. monogyna* [43,56], compounds 3, 5, 13 and 15 have been reported here for the first time.

Peak 19 (t_R 17.6 min) revealed a deprotonated molecule at m/z 325.0927 and MS/MS fragment ions at m/z 163.0401 and m/z 119.0503 corresponding to the neutral loss of a hexosyl moiety $[M–H^-162]^-$ followed by the neutral loss of carbon dioxide molecule (CO_2). In line with the previous reported MS data [32], this compound was assigned as p-coumaric acid O-hexoside (Figure 3H). Assignment of the position of the hexose (glucose or galactose) was not possible because of the lack of a commercial standard.

At m/z 335.0773 ($C_{16}H_{16}O_9$) one peak was detected at retention time of 29.7 min in the EIC. It generated MS^2 fragment ions at m/z 179, 173, 135 and 191 (Table 2). Based on this fragmentation pattern this compound was tentatively assigned as 3-O-caffeoylshikimic acid 41, apart from the ion at m/z 191, interestingly, it showed however similar MS^2 and MS^3 spectra if compared to the MS^2 and MS^3 spectra of 3-O-caffeoylshikimic acid previously reported [21,78].

Characterization of chlorogenic acids glycosides: The Q-TOF-MS spectra of peaks 6-9 (t_R 11.8, 13.5, 16.5 and 18.1 min) displayed $[M–H]^-$ pseudomolecular ion $[M–H]^-$ at m/z 515.1406. These compounds with the identical molecular formula $C_{22}H_{27}O_{14}$ were proposed to be caffeoylquinic acid glucosides (Table 1 and 2). In addition, they showed a typical fragmentation patterns and UV spectra of chlorogenic acids (Figure 6). Thus, these peaks were tentatively proposed as 3-O-(4'-O-Caffeoyl glucosyl)quinic acid 6, 4-O-(4'-O-Caffeoyl glucosyl)quinic acid 7, 5-O-(3'-O-Caffeoyl glucosyl)quinic acid 8, 5-O-(4'-O-Caffeoyl glucosyl)quinic acid 9. Recently, we have reported these compounds in *Lonicera henryi* [79] and *Ilex glabra* [80].

With retention time of 22.2 min and *m/z* of 515.1407 ($C_{22}H_{28}O_{14}$), a further small peak (10) was observed. Compound 10 exhibited fragmentation patterns identical to the 5-O-(3'-O-Caffeoyl glucosyl) quinic acid (8) and we assumed that it might be a cis isomer of compound 8. For confirmation of this isomer, the extract of *C. monogyna* leaves was irradiated with UV light at 245 nm for 60 min. After irradiation, we found that the cis isomer in the chromatogram as peak with significantly increased intensities if compared to trans isomer from the original plant extract, which confirmed the presence of the cis-5-O-(3'-O-Caffeoyl glucosyl)quinic acid 10 [24]. We have reported the presence of cis derivatives of chlorogenic acids earlier in *L. henryi*, *I. coccinea,* quince fruits, coffee leaves, *Carlina acaulis*, *Helianthus tuberosus*, *Symphyotrichum novae-angliae* and *Rudbeckia hirta,* [21,22,24,25,79]. It is important noting that caffeoylquinic acid glucosides have been reported in hawthorn for the first time.

Conclusion

Crataegus species are medicinal plants used extensively in traditional medicine for the treatment of cardiovascular diseases, arrhythmia and hypertension. Using UPLC-ESI-Q-TOF-MS/MS and HPLC-ESI-MS^n, a total of 113 compounds were tentatively identified. To the best of our knowledge 63 of them are described for the first time

in Crataegus species and two for the first time in nature. In this context, the obtained results indicate that Crataegus species are rich source of phenolic constituents including simple phenolic acids, chlorogenic acids, proanthocyanidins, flavonoids and flavonoids glycosides.

The current study clearly emphasis the need for re-investigation of medicinal plants using powerful state of the art analytical instrumentation, providing additional information on minor secondary metabolites previously not obtainable.

Acknowledgement

This work was supported by Jacobs University Bremen and Deutscher Akademischer Austausch Dienst (DAAD). We are grateful to Mss Anja Müller for excellent technical support.

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Citation: Karar MGE, Kuhnert N (2015) UPLC-ESI-Q-TOF-MS/MS Characterization of Phenolics from *Crataegus monogyna* and *Crataegus laevigata* (Hawthorn) Leaves, Fruits and their Herbal Derived Drops (Crataegutt Tropfen). J Chem Biol Ther 1: 102. doi: 10.4172/2572-0406.1000102