Supplementary information

An untargeted metabolomics approach applied to the study of the bioavailability and metabolism of bioactive compounds. A human intervention study with three different bioactive extracts.

María del Carmen Villegas-Aguilar[1,*], María de la Luz Cádiz-Gurrea[1], María Herranz-López[2], Enrique Barrajón-Catalán[2], David Arráez-Román[1], Álvaro Fernández-Ochoa[1,*,#] and Antonio Segura-Carretero[1,#]

[1]Department of Analytical Chemistry, University of Granada, 18071 Granada, Spain

[2]Institute of Research, Development and Innovation in Biotechnology of Elche (IDiBE) and Molecular and Cell Biology Institute (IBMC), Miguel Hernández University (UMH), 03202 Elche, Spain

Corresponding authors:

María del Carmen Villegas-Aguilar, Department of Analytical Chemistry, University of Granada, 18071 Granada, Spain, marivillegas@ugr.es

Álvaro Fernández-Ochoa, Department of Analytical Chemistry, University of Granada, 18071 Granada, Spain, Tel. +34 958240794, alvaroferochoa@ugr.es

[#] These authors shared author co-senior ship.

Table S1. Identification of phytochemical compounds in *H. sabdariffa* extract by HPLC-ESI-qTOF-MS.

RT (min)	Observed m/z $[M-H]^-$	Theoretical m/z $[M-H]^-$	Mass error (ppm)	Molecular Formula	Level of annotation	Compound name	MS/MS fragments	Relative area	References
0.96	132.0302	132.0302	0.00	$C_4H_7NO_4$	2	Aspartic Acid	88/71	1.7E5	HMDB0000191
0.99	294.0807	294.0831	-8.16	$C_{10}H_{17}NO_9$	2	Fructose-aspartic acid	132/88	1.6E5	[1]
1.07	207.0150	207.0146	1.93	$C_6H_8O_8$	2	Hibiscus acid lactone isomer 1	127	1.3E5	[2]
1.15	207.0159	207.0146	6.28	$C_6H_8O_8$	2	Hibiscus acid lactone isomer 1	127	3.8E4	[2]
1.32	189.0078	189.0041	19.58	$C_6H_6O_7$	2	Hibiscus acid	127	1.6E6	[3]
1.66	203.0215	203.0197	8.87	$C_7H_8O_7$	2	Hibiscus acid monomethyl ester isomer 1	142/157/201	1.5E5	[4]
1.82	203.0215	203.0197	8.87	$C_7H_8O_7$	2	Hibiscus acid monomethyl ester isomer 2	142/157/201	3.0E5	[4]
2.63	217.0332	217.0354	-10.14	$C_8H_{10}O_7$	2	Hibiscus acid dimethylester	155/125	2.5E5	[5]
4.02	353.0867	353.0878	-3.12	$C_{16}H_{18}O_9$	2	Chlorogenic acid isomer 1	191/135	7.7E4	HMDB0003164
4.20	297.0266	297.0252	4.71	$C_{12}H_{10}O_9$	2	Methyl gallate derivative isomer 1	125/82	6.2E4	[6]
4.98	297.0266	297.0252	4.71	$C_{12}H_{10}O_9$	2	Methyl gallate derivative isomer 2	125/82	6.9E4	[6]
6.34	353.0867	353.0878	-3.12	$C_{16}H_{18}O_9$	2	Chlorogenic acid isomer 2	191/135	6.3E4	HMDB0003164
6.89	353.0867	353.0878	-3.12	$C_{16}H_{18}O_9$	2	Chlorogenic acid isomer 3	191/135	8.2E4	HMDB0003164
8.56	335.0771	335.0772	-0.30	$C_{16}H_{16}O_8$	2	5-O-Caffeoylshikimic acid	161/191/133	3.4E4	[5]

8.90	367.1030	367.1035	-1.36	$C_{17}H_{20}O_9$	2	3-O-Feruloylquinic acid	135/179	1.4E4	HMDB0030669
9.60	609.1458	609.1461	-0.49	$C_{27}H_{30}O_{16}$	3	Quercetin 3-O-rutinoside	-	4.1E5	HMDB0037934
9.84	463.0884	463.0882	0.43	$C_{21}H_{20}O_{12}$	2	Quercetin 7-glucoside	301/463	1.0E4	HMDB0302151
10.23	539.1751	539.1770	-3.52	$C_{25}H_{32}O_{13}$	3	Oleuropein	-	1.6E4	HMDB0035872
10.96	317.0294	317.0303	-2.84	$C_{15}H_{10}O_8$	2	Myrecetin	151/107	1.2E4	HMDB0002755
11.07	312.1239	312.1241	-0.64	$C_{18}H_{19}NO_4$	2	N-Feruloyltyramine	148/190	1.9E4	Pubchem: 6440659
12.04	522.3276	522.3283	-1.34	$C_{25}H_{49}NO_{10}$	4	Unknown	-	1.1E4	-
12.15	301.0339	301.0354	-4.98	$C_{15}H_{10}O_7$	1	Quercetin	151/121/65	4.3E4	HMDB0005794
12.95	329.0654	329.0667	-3.95	$C_{17}H_{14}O_7$	2	3,7-Dimethylquercetin	285/299/314	2.4E4	HMDB0029263
13.05	299.0538	299.0561	-7.69	$C_{16}H_{12}O_6$	3	Kaempferide	-	2.4E4	Pubchem: 5281666
13.13	394.2945	394.2936	2.28	$C_{20}H_{43}O_7$	4	Unknown	-	2.9E4	-
13.56	343.0821	343.0823	-0.58	$C_{18}H_{16}O_7$	3	Eupatorin isomer 1	-	1.4E4	HMDB0252128
13.61	343.0818	343.0823	-1.46	$C_{18}H_{16}O_7$	3	Eupatorin isomer 2	-	2.8E4	HMDB0252128
13.69	313.0684	313.0718	-10.86	$C_{17}H_{14}O_6$	3	Cirsimaritin	-	2.6E4	Pubchem: 188323
13.92	293.1767	293.1758	3.07	$C_{17}H_{26}O_4$	3	Gingerol	236/221	3.0E4	HMDB0005783
15.74	293.2107	293.2122	-5.12	$C_{18}H_{30}O_3$	3	Hydroxylinolenic acid isomer 1	-	3.8E4	HMDB0011108
15.81	293.2119	293.2122	-1.02	$C_{18}H_{30}O_3$	3	Hydroxylinolenic acid isomer 2	-	3.5E4	HMDB0011108

15.85	293.2113	293.2122	-3.07	$C_{18}H_{30}O_3$	3	Hydroxylinolenic acid isomer 3	-	4.7E4	HMDB0011108
16.41	295.2265	295.2279	-4.74	$C_{18}H_{32}O_3$	3	Hydroxylinoleic acid	-	5.5E4	HMDB0247599
18.46	277.2159	277.2173	-5.05	$C_{18}H_{30}O_2$	2	Linolenic acid	205/97	1.2E5	HMDB0001388
18.73	375.2712	375.2752	-10.66	$C_{20}H_{40}O_6$	2	Myristyl glucoside	291/311	3.8E4	Pubchem: 6453025
19.15	279.2328	279.2330	-0.72	$C_{18}H_{32}O_2$	3	Linoleic acid	-	5.8E4	HMDB0000673
19.86	403.3052	403.3065	-3.22	$C_{22}H_{44}O_6$	3	Hexadecyl D-glucoside	-	9.0E4	Pubchem: 171356
19.94	281.2508	281.2486	7.82	$C_{18}H_{34}O_2$	2	Oleic Acid	211/101	2.9E4	HMDB0000207
20.81	443.2490	443.2439	11.51	$C_{26}H_{36}O_6$	3	Bufotalin	-	2.1E4	Pubchem: 12302120
20.97	383.1916	383.1923	-1.83	$C_{16}H_{32}O_{10}$	3	Hexanedioic acid derivative	-	5.6E4	Pubchem: 88032455

RT: Retention Time.

Table S2. Identification of phytochemical compounds in *S. marianum* extract by HPLC-ESI-qTOF-MS.

RT (min)	Observed m/z $[M-H]^-$	Theoretical m/z $[M-H]^-$	Mass error (ppm)	Molecular Formula	Level of annotation	Compound name	MS/MS fragments	Relative area	References
0.80	112.9866	112.9880	-12.39	$C_4H_2O_4$	3	Acetylenedicarboxylic acid	-	1.9E4	HMDB0247933
0.90	134.0479	134.0472	5.22	$C_5H_5N_5$	3	Adenine	-	1.1E4	HMDB0000034
0.99	179.0556	179.0561	-2.79	$C_6H_{12}O_6$	3	Fructose	-	4.4E4	HMDB0000660
1.06	266.0883	266.0894	-4.13	$C_{10}H_{13}N_5O_4$	3	Adenosine	-	7.9E2	HMDB0000050
1.09	117.0130	-	-	-	4	Unknown	-	1.5E4	-
1.32	197.8088	-	-	-	4	Unknown	-	2.5E4	-
6.57	303.0492	303.0510	-5.94	$C_{15}H_{12}O_7$	2	Dihydroquercetin	285/125	2.7E4	[7]
6.87	353.0881	353.0878	0.85	$C_{16}H_{18}O_9$	2	Chlorogenic acid	191/161	2.3E4	HMDB0003164
7.75	373.1487	373.1504	-4.56	$C_{17}H_{26}O_9$	2	Deoxyloganin	165/135	6.7E4	Pubchem: 440906
7.93	441.1949	441.1978	-6.57	$C_{18}H_{34}O_{12}$	2	Hexo-Glucose	395/249	4.9E4	Pubchem: 129630443
8.05	505.1915	505.1927	-2.38	$C_{22}H_{34}O_{13}$	2	Oleuropeic acid derivative isomer 1	459/293	3.6E4	[8]
8.41	475.1843	475.1821	4.63	$C_{21}H_{32}O_{12}$	3	Cistanoside E	-	2.5E4	Pubchem: 21632979
8.58	505.1935	505.1927	1.58	$C_{22}H_{34}O_{13}$	2	Oleuropeic acid derivative isomer 2	459/293	3.1E4	[8]
8.92	459.1856	459.1872	-3.48	$C_{21}H_{32}O_{11}$	2	Apiosylepirhododendrin	89/59	8.9E4	Pubchem: 101287073
8.99	163.0389	163.0401	-7.36	$C_9H_8O_3$	2	4-Hydroxycinnamic acid	119	6.3E4	HMDB0002035

9.12	193.0141	193.0142	-0.52	$C_9H_6O_5$	2	Trihydroxycoumarin	137/93	2.7E5	[9]
9.26	481.1161	481.1140	4.36	$C_{25}H_{22}O_{10}$	2	Silybin isomer 1	125/178	2.6E5	MoNA:VF-NPL-QTOF009680
9.42	455.2130	455.2134	-0.88	$C_{19}H_{36}O_{12}$	3	Alkyl glicoside	-	1.3E5	[10]
9.58	303.0512	303.0510	0.66	$C_{15}H_{12}O_7$	2	Dihydroquercetin	285/125	5.1E5	[7]
9.73	607.1081	607.1093	-1.98	$C_{30}H_{24}O_{14}$	2	(Epi)gallocatechin-A-(epi)gallocatechin	285/303	4.5E5	[11]
9.82	187.0957	187.0976	-10.16	$C_9H_{16}O_4$	3	Azelaic acid	-	5.3E4	HMDB0000784
9.87	433.1123	433.1140	-3.93	$C_{21}H_{22}O_{10}$	2	Naringenin 4'-O-glucoside	271/151	5.1E4	Pubchem: 42607906
10.12	481.1125	481.1140	-3.12	$C_{25}H_{22}O_{10}$	2	Silybin isomer 2	125/178	7.0E4	MoNA:VF-NPL-QTOF009680
10.25	483.1293	483.1297	-0.83	$C_{25}H_{24}O_{10}$	2	Silybin hydrogenated	125/151/285	1.4E5	[12]
10.35	813.3162	813.3187	-3.07	$C_{38}H_{54}O_{19}$	2	Tricrocin	767/473	9.1E4	Pubchem: 22833598
10.46	383.1604	383.1612	-2.09	$C_{21}H_{24}N_2O_5$	3	Alschomine	-	1.3E5	Pubchem: 11969856
10.54	675.3212	675.3174	5.63	$C_{39}H_{48}O_{10}$	2	Gambogoic acid A	629/293	1.5E5	ChemBK: 1592842-93-7
10.61	481.1120	481.1140	-4.16	$C_{25}H_{22}O_{10}$	2	Silybin isomer 3	125/178	3.5E5	MoNA:VF-NPL-QTOF009680
10.69	287.0516	287.0561	-15.68	$C_{15}H_{12}O_6$	2	Aromadendrin	125/259	3.8E5	HMDB0030847
10.75	317.0655	317.0667	-3.78	$C_{16}H_{14}O_7$	2	(+)-Dihydroisorhamnetin	245/259	1.7E5	HMDB0037501
10.96	481.1114	481.1140	-5.40	$C_{25}H_{22}O_{10}$	2	Silychrystin isomer 1	151/125	1.8E5	MoNA:RIKENPlaSMA007

									844
11.00	481.1139	481.1140	-0.21	$C_{25}H_{22}O_{10}$	2	Silybin isomer 3	125/178	1.7E5	MoNA:VF-NPL-QTOF009680
11.03	481.1150	481.1140	2.08	$C_{25}H_{22}O_{10}$	2	Silychrystin isomer 2	107/125/151/325/463	2.9E5	MoNA:RIKENPlaSMA007844
11.08	481.1146	481.1140	1.25	$C_{25}H_{22}O_{10}$	2	Silychrystin isomer 3	125/151/178/325/355/433	2.4E5	MoNA:MetaboBASE0687
11.54	677.1892	677.1876	2.36	$C_{35}H_{34}O_{14}$	4	Unknown	-	1.5E5	-
11.85	685.3060	685.3077	-2.48	$C_{33}H_{50}O_{15}$	2	Pterocecide B	477/639	6.1E4	Pubchem: 122228272
11.90	659.1765	659.1770	-0.76	$C_{35}H_{32}O_{13}$	2	Phylloflavanine	178/125	1.1E5	[13]
12.06	481.1130	481.1140	-2.08	$C_{25}H_{22}O_{10}$	2	Silybin isomer 4	125/152/301	1.3E5	MoNA:VF-NPL-QTOF009680
12.12	481.1176	481.1140	7.48	$C_{25}H_{22}O_{10}$	2	Silybin isomer 5	125/152/301	2.5E5	MoNA:VF-NPL-QTOF009680
12.26	481.1172	481.1140	6.65	$C_{25}H_{22}O_{10}$	2	Silybin isomer 6	125/152/273	2.6E5	MoNA:VF-NPL-QTOF009680
12.34	481.1158	481.1140	3.74	$C_{25}H_{22}O_{10}$	2	Silybin isomer 7	125/152/301	3.3E5	MoNA:VF-NPL-QTOF009680
12.44	481.1120	481.1140	-4.16	$C_{25}H_{22}O_{10}$	2	Silybin isomer 8	125/152/301	2.1E5	MoNA:VF-NPL-QTOF009680
12.48	481.1156	481.1140	3.33	$C_{25}H_{22}O_{10}$	2	Silybin isomer 9	125/152/301	2.2E5	MoNA:VF-NPL-QTOF009680
12.67	381.2269	381.2282	-3.41	$C_{21}H_{34}O_6$	3	Sarcostin	-	6.2E4	Pubchem: 46173994

12.94	477.0837	477.0827	2.10	$C_{25}H_{18}O_{10}$	3	Pradinone I	-	2.0E4	Pubchem: 460846
13.01	477.2489	477.2494	-1.05	$C_{26}H_{38}O_8$	3	Adenanthin B isomer 1	-	3.4E4	[14]
13.12	811.1896	811.1938	-5.18	$C_{35}H_{40}O_{22}$	3	Kaempferol 3-[2''-glucosyl-6''-acetylgalactoside] 7-glucoside	-	8.7E4	FDB016214
13.42	989.2502	989.2510	-0.81	$C_{52}H_{46}O_{20}$	4	Unknown	-	2.8E5	-
13.70	523.1249	523.1246	0.57	$C_{27}H_{24}O_{11}$	3	Acetylsilybin A/B	-	1.0E5	Pubchem: 10236891
13.78	477.2522	477.2493	6.08	$C_{26}H_{38}O_8$	3	Adenanthin B isomer 2	-	1.2E5	[14]
14.14	479.0975	479.0984	-1.88	$C_{25}H_{20}O_{10}$	2	Dehydrosilybin	125/177/179/283/327/449	6.2E4	HMDB0040513
14.87	627.1569	627.1567	0.32	$C_{27}H_{32}O_{17}$	2	Vitexia-glucoside	152/271/435/463	4.2E4	Pubchem: 56933064
15.02	313.2378	313.2384	-1.92	$C_{18}H_{34}O_4$	3	Octadecanedioic acid	-	4.2E4	Pubchem: 70095
15.61	564.3351	-	-	-	4	Unknown	-	8.3E4	-
15.71	476.2772	476.2780	-1.68	$C_{27}H_{41}O_7$	3	Sinapoyloxypalmitate	-	3.1E4	Pubchem: 90657145
15.83	564.3364	-	-	-	4	Unknown	-	2.4E5	-
16.04	540.3289	540.3304	-2.78	$C_{29}H_{49}O_9$	3	Steroid compound	-	3.7E4	[15]
16.30	540.3306	540.3304	0.37	$C_{29}H_{49}O_9$	3	Steroid compound	-	1.6E5	[15]
16.37	566.3453	-	-	-	4	Unknown	-	6.0E4	-
16.60	566.3530	-	-	-	4	Unknown	-	2.1E5	-
18.66	591.4117	591.4114	0.51	$C_{31}H_{60}O_{10}$	4	Unknown	-	6.6E4	-

18.73	375.2712	375.2752	-10.66	$C_{20}H_{40}O_6$	2	Myristyl glucoside	291/311	2.9E4	Pubchem: 6453025
19.16	279.2328	279.2330	-0.72	$C_{18}H_{32}O_2$	3	Linoleic acid	-	5.6E4	HMDB0000673
19.74	577.3730	577.3746	-2.77	$C_{33}H_{54}O_8$	3	Timosaponin A	-	4.1E4	[16]
19.85	403.3052	403.3065	-3.22	$C_{22}H_{44}O_6$	3	Hexadecyl D-glucoside	-	8.5E4	Pubchem: 171356
19.94	281.2508	281.2486	7.82	$C_{18}H_{34}O_2$	2	Oleic Acid	211/101	6.4E4	HMDB0000207
20.62	605.4040	605.4059	-3.14	$C_{35}H_{58}O_8$	3	Deoxybafilomycin A1	-	3.5E4	CAS: 1883587-79-8
20.98	621.4417	621.4372	7.24	$C_{36}H_{62}O_8$	3	Ginsenoside Rh2	-	2.1E5	Pubchem:119307
21.56	633.4375	633.4371	0.63	$C_{37}H_{62}O_8$	3	Karaviloside III	-	5.4E4	CHEMBL2335924
22.92	371.2592	371.2592	0.00	$C_{24}H_{36}O_3$	3	Pelandjauic acid	-	1.6E5	Pubchem: 178575
24.49	373.2751	373.2748	0.80	$C_{24}H_{38}O_3$	3	Dehydrolithocholic acid	-	1.4E5	Pubchem: 4446994

RT: Retention Time.

Table S3. Identification of phytochemical compounds in *T. cacao* extract by HPLC-ESI-qTOF-MS.

RT (min)	Observed m/z $[M-H]^-$	Theoretical m/z $[M-H]^-$	Mass error (ppm)	Molecular Formula	Level of annotation	Compound name	MS/MS fragments	Relative area	Phenolic content (mg/g dry extract)	References
0.75	272.9512	-	-	-	4	Unknown	-	5.5E4		-
0.91	203.1035	203.1037	-0.98	$C_8H_{16}N_2O_4$	2	Valylserine	74/141/173	5.9E4		Pubchem: 18218236
0.92	217.1187	217.1194	-3.22	$C_9H_{18}N_2O_4$	2	Leucylserine	74/129/173	7.7E4		Pubchem: 3621685
1.01	195.0519	195.0510	4.61	$C_6H_{12}O_7$	2	Gluconic acid	75/129/177	4.3E5		HMDB0000625
1.13	191.0207	191.0197	5.24	$C_6H_8O_7$	2	Citric acid isomer 1	87/111	3.9E4		HMDB0000094
1.33	191.0228	191.0197	16.23	$C_6H_8O_7$	2	Citric acid isomer 2	87/111	1.8E5		HMDB0000094
1.69	96.9605	96.9601	4.13	H_2O_4S	3	Sulfate	-	7.1E5		HMDB0001448
2.67	117.0554	117.0557	-2.56	$C_5H_{10}O_3$	2	3-Hydroxyvaleric acid	71/99	6.4E4		HMDB0000531
4.66	294.0606	294.0619	-4.42	$C_{13}H_{13}NO_7$	2	N-caffeoyl-L-aspartate isomer 1	88/132/179	9.7E4		[17]
5.05	294.0610	294.0619	-3.06	$C_{13}H_{13}NO_7$	2	N-caffeoyl-L-aspartate isomer 2	88/132/179	1.2E5		[17]
5.40	131.0704	131.0714	-7.63	$C_6H_{12}O_3$	2	L-Leucate	85	2.4E5		Pubchem: 69955
5.61	407.1554	407.1559	-1.23	$C_{17}H_{28}O_{11}$	3	Secoiridoid derived	99/263/305	3.1E4		[18]
6.01	289.0721	289.0718	1.04	$C_{15}H_{14}O_6$	1	Catechin	161/163/174/192	2.0E4	0.18	HMDB0001871
6.88	278.0670	278.0670	0.00	$C_{13}H_{13}NO_6$	2	L-Aspartic acid. N-[3-(4-hydroxyphenyl)-1-oxo-2-	71/93/119	1.3E5		[17]

						propenyl]				
7.01	165.0554	165.0557	-1.82	$C_9H_{10}O_3$	2	Dihydro-p-coumaric acid	72/103/147	2.2E5		HMDB0002199
7.60	289.0716	289.0718	-0.69	$C_{15}H_{14}O_6$	2	Epicatechin	161/163/174/192	1.4E5	1.95	HMDB0002780
7.80	275.1006	-	-	-	4	Unknown	-	8.7E4		-
8.01	577.1352	577.1352	0.00	$C_{30}H_{26}O_{12}$	2	Procyanidin B1	-	4.6E4	0.79	[17]
8.37	437.2040	437.2028	2.74	$C_{19}H_{34}O_{11}$	2	Ebracteatoside D isomer 1	57/99/161	6.5E4		-
8.61	167.0366	167.0350	9.58	$C_8H_8O_4$	3	Vanillic acid	-	1.0E5		HMDB0000484
8.79	865.1988	865.1985	0.35	$C_{45}H_{38}O_{18}$	2	Procyanidin C	289/577	2.6E4	0.47	[17]
9.18	305.0690	305.0700	-3.28	$C_{12}H_{18}O_7S$	2	Hydroxy-jasmonic acid sulfate	225/67/59	7.6E5		[19]
9.51	437.2057	437.2028	6.63	$C_{19}H_{34}O_{11}$	2	Ebracteatoside D isomer 2	57/99/161	2.2E4		-
9.70	737.1750	737.1723	3.66	$C_{36}H_{34}O_{17}$	2	(Epi)catechin dimer hexose	449/611/539/289	1.9E4		[17]
9.85	463.0878	463.0882	-0.86	$C_{21}H_{20}O_{12}$	1	Quercetin glucoside	149/242/271/285 /300/335	3.3E4		HMDB0037362
9.93	707.1787	707.1828	-5.80	$C_{32}H_{36}O_{18}$	2	Kalambroside A	449/539/581/289	1.9E4		Pubchem: 10349838
9.99	516.2476	516.2450	5.04	$C_{24}H_{39}NO_{11}$	4	Unknown	-	2.8E4		[17]
10.18	326.1075	326.1034	12.57	$C_{18}H_{17}NO_5$	2	Deoxyclovamide (N-[(2E)-3-(3.4-Dihydroxyphenyl)-1-oxo-2-propen-1-yl]-L-tyrosine)	206/282/119	1.7E4		[17]
10.24	433.0806	433.0776	6.93	$C_{20}H_{18}O_{11}$	2	Quercetin arabinoside	300/271	3.5E4		[17]
10.45	238.1091	238.1085	2.52	$C_{12}H_{17}NO_4$	3	Dihydroisoflavipucine	-	1.3E4		Pubchem:

										38354263
10.75	272.0935	272.0928	2.57	$C_{15}H_{15}NO_4$	2	L-Thyronine	94/124	1.4E4		HMDB0000667
11.09	421.2064	421.2079	-3.56	$C_{19}H_{34}O_{10}$	2	1-Octen-3-yl primeveroside	57/97/277	1.3E5		HMDB0032960
12.16	301.0339	301.0354	-4.98	$C_{15}H_{10}O_7$	1	Quercetin	151/121/65	1.3E4		HMDB0005794
12.36	391.1196	391.1187	2.30	$C_{23}H_{20}O_6$	2	Dehydrodeguelin	117/62	1.6E4		[20]
12.46	329.2329	329.2333	-1.21	$C_{18}H_{34}O_5$	2	5,8,12-Trihydroxy-9-octadecenoic acid isomer 1	211	4.1E4		HMDB0030936
12.57	329.2358	329.2333	7.59	$C_{18}H_{34}O_5$	2	5,8,12-Trihydroxy-9-octadecenoic acid isomer 2	211	1.0E4		HMDB0030936
12.79	357.1215	357.1191	6.72	$C_{16}H_{22}O_9$	2	Sweroside	97/198	1.5E5		[17]
13.13	394.2954	394.2963	-2.28	$C_{23}H_{41}NO_4$	4	Unknown	-	2.5E4		-
13.47	276.1227	276.1241	-5.07	$C_{15}H_{19}NO_4$	3	Barmumycin	-	4.1E4		[21]
13.92	293.1748	293.1758	-3.41	$C_{17}H_{26}O_4$	2	Gingerol	236/221	2.9E4		HMDB0005783
14.07	333.1815	333.1820	-1.50	$C_{18}H_{26}N_2O_4$	3	Hydroxyhuperzine	-	1.9E4		[22]
14.20	367.1671	367.1663	2.18	$C_{21}H_{24}N_2O_4$	3	3,4-dimethoxy-N-[4-[oxo(1-piperidinyl)methyl]phenyl]benzamide	-	2.3E4		CHEBI:114263
14.77	195.0686	195.0663	11.79	$C_{10}H_{12}O_4$	2	Ethyl vanillate	123	6.9E4		[23]
15.61	564.3295	-	-	-	4	Unknown	-	3.1E4		-
15.71	476.2780	476.2780	0.00	$C_{27}H_{41}O_7$	2	Sinapoyloxypalmitate	279	5.1E4		Pubchem: 90657145

15.83	564.3292	-	-	-	4	Unknown	-	1.8E5		-
16.30	540.3288	-	-	-	4	Unknown	-	1.1E5		-
16.60	566.3492	-	-	-	4	Unknown	-	2.3E5		-
17.58	299.2585	299.2592	-2.34	$C_{18}H_{36}O_3$	3	Hydroxyoctadecanoic acid	-	7.3E4		HMDB0112182
17.81	297.2424	297.2435	-3.70	$C_{18}H_{34}O_3$	2	Oxooctadecanoic acid	253/155	6.1E4		HMDB0030981
18.46	277.2159	277.2173	-5.05	$C_{18}H_{30}O_2$	2	Linolenic acid	205/97	6.7E4		HMDB0001388
18.73	375.2712	375.2752	-10.66	$C_{20}H_{40}O_6$	2	Myristyl glucoside	291/311	4.5E4		Pubchem: 6453025
19.16	279.2328	279.2330	-0.72	$C_{18}H_{32}O_2$	3	Linoleic acid	-	1.4E5		HMDB0000673
19.82	255.2325	255.2330	-1.96	$C_{16}H_{32}O_2$	2	Palmitic acid	190/110	1.6E5		HMDB0000220
19.87	403.3052	403.3065	-3.22	$C_{22}H_{44}O_6$	3	Hexadecyl D-glucoside	-	9.9E4		Pubchem: 171356
19.95	281.2482	281.2486	-1.42	$C_{18}H_{34}O_2$	2	Oleic Acid	211/101	1.6E5		HMDB0000207
20.98	383.1934	383.1923	2.87	$C_{16}H_{32}O_{10}$	3	Hexanedioic acid derivative	-	1.2E5		Pubchem: 88032455

RT: Retention Time.

Table S4. Unknown compounds detected in plasma samples obtained after intake of an extract of *H. sabdariffa*, *S. marianum* and *T. cacao*.

RT (min)	*H. sabdariffa**	*S. marianum**	*T. cacao**	Observed m/z	Adduct	Theoretical m/z	Mass error (ppm)	Molecular formula	MS/MS fragments
1.2	8	0	0	83.0139	$(M-H)^-$	83.0139	1.14	$C_4H_4O_2$	-

1.2	6	0	0	503.0761	$(M-H)^-$	503.0772	-2.23	$C_{30}H_{16}O_8$	313.0631; 189.0036
1.4	6	0	0	203.9975	$(M-H)^-$	203.9945	14.46	$C_3H_9O_8S$	-
2.0	6	0	0	141.0192	$(M-H)^-$	141.0193	-0.65	$C_6H_6O_4$	-
4.1	0	0	7	181.0366	$(M-H)^-$	181.0354	6.63	$C_5H_{10}O_7$	134.0356; 107.0499; 164.0357
5.5	8	0	0	141.0192	$(M-H)^-$	141.0193	-1.05	$C_6H_6O_4$	-
10.4	0	0	8	167.0378	$(M-H)^-$	167.0384	-3.39	$C_5H_{12}O_4S$	96.9588
11.8	0	5	0	368.0475	$(M-H)^-$	368.0538	-16.93	$C_{19}H_{13}O_8$	175.0267
12.2	0	5	0	833.1774	$(M-H)^-$	-	-	-	-
12.8	0	5	0	833.1775	$(M-H)^-$	-	-	-	-
13.0	0	5	0	245.0487	$(M-H)^-$	245.0489	-1.03	$C_{10}H_{14}O_5S$	
13.0	0	4	0	368.5492	$(M-H)^-$	-	-	-	280.0324; 113.0260
13.1	0	8	0	368.0477	$(M-H)^-$	368.0538	-16.53	$C_{19}H_{13}O_8$	175.0267
13.2	0	4	0	404.9896	$(M-H)^-$	404.9922	-6.36	$C_{17}H_{10}O_{10}S$	-
13.4	0	6	0	368.5492	$(M-H)^-$	-	-	-	280.0324; 113.0261
13.4	0	4	0	535.1814	$(M-H)^-$	535.1821	-1.28	$C_{26}H_{32}O_{12}$	175.0222; 113.0299
13.5	0	8	0	368.0477	$(M-H)^-$	368.0538	-16.57	$C_{19}H_{13}O_8$	175.0267
13.5	0	5	0	759.0845	$(M-H)^-$	-	-	-	-
13.5	0	5	0	402.0405	$(M-H)^-$	-	-	-	-
13.5	0	6	0	549.1608	$(M-H)^-$	549.1614	-0.97	$C_{26}H_{30}O_{13}$	459.3905; 174.0624
14.5	0	5	0	549.1016	$(M-H)^-$	-	-	-	371.1065;
14.7	0	5	0	747.1153	$(M-H)^-$	747.1143	1.39	$C_{19}H_{40}O_{26}S_2$	-
15.6	0	6	0	549.1015	$(M-H)^-$	-	-	-	371.1065
15.6	0	5	0	503.0954	$(M-H)^-$	503.0984	-5.89	$C_{27}H_{20}O_{10}$	-
15.6	0	4	0	511.2542	$(M-H)^-$	-	-	-	175.0208; 113.0194
15.7	0	6	0	549.1013	$(M-H)^-$	-	-	-	371.1065

15.9	0	7	0	511.2543	$(M-H)^-$	511.2549	-1.08	$C_{26}H_{40}O_{10}$	175.0208; 113.0194
16.0	0	5	0	549.1013	$(M-H)^-$	-	-	-	371.1065
17.1	4	0	0	227.1285	$(M-H)^-$	227.1289	-1.78	$C_{12}H_{20}O_4$	157.1219;182.0693
17.5	0	0	5	326.1222	$(M-H)^-$	-	-	-	-
17.5	0	0	4	416.0911	$(M-H)^-$	-	-	-	240.1475; 295.2614; 159.0587
17.5	0	0	5	348.1038	$(M-H)^-$	348.1037	0.38	$C_{18}H_{21}O_5S$	-
17.6	0	0	4	511.3020	$(M-H)^-$	-	-	-	-
17.6	0	0	4	788.2430	$(M-H)^-$	-	-	-	-
18.5	0	0	4	314.2404	$(M-H)^-$	-	-	-	-
18.6	0	0	5	435.2759	$(M-H)^-$	-	-	-	-
19.3	0	0	5	495.2574	$(M-H)^-$	-	-	-	287.9892; 147.0447
20.5	0	0	4	634.2370	$(M-H)^-$	-	-	-	-
29.0	5	0	0	489.3554	$(M-H)^-$	489.3585	-6.36	$C_{30}H_{50}O_5$	-
29.3	5	0	0	690.3819	$(M-H)^-$	-	-	-	-
29.4	4	0	0	675.4551	$(M-H)^-$	-	-	-	-
29.5	5	0	0	930.6609	$(M-H)^-$	-	-	-	-
32.7	5	0	0	774.5459	$(M-H)^-$	-	-	-	-

*Number of volunteers in whom the metabolite appears; RT: retention time.

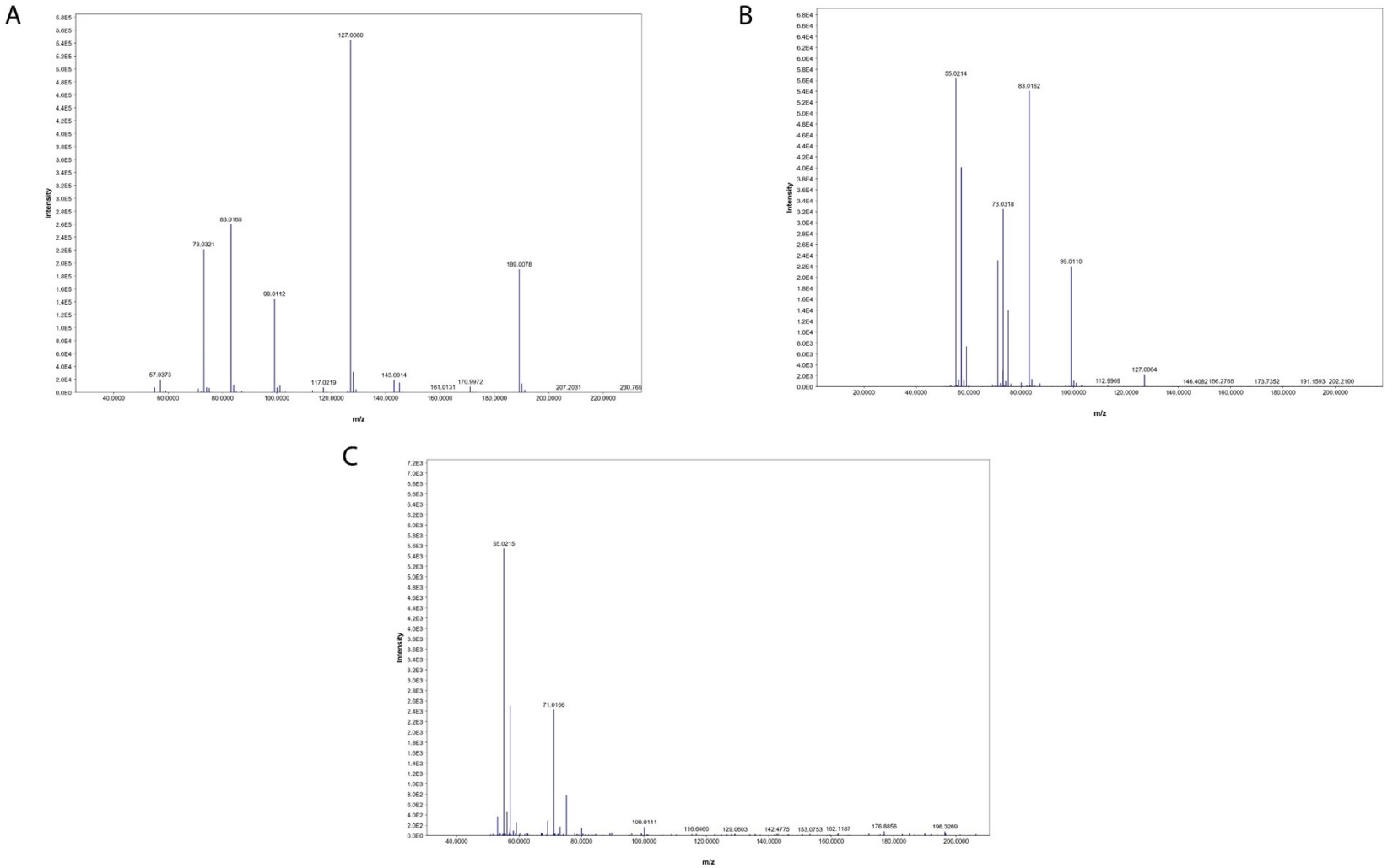

Figure S1. Mass fragmentation spectrum of analytical standard Hydroxycitric acid lactone (Hibiscus acid) (m/z: 189.0078). A: collision energies: 10 eV. B: collision energies: 30 eV. C: collision energies: 60 eV.

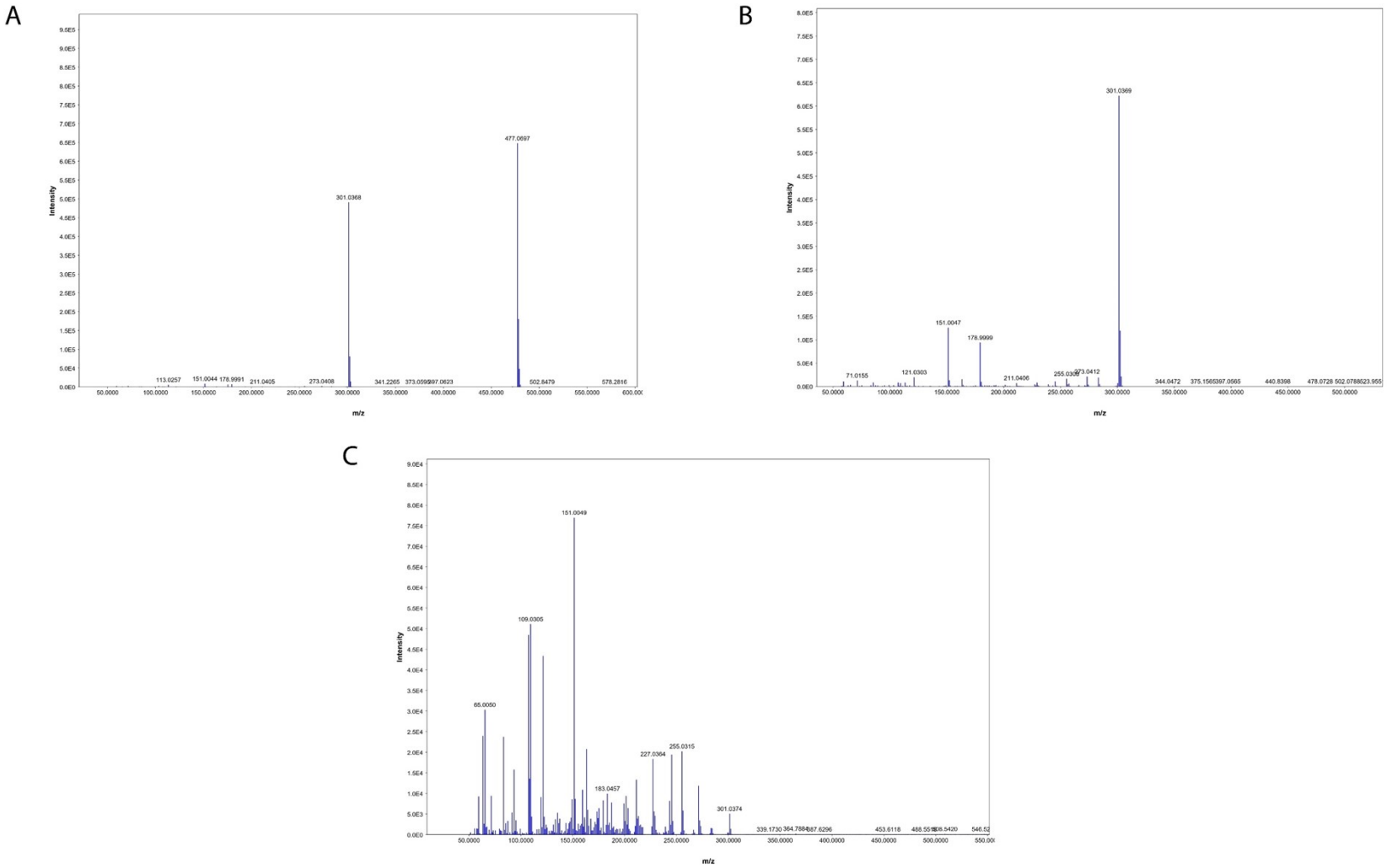

Figure S2. Mass fragmentation spectrum of analytical standard Quercetin glucuronide (m/z: 477.0697). A: collision energies: 10 eV. B: collision energies: 30 eV. C: collision energies: 60 eV.

Quantification of flavan-3-ols and proanthocyanidins by HPLC-ESI-QTOF-MS in *T. cacao* extract

To justify the low number of circulating metabolites detected in plasma samples from the TC extract, the content of catechin and epicatechin in the original extract was quantified, as these compounds have been identified in previous studies as the common bioavailable compounds from the cocoa matrix [24–26]. In addition, the quantification of the two proanthocyanidins identified in the cocoa extract has been included, as these compounds are composed of flavan-3-ol monomers. The quantification was performed using the same HPLC-ESI-QTOF-MS method that had been previously used for the qualitative characterization of the extract [27]. A calibration curve using the commercial analytical standard of catechin (catechin ≥97% from Sigma-Aldrich (Steinheim, Germany)) was used for all compounds. Different standard solution at different concentrations (from 0.39 to 6.25 mg/L) and the TC extract at 1000 mg/L were prepared and analysed by the HPLC-ESI-QTOF-MS. The analytical parameters of the catechin calibration curve are summarised in **Table S5**. Based on the calibration curve and the chromatographic areas for the TC extract, concentrations of 0.18 mg/(g of extract), 1.96 mg/(g of extract), 0.79 mg/(g of extract) and 0.47 mg/(g of extract) were obtained for catechin, epicatechin, procyanidin B1 and procyanidin C, respectively.

Table S5. Standard calibration curve used for the quantification of the compounds of interest in TC extract.

Analytical standard	LOD (mg/L)	LOQ (mg/L)	Calibration Range (mg/L)	Calibration Equation	R^2
Catechin	0.074 ± 0.004	0.25 ± 0.01	(LOQ – 6.25)	y = 29638x - 4779.6	0.998

LOD: Limit of Detection; LOQ: Limit of Quantification, R^2: Coefficient of variation.

References

1 Z. Luo, G. Yu, X. Chen, Y. Liu, Y. Zhou, G. Wang and Y. Shi, Integrated phytochemical analysis based on UHPLC-LTQ-Orbitrap and network pharmacology approaches to explore the potential mechanism of: Lycium ruthenicum Murr. for ameliorating Alzheimer's disease, *Food Funct*, 2020, **11**, 1362–1372.

2 I. C. Rodríguez-Medina, R. Beltrán-Debón, V. M. Molina, C. Alonso-Villaverde, J. Joven, J. A. Menéndez, A. Segura-Carretero and A. Fernández-Gutiérrez, Direct characterization of aqueous extract of Hibiscus sabdariffa using HPLC with diode array detection coupled to ESI and ion trap MS, *J Sep Sci*, 2009, **32**, 3441–3448.

3 S. Pimentel-Moral, I. Borrás-Linares, J. Lozano-Sánchez, D. Arráez-Román, A. Martínez-Férez and A. Segura-Carretero, Microwave-assisted extraction for Hibiscus sabdariffa bioactive compounds, *J Pharm Biomed Anal*, 2018, **156**, 313–322.

4 M. S. Hifnawy, M. A. Aboseada, H. M. Hassan, A. M. AboulMagd, A. F. Tohamy, S. H. Abdel-Kawi, M. E. Rateb, E. M. B. El Naggar, M. Liu, R. J. Quinn, H. A. Alhadrami and U. R.

Abdelmohsen, Testicular Caspase-3 and β-Catenin Regulators Predicted via Comparative Metabolomics and Docking Studies, *Metabolites*, 2020, **10**, 31.

5 M. del C. Villegas-Aguilar, F. J. Leyva-Jiménez, M. de la L. Cádiz-Gurrea, A. Segura-Carretero and D. Arráez-Román, Comprehensive Analysis of Antioxidant Compounds from Lippia citriodora and Hibiscus sabdariffa Green Extracts Attained by Response Surface Methodology, *Antioxidants*, 2020, **9**, 1175.

6 J. T. Pierson, G. R. Monteith, S. J. Roberts-Thomson, R. G. Dietzgen, M. J. Gidley and P. N. Shaw, Phytochemical extraction, characterisation and comparative distribution across four mango (Mangifera indica L.) fruit varieties, *Food Chem*, 2014, **149**, 253–263.

7 K. Ferysiuk, K. M. Wójciak and M. Materska, Phytochemical profile of *Silybum marianum* (L.) *Gaertn* . and *Graminis rhizoma* and its influence on the bioactivity and shelf life of industrially produced pâté, *Int J Food Sci Technol*, 2020, **55**, 1586–1598.

8 L. W. Tian, Y. J. Zhang, Y. F. Wang, C. C. Lai and C. R. Yang, Eucalmaidins A-E, (+)-oleuropeic acid derivatives from the fresh leaves of Eucalyptus maideni, *J Nat Prod*, 2009, **72**, 1608–1611.

9 M. Waseem, R. Basra and M. Asim, *Molecular Docking and Computational Exploration of Isolated Drugs from Daphne Species Against COVID-19*, 2021, vol. 40.

10 G. Vidal and C. Ccapatinta, 2018.

11 A. Singh, S. Kumar and B. Kumar, LC-MS Identification of Proanthocyanidins in Bark and Fruit of six Terminalia species, *Nat Prod Commun*, 2018, **13**, 1934578X1801300.

12 J. Vrba, B. Papoušková, L. Roubalová, M. Zatloukalová, D. Biedermann, V. Křen, K. Valentová, J. Ulrichová and J. Vacek, Metabolism of flavonolignans in human hepatocytes, *J Pharm Biomed Anal*, 2018, **152**, 94–101.

13 L. Y. Foo, Phenylpropanoid derivatives of catechin, epicatechin and phylloflavan from Phyllocladus trichomanoides, *Phytochemistry*, 1987, **26**, 2825–2830.

14 J. Hu, X. Li, W. Tian, Y. Lu, Y. Xu, F. Wang, W. Qin, X. Ma, P.-T. Puno and W. Xiong, Adenanthin, a Natural ent-Kaurane Diterpenoid Isolated from the Herb Isodon adenantha Inhibits Adipogenesis and the Development of Obesity by Regulation of ROS, *Molecules*, 2019, **24**, 158.

15 R. Hendriani and E. Yulinah Sukandar, *Vitro Evaluation of Xanthine Oxidase Inhibitory Activity of Selected Medicinal Plants*, 2016, vol. 8.

16 H. Q. Wang, F. Lan, Y. H. Zhang, J. E. Xia, X. M. Gong and M. Liu, Identification and pharmacokinetics of saponins in Rhizoma Anemarrhenae after oral administration to rats by HPLC-Q-TOF/MS and HPLC-MS/MS, *Acta Pharmaceutica*, 2021, **71**, 567–585.

17 M. L. Cádiz-Gurrea, J. Lozano-Sanchez, M. Contreras-Gámez, L. Legeai-Mallet, S. Fernández-Arroyo and A. Segura-Carretero, Isolation, comprehensive characterization and antioxidant activities of Theobroma cacao extract, *J Funct Foods*, 2014, **10**, 485–498.

18 M. de la L. Cádiz-Gurrea, Á. Fernández-Ochoa, F. J. Leyva-Jiménez, N. Guerrero-Muñoz, M. del C. Villegas-Aguilar, S. Pimentel-Moral, F. Ramos-Escudero and A. Segura-Carretero,

LC-MS and Spectrophotometric Approaches for Evaluation of Bioactive Compounds from Peru Cocoa By-Products for Commercial Applications, *Molecules*, 2020, **25**, 3177.

19 M. Greño, M. Herrero, A. Cifuentes, M. L. Marina and M. Castro-Puyana, Assessment of cocoa powder changes during the alkalization process using untargeted metabolomics, *LWT*, 2022, **172**, 114207.

20 K. K. N. Bini, K. C. Kobenan, M. Kouakou, I. S. Kouadio, G. Zengin, J. Jekő, Z. Cziáky, M. Danho and O. G. Ochou, Phytochemical profiling, antioxidant activities, enzymatic activities and insecticidal potential of aqueous extracts of four plants on the larvae of Helicoverpa armigera (Lepidoptera: Noctuidae), the main pest of cotton plant in Ivory Coast, *Arch Insect Biochem Physiol*, , DOI:10.1002/arch.22017.

21 A. Lorente, D. Pla, L. M. Cañedo, F. Albericio and M. Álvarez, Isolation, structural assignment, and total synthesis of barmumycin, *Journal of Organic Chemistry*, 2010, **75**, 8508–8515.

22 J. Jiang, Y. Liu, K. Min, B. Jing, L. Wang, Y. Zhang and Y. Chen, Two New Lycopodine Alkaloids from Huperzia serrata, *Helv Chim Acta*, 2010, **93**, 1187–1191.

23 M. Schwarz, F. Weber, E. Durán-Guerrero, R. Castro, M. D. C. Rodríguez-Dodero, M. V. García-Moreno, P. Winterhalter and D. Guillén-Sánchez, HPLC-DAD-MS and Antioxidant Profile of Fractions from Amontillado Sherry Wine Obtained Using High-Speed Counter-Current Chromatography, *Foods*, 2021, **10**, 131.

24 L. M. Ostertag, P. A. Kroon, S. Wood, G. W. Horgan, E. Cienfuegos-Jovellanos, S. Saha, G. G. Duthie and B. de Roos, Flavan-3-ol-enriched dark chocolate and white chocolate improve acute measures of platelet function in a gender-specific way—a randomized-controlled human intervention trial, *Mol Nutr Food Res*, 2013, **57**, 191–202.

25 F. A. Tomas-Barberán, E. Cienfuegos-Jovellanos, A. Marín, B. Muguerza, A. Gil-Izquierdo, B. Cerdá, P. Zafrilla, J. Morillas, J. Mulero, A. Ibarra, M. A. Pasamar, D. Ramón and J. C. Espín, A New Process To Develop a Cocoa Powder with Higher Flavonoid Monomer Content and Enhanced Bioavailability in Healthy Humans, *J Agric Food Chem*, 2007, **55**, 3926–3935.

26 M. Gómez-Juaristi, B. Sarria, S. Martínez-López, L. Bravo Clemente and R. Mateos, Flavanol Bioavailability in Two Cocoa Products with Different Phenolic Content. A Comparative Study in Humans, *Nutrients*, 2019, **11**, 1441.

27 M. del C. Villegas-Aguilar, N. Sánchez-Marzo, Á. Fernández-Ochoa, C. Del Río, J. Montaner, V. Micol, M. Herranz-López, E. Barrajón-Catalán, D. Arráez-Román, M. de la L. Cádiz-Gurrea and A. Segura-Carretero, Evaluation of Bioactive Effects of Five Plant Extracts with Different Phenolic Compositions against Different Therapeutic Targets, *Antioxidants*, 2024, **13**, 217.