Phytochemical profile of eleven peruvian Mentheae. Isolation of ursolic acid from Clinopodium revolutum (Ruiz & Pavon) Govaerts and analysis of the aqueous infusions

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Abstract

The profile of secondary metabolites in eleven *Mentheae* (*Nepetoideae*, *Lamiaceae*) from Peru by liquid chromatography associated with Orbitrap mass spectrometry, UHPLC-OT-MS is presented. Precursors of and salvianolic acids have been found, particularly rosmarinic acid, as well as a diversity of free and glycosylated flavonoids as main substances, 127 tentatively identified structures. In addition, a method to obtain ursolic acid from *Clinopodium revolutum* (R. & P.) Govaerts and a quantitative analysis for rosmarinic acid and triterpenic acids in aqueous infusions of these plants are presented.

Introduction

The tropical Andes are considered one of the most diverse areas on the planet in terms of vascular plants. The flora of Peru is extremely rich, its territory is home to some 25,000 species, almost 10% of all plants in the world. The large family *Lamiaceae* has twelve subfamilies. The *Nepetoideae* subfamily with 3400 species and 105 genera has three tribes [1]: *Elsholtzieae*, *Ocimeae* and *Mentheae*, the latter with 65 genera. The *Mentheae* tribe is chemically characterized by having volatile terpenoids and a phenolic acid called rosmarinic acid that makes these plants aromatic and with medicinal properties [2, 3]. The *Mentheae* can also be classified into 3 subtribes: *Menthinae* (43 genera), *Salviinae* (10 genera) and *Nepetiinae* (12 genera) [4, 5]. In Peru (Herbario Nacional Universidad de San Marcos-Perú, October 2017), the main genera of *Mentheae* are *Clinopodium* (29 species); *Hedeoma* (1 species); *Lepechinia* (11 species); *Minthostachys* (7 species) and *Salvia* (59 species). *Clinopodium*, *Hedeoma*, and *Minthostachys* belong to the *Menthinae* subtribe, while *Lepechinia* and *Salvia* belong to the *Salviinae* subtribe. In a previous work [6] the content of rosmarinic acid and triterpenic acids, oleanolic and ursolic was quantified in thirteen Peruvian *Mentheae*: The highest content of rosmarinic acid was observed in *Lepechina meyenii* (Walp.) Epling and the highest content of triterpenic acids in *Clinopodium revolutum* (Ruiz & Pavón) Govaerts. Subsequently [7], the metabolite profile was obtained in two *Lepechinia* species: *L. meyenii* and *L. floribunda* (Benth.) Epling, by UHPLC-Q-Exactive Orbitrap - MS, the presence of salvianolic acids and diterpenoids being notable, as well as a preparative method for rosmarinic acid by cold evaporative crystallization. In the present communication, the profile of secondary metabolites by UHPLC-Q-Exactive Orbitrap-MS is also described in eleven *Mentheae*: *Clinopodium* (4), *Salvia* (5), *Hedeoma* (1) and *Minthostachys* (1) and also a preparative method for ursolic acid from *Clinopodium revolutum*. In addition, the content of rosmarinic acid and triterpenic acids in the aqueous infusions of these plants was evaluated.

Results

Phytochemical Profile

The metabolite profile of the ethanolic extracts of the eleven *Mentheae* was obtained in the negative mode and the detected compounds appear in Table 1. Assignments were made based on the literature [8–40]. Isomers of quinic acid (*m/z* 191), danshensu (*m/z* 197), protocatechuic aldehyde (*m/z* 137), and caffeic acid (*m/z* 179) occur in most plants. Equally abundant are the monocaffeoylquinic acids present in seven species. *Minthostachys mollis* contains four different monocaffeoylquinic acids. Furthermore, diversity of flavonoids (flavonols, flavones, flavanones, flavanonols) has been found in all the samples, both free and glycosylated. *Minthostachys mollis*, *Clinopodium sericeum* and *Clinopodium pulchellum* are the most diverse with respect to their flavonoids. In *Salvia dombeyi*, the presence of polymethoxylated flavones is notable. All samples contain rosmarinic acid (*m/z* 359). In *Clinopodium revolutum*, salvianic acid C (*m/z* 377) has been detected, which is the result of hydrating the double bond of rosmarinic acid, and in *Salvia sagitatta*, teucrol (m/z 315), which is a decarboxylated rosmarinic acid. Isorinic acid (*m/z* 343) which is a rosmarinic acid without the 3-OH is present in *Clinopodium brevicalyx*, *Salvia sagitatta*, *Salvia cuspidata* and *Hedeoma mandoniana*. Methyl (*m/z* 373) and ethyl (*m/z* 387) esters of rosmarinic acid are present in *Salvia cuspidata* and in *Clinopodium brevicalyx*. In *Salvia cuspidata* and *Clinopodium revolutum*, the dimer of rosmarinic acid, sagerinic acid (*m/z* 719), which is a molecule with a stabilized cyclobutane ring was found. In *Clinopodium brevicalyx* and *Hedeoma mandoniana*, the presence of salvianolic acid B (*m/z* 717) was observed, a particularly important substance due to its effect on neurodegenerative diseases [41]. *Clinopodium pulchellum* contains salvianolic acid A (*m/z* 493) and salvianolic acid F (*m/z* 313). But the plant with the greatest diversity of salvianolic acids is *Clinopodium sericeum*, "romero de jalca", since lithospermic acid (m/z 537), salvianolic acid B, two isomers of salvianolic acid A and two isomers of salvianolic acid F have been found in it. This type of substances is very important due to its effect on cell fibrosis (scar formation) in direct relation to cancer [42]. Among the other substances found, it should be noted that the *Rosmarinus* type diterpenoids, common in *Lepechinia* [7, 43] are scarce in this work, only *Salvia sagitatta* and *Salvia cuspidata* show the presence of carnosol (*m/z* 285) and the phenolic diterpenoid, rosmadial (*m/z* 343) in the last one. *Salvia dombeyi* turns out to be the most dissimilar plant in this work, since apart from

a very low content of rosmarinic acid [6] and the presence of polymethoxylated flavones, it shows substances such as cimifugin (*m/z* 305), a substance with antiallergic and antipruritic activity [51, 52] and the lignan lariciresinol (*m/z* 359), common in oilseeds and in the *Brassica* and *Allium* genera [53]. *Salvia haenkei* contains the ent-(5R,9R)-15,16-epoxy-10S-hydroxycleroda-3,7,13(16),14-tetraene-17,12S; 18,19 diolide (*m/z* 355) [49], while *Salvia cuspidata* contains other lignan, isolariciresinol (*m/z* 359) present in *Linum* seeds [54], and also 5-epi-icetexone (*m/z* 341) described as anti Trypanosoma cruzii molecule [40]. Oleanolic and ursolic triterpenic acids, quantified in [6], do not appear in this analysis due to the elution program used, which does not reach 100% acetonitrile. Figure 1 shows the ESI(-) chromatogram of *Salvia sagitatta* and Fig. 2, the chromatogram of *Clinopodium sericeum*. Table 1 shows the 129 substances detected.

Table 1
Detected compounds in eleven peruvian Mentheae

N° peak	assignment	Rt	[M-H]-	experimental mass (error)	fragments	Detected in	Reference
1	Quinic acid	1.33	$C_7H_{11}O_6$	191.0559(1.57)	127.0394	*Cb, So, Mm, Sc, Cr, Cs, Sd, Cp.*	[8]
2	Malic acid	1.36	$C_4H_5O_5$	133.0139 (1.5)		*Ss, Sc, Sd*	[8]
3	Quinic acid isomer	1.44	$C_7H_{11}O_6$	191.056(2.09)	127.8695	*Cb, Mm, Cr, Cs, Cp*	[8]
4	Citric acid	1.77	$C_6H_7O_7$	191.0196(2.09)	111.0081	*So*	[8]
5	Pyroglutamic acid	1.87	$C_5H_6O_3N$	128.0348 (0)		*Sh*	[8]
6	Succinic acid	1.98	$C_4H_5O_4$	117.0187(0.85)		*So, Mm, Ss, Sc, Cr, Cs, Sh, Sd, Hm*	[8]
7	monoacetylglycerol	2.09	$C_5H_9O_4$	133.0502 (0.75)		*Ss*	
8	Mesaconic acid	2.96	$C_5H_5O_4$	129.0190(1.55)		*Cp*	
9	3,4-dihydroxyphenyl lactic acid "danshensu"	4.05	$C_9H_9O_5$	197.0454(2.02)	135.0446[M-H-H_20-CO_2]$^-$, 179.0346 [M-H-H_2O]$^-$, 123.0445	*So, Cb, So, Mm, Sc, Cr,Cs, Hm, Sh*	[9], [10]
10	Protocatechuic acid	4.64	$C_7H_5O_4$	153.019(1.31)	135.0448[M-H-H_2O]$^-$, 109.0289[M-H-CO_2]$^-$	*So, Sc, Hm, Cp*	[9]
11	Caffeoyl quinic acid	6.39	$C_{16}H_{17}O_9$	353.0883(2.83)	135.0447, 179.0347[caffeic acid – H]$^-$, 191.0559[quinic acid – H]$^-$	*Mm*	[11], [12], [13], [50], [14], [15]
12	protocatechuic aldehyde	7.73	$C_7H_5O_3$	137.0239(0)	109.0289 [M-2H-CO]$^-$	*Cb,So, Mm,Ss, Cr, Cs, Sh, Hm, Cp*	[9]
13	Hydroxyheptandioic acid	8.78	$C_7H_{11}O_5$	175.0611(2.28)		*So, Ss*	
14	Coumaroyl quinic acid	8.83	$C_{16}H_{17}O_8$	337.0934(2.96)	119.0496, 163.0398, 173.0453, 191.0559	*Mm*	
15	Caffeoyl quinic acid	8.99	$C_{16}H_{17}O_9$	353.0883(2.83)	173.0453, 179.0559[caffeic acid – H]$^-$, 191.056[quinic acid – H]$^-$	*Mm*	[11], [12], [13], [50], [14], [15]
16	Caffeic acid –O-hexoside	9.02	$C_{15}H_{17}O_9$	341.0883(2.93)	179.0347[caffeic acid –H]$^-$, 251.0564, 233.0458, 281.0670	*So, Ss, Sc*	[16], [17]

N° peak	assignment	Rt	[M-H]-	experimental mass (error)	fragments	Detected in	Reference
17	Caffeoyl quinic acid	9.04	$C_{16}H_{17}O_9$	353.0883(2.83)	191.056[quinic acid-H]$^-$	*Mm, Cr*	[11], [12], [13], [50], [14], [15]
18	Coumaric acid	9.28	$C_9H_7O_3$	163.0399 (2.45)	119.0497 [M-carboxyl]$^-$	*Ss*	[11], [12]
19	Caffeoyl quinic acid	9.41	$C_{16}H_{17}O_9$	353.0883(2.83)	179.0347[caffeic acid-H]$^-$, 173.0452	*Cb, So, Mm, Cr, Hm, Cp*	[11], [12], [13], [50], [14], [15]
20	Eucomic acid	9.54	$C_{11}H_{11}O_6$	239.0561(2.09)	195.0660, 178.0586	*Cb*	
21	Caffeoylquinic acid	9.56	$C_{16}H_{17}O_9$	353.0882(2.55)	135.0446 [caffeic acid-H-CO_2]$^-$, 179.0346 [caffeic acid-H]$^-$, 191.055 [quinic acid-H]$^-$	*Hm*	[11], [12], [13], [50], [14], [15]
22	Caffeic acid	9.64	$C_9H_7O_4$	179.0348(1.67)	135.0446 [M-H-CO_2]$^-$, 161.0446 [6,7-dihydroxy-1H-inden-1-one – H]$^-$	*So,Mm, Ss, Sc, Cr, Cs, Sh, Sd*	[11], [12], [17]
23	Caffeic acid –O-hexoside I	9.77	$C_{15}H_{17}O_9$	341.088 (2.05)	179.0345[caffeic acid –H]$^-$, 281.0667, 251.0561,235.0453	*Sc*	[16], [17]
24	Tuberonic acid glucoside	9.86	$C_{18}H_{27}O_9$	387.1665(2.58)	206.9725, 163.0033, 101.5668	*So, Ss, Cr, Sh, Sd*	[8]
25	coumaroylquinic acid	10.1	$C_{16}H_{17}O_8$	337.0934(2.97)	173.0454, 163.0397[coumaric acid – H]$^-$	*So, Mm*	[11], [12], [15], [18]
26	salvianic acid C	10.21	$C_{18}H_{17}O_9$	377.0882(2.39)	359.0776 [M-H_2O]$^-$, 161.0240[6,7-dihydroxy-1H-inden-1-one – H]$^-$, 179.0347 [caffeic acid –H]$^-$	*Cr*	[17]
27	p-coumaroyl hexoside	10.38	$C_{15}H_{17}O_8$	325.093(1.85)	119.0496 [coumaric acid-H-CO_2]$^-$, 163.0396 [coumaric acid-H]$^-$	*Sc*	[16]
28	Feruloylquinic acid	10.38	$C_{17}H_{19}O_9$	367.104(3.0)	149.0240 [ferulic acid-H-CO_2]$^-$, 191.0560 [quinic acid-H]$^-$, 193.0504 [ferulic acid – H]$^-$, 173.0453	*Mm*	
29	Quercetin-3,7-di-O-glucoside	10.43	$C_{27}H_{29}O_{17}$	625.1407(0.32)	303.1084, 463.0882	*Cs, Cp*	[19]
30	Caffeoylquinic acid	10.45	$C_{16}H_{17}O_9$	353.088 (1.12)	135.0445 caffeic acid-H-CO_2]$^-$, 179.0345 [caffeic acid –H]-, 191.0557[quinic acid-H]$^-$	*Sc*	[11], [12], [13], [50], [14], [15]

N° peak	assignment	Rt	[M-H]-	experimental mass (error)	fragments	Detected in	Reference
31	p-coumaroyl hexoside I	10.59	$C_{15}H_{17}O_8$	325.093(1.85)	119.0496, 163.0396	*Sc*	
32	Quercetin-O-vicianoside	10.64	$C_{26}H_{27}O_{16}$	595.1301 (0.34)	301.0356 [M-vicianose-H]-, 433.2081	*Sd*	
33	Salvianic acid C isomer	10.67	$C_{18}H_{17}O_9$	377.0881(2.12)	359.0775 $[M-H_2O]^-$, 347.1708, 197.0453 [HOOC-CHOH-CH_2-$C_6H_3(OH)_2$ – H]$^-$	*Cr*	[17]
34	Tuberonic acid	10.67	$C_{12}H_{17}O_4$	225.1132(2.22)	134.8648, 146.9382, 168.8359, 187.9417, 213.0961	*Mm, Sh, Sd*	[8]
35	Quercetin-O-rutinoside	10.78	$C_{27}H_{29}O_{16}$	609.1458 (0.33)	301.0363 [M-rutinosa-H]$^-$, 447.1302	*Mm, Sd, Cp*	[16]
36	Eriodyctiol rutinoside (eriocitrin)	10.89	$C_{27}H_{31}O_{15}$	595.1661 (0.34)	287.0562, 151.0397	*Cs, Sh, Hm*	[20]
37	Luteolin rutinoside	11.00	$C_{27}H_{29}O_{15}$	593.1504 (0.51)	285.0403 [M-rutinose-H]$^-$, 447.0928	*Cb, Sc, Cr, Cp*	[21]
38	Apigenin rutinoside	11.01	$C_{27}H_{29}O_{14}$	577.1556 (0.35)	269.103 [M-rutinose-H]$^-$	*Sc, Cr*	[15], [21]
39	Luteolin-O-hexoside	11.02	$C_{21}H_{19}O_{11}$	447.0936(1.78)	285.0406 [M-hexose-H]$^-$	*Cb, Ss, Cs*	[22]
40	Quercetin-O-hexoside	11.02	$C_{21}H_{19}O_{12}$	463.0886(1.94)	301.0358 [M-hexose-H]$^-$	*So, Mm, Ss, Sc, Cp*	[16], [23]
41	Quercetin-O-glucuronide	11.09	$C_{21}H_{17}O_{13}$	477.0679(2.1)	301.0356 [M-GlcA-H]$^-$	*So, Ss, Sd*	[23]
42	Feruoyl hexoside	11.12	$C_{16}H_{19}O_9$	355.1036 (1.97)	193.0502 [ferulic acid – H]$^-$, 149.024043[ferulic acid-H-CO_2]$^-$	*Sc*	[16]
43	Pentahydroxymethoxyflavone glycoside	11.12	$C_{22}H_{21}O_{13}$	493.0989(1.42)	331.0827, 315.1089, 163.0397, 162.8387	*Cs*	
44	Isorhamnetin-O-glucoside	11.23	$C_{22}H_{21}O_{12}$	477.1046 (2.72)	315.0824 [isorhamnetin-H]$^-$, 357.0352, 462.0768	*Ss*	[22]
45	Naringenin-7-O-Glc-Rha (narirutin)	11.33	$C_{27}H_{31}O_{14}$	579.1714 (0)	271.0612, 235.0611, 151.0030	*Mm, Cb, Cs, Hm, Cp*	
46	Eriodictiol-O-neohesperidoside (neoeriocitrin)	11.40	$C_{27}H_{31}O_{15}$	595.1665(0.34)	287.0564, 151.0033	*Cs*	
47	Luteolin-O-glucuronide	11.56	$C_{21}H_{17}O_{12}$	461.0729(1.95)	285.0407 [M-GlcA-H]$^-$, 151.0395, 133.0290	*So, Ss, Cr, Sh*	[23]
48	Kaempferol-O-hexoside	11.57	$C_{21}H_{19}O_{11}$	447.0937 (2.01)	285.0407 [Kf-hexose]$^-$	*Mm, Cr, Sd*	[16]

N° peak	assignment	Rt	[M-H]-	experimental mass (error)	fragments	Detected in	Reference
49	dihydrobaicalin	11.57	$C_{21}H_{19}O_{11}$	447.0936 (1.79)	403.1613 [M-H-CO2]$^-$, 271.0250[M-glucuronic acid]$^-$	*Sc*	
50	Sagerinic acid	11.58	$C_{36}H_{31}O_{16}$	719.16(1.67)	539.1186 [M – HOOC-CH-CH_2-$C_6H_3(OH)_2$]$^-$, 359.0715 [M-rosmarinic - H]$^-$, 179.0348, 161.0239 [6,7-dihydroxy-1H-inden-1-one – H]$^-$	*Sc, Cr*	[11], [12], [24], [25], [26]
51	Caffeoyldihydroxyhexyl ester	11.63	$C_{15}H_{19}O_6$	295.1193(3.73)	161.0242 [$C_9H_5O_3$]-	*Ss*	
52	Hesperetin-7-O-rutinoside	11.64	$C_{28}H_{33}O_{15}$	609.1819(0.16)	301.0718	*Mm, Cb, Hm, Cp*	
53	Apigenin-O-rutinoside	11.66	$C_{27}H_{29}O_{14}$	577.1557(0.17)	269.0453	*Cr*	
54	Dimethylrosmarinic acid	11.67	$C_{20}H_{19}O_8$	387.1091(2.84)	179.0347 [caffeic acid – 1]-, 135.0447 [caffeic acid-H-CO_2]$^-$„ 161.0452 [6,7-dihydroxy-1H-inden-1-one – H]$^-$	*So, Sh*	
55	Isorhamnetin-3-O-glucuronide	11.7	$C_{22}H_{19}O_{13}$	491.0834(1.62)	299.0565, 301.0358, 302.0388	So	
56	Salvianolic acid A isomer	11.75	$C_{26}H_{21}O_{10}$	493.1142(1.42)	179.0344 [caffeic acid-H]$^-$, 197.0450 [danshensu-H]-, 269.0821, 295.1192 [salvianolic acid F – OH]$^-$, 313.0723 [salvianolic acid F-H]$^-$, 359.0778 [rosmarinic acid –H]$^-$	Cs	[9], [10], [17], [33]
57	Tetrahydroxymethoxyflavone –O-hexoside	11.78	$C_{22}H_{21}O_{12}$	477.1041(1.68)	315.1451, 163.8391, 162.8398	Cr	
58	Trihydroxymethoxyflavone hexoside	11.89	$C_{22}H_{21}O_{11}$	461.1093 (1.95)	299.0559[M-H-glucose]-	*Sh, Sd*	
59	Caffeoyldihydroxylhexyl ester	11.93	$C_{15}H_{19}O_6$	295.1193(3.73)	161.0245 [$C_9H_5O_3$]-	*Ss*	
60	Acetylglycitin	11.96	$C_{24}H_{23}O_{11}$	487.1250(1.85)	267.0720, 241.1080, 444.0807	*Hm*	
61	Salvianolic acid B isomer	11.99	$C_{36}H_{29}O_{16}$	717.1443 (1.81)	321.0616 [M – 2 danshensu- 3H]$^-$, 519.0945 [M-danshensu-2H]$^-$	*Cs*	[10], [15], [17], [26], [27]
62	Apigenin-6-C-hexósido	12.01	$C_{21}H_{19}O_{10}$	431.0984(1.16)	331.1396, 341.1960, 283.1192	*Cr*	
63	cimifugin	12.03	$C_{16}H_{17}O_6$	305.1035 (3.28)	274.1170[M-hydroxymethyl-H]$^-$, 215.0098[M-CH3COH-CH3-H]$^-$	*Sd*	[28]

N° peak	assignment	Rt	[M-H]-	experimental mass (error)	fragments	Detected in	Reference
64	Rosmarinic acid	12.04	$C_{18}H_{15}O_8$	359.0775(2.23)	161.0240 [6,7-dihydroxy-1H-inden-1-one – H]$^-$, 179.0345 [caffeic acid-H]$^-$, 197.0452 [danshensu-H]$^-$	*Cb, So, Mm, Ss, Sc, Cr, Cs, Sh, Sd, Hm, Cp*	[10–12], [24–25], [27]
65	isorhamnetin	12.50	$C_{16}H_{11}O_7$	315.0513 (2.54)	301.0306, 300.0272 [M-Me-H]$^-$, 271.1550[(M-CH3-H)-(H + CO)]$^-$,179.0346, 151.0394[A]$^-$	*So, Sh*	[29–30]
66	Salvianolic acid B isomer	12.53	$C_{36}H_{29}O_{16}$	717.1443(1.81)	295.0611, 321.0408 [M – 2 danshensu-3H]$^-$, 339.0512, 493.1137, 519.0930[M-danshensu-2H]$^-$, 537.1024 [M-caffeic acid- 2H]$^-$	*Cb, Hm*	[10], [15], [17], [26], [27]
67	isorhamnetin acetyldihydroxybutyl ester	12.61	$C_{22}H_{21}O_{10}$	445.1144 (2.02)	387.1090[M-acetyl]$^-$, 315.0514[M-acetyl,hydroxybutyl]$^-$, 151.0396 [A]$^-$	*Sd*	
68	Luteolin-O-acetylglucoside	12.71	$C_{23}H_{21}O_{12}$	489.1039(1.23)	285.0404, 447.0935	*Cr*	
69	artemetin	12.80	$C_{20}H_{19}O_8$	387.1089 (2.32)	372.1184[M-H-Me]$^-$, 357.0992[M-H-2Me]$^-$, 342.1067[M-H-3Me]$^-$[70]327.1241[M-H-4Me]$^-$	*Sh, Sd*	
70	heptamethoxyflavone	12.81	$C_{22}H_{23}O_9$	431.1352 (2.32)	417.1189, 402.1563, 387.1089, 372.1174	*Sd*	
71	caffeoil-4'-hydroxyphenyllactic acid (isorinic acid)	12.95	$C_{18}H_{15}O_7$	343.0827(2.62)	161.0241 [6,7-dihydroxy-1H-inden-1-one – H]$^-$, 327.2181[M-OH]$^-$	*Cb, Ss, Sc Hm*	[17], [31]
72	Lithospermic acid	13.03	$C_{27}H_{21}O_{12}$	537.1038(0.93)	493.1147 [M-CO_2-H]$^-$, 295.0610 [M-CO_2-H – danshensu]$^-$	*Cs*	[9], [17]
73	Isosakuranetin-O-rutinoside (Neoponcirin)	13.15	$C_{28}H_{33}O_{14}$	593.1874(0.51)	594.1905, 593.1873, 285.077	*Mm, Cp*	
74	Caffeoyloxohydroxyhexyl ester	13.23	$C_{15}H_{17}O_6$	293.1035 (3.41)	161.0242 [$C_9H_5O_3$]-	*Ss*	
75	Methyl rosmarinate	13.28	$C_{19}H_{17}O_8$	373.0935 (2.95)	359.0778 [rosmarinic acid –H]-, 194.0540, 179.0347 [danshensu –H]$^-$	*Sc*	[11], [12], [21]
76	pentamethoxyflavone	13.36	$C_{20}H_{19}O_7$	371.114 (2.43)	357.1349, 327.1241, 311.0934	*Sd*	

N° peak	assignment	Rt	[M-H]-	experimental mass (error)	fragments	Detected in	Reference
77	Quercetin-3-O-(3'-O-p-coumaroyl)-hexoside (Helichrisroside)	13.51	$C_{30}H_{25}O_{14}$	609.1242 (0.49)	301.0719, 447.0940, 462.0747, 594.1343	*Cr*	
78	Eriodictiol	13.60	$C_{15}H_{11}O_6$	287.0563(2.44)	135.0446, 151.0030	*Cb, Cp*	
79	Luteolin	13.62	$C_{15}H_9O_6$	285.0408(3.16)	133.0289, 151.0032	*Cb, Hm*	
80	dihydrophilonotisflavone	13.63	$C_{30}H_{19}O_{12}$	571.0883 (1.05)	285.0410, 286.0441[M-luteolin-H]$^-$	*So, Ss*	
81	Ferulic acid	13.68	$C_{10}H_9O_4$	193.0504 (1.55)	134.0367, 149.0239 [M-H-CO_2]$^-$, 178.022 [M-H-CH_3]$^-$	*Sc*	[10], [21], [32]
82	pentamethoxyflavone	13.79	$C_{20}H_{19}O_7$	371.114 (2.43)	357.1339, 327.1240	*Sd*	
83	Salvianolic acid A isomer	13.87	$C_{26}H_{21}O_{10}$	493.1141(1.22)	159.8595, 179.0345 [caffeic acid – H]$^-$, 197.0451[danshensu-H]$^-$, 269.0821, 295.0612, 313.0719, 359.0774 [rosmarinic acid –H]$^-$	*Cs, Cp*	[9], [10], [17], [33]
84	Protocatechuic acid – 7-O-(4-hydroxybenzoyl)glucoside	13.94	$C_{20}H_{19}O_{11}$	435.0935(1.61)	297.1346, 153.0191, 315.1452, 137.0239	*Cr*	
85	Chrysoeriol (trihydroxymethoxyflavone)	14.00	$C_{16}H_{11}O_6$	299.0565 (3.01)	285.0413 [M-H-methyl]$^-$, 151.0397 [A]$^-$, 255.0698 [M-H-CO_2]$^-$	*So, Mm, Ss, Sh*	
86	Hesperetin-O-hexoside	14.27	$C_{22}H_{23}O_{11}$	463.1250(2.06)	285.0773, 301.0720	*Cp*	
87	pentamethoxyflavone	14.38	$C_{20}H_{19}O_7$	371.114 (2.43)	357.1348, 327.1241	*Sd*	
88	caffeic acid ethyl ester	14.66	$C_{11}H_{11}O_4$	207.0661(1.44)	179.0347	*Cb, Sc*	
89	kaempferol	14.72	$C_{15}H_9O_6$	285.0406 (2.46)	161.0240, 151.0396 [A]$^-$, 135.0444	*Sc, Cr*	[29]
90	lariciresinol	14.84	$C_{20}H_{23}O_6$	359.1505 (2.78)	419.1715	*Sd*	[19]
91	quercetin	14.97	$C_{15}H_9O_7$	301.0356 (2.33)	179.0344, 121.0288,273.1719 92	*Sc*	[29]
92	Caffeic acid dimethyl derivative	15.01	$C_{11}H_{11}O_4$	207.0661(1.45)	16931.0239, 151.940396, 147.069552	*Cs*	
93	salvianolic acid F isomer	15.46	$C_{17}H_{13}O_6$	313.0721(2.88)	269.082196 [M-CO_2-H]$^-$, 15979.0656	*Sc, Cs*	[17]

N° peak	assignment	Rt	[M-H]-	experimental mass (error)	fragments	Detected in	Reference
94	dimethylquercetin	15.49	$C_{17}H_{13}O_7$	329.0673 (3.34)	314.0756[M-H98-methyl]$^-$, 9996299.0200[M-H-2 100methyl]-	*Cb*	
95	Trihydroxydimethoxyflavone	15.53	$C_{17}H_{13}O_7$	329.0672(3.04)	151.0398, 201.8020, 257.8197, 283.0612, 313.0722, 314.0754, 299.0201	*Mm*	
96	Trihydroxylinoleic acid	16.03	$C_{18}H_{31}O_5$	327.2183(2.75)	269.0457	*Cb, Mm, Ss, Sc, Hm*	
97	Ethyl caffeate	16.14	$C_{11}H_{11}O_4$	207.0660(0.97)	179.0346 [caffeic acid-H]$^-$	*Ss*	
98	apigenin	16.15	$C_{15}H_9O_5$	269.0459 (3.35)	151.0396 A$^-$, 117.0187 B$^-$	*Ss, Sc, Cr*	[15], [29]
99	naringenin	16.39	$C_{15}H_{11}O_5$	271.0616 (3.32)	151.0397 A$^-$, 177.0190 [M-H-ring B]$^-$	*Ss, Cp*	[34]
100	Salvianolic acid F isomer	16.87	$C_{17}H_{13}O_6$	313.0719(2.23)	269.0822 [M-CO_2-H]$^-$, 159.0658	*Sc*	[17]
101	Ethyl rosmarinate	17.23	$C_{20}H_{19}O_8$	387.1088(2.07)	359.0777 [M-ethyl]$^-$, 206.9724 [M – caffeic acid]$^-$, 179.0344 [caffeic acid-H]$^-$	*Cb*	[11], [12], [21]
102	3,7-quercetindimethylether	17.59	$C_{17}H_{13}O_7$	329.0673 (3.34)	314.0756[M-H-methyl]$^-$, 299.0200[M-H-2 methyl]-	*Ss, Sd, Cp*	[32]
103	Hesperetin	17.66	$C_{16}H_{13}O_6$	301.0722(3.32)	286.0495, 257.0822, 241.7579	*Cp*	
104	Salvianolic acid F isomer	17.87	$C_{17}H_{13}O_6$	313.0721(2.87)	269.0821 [M-CO_2-H]$^-$, 159.0448	*Cs*	[17]
105	15,16-epoxi-10S-hidroxicleroda-3,7, 13(16), 14 tetraeno-17, 12S; 18,19 diolido	17.94	$C_{20}H_{19}O_6$	355.119 (2.25)	311.1291[M-H-CO2]$^-$	*Sh*	[49]
106	Sanleng acid (6,9,10-trihydroxy-7-octodecenoic acid)	18.13	$C_{18}H_{33}O_5$	329.2336(2.43)	171.0195, 224.7632, 250.1448	*Mm, Cb Cs*	
107	Hydroxypentamethoxyflavone	18.47	$C_{20}H_{19}O_8$	387.1087(1,81)	285.0403	*Sc*	
108	8-Hydroxyhexadecandioic acid	18.63	$C_{16}H_{29}O_5$	301.2025(3.32)		*Cs*	
109	Trihydroxy-trimethoxyflavone	18.73	$C_{18}H_{15}O_8$	359.0766(0.28)	344.0546, 329.0299, 314.2232, 301.6655	*Mm, Cb, Cp*	
110	10-(1-hydroxyhexoxy)-10-oxodecanoic acid	18.80	$C_{16}H_{29}O_5$	301.2023(2.66)	201.8017	*Cr*	
111	Trihydroxymethoxyflavanone (hesperetin isomer)	19.15	$C_{16}H_{13}O_6$	301.0721(2.87)	161.0240, 139.0032	*Cp*	

N° peak	assignment	Rt	[M-H]-	experimental mass (error)	fragments	Detected in	Reference
112	trihydroxymethoxyflavone	19.23	$C_{16}H_{11}O_6$	299.0565(3.01)	151.0397 $[A]^-$, 179.0346, 284.0327 $[M\text{-}CH3\text{-}H]^-$	*So, Mm, Cr, Sh, Cp*	[35]
113	cirsimaritin	19.34	$C_{17}H_{13}O_6$	313.0724 (3.19)	298.0488[M-H-methyl]-, 283.0249[M-H-2methyl]-	*Ss, Cr*	[36]
114	isolariciresinol	19.61	$C_{20}H_{23}O_6$	359.1502 (1.95)	345.1346$[M\text{-}Me]^-$, 344.1582$[M\text{-}H\text{-}Me]^-$, 313.0714	*Sc*	[16], [37]
115	Salvianolic acid F isomer	19.77	$C_7H_{13}O_6$	313.0722(3.19)	269.0459 $[M\text{-}CO_2\text{-}H]^-$, 159.8597	*Cp*	[17]
116	rosmadial	20.03	$C_{20}H_{23}O_5$	343.1552 (1.75)	299.1652 $[M\text{-}H\text{-}CO_2]^-$, 315.1598 $[M\text{-formyl}]^-$	Sc	
117	eupatorin	20.06	$C_{18}H_{15}O_7$	343.0829 (3.21)	328.0595$[M\text{-}H\text{-}Me]^-$, 313.0359$[M\text{-}H\text{-}2Me]^-$, 298.0125$[M\text{-}H\text{-}3Me]^-$	*Cb, Mm, Ss, Cr, Cp*	[27], [38]
118	teucrol	20.3	$C_{17}H_{15}O_6$	315.088 (3.5)	179.0349[Caffeic acid-H]$^-$, 135.0447[caffeic acid -H-CO2]$^-$, 161.0244 [6,7-dihydroxy-1H-inden-1-one – H]$^-$	*Ss*	[39]
119	Dihydroxymethoxyflavone	20.32	$C_{16}H_{11}O_5$	283.0617(3.53)	268.0386, 151.0034, 107.0327	*Mm,Cp*	
120	Dihysroxydimethoxyflavanone	20.36	$C_{16}H_{13}O_5$	285.0773(3.51)	153.0190, 161.0453, 179.0349, 151.0397, 243.0668, 270.0535, 164.0012	*Mm*	
121	genkwanin	20.47	$C_{16}H_{11}O_5$	283.0616 (3.18)	268.0386$[M\text{-}H\text{-}Me]^-$, 151.0398 A^-	*Ss, Cr, Sh*	[22]
122	Sakuranetin	20.57	$C_{16}H_{13}O_5$	285.0771(2.81)	119.0497, 165.0188, 151.0396	*Cr*	
123	Octadecendioic acid	20.68	$C_{18}H_{31}O_4$	311.2232(2.89)	310.2107	*So, Sh, Sd*	[8]
124	Octadihydroxyoctadecadienoic acid	21.15	$C_{18}H_{31}O_4$	311.2229 (1.93)	197.8076	*Sc*	[8]
125	Carnosol	22.2	$C_{20}H_{25}O_4$	329.1761(2.7)	285.1861$[M\text{-}H\text{-}CO_2]^-$	*Ss, Sc*	[11–12]
126	5-epi-icetexone	22.45	$C_{20}H_{21}O_5$	341.1396 (0.88)	297.1500 $[M\text{-}H\text{-}CO2]^-$, 299.1652 $[M\text{-}H\text{-}CO]^-$	*Sc*	[40]
127	9,10-dihydroxystearic acid	23.47	$C_{18}H_{35}O_4$	315.2547 (3.47)		*Ss*	[8]

Ursolic acid purification from Clinopodium revolutum

In a previous work [6] the content of triterpenic acids was analyzed in thirteen plants of the *Mentheae* tribe, finding that *Clinopodium revolutum* "Flor de Arena" is the one with the highest ursolic acid content, suggesting that this substance could be isolated from it. Therefore, from dry, pulverized and defatted material, by alcoholic extraction and subsequent filtration of the extract on SiGel with increasing polarity gradient, ursolic acid crystals of approximately 90% purity were obtained. The purification of this material, by recrystallization from ethanol, was done taking into account the solubilities described in [44] as shown in Table 2.

Table 2
Ursolic acid (UA) solubility in ethanol [44].

temperature °C	solubility (mg UA/g ethanol)	solubility (mg UA/mL ethanol)	g of ethanol to disolve 100 mg of UA	mL of ethanol to disolve 100 mg of UA
20.45	5.10	4.08	19.61	24.51
26.95	7.61	6.09	13.14	16.43
32.05	7.30	5.84	13.70	17.12
36.85	7.56	6.05	13.23	16.53
46.95	10.39	8.31	9.62	12.03
50.05	10.41	8.33	9.61	12.00
56.55	16.34	13.07	6.12	7.65

Experiments with 50 mg of 90% ursolic acid at concentrations of 0.8 to 1.4% solute:solvent ratio show that at a percentage of 0.99 approximately 50% of the acid used is recovered with a purity greater than 98% as seen in table 3 and Fig. 3.

Tabla3. Recovery and purity of recrystallized ursolic acid according to the material/solvent volume ratio. (crystallizand with 91.15% ursolic acid and 4.65% oleanolic acid).

% (mass/volume) of crystallizand in ethanol	Recovery %	UA Purity % in the recrystallized
0.84	39.53	95.51
0.88	40.46	95.57
0.99	51.09	98.52
1.39	38.07	99.47

It is then seen that 1 g of ursolic acid of approximately 90% purity by recrystallization in 100 mL of 96% ethanol, will give us 0.5 g of ursolic acid with a purity greater than 98%.

Quantification of rosmarinic acid and triterpenic acids in the aqueous infusions of eleven Mentheae

As the form of use of the majority of medicinal plants is the aqueous infusion, it is interesting to know the amount of phenolics, rosmarinic acid and triterpenic acids in said infusions. Thus, the content of total phenols (expressed as µg of gallic acid/10 mL of infusion), the total antioxidant capacity (as µg of ascorbic acid/10 mL of infusion) and the mg of rosmarinic acid/10 mL of infusion were determined. [6]. The results appear in Table 4.

Table 4
Total phenols, antioxidant capacity and rosmarinic acid content of the eleven *Mentheae* infusions (three repetitions).

Plant	Total phenols (mg gallic acid/10mL)	Antioxidant capacity (ascorbic acid mg/10 mL)	Rosmarinic acid mg/10 mL
Clinopodium brevicalyx	4.08 ± 0.05	2.72 ± 0.02	0.90 ± 0.01
Salvia oppositiflora	2.19 ± 0.01	1.82 ± 0.26	0.21 ± 0.01
Minthostachys mollis	2.07 ± 0.00	1.53 ± 0.24	0.26 ± 0.00
Salvia sagitatta	3.25 ± 0.05	2.19 ± 0.04	0.79 ± 0.01
Salvia cuspidata	3.96 ± 0.09	2.84 ± 0.16	0.41 ± 0.00
Clinopodium revolutum	3.72 ± 0.03	2.46 ± 0.17	0.32 ± 0.01
Clinopodium sericeum	4.20 ± 0.12	2.96 ± 0.06	0.39 ± 0.01
Salvia haenkei	2.08 ± 0.01	1.63 ± 0.18	0.22 ± 0.00
Salvia dombeyi	1.65 ± 0.02	0.88 ± 0.13	undetected
Hedeoma mandoniana	2.00 ± 0.03	1.65 ± 0.22	0.02 ± 0.00
Clinopodium pulchellum	1.49 ± 0.01	1.14 ± 0.15	0.05 ± 0.00

Also, in all cases the content of triterpenic acids was analyzed but it was not found in any of the eleven aqueous infusions.

Discussion

It is the first time that the phytochemical profile has been obtained in these Peruvian *Mentheae* (*Lamiaceae*). The genera studied were *Salvia* (*Salviinae*), *Clinopodium*, *Hedeoma* and *Minthostachys* (*Menthinae*). While *Salvia* and *Clinopodium* are genera of worldwide distribution, *Hedeoma* and *Minthostachys* are American and South American genera, respectively. All *Salvia* species in this work belong to the *Salvia* subgenus: *Calosphace* Benth. (Epling) [46]. The phytochemical profile of these *Mentheae* are quite similar to their European and Asian relatives. All the species analyzed show the presence of rosmarinic acid and, quinic acid, 3,4-dihydroxyphenyl-lactic acid “danshensu”, protocatechuic aldehyde and caffeic acid are present in most of the samples. Monocaffeoylquinic acids, also called chlorogenic acids, are also frequent but better expressed in *Minthostachys*. Di or tricaffeoylquinic acids have not been detected. All samples contain flavonoids with more diversity in *Minthostachys* and *Clinopodium*. Flavonoid-free aglycones predominate in several plants: In *Salvia sagitatta*, cirsimaritin is abundant [58] while eupatorin predominates in *Clinopodium revolutum* [38], chrysoeriol in *Salvia haenkei* [59], 3,7-quercetindimethylether [60] in *Salvia dombeyi* and hesperetin in *Clinopodium pulchellum* [61]. In several plants, rosmarinic acid is the main peak: *Clinopodium brevicalyx*, *Salvia oppositiflora*, *Clinopodium sericeum* and *Hedeoma mandoniana*. Some type of salvianolic acid is present in all the samples, although in some cases they are very small modifications of the rosmarinic acid molecule. Dimers and trimers of rosmarinic acid are present in *Clinopodium brevicalyx*, *Salvia oppositiflora*, *Salvia cuspidata*, *Clinopodium sericeum*, *Hedeoma mandoniana* and *Clinopodium pulchellum*. In *Clinopodium sericeum*, not only is the diversity of salvianolic acids important, but also their abundance in salvianolic acid A, which would allow the preparation of said substance from it [62].

In all cases, triterpenic acids, oleanolic and ursolic are present, which do not appear with the present methodology used, but did appear in a previous work [6], where it is reported that *Clinopodium revolutum* contains 2.55 and 4.81 percent of these acids. As it is now known that ursolic acid has great pharmacological importance [47], it was decided to study the purification of this substance [48], now more focused on its recrystallization in ethanol. These procedures serve to have a ursolic acid chromatographic standard that could be applicable on a larger scale, to treat cheaper raw materials such as apple peels (*Malus*), eucalyptus (*Eucalyptus*) or olive (*Olea*) leaves [55].

The aqueous infusion of these plants shows the presence of phenolic substances with antioxidant capacity and specifically the presence of rosmarinic acid, with *Clinopodium brevicalyx* and *Clinopodium sericeum* being the ones with the highest rosmarinic acid content and also the highest total antioxidant capacity. In none of the aqueous infusions could the presence of triterpenic acids be detected. It means that the effect of the infusion of these plants is due to the action of salvianolic acids and other water-soluble compounds and not due to the presence of triterpenic acids.

Methods

Plant material

The plants used in this study are: *Clinopodium brevicalyx* Epling (Harley & Granda) (*Menthinae*), *Salvia oppositiflora* (R. and P.) (*Salviinae*), *Minthostachys mollis* Griseb. (*Menthinae*), *Salvia sagittata* R. and P. (*Salviinae*), *Salvia cuspidata* subsp. *cuspidata* (R. and P.) (*Salviinae*), *Clinopodium revolutum* (R. and P.) (*Menthinae*), *Clinopodium sericeum* (Briq. et Benth) Govaerts (*Menthinae*), *Salvia haenkei* Benth. (*Salviinae*), *Salvia dombeyi* Epl. (*Salviinae*), *Hedeoma mandoniana* Wedd. (*Menthinae*), *Clinopodium pulchellum* Kunth (Govaerts) (*Salviinae*). All of them were collected in Peru (2014-2018) by the author (C.S.) according to the Universidad San Antonio Abad procedures and following the guidelines of the Herbarium Truxillense of the Universidad Nacional de Trujillo-Perú https://facbio.unitru.edu.pe. Specimens were identified and deposited by the botanist Eric Frank Rodríguez.

Sample preparation for metabolite profiling

50 mg of pulverized aerial parts are subjected to an ultrasonic bath for five minutes with 1 mL of ethanol three times. The filtrates are evaporated in vacuo and stored at 4°C until use.

UHPLC- Q- Exactive – Orbitrap – MS

Chromatographic separation was performed on a Thermo Scientific Dionex Ultimate 3000 UHPLC system with an Acclaim RPC18 150 x 4.6 mm x 1.8 µm chromatographic column at 25°C and a gradient of a) 0.1% H2CO2 in water and b) acetonitrile: (time, % b)): (0.5); (5,5); (10.30); (15.30); (20,70); (25.70); (35.5) and 12 min of equilibration before each injection. The flow was 1 mLmin-1 and the injection volume was 10 µL. The extracts are dissolved in 1.5 mL of methanol and filtered through 0.22 µm PTFE. For mass spectrometry, a Q-Exactive MS (Thermo Fisher Germany) equipped with electrospray ionization (ESI) in negative mode was used. The MS collection parameters were: spray voltage 2500 V. Capillary temperature 400°C. Sheath gas flowed at a rate of 75 units. Auxiliary gas flowed at 20 units. Scanning range of 100/1500 m/z. Resolution 35,000. The mass tolerance threshold was 5 ppm. Data acquisition and processing was done with XCalibur 2.3 (Thermo Fisher Scientific).

Isolation of ursolic acid from *Clinopodium revolutum*

Dry and ground material with a content of 4.48% ursolic acid and 1.27% oleanolic acid was used. 53.9 g are Soxhlet defatted with petroleum ether and then extracted with 96% ethanol until exhausted. The dry ethanolic extract (15 g) is impregnated in 40-63 µm Si Gel and placed on a 1 cm high bed of the same sorbent in a 9 cm diameter Büchner funnel and eluted with 1.- ether of petroleum, 2.- ethyl acetate and 3.- 96% ethanol. The 7.5 g of alcoholic eluate by dissolution in 80 mL of boiling ethanol in 24 hours produces 1200 mg of ursolic acid needles of 90.91% purity. Recrystallization of this product from 100 mL of ethanol produced 900 mg of 95.34% pure crystals. The purity of ursolic acid was verified by HPLC [45] and its identity by ^{1}HNMR and ^{13}CNMR spectroscopy.

^{1}H-NMR (in CD3OD, 400 MHz): δ 5.22, 1H, m, H-12; δ 3.15, 1H, m, H-3; δ 2.20, 1H, J = 11.2 Hz, H-18; δ 2.02-1.15, m, H-22; δ 1.12, 3H, s, H-23; δ 0.972, 3H, s, H-27; δ 0.966, 3H, s, H-26; δ 0.956, 3H, s, H-24; δ 0.88, 3H, d, H-29; δ 0.84, 3H, d, H-30; δ 0.77, 3H, s, H-25. ^{13}C-NMR (in CD3-OD, 101 MHz): δ 38.12 (C-1), 27.91 (C-2), 79.71 (C-3), 40.43 (C-4), 58.33 (C-5), 19.49 (C-6), 34.35 (C-7), 40.80 (C-8), 49.04 (C-9), 38.15 (C-10), 17.82 (C-11), 126.90 (C-12), 139.64(C-13), 43.26 (C-14), 29.23(C-15), 25.34(C-16), 48.37(C-17), 54.38(C-18), 40.01(C-19), 39.85 (C-20), 31.78 (C-21), 32.70 (C-22), 28.78 (C-23), 16.03 (C-24), 16.39 (C-25), 17.65 (C-26), 24.10 (C-27), 181.64 (C-28), 16.39 (C-29), 21.58 (C-30). [56, 57].

This last recrystallization was found to be crucial. Subsequent experiments showed that 50 mg samples of 90% purity ursolic acid recrystallized in 6.3-3.6 mL (0.8-1.4%) of ethanol in sealed vials at 60°C and then cooled to 15.8°C over a period of 24 hours produce 25 mg of ursolic acid with a purity greater than 98%.

Preparation and Characterization of aqueous infusions in eleven *Mentheae*

A 50 mg plant sample was infused with 10 mL of boiling water for 10 minutes. It was filtered (0.22 µm) and in the filtrate total phenols (Folin Ciocalteau) [6], antioxidant capacity (Blue Mo(V)) [6] and the content of rosmarinic acid and triterpenic acids were quantified by UHPLC [6, 45].

For rosmarinic acid quantification, DAD-UHPLC (Dionex Thermoscientific Ultimate 3000 UHPLC with Chromeleon 7.2 software) was used with a 100 x 2.1 mm x 1.8 µm RPC_{18} Zorbax Eclipse Rapid Resolution column and a 1.5 x 2.1 mm x 1.8 µm UHPLC Zorbax Eclipse column guard. The working conditions were: Separation temperature: 40°C, Flow: 0.4 mL/minute. Gradient: a) 0.1% H2CO2; b) acetonitrile; (time, %b)): (0,0); (1,0); (6.40); (9,100); (13,100); (14,0); (17.0). The quantification was done with the readings obtained at 330 nm. In parallel, a calibration curve was made with standard rosmarinic acid (Aldrich) of 2, 1.5, 1, 0.5 and 0.2 mg/mL with a linearity of 0.999. The analysis was run in triplicate.

Quantification of triterpenic acids by HPLC [45] (Dionex Thermoscientific Ultimate 3000 UHPLC, with Chromeleon 7.2 software) was performed using a Phenomenex Lichrospher RPC_{18} 25 x 0.46 cm x 5 µm column and Zorbax Eclipse 1.5 x 4.6 mm x 5 µm HPLC column guard. . The analysis conditions were as follows: Analysis time: 20 minutes. Temperature: 30°C. Elution mode: isocratic (acetonitrile:water, 8:2). Detection at 209 nm. A calibration curve was made with a mixed standard of oleanolic acid and ursolic acid (Chromadex) of 2, 1, 0.5 and 0.3 mg/mL of AO and AU. The analysis was run in triplicate.

Declarations

Data Availability Statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing Interest Statement

The authors declare no conflict of interest.

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Figures

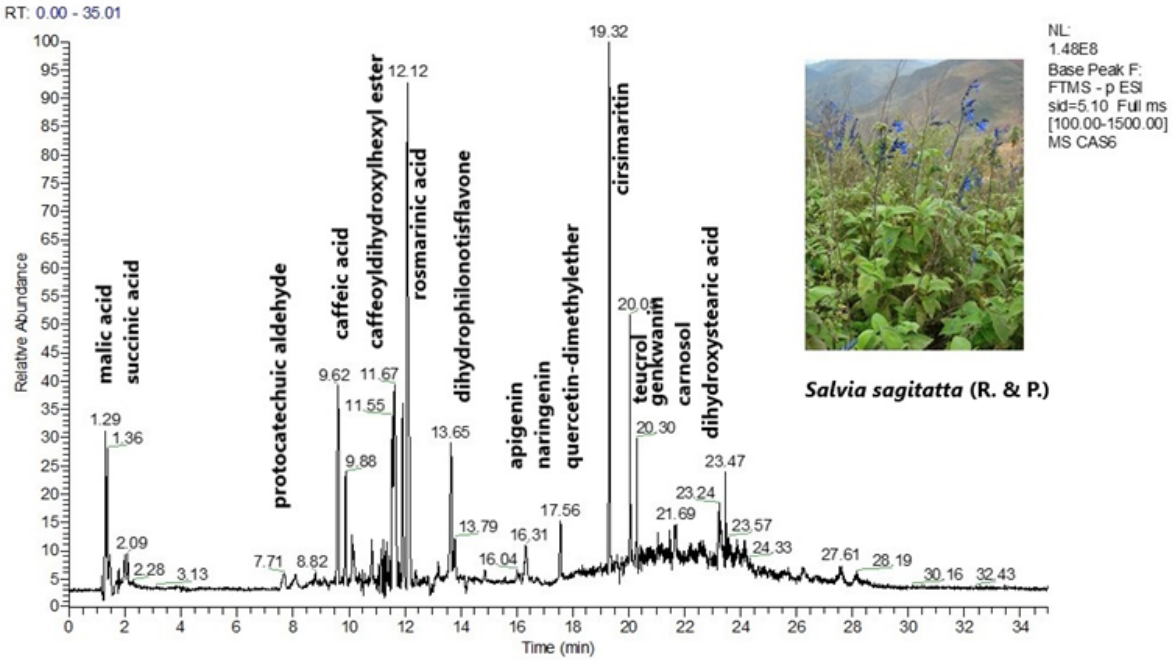

Figure 1

ESI(-) chromatogram of *Salvia sagitatta*.

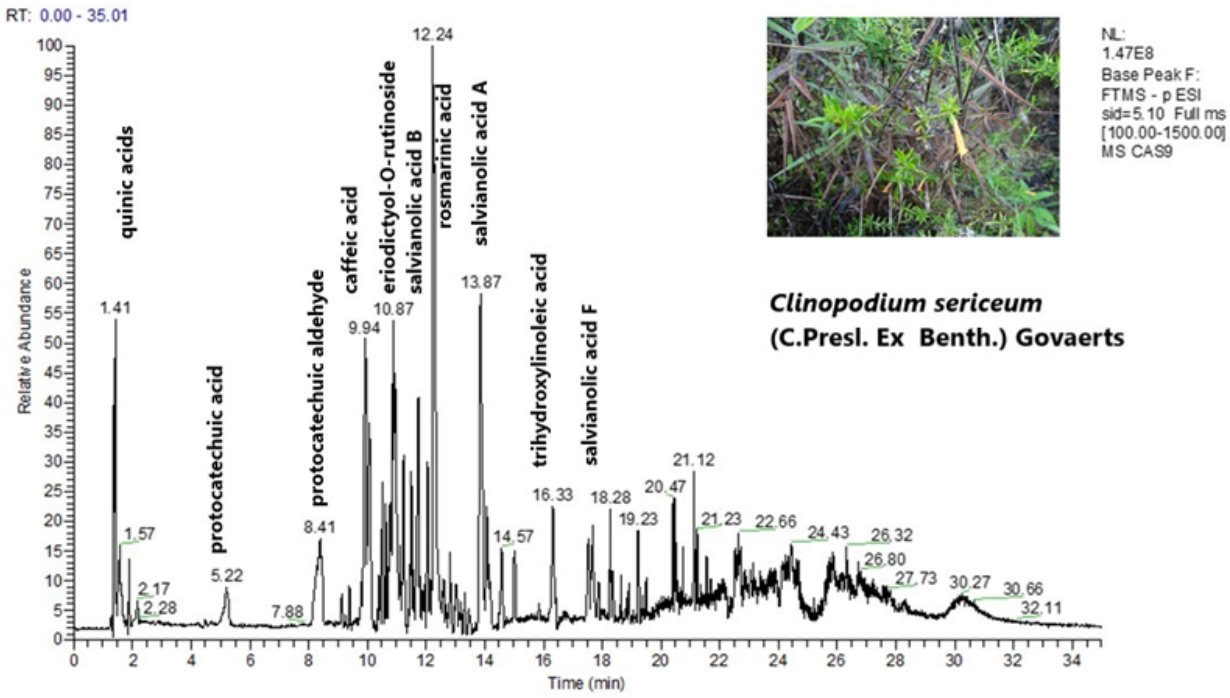

Figure 2

ESI(-) chromatogram of *Clinopodium sericeum*.

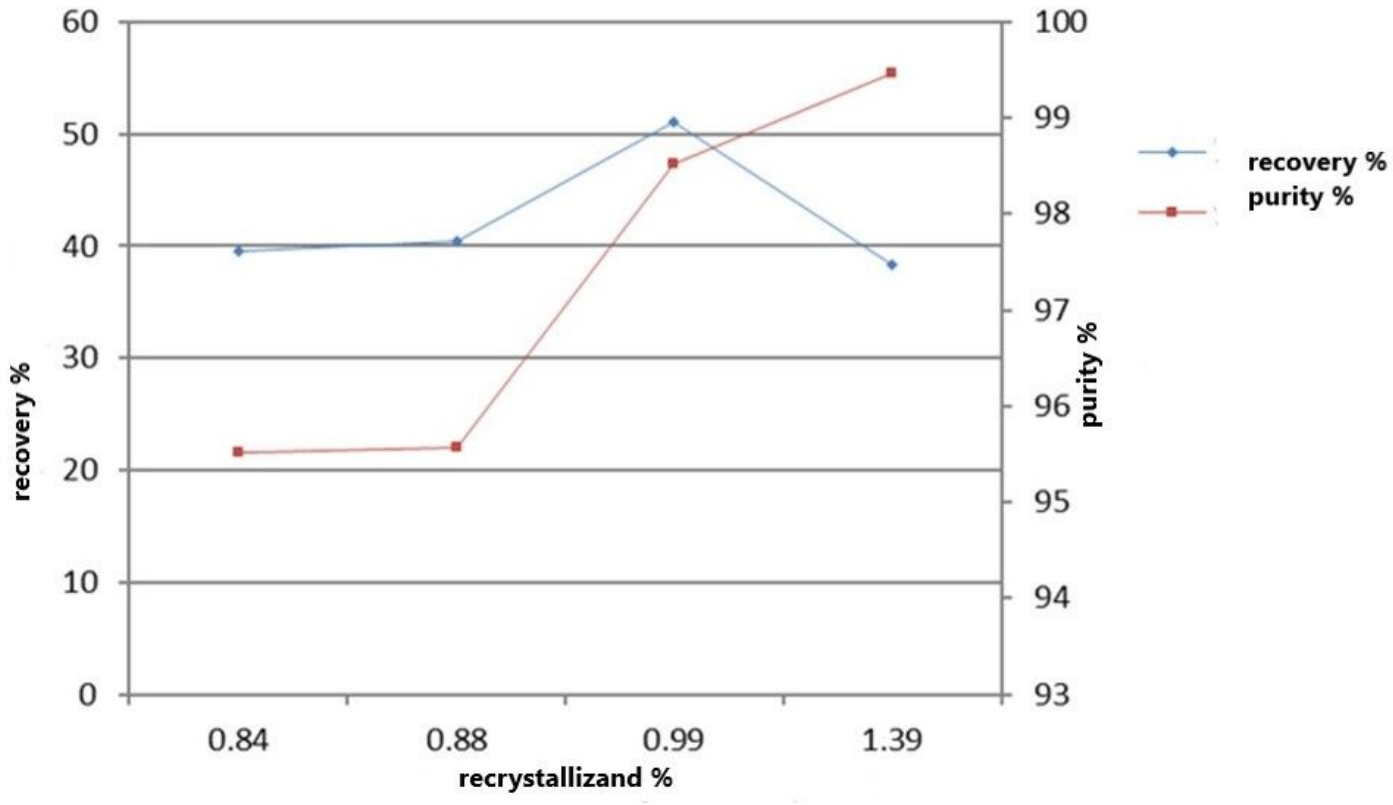

Figure 3

Recovery and purity in ursolic acid recrystallized from ethanol.

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