Non-targeted secondary metabolites screening of *Thymus capitatus* growing in Palestine

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Methods:

0.5 gram of lyophilized sample was extracted by aqueous methanol. Then the mixture was vortexed, sonicated for 30 min, and finally centrifuged for 15 min at 4000 rpm. The supernatant was collected in a round bottom flask and thoroughly evaporated. The final extract was resolved in 0.5 ml of aqueous methanol which was filtered and finally injected in the instrument. The analysis were performed by using an HPLC coupled to QTOF-MS equipped with an ESI interface operating in negative ionization mode using a C18 column and acidified water and acetonitrile as mobile phases at 0.8 mL/min.[1]

HPLC Conditions

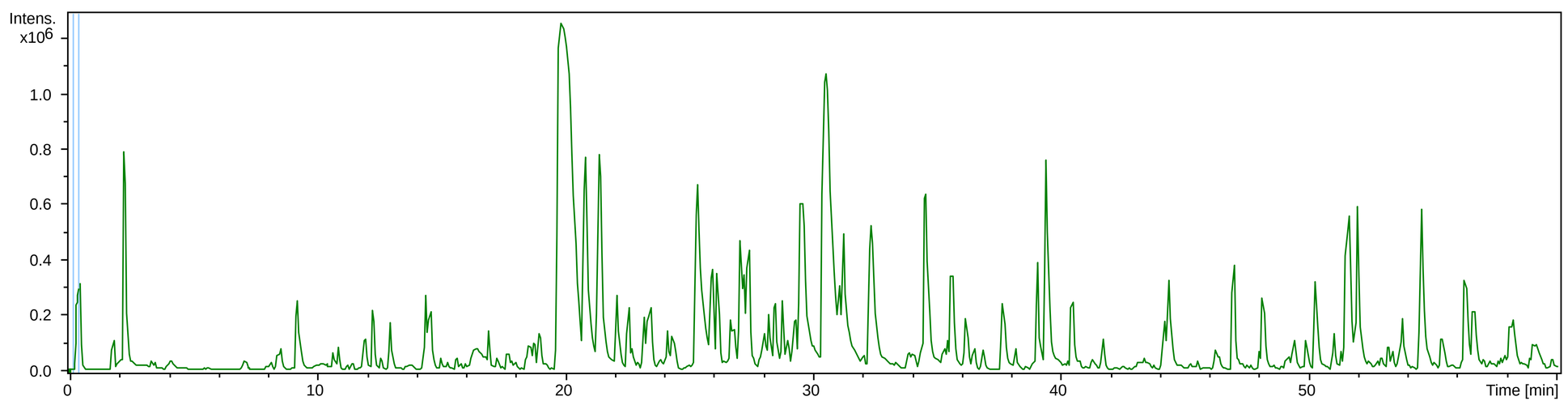
- **Instrument**: Agilent 1200 RRLC system.
- **Column:** C_{18} 4.6 x 150 mm, 1.8 µm.
- **Temperature:** 25 ºC.
- **Injection volume:** 10 µl.
- **Flow:** 0.8 ml/min.
- **Mobile phase A:** (H_2O+ Acetic Acid) 0.05%.
- **Mobile phase B:** (100%, v/v) Acetonitrile.

ESI-QTOF-MS Conditions

- **Instrument Bruker Daltonics Q-TOF**
- **Mode:** Negative
- **Mass range:** 50-1100 m/z
- **Nebulizing gas pressure:** 29 psi
- **Drying gas flow:** 9 L/min
- **Flow split ratio:** 1:3
- **Drying gas temperature:** 190 ºC
- **Capillary:** 4.5 kV
- **Collision RF:** 150 Vpp.
- **Transfer Time:** 70 µs
- **Pre-pulse storage:** 5 µs

Results

The coupling of HPLC to ESI-QTOF-MS has demonstrated to be a useful tool for the tentative characterization of 41 phenolic compounds and other polar compounds; most of them have not been described so far in *Thymus capitatus*.



Peak No.	Rt (min)	Observed m/z	Calculated m/z	Empirical formula	Error (ppm)	mSigma	Major MS/MS fragments	Proposed Compound
Hydroxybenzoic acid derivatives								
3	8.23	315.0715	315.0722	$C_{13}H_{15}O_9$	2.2	38.8	153.0195, 109.0277	Protocatechuic acid glucoside I
6	9.84	301.0571	301.0565	$C_{12}H_{13}O_9$	-2.0	41.7	125.0235	Phloroglucinol glucuronide
8	10.48	299.0770	299.0772	$C_{13}H_{15}O_8$	0.7	1.3	137.0236	Salicylic acid-O-hexoside I
9	10.68	315.1072	315.1085	$C_{14}H_{19}O_8$	4.3	5.0	151.0397	Vanillin hexoside I
12	11.30	659.1826	659.1829	$C_{28}H_{35}O_{18}$	0.5	15.7	497.1281, 335.0764	Tri-O-caffeoylshikimic acid I
15	11.64	329.0880	329.0878	$C_{14}H_{17}O_9$	-0.6	9.2	167.0349	Vanillic acid hexoside
39	18.20	389.1249	389.1242	$C_{20}H_{21}O_8$	-1.7	35.3	Nd	Salicin 2-benzoate
Hydroxycinnamic acid derivatives								
5	9.67	325.0927	325.0929	$C_{15}H_{17}O_8$	0.4	21.1	163.0416, 145.0296	p-coumaric acid glucoside I
10	10.82	355.1028	355.1035	$C_{16}H_{19}O_9$	1.8	5.5	193.0512, 175.0396	Ferulic acid hexoside I
11	10.85	517.1556	517.1563	$C_{22}H_{29}O_{14}$	1.3	11.6	193.0517	3-O-feruloylsucrose
18	12.56	341.0877	341.0878	$C_{15}H_{17}O_9$	0.3	1.7	179.0351, 135.0421	Caffeoylhexose I
20	13.43	461.1646	461.1664	$C_{20}H_{29}O_{12}$	4.1	7.4	133.0502	Decaffeoylacetoside or Descaffeoylverbascoside
21	13.57	497.1278	497.1301	$C_{22}H_{25}O_{13}$	4.5	2.0	335.0767, 179.0344	Dicaffeoylshikimic acid I
32	16.59	385.1134	385.1140	$C_{17}H_{21}O_{10}$	1.5	3.4	223.0619, 205.0506	Sinapic acid glucoside
37	18.15	471.1510	471.1508	$C_{21}H_{27}O_{12}$	-0.5	21.3	193.0497	O-(trans)feruloyl-arabinofuranosyl-xylopyranose I
40	18.30	335.0749	335.0772	$C_{16}H_{15}O_8$	-1.0	6.5	179.0351, 161.0272, 135.0451	O-Caffeoylshikimic acid I
59	22.94	349.0931	349.0929	$C_{17}H_{17}O_8$	-0.5	23.5	193.0502	O-feruloylquinide
Flavonoids derivatives								
31	16.56	593.1527	593.1512	$C_{27}H_{29}O_{15}$	-2.6	23.3	431.0987	Kaempferol rhamnoside-hexoside I
35	18.11	623.1596	623.1618	$C_{28}H_{31}O_{16}$	3.4	53.8	Nd	Isorhamnetin 3-O-rutinoside or Lucenin-2-methyl ether
41	18.44	449.1097	449.1089	$C_{21}H_{21}O_{11}$	-1.7	12.3	287.0786	Eriodictyol 7-glucoside or Dihydrokaempferol 7-glucoside I
42	18.46	609.1488	609.1461	$C_{27}H_{29}O_{16}$	-4.4	40.7	447.0911	Quercetin 3-rutinoside (rutin)
44	18.54	627.1557	627.1567	$C_{27}H_{31}O_{17}$	1.6	16.3	465.1038, 303.0515	Taxifolin dihexoside (Calodendroside A)
47	19.63	447.0934	447.0933	$C_{21}H_{19}O_{11}$	6.4	12.7	357.0591, 327.0519	Luteolin 6-C-glucoside (Isoorientin)
48	19.85	579.1736	579.1719	$C_{27}H_{31}O_{14}$	-2.9	14.7	271.0722	Naringenin 7-neohesperidoside or Naringenin 7-rutinoside I
51	20.84	447.0943	447.0933	$C_{21}H_{19}O_{11}$	-2.2	31.2	285.0437	Luteolin-O-hexoside I

Conclusions:

To the best of our knowledge, this study reports the first characterization work of phenolic compounds (Flavonoids, phenolic acids) and other polar compounds in *Thymus capitatum*. The obtained data may facilitate the quantitative analysis of this important medicinal plant.