

PHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITY OF MINT (MENTHA SPP.) HONEY

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The composition of honey is influenced by the **botanical source** and **geographical area** of the nectar from which it is derived. Unifloral honeys reach higher market values than multifloral ones due to their specific and reproducible aromas, which result from volatile and phenolic compounds.

The aim of our study was to characterize the phenolic composition of a rare unifloral variety of honey- *Mentha spp.* honey.

Keywords: *Mentha spp.* honey, antioxidant activity, LC-MS/MS, phenols, phenolic profiling

Methodology

Seven samples of *Mentha spp.* honey were collected directly from local Croatian producers. Samples were collected between years 2015 and 2020 at different geographical locations in Croatia (Figure 3). For all the samples, total phenolic content (Folin method), flavonoid content, and antioxidant activity (DPPH and ABTS assays) were determined. In addition, by use of LC-MS/MS, qualitative and quantitative analysis of phenolic compounds were obtained.



Figure 1. *Mentha aquatica* L.



Figure 2. *Mentha pulegium* L.

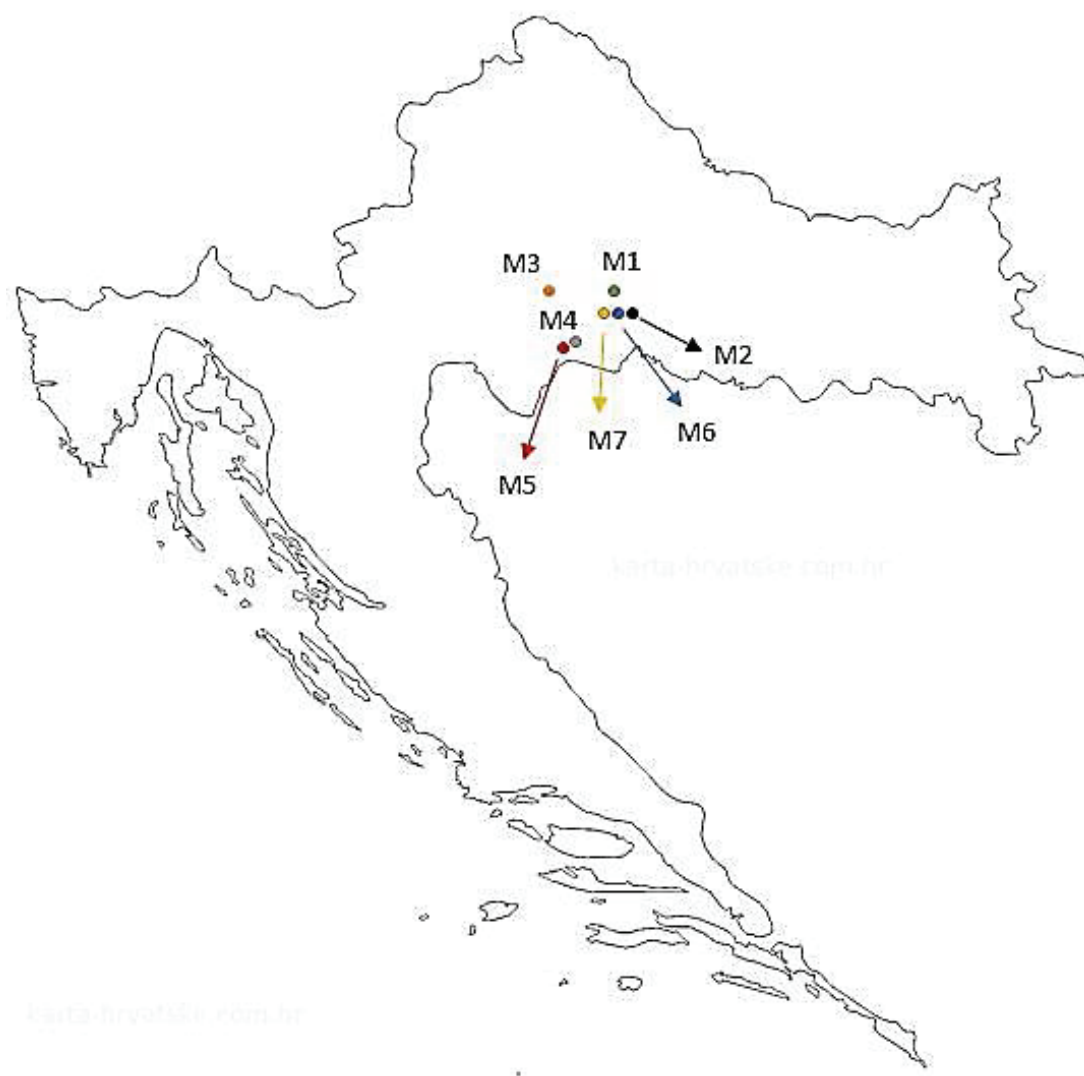


Figure 3. Sample locations

Results

Our results indicate that *Mentha spp.* honey have high phenolic content, ranging from 76.66±0.56 to 121.14±3.81 mgGAEeq phenols/100g and 6.70±0.62 to 17.10±0.68 mgQUEeq flavonoids/100g (Table 1). These honeys also exhibit strong antioxidant activity ranging from 33.58±2.80 to 57.86±1.24 mg Trolox eq/100g and 14.37±0.85 to 57.83±0.18 mg Trolox eq/100g when analysed using DPPH and ABTS assays, respectively (Table 1). Quantitative LC-MS/MS analysis revealed that the most abundant phenols in all samples were chrysin, apigenin and *p*-coumaric acid. Qualitative LC-MS/MS analysis identified the presence of kaempferide, diosmetin, acacetin and several caffeic acid derivatives.

Table 1. Total phenolic content, total flavonoid content and antioxidant activity of *Mentha* L honey. Results are expressed as mean ± standard deviation.

Sample	Total phenols mg eqGAE/100 g honey	Total flavonoids mg eqQUE/100 g honey	DPPH mg eqTROLOX/ 100 g honey	ABTS mg eqTROLOX/ 100 g honey
M1	121.14 ± 3.81	13.65 ± 0.58	57.40 ± 0.81	47.18 ± 0.31
M2	89.76 ± 0.16	11.59 ± 0.35	40.89 ± 1.90	38.42 ± 0.62
M3	78.35 ± 1.25	10.60 ± 0.54	42.72 ± 1.87	30.36 ± 0.74
M4	90.06 ± 1.05	12.52 ± 0.81	51.26 ± 1.20	44.81 ± 1.90
M5	118.60 ± 2.09	17.10 ± 0.68	57.86 ± 1.24	57.83 ± 0.18
M6	78.44 ± 0.34	7.77 ± 0.24	33.58 ± 2.80	14.37 ± 0.85
M7	76.66 ± 0.56	6.70 ± 0.62	36.73 ± 2.00	55.07 ± 2.37

Table 2. Quantified phenolic compounds in *Mentha* L honey. Results are expressed as mean mg/100 g honey ± standard deviation.

	M1	M2	M3	M4	M5	M6	M7
2,5-DHBA	0.0130 ± 0.0184	<LOQ*	<LOQ	0.0450 ± 0.0080	<LOQ	0.0136 ± 0.0017	0.0133 ± 0.0008
3,4-DHBA	0.0786 ± 0.0079	0.1776 ± 0.0014	0.0726 ± 0.0024	0.5652 ± 0.1604	0.1235 ± 0.0027	0.3425 ± 0.1226	NA
apigenin	0.2199 ± 0.0168	0.2424 ± 0.0176	0.6393 ± 0.0149	0.1093 ± 0.0010	0.1098 ± 0.0013	0.1789 ± 0.0098	0.1065 ± 0.0002
caffeic acid	0.1299 ± 0.0113	0.1907 ± 0.0084	0.1284 ± 0.0205	0.1541 ± 0.0504	<LOQ	0.1148 ± 0.0089	0.2772 ± 0.2083
chrysin	1.3297 ± 0.5159	0.4760 ± 0.0957	0.2898 ± 0.0181	0.1119 ± 0.0049	0.2205 ± 0.0404	0.5137 ± 0.0682	0.2506 ± 0.0073
kaempferol	0.0352 ± 0.0006	0.0587 ± 0.0020	0.0722 ± 0.0034	0.0337 ± 0.0005	0.0327 ± 0.0027	0.0661 ± 0.0239	0.0348 ± 0.0003
luteolin	0.0572 ± 0.0017	0.0164 ± 0.0003	0.0108 ± 0.0002	0.0142 ± 0.0004	0.0223 ± 0.0006	0.0096 ± 0.0002	0.0179 ± 0.0010
myricetin	0.0008 ± 0.0002	0.0042 ± 0.0001	0.0016 ± 0.0018	0.0017 ± 0.0005	0.0019 ± 0.0001	0.0004 ± 0.0004	0.0058 ± 0.0001
naringenin	0.0656 ± 0.0016	0.0314 ± 0.0002	0.0315 ± 0.0000	0.0131 ± 0.0151	0.0232 ± 0.0006	0.0254 ± 0.0002	0.0255 ± 0.0006
p-coumaric acid	0.6790 ± 0.1075	0.5338 ± 0.0443	0.3732 ± 0.0248	0.7105 ± 0.1523	0.4314 ± 0.0633	0.8166 ± 0.2376	0.3400 ± 0.0151
quercetin	0.0320 ± 0.0006	0.1015 ± 0.0006	0.1279 ± 0.0001	0.0580 ± 0.0004	0.0573 ± 0.0012	0.0960 ± 0.0004	0.1495 ± 0.0228

Table 3 Some of the compounds in *Mentha* L. honey identified via LC-MS/MS. In the table, tentative identification based on the retention time (RT), precursor mass and fragmentation pattern are given. Level of confirmation: 1- standard, 2- literature

Tentative identification	RT	Precursor mass	Fragmentation pattern	Molecular formula	Level of confirmation
Dimethyl caffeic acid	8.6	209.0 (+)	190.7 [C11H11O3] ⁺ , 162.9 [C9H7O3] ⁺ , 133.0 [C9H9O] ⁺ , 118.9 [C7H3O2] ⁺	C ₉ H ₉ O ₄	2
Abscicic acid	8.9	263.0 (-)	219.1 [C14H19O2] ⁻ , 203.8 [C13H15O2] ⁻ , 152.9 [C9H13O2] ⁻	C ₁₅ H ₁₅ O ₄	2
Sebacic acid	9.2	200.9 (-)	182.8 [C10H15O3] ⁻ , 138.8 [C9H15O] ⁻ , 110.9 [C8H15] ⁻	C ₁₆ H ₃₁ O ₄	2
Quercetin methyl ether	10	315.0 (-)	300.0 [M-CH3] ⁻ , 270.7 [M-CH3-CO] ⁻ , 255.0	C ₁₆ H ₁₅ O ₆	2
Diosmetin	10.9	301.0 (+)	285.9 [C15H9O6] ⁺ , 257.9 [C14H9O5] ⁺ , 228.7 [C13H9O4] ⁺	C ₁₆ H ₁₁ O ₆	1
Kaempferide	11	299.0 (-)	284.0 [C15H8O6] ⁻ , 255.0, 227.0 [C13H7O4] ⁻	C ₁₆ H ₁₂ O ₆	2
Caffeic acid prenyl ester (prenyl caffeate)	11.4	247.0 (-)	246.7, 178.8 [C9H7O4] ⁻ , 160.8 [C9H5O3] ⁻ , 135.0 [C9H7O4-CO2] ⁻	C ₁₈ H ₁₆ O ₄	2
Caffeic acid phenylethyl ether (CAPE)	11.5	283.0 (-)	179.0 [C9H7O4] ⁻ , 135.0 [C9H7O4-CO2] ⁻	C ₁₇ H ₁₆ O ₄	2
Acacetin	11.7	283.0 (-)	268.0	C ₁₈ H ₁₈ O ₄	2

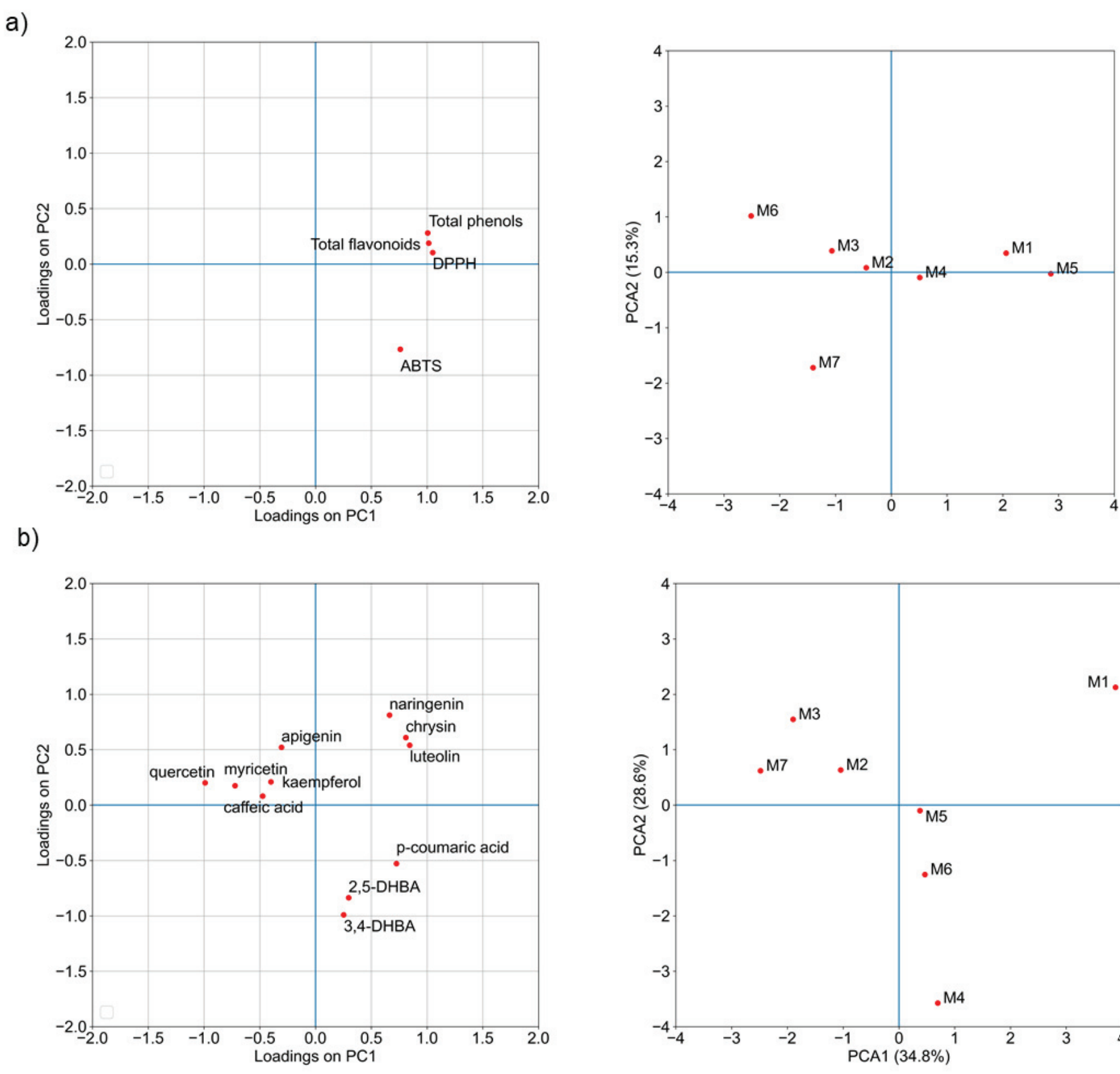


Figure 4. Distribution of samples in the space of principal component 1 (PC1) and principal component 2 (PC2) for a) total phenolic content, total flavonoid content, DPPH and ABTS antioxidant activities, and b) quantified phenolic compounds (LC-MS/MS) in *Mentha* L. honeys.

Conclusion

Our study indicates that *Mentha spp.* honeys contain unique phenolic profiles, which likely contribute to their distinctive aroma and strong antioxidant activity.

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