Phytochemical Profile, Chemotaxonomic Studies, and *In Vitro* Antioxidant Activities of Two Endemisms from Madeira Archipelago: *Melanoselinum decipiens* and *Monizia edulis* (Apiaceae)

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Melanoselinum decipiens and *Monizia edulis* (Apiaceae) are two endemic plants from Madeira archipelago, phytochemical compositions of which remains little explored, despite their use in folk medicine. Using liquid chromatography with diode array and electrospray ionization/mass spectrometry analysis, their polyphenolic profile was established for the first time. Fifty-six compounds were identified with 5-*O*-caffeoylquinic acid, quercetin-*O*-(malonyl)hexoside, luteolin diacetyl, and quercetin-*O*-hexoside being the major constituents in the leaves of both plant species ($\geq$ 0.76 mg/g of dry extract). Principal component analysis provided a suitable tool to differentiate targeted plants. Naringenin-6,8-di-*C*-glucoside, quercetin 3-*O*-pentosylhexoside, and 1,5-*O*-dicaffeoylquinic acid can be used as discriminatory taxonomic/geographical markers for *M. edulis* subspecies from Madeira and Porto Santo populations. This methodology of using polyphenols as chemotaxonomic markers proved to be useful for identification of plant species since the results are consistent with previous taxonomical data. The free-radical scavenging activities of the *M. decipiens* extracts proved to be higher than those of *M. edulis*, which correlated well with their phenolic content ($R^2 > 0.906$).

Keywords: *Melanoselinum decipiens*, *Monizia edulis*, Polyphenols, Chemotaxonomy, Antioxidant activities.

Introduction

The study of the phytochemical chemical profile is rather important for herbal identification, clarification of their bioactivities and possible side effects, and enhancing product quality control.[1] High-performance liquid chromatography has been widely used for the chemical identification of plant secondary metabolites and discrimination of many closely related herbs, since it can provide accurate information on complex samples and mixtures.[2] Flavonoids and phenolic acids in particular are highly relevant as bioactive components of medicinal and edible plants. One important feature of polyphenols is their usage in chemotaxonomic studies, mainly due to their abundant occurrence in vascular plants and their structural variability and stability.[3 – 5] These secondary constituents have been used to solve taxonomic problems that have arisen as a result of morphological classification.[1][2][6][7] Principle component analysis (PCA) has been successfully applied for distinguishing, for instance, *Angelica sinensis* from related Apiaceae herbs based on their chromatographic profiles and coniferyl ferulate amount.[8] *Olivier et al.*[9] used rosmarinic acid and its derivatives for the chemotaxonomical differentiation in Apiaceae subfamilies. Chemotaxonomic classification has also been applied for quality control of grapevines, tomatoes, flowers, and medicinal herbs.[1][7][10 – 12]

Apiaceae (or Umbelliferae) is a large family represented by 2500 – 3700 species, which includes known plants used in culinary like parsley, carrot, celery, coriander, fennel, and cumin.[13] *Melanoselinum decipiens* (Schrad. & J. C. Wendl.) Hoffm and *Monizia edulis* Lowe (Apiaceae) are two endemic plants from Madeira archipelago (Portugal),[14][15] phytochemical compositions of which have been paid little attention.

Melanoselinum decipiens ('Madeira giant black parsley' or 'cattle celery') inhabits shady rocks and banks in Laurissilva (Madeira Laurel Forest) and is an herbaceous monocarpic perennial shrub, resembling giant parsley, that can grow up to 3 m high.[15] It was once cultivated for cattle fodder and their leaf extracts were used for skin diseases.[16]

Monizia edulis ('carrot tree') is a monocarpic, perennial shrub growing at clefts or hollows and hedges of the islands, that resembles a large arborescence carrot (about 1.2 m tall).[15] Formerly, it has

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been used as vegetable (stout roots) by fishermen and goat-herders in lack of other food supplies. This species was also gathered from the wild for medicinal digestive properties of the leaves.[17] Based on morphological traits, four subspecies of *M. edulis* have been recently designated from Madeira archipelago[18]: two from Madeira Island (*M. edulis* subsp. *isambertoi* and *M. edulis* subsp. *giranus*); one on Deserta Grande Island (*M. edulis* subsp. *edulis*) and another on Porto Santo Island (*M. edulis* subsp. *santosii*).

Previous studies have reported β-pinene as the predominant volatile from *M. decipiens* (essential oils and aerial parts)[19][20] and γ-terpinene and myristicin as main components from *M. edulis* essential oils.[21] The presence of some sesquiterpene lactones was also described in *M. decipiens* leaves.[22][23]

Despite the existence of the mentioned works, they addressed only essential oils components and overlooked phenolic characterization. Therefore, the aim of this study was to establish, for the first time, the phytochemical profile of MeOH leaf extracts from *M. decipiens* and *M. edulis*. Also, a comparison was made for *M. edulis* populations collected in two different geographical locations. PCA was used to investigate whether polyphenolic composition of *M. edulis* subspecies provide any correspondence with and support for the establishment of currently recognized described *taxa*. Additionally, correlation of the polyphenols with the *in vitro* antioxidant activity of these plants' extracts was determined, in order to evaluate their interest as possible novel foodstuffs.

Results and Discussion

Qualitative Phytochemical Analysis

In this study, MeOH extracts from two plant species were submitted to liquid chromatography with diode array and electrospray ionization/mass spectrometry (HPLC-DAD-ESI$^-$/MS^n) analysis in order to establish their phytochemical profile and identify potential chemotaxonomic markers. Representative chromatograms of the MeOH extracts are shown in *Fig. 1*.

In general, in the negative ionization mode (ESI$^-$) MS^1 spectrum, the most intense peak corresponded to the deprotonated molecular ion [M − H]$^-$; this permitted to perform MS^n analysis. The mass spectra of the conjugated phenolic compounds showed the aglycone ion as a result of the loss of sugar moieties like hexosyl, caffeoyl, and pentosyl (−162, −162, −132 Da, resp.). Compounds were numbered by their order of elution and this numeration was kept identical for both species (*Table 1*).

Among the 56 identified compounds, there were 23 phenolic acids (hydroxycinnamic and hydroxybenzoic acids), 18 *O*-flavonoids (flavones, flavonols, and flavanones type), and 15 other compounds (terpenoid, lignan, coumarin, organic acids, and saccharides). As expected, different species exhibited different phytochemical profiles. Within the same species, quantitative variations were more relevant than qualitative ones, but still there are statistically significant differences. Nevertheless, most of the identified compounds were common to both species.

Phenolic Acids

The presence of mono- and dicaffeoylquinic acids isomers was confirmed by comparison of their MS^n spectra with standards and information from previous reports.[24][25]

Compounds **9** and **17** gave [M − H]$^-$ ions at *m/z* 353 and showed fragment ion at *m/z* 191 as MS^2 base peak. Compound **9** showed an intense fragment ion at *m/z* 179 (> 40% of base peak) and was characterized as 3-*O*-caffeoylquinic acid. The isomer 5-*O*-caffeoylquinic acid, with higher retention time, was assigned to compound **17**.

Compound **10** displayed [M − H]$^-$ ion at *m/z* 707 and the presence of fragment ions at *m/z* 515 and 353, characteristic of caffeoylquinic acid (CQA) derivatives, was observed at MS^2. However, **10** was not completely characterized being assigned as a dicaffeoylquinic acid derivative.

Compound **31** displayed fragmentation 367 → 179 and was identified as methyl-(5-caffeoyl)quinate according to literature.[26]

Compounds **45**, **48**, **52**, and **54** all exhibited [M − H]$^-$ ions at *m/z* 515 and were identified as 3,4-1,5-, 3,5-, and 4,5-*O*-dicaffeoylquinic acid, respectively, by comparison with analytical standards. The presence of caffeoylquinic acid derivatives was previously reported in other Apiaceae species.[27]

Compound **5** with [M − H]$^-$ at *m/z* 395 suffered loss of 36 Da at MS^2, followed by typical fragmentation of rosmarinic acid 359 → 161.[4] Thus, **5** was identified as a derivative of rosmarinic acid.

Compound **6** exhibited [M − H]$^-$ ion at *m/z* 349 and produced MS^2 base peak at *m/z* 313 (by loss of 36 Da). After loss of a sugar moiety, it gave origin to fragment ions at *m/z* 177 and 151 typical of vanillin.[28] Since additional data were not available, **6** was characterized as a derivative of vanillin-*O*-hexoside.

Sinapic acid-*O*-hexoside was assigned to compound **12** with typical fragmentation pattern 385 → 223.[29]

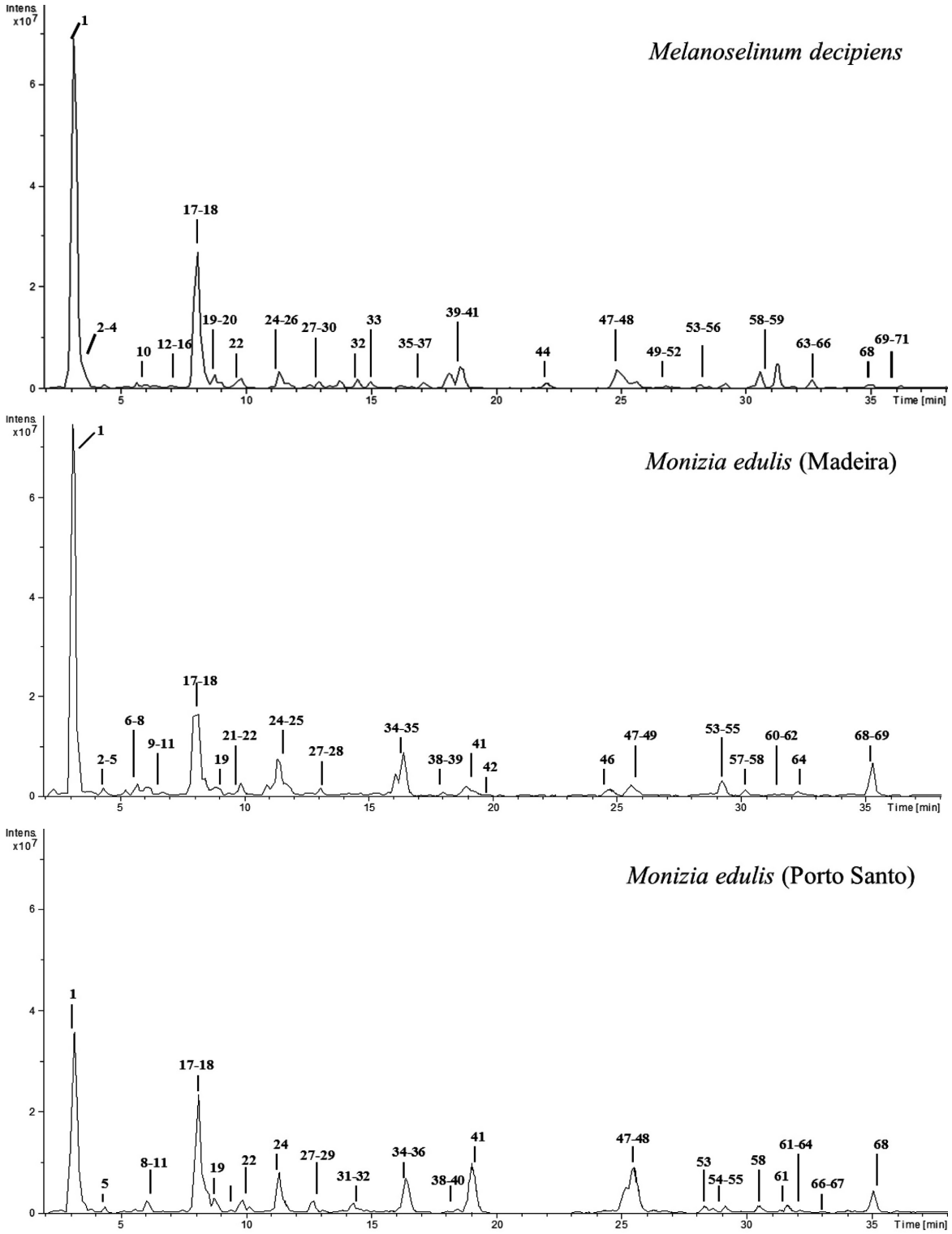

Figure 1. HPLC-DAD-ESI/MS^n base peak chromatograms (BPC) of *Melanoselinum decipiens* and *Monizia edulis* MeOH extracts (for peak identification see *Table 1*).

Compound **15** displayed $[M - H]^-$ ion at *m/z* 325, and after loss of 162 Da showed typical fragmentation of coumaric acid (163 → 119), thus was identified as coumaric acid-*O*-hexoside.[4]

Compounds **26** and **27** were identified as coumaroylquinic and feruloylquinic acids, respectively.[30] Other coumaric acid derivatives, *p*-coumaroyl-*O*-caffeoylquinic acid (compound **61**) and feruloyl-*O*-caffeoylquinic

Table 1. Characterization of phytochemical compounds from the leaves of *Melanoselinum decipiens* and *Monizia edulis*

No.	t_R [min]	$[M - H]^-$ [m/z]	HPLC-DAD-ESI/MS^n m/z [% base peak]	Assigned identity	*M. decipiens*	*M. edulis*	
					Madeira	Madeira	P. Santo
1	3.2	683	MS^2 [683]: 342 (12.0), 341 (100) MS^3 [683 → 341]: 179 (100), 161 (19.4), 113 (36.9), 131 (16.2) 119 (13.1), 101 (18.6) MS^4 [683 → 341 → 179]: 161 (23.5), 149 (20.0), 143 (34.0), 119 (21.7), 89 (100)	Hexose polymer	+	+	+
2	3.5	533	MS^2 [533]: 191 (100) MS^3 [533 → 191]: 173 (100), 127 (46.7), 109 (17.8), 85 (10.4)	Quinic acid derivative	+	+	+
3	3.7	133	MS^2 [133]: 115 (100) MS^3 [133 → 115]: 71 (100)	Malic acid	+	+	+
4	3.8	191	MS^2 [191]: 173 (36.7), 127 (48.4), 111 (33.7), 85 (100)	Quinic acid	+	+	+
5	4.4	395	MS^2 [395]: 359 (100), 345 (51.9), 335 (60.4), 226 (17.2), 189 (23.8), 179 (38.2), 161 (30.0), 135 (16.2) MS^3 [395 → 359]: 179 (43.5), 161 (100), 135 (45.3)	Rosmarinic acid derivative		+	+
6	5.1	349	MS^2 [349]: 313 (91.6), 313 (100), 151 (12.3), 145 (11.0), 143 (22.8) MS^3 [349 → 313]: 205 (10.7), 178 (55.6), 177 (35.0), 151 (100), 113 (41.2)	Vanillin-*O*-hexoside derivative		+	+
7	5.4	393	MS^2 [393]: 348 (46.7), 345 (100), 331 (75.4), 313 (48.8), 300 (99.4), 297 (13.0), 283 (39.4) MS^3 [393 → 345]: 330 (100), 300 (38.6), 287 (55.9), 283 (86.7), 269 (13.1), 265 (11.2), 149 (16.7)	Quercetagetin-dimethyl ether derivative		+	+
8	5.8	467	MS^2 [467]: 431 (100) MS^3 [467 → 431]: 299 (51.9), 191 (27.9), 161 (21.9), 149 (100), 143 (15.2), 131 (38.3), 125 (13.4) MS^4 [467 → 431 → 149]: 131 (81.2), 119 (22.6), 113 (100), 89 (87.2), 71 (43.7) MS^4 [467 → 431 → 299]: 161 (100), 119 (45.6), 97 (49.0)	Saccharide 2		+	+
9	6.3	353	MS^2 [353]: 191 (100), 179 (59.4), 135 (15.7) MS^3 [353 → 191]: 173 (80.9), 127 (100), 111 (71.7), 93 (59.4), 85 (46.7) MS^4 [353 → 191 → 173]: 111 (100)	3-*O*-Caffeoylquinic acid		+	+
10	6.4	707	MS^2 [707]: 533 (85.7), 515 (49.5), 463 (48.1), 353 (16.3), 323 (100) MS^3 [707 → 323]: 295 (43.7), 191 (100), 179 (28.9), 135 (17.2) MS^3 [707 → 533]: 359 (22.0), 297 (44.5), 271 (47.8), 191 (100) MS^4 [707 → 533 → 191]: 173 (100)	Dicaffeoylquinic acid derivative	+	+	+
11	6.8	415	MS^2 [415]: 379 (100) MS^3 [415 → 379]: 247 (31.5), 179 (21.9), 149 (100), 143 (51.7), 131 (53.9) MS^4 [415 → 379 → 149]: 131 (100), 113 (78.7)	Saccharide 3		+	+

Table 1. (cont.)

No.	t_R [min]	$[M - H]^-$ [m/z]	HPLC-DAD-ESI/MS^n m/z [% base peak]	Assigned identity	*M. decipiens*	*M. edulis*	
					Madeira	Madeira	P. Santo
12	7.1	385	MS^2 [385]: 223 (100), 208 (33.7), 179 (25.5), 135 (25.9) MS^3 [385 → 223]: 208 (100), 205 (30.4), 179 (29.3), 164 (43.1)	Sinapic acid-*O*-hexoside	+		
13	7.4	595	MS^2 [595]: 537 (40.6), 487 (27.0), 475 (42.2), 457 (33.2), 415 (23.8), 397 (36.1), 386 (28.8), 385 (100); 356 (41.4), 355 (74.5) MS^3 [595 → 385]: 355 (29.3), 313 (100), 193 (35.4) MS^4 [595 → 385 → 313]: 193 (100)	Naringenin-6,8-di-*C*-glucoside	+	+	
14	7.4	399	MS^2 [399]: 191 (100), 176 (14.3) MS^3 [399 → 191]: 176 (100) MS^3 [399 → 191 → 176]: 148 (100)	Scopoletin-*O*-hexoside (formate adduct)			+
15	7.6	325	MS^2 [325]: 163 (100) MS^3 [325 → 163]: 119 (100)	Coumaric acid-*O*-hexoside	+	+	+
16	7.8	285	MS^2 [285]: 153 (100), 108 (12.1) MS^3 [285 → 153]: 109 (100)	1-O-Protocatechuyl-beta-xylose	+	+	+
17	8.1	353	MS^2 [353]: 191 (100), 179 (12.7), 173 (18.2) MS^3 [353 → 191]: 173 (100), 171 (27.1), 155 (25.1), 145 (11.3), 127 (86.6), 111 (63.9), 93 (80.6), 85 (96.9) MS^4 [353 → 191 → 173]: 111 (63.1), 109 (48.1), 93 (100)	5-*O*-Caffeoylquinic acid	+	+	+
18	8.2	707	MS^2 [707]: 353 (100) MS^3 [707 → 353]: 191 (100) MS^4 [707 → 353 → 191]: 173 (100), 127 (71.7), 111 (14.1), 93 (80.9); 85 (27.1)	5-*O*-Caffeoylquinic acid dimer	+	+	+
19	8.8	431	MS^2 [431]: 385 (100), 223 (16.7), 153 (11.8) MS^3 [431 → 385]: 224 (18.0), 205 (15.6), 161 (31.3), 153 (100), 139 (33.4), 147 (32.6), 125 (26.0) MS^4 [431 → 385 → 153]: 135 (100)	Drovomifoliol-*O*-glucoside (formate adduct)	+	+	+
20	9.1	593	MS^2 [593]: 503 (43.9), 473 (100), 455 (22.3), 383 (36.0), 353 (55.2), 337 (10.3), 325 (12.9) MS^3 [593 → 473]: 353 (100) MS^4 [593 → 473 → 353]: 325 (100), 307 (14.5), 297 (97.4), 265 (36.1)	Apigenin-6,8-di-C-glucoside (vicenin-2)	+		
21	9.4	433	MS^2 [433]: 387 (100) MS^3 [433 → 387]: 369 (100), 225 (50.5), 205 (22.7), 187 (11.5), 161 (69.3), 153 (10.8), 147 (32.6), 125 (26.0)	Dihydrovomifoliol-*O*-glucoside (formate adduct)		+	+
22	9.9	353	MS^2 [353]: 191 (100) MS^3 [353 → 191]: 173 (86.7), 127 (76.4), 111 (53.2), 93 (46.2), 85 (100)	Caffeoylquinic acid	+	+	+
23	10.9	433	MS^2 [433]: 387 (100) MS^3 [433 → 387]: 369 (71.3), 225 (100), 223 (15.6), 187 (12.4), 179 (35.8), 161 (49.1), 147 (21.2)	Dihydrovomifoliol-*O*-glucoside isomer (formate adduct)		+	+
24	11.5	361	MS^2 [361]: 199 (100), 155 (50.6) MS^3 [361 → 199]: 155 (100), 137 (70.2), 73 (23.1)	Unknown 1	+	+	+

Table 1. (cont.)

No.	t_R [min]	$[M - H]^-$ [m/z]	HPLC-DAD-ESI/MS^n m/z [% base peak]	Assigned identity	*M. decipiens*	*M. edulis*	
					Madeira	Madeira	P. Santo
25	11.7	377	MS^2 [377]: 331 (100), 179 (37.3), 161 (11.1), 119 (15.5) MS^3 [377 → 331]: 179 (100), 161 (20.7), 143 (27.3), 131 (12.5), 119 (23.3), 113 (32.5), 101 (47.4), 89 (26.2)	Saccharide 4		+	
26	11.7	337	MS^2 [337]: 191 (100), 179 (80.6), 135 (20.7) MS^3 [337 → 179]: 135 (100) MS^3 [337 → 191]: 173 (42.7), 127 (31.3), 111 (100), 85 (31.7)	Coumaroylquinic acid	+	+	+
27	12.9	367	MS^2 [367]: 191 (100) MS^3 [367 → 191]: 173 (15.7), 127 (100), 109 (66.9); 93 (28.7), 85 (94.5)	Feruloylquinic acid	+	+	+
28	13.1	371	MS^2 [371]: 249 (100), 121 (11.0) MS^3 [371 → 249]: 231 (54.2), 113 (36.1), 111 (22.7), 85 (10.9)	Unknown 2		+	+
29	13.4	337	MS^2 [337]: 191 (100) MS^3 [337 → 191]: 173 (100), 127 (44.7), 109 (41.3), 93 (32.6)	Coumaroylquinic acid isomer	+	+	+
30	13.8	377	MS^2 [377]: 331 (100), 179 (26.6); 113 (10.3) MS^3 [377 → 331]: 179 (100), 161 (17.5), 149 (17.1), 143 (22.9), 119 (25.6), 113 (25.2), 101 (16.5)	Saccharide 4 isomer	+		
31	13.9	367	MS^2 [367]: 191 (10.3), 179 (100), 161 (19.4), 135 (43.2) MS^3 [367 → 179]: 135 (100)	Methyl-5-*O*-caffeoylquinic acid		+	+
32	14.5	595	MS^2 [595]: 563 (17.0), 463 (29.2), 401 (21.4), 301 (100), 272 (27.9); 255 (15.0), 179 (20.4) MS^3 [595 → 301]: 271 (36.3), 257 (29.2), 255 (100), 243 (27.6), 151 (31.1)	Quercetin 3-*O*-pentosylhexoside	+	+	+
33	15.0	489	MS^2 [489]: 327 (13.3.), 308 (17.9), 307 (100), 293 (19.1), 265 (84.7), 239 (88.1), 203 (11.2) MS^3 [489 → 239]: 133 (100), 93 (12.0) MS^3 [489 → 307]: 279 (23.5), 265 (16.6), 201 (37.8), 200 (10.4), 187 (12.2), 174 (25.9), 173 (100), 143 (18.4) MS^4 [489 → 307 → 173]: 159 (100)	Unknown 3	+		
34	16.2	509	MS^2 [509]: 473 (100) MS^3 [509 → 473]: 293 (100), 233 (30.8), 191 (11.7), 180 (72.3), 164 (24.6) MS^4 [509 → 473 → 293]: 233 (22.2), 191 (15.5), 149 (65.4), 131 (21.7), 119 (100), 101 (54.8), 99 (45.5)	Unknown 4		+	+
35	16.4	431	MS^2 [431]: 385 (20.0), 372 (14.1), 341 (23.0), 312(19.3), 311 (100), 179 (15.2) MS^3 [431 → 311]: 311 (11.5), 284 (12.2), 283 (100)	Apigenin-8-*C*-hexoside	+	+	+
36	16.8	523	MS^2 [523]: 508 (16.8), 488 (43.2), 361 (100), 343 (15.9), 299 (15.7), 165 (32.1), 147 (12.0) MS^3 [523 → 361]: 347 (43.1), 346 (96.1), 313 (80.4), 222 (47.1), 205 (85.0), 192 (84.3), 166 (100), 165 (80.4), 109 (94.8) MS^4 [523 → 361 → 165]: 148 (100)	Secoisolariciresinol-*O*-hexoside			+

Table 1. (cont.)

No.	t_R [min]	$[M - H]^-$ [m/z]	HPLC-DAD-ESI/MS^n m/z [% base peak]	Assigned identity	*M. decipiens*	*M. edulis*	
					Madeira	Madeira	P. Santo
37	17.1	469	MS^2 [469]: 423 (100) MS^3 [469 → 423]: 261 (41.7), 233 (22.2), 217 (23.5), 199 (100), 189 (26.5), 143 (10.8) MS^4 [469 → 423 → 199]: 184 (100)	Unknown 5	+		
38	18.0	341	MS^2 [341]: 179 (100), 164 (25.3) MS^3 [341 → 179]: 164 (100)	Unknown 6		+	+
39	18.4	463	MS^2 [463]: 301 (100) MS^3 [463 → 301]: 272 (14.7), 271 (17.6), 255 (13.5), 179 (97.9), 151 (100), 107 (10.5)	Quercetin-*O*-hexoside	+	+	+
40	18.8	447	MS^2 [447]: 285 (100) MS^3 [447 → 285]: 256 (10.3), 243 (25.2), 241 (85.7), 230 (50.6), 223 (11.4), 217 (100), 201 (27.1), 200 (32.7), 199 (52.6), 198 (12.8)	Luteolin-*O*-hexoside	+		+
41	19.0	463	MS^2 [463]: 301 (100) MS^3 [463 → 301]: 272 (14.7), 271 (17.6), 255 (13.5), 179 (97.9), 151 (100), 121 (30.5)	Quercetin-*O*-hexoside isomer	+	+	+
42	19.8	433	MS^2 [433]: 347 (100) MS^3 [433 → 347]: 301 (50.8), 229 (24.4), 203 (100), 185 (64.9), 172 (26.1), 143 (32.9), 111 (16.8) MS^4 [433 → 347 → 185]: 156 (100)	Unknown 7		+	
43	21.2	433	MS^2 [433]: 347 (100), 196 (18.9), 172 (11.0) MS^3 [433 → 347]: 185 (100)	Unknown 7 isomer		+	
44	22.0	473	MS^2 [473]: 427 (100) MS^3 [473 → 427]: 265 (25.2), 247 (79.5), 186 (91.9), 185 (100), 179 (27.7), 174 (38.5), 161 (19.3)	Unknown 8	+		
45	23.5	515	MS^2 [515]: 471 (11.0), 463 (19.4), 369 (12.9), 353 (100), 335 (15.2), 203 (14.5), 255 (16.1), 191 (16.6), 179 (25.3), 173 (54.2) MS^3 [515 → 353]: 191 (32.8), 179 (63.7), 173 (100), 135 (27.4)	3,4-*O*-Dicaffeoylquinic acid		+	+
46	24.5	413	MS^2 [413]: 367 (67.7), 353 (100), 221 (91.3), 143 (44.9)	Unknown 9		+	+
47	25.5	549	MS^2 [549]: 505 (100) MS^3 [549 → 505]: 463 (17.3), 301 (100), 299 (47.9) MS^4 [549 → 505 → 301]: 271 (36.1), 255 (37.9); 179 (91.4), 151 (100)	Quercetin-*O*-malonyl(hexoside)	+	+	+
48	25.9	515	MS^2 [515]: 353 (100), 191 (25.3) MS^3 [515 → 353]: 191 (100), 179 (19.7) MS^4 [515 → 353 → 191]: 173 (76.9), 171 (41.5), 127 (100), 109 (23.1), 93 (64.2), 85 (90.0)	1,5-*O*-Dicaffeoylquinic acid	+		+
49	26.2	447	MS^2 [447]: 285 (100), 255 (25.7) MS^3 [447 → 285]: 255 (100) MS^4 [447 → 285 → 255]: 229 (37.9), 227 (31.4), 213 (23.2), 211 (52.6), 163 (100)	Kaempferol-*O*-hexoside	+	+	+

Table 1. (cont.)

No.	t_R [min]	$[M - H]^-$ [m/z]	HPLC-DAD-ESI/MS^n m/z [% base peak]	Assigned identity	*M. decipiens*	*M. edulis*	
					Madeira	Madeira	P. Santo
50	26.4	431	MS^2 [431]: 269 (100), 225 (14.2), 181 (16.2) MS^3 [431 → 269]: 225 (100)	Apigenin-*O*-hexoside	+		
51	26.8	477	MS^2 [477]: 315 (100) MS^3 [477 → 315]: 301 (51.4), 300 (65.5), 299 (13.2), 285 (78.1), 271 (100), 255 (13.0), 243 (53.1), 215 (32.1), 164 (13.4)	Isorhamnetin-*O*-hexoside		+	
52	26.8	515	MS^2 [515]: 353 (100), 191 (25.3) MS^3 [515 → 353]: 191 (100), 179 (53.7), 173 (30.5), 135 (12.9) MS^4 [515 → 353 → 191]: 127 (33.7), 111 (33.7), 109 (42.4), 93 (100)	3,5-*O*-Dicaffeoylquinic acid	+		+
53	28.3	505	MS^2 [505]: 463 (56.5), 301 (100), 299 (71.7) MS^3 [505 → 301]: 273 (15.3), 271 (42.1), 256 (20.4), 255 (41.7), 239 (12.8), 179 (54.1), 151 (100)	Quercetin-*O*-acetylhexoside	+	+	+
54	28.8	515	MS^2 [515]: 353 (100), 255 (10.5), 203 (19.4), 191 (12.0), 179 (10.5), 173 (19.6) MS^3 [515 → 353]: 191 (57.8), 179 (72.1), 173 (100), 135 (27.7)	4,5-*O*-Dicaffeoylquinic acid	+	+	+
55	29.1	383	MS^2 [383]: 179 (100), 164 (37.2) MS^3 [383 → 179]: 164 (100)	Unknown 10		+	+
56	29.1	533	MS^2 [533]: 489 (100) MS^3 [533 → 489]: 285 (100) MS^4 [533 → 489 → 285]: 243 (100), 226 (93.8), 223 (58.5), 217 (93.7), 202 (97.1), 198 (59.9), 175 (64.9); 133 (41.5), 129 (46.6)	Luteolin-*O*-diacetylhexoside	+		
57	30.1	493	MS^2 [493]: 447 (100) MS^3 [493 → 447]: 315 (100), 300(44.9), 251 (10.4), 221 (27.3), 191 (48.1), 161 (49.8), 151 (93.5), 149 (76.2), 131 (10.3)	Isorhamnetin-*O*-pentoside (formate adduct)		+	
58	30.5	489	MS^2 [489]: 427 (10.7), 285 (100) MS^3 [489 → 285]: 243 (15.9), 241 (59.8), 217 (90.3), 215 (64.4), 201 (99.7), 185 (43.3), 151 (100)	Luteolin-*O*-acetylhexoside	+	+	+
59	31.1	597	MS^2 [597]: 553 (100), 427 (47.0), 265 (28.1), 239 (12.6) MS^3 [597 → 553]: 427 (60.1), 367 (19.1), 349 (13.2), 323 (17.4), 307 (16.6), 281 (13.2), 265 (100), 239 (28.6) MS^4 [597 → 553 → 265]: 237 (24.2), 221 (11.7), 159 (100), 158 (57.8)	Unknown 11	+		
60	31.3	519	MS^2 [519]: 315 (100), 300 (14.7) MS^3 [519 → 315]: 301 (17.5), 300 (100) MS^4 [519 → 315 → 300]: 271 (72.1), 255 (100), 254 (44.6), 244 (21.9)	Methylellagic acid acetyl(hexoside)		+	
61	31.3	499	MS^2 [499]: 338 (13.6), 337 (100), 335 (6.8), 173 (14.4), 163 (24.0) MS^3 [499 → 337]: 191 (6.3), 173 (44.8), 163 (100), 119 (10.1) MS^4 [499 → 337 → 163]: 120 (5.7), 119 (100), 118 (4.8)	*p*-Coumaroyl-*O*-caffeoylquinic acid			+
62	31.5	413	MS^2 [413]: 353 (100), 161 (26.3)	Unknown 12		+	
63	31.9	489	MS^2 [489]: 429 (27.5), 285 (100) MS^3 [489 → 285]: 267 (24.8), 259 (22.9), 257 (100), 255 (39.4), 229 (59.5), 213 (49.8), 119 (24.2), 108 (24.0)	Kaempferol-*O*-acetylhexoside	+		+

Table 1. (cont.)

No.	t_R [min]	$[M - H]^-$ [m/z]	HPLC-DAD-ESI/MS^n m/z [% base peak]	Assigned identity	*M. decipiens*	*M. edulis*	
					Madeira	Madeira	P. Santo
64	32.0	529	MS^2 [529]: 367 (100), 353 (87.3), 191 (43.4), 179 (15.9) MS^3 [529 → 353]: 191 (100), 179 (37.1), 135 (16.4)	Feruloyl-*O*-caffeoylquinic acid	+	+	+
65	32.6	597	MS^2 [597]: 553 (100), 473 (40.7), 427 (77.2), 367 (11.8), 265 (61.2), 239 (15.3), 203 (10.9) MS^3 [597 → 553]: 427 (45.7), 393 (15.5), 367 (20.8), 349 (35.8), 307 (32.5), 283 (24.1), 265 (100), 239 (36.8), 175 (11.8), 157 (11.9) MS^4 [597 → 553 → 265]: 159 (100), 131 (95.0), 93 (37.3)	Unknown 11 isomer	+		
66	32.9	497	MS^2 [497]: 337 (74.7), 335 (81.9), 255 (22.3), 179 (100), 173 (37.8) MS^3 [497 → 179]: 136 (4.3), 135 (100)	Dicaffeoylshikimic acid	+		+
67	33.1	529	MS^2 [529]: 367 (100), 353 (87.3), 191 (43.4), 179 (15.9) MS^3 [529 → 353]: 191 (100), 179 (37.1), 135 (16.4)	Feruloyl-*O*-caffeoylquinic acid isomer		+	+
68	35.2	325	MS^2 [325]: 281 (100) MS^3 [325 → 281]: 221 (100), 177 (27.0) MS^4 [325 → 281 → 221]: 177 (100), 161 (37.0), 135 (85.2), 121 (22.7), 95 (41.9)	Unknown 14	+	+	+
69	35.9	497	MS^2 [497]: 453 (16.3), 343 (100), 335 (63.0), 331 (84.6), 255 (13.7), 179 (56.6), 163 (16.3), 143 (13.4) MS^3 [497 → 335]: 317 (19.7), 179 (100), 161 (23.3), 135 (51.8)	Dicaffeoylshikimic acid isomer	+	+	+
70	36.0	659	MS^2 [659]: 627 (100), 615 (30.4), 587 (10.5), 584 (15.3), 583 (48.6) MS^3 [659 → 627]: 610 (100), 583 (26.4), 565 (46.1), 539 (12.8), 506 (67.8), 505 (73.6), 462 (91.7) MS^4 [659 → 627 → 461]: 443 (100), 417 (41.8), 393 (72.0), 387 (29.3), 373 (26.6), 253 (26.2), 175 (14.7)	Unknown 15			+
71	36.1	509	MS^2 [509]: 463 (100), 317 (10.1) MS^3 [509 → 463]: 317 (100), 161 (46.2) MS^4 [509 → 463 → 317]: 161 (100), 143 (28.8), 113 (25.6), 101 (20.7), 97 (52.2)	Saccharide 5	+		

t_R, retention time; P. Santo, Porto Santo.

acid isomers (compounds **64** and **67**) were identified according to previous reports.[24][30]

1-*O*-Protocatechuyl-β-xylose was assigned to compound **16** according to bibliographic data.[31]

Compounds **66** and **69** showed both $[M - H]^-$ ions at *m/z* 497. Under MS^2 fragmentation, they gave rise to fragment ion at *m/z* 179 (by loss of 318 Da), which suggests the presence of a caffeic acid. Also, in the MS^2 spectrum, a base peak ion at *m/z* 335 was observed (by loss of 162 Da) indicating the presence of a caffeic acid residue. Further fragmentation showed the [caffeic acid $- H]^-$ ion at *m/z* 179 and its typical fragment ions. This fragmentation pattern has been previously described for dicaffeoylshikimic acid.[32]

Flavonoids

Compound **7** with $[M - H]^-$ at *m/z* 393 showed fragmentation 345 → 330 at MS^3, and based on literature data, it was identified as quercetagetin-dimethyl ether.[33]

Compound **13** displayed $[M - H]^-$ ion at *m/z* 595 and its fragmentation pattern matches that of naringenin-6,8-di-*C*-glucoside.[34]

Compound **20** exhibited $[M - H]^-$ at *m/z* 593 and successive neutral losses of 120 Da typical of *C*-glycoside flavonoids, being identified as apigenin-6,8-di-*C*-glucoside (vicenin-2).[4]

Compound **35** displayed $[M - H]^-$ at *m/z* 431 followed by typical fragments $[M - H - 120]^-$, $[M - H - 90]^-$, $[M - H - 60]^-$ and was characterized as apigenin-*8*-*C*-glucoside, according to previous publications.[35]

Compound **32** showed $[M - H]^-$ at *m/z* 595 and gave origin to quercetin aglycone at *m/z* 301 (after loss of 295 Da), indicating that **32** was quercetin-*O*-pentosylhexoside.[27] Quercetin-*O*-hexoside isomers were assigned to compounds **39** and **41**. Compounds **47** and **53** exhibited $[M - H]^-$ ions at *m/z* 549 and 505 and were identified as quercetin-*O*-malonyl(hexoside) and quercetin-*O*-acetylhexoside, respectively, based on their fragmentation patterns.[36]

Compounds **40** and **49** displayed both $[M - H]^-$ ions at *m/z* 447, and after loss of 162 Da gave origin to different fragmentation patterns. Based on previous data, **40** and **49** were identified as luteolin-*O*-hexoside and kaempferol-*O*-hexoside.[27][34] Compound **56** with $[M - H]^-$ ion at *m/z* 533 suffered a loss of 44 Da residue to form a MS^2 base peak at *m/z* 489. Further fragmentation of this ion gave [luteolin $- H]^-$ ion at *m/z* 285 (by loss of 204 Da), being identified as luteolin-*O*-diacetylhexoside.[37] Both luteolin-*O*-acetylhexoside and kaempferol-*O*-acetylhexoside displayed $[M - H]^-$ ion at 489 and were assigned to compounds **58** and **63**, respectively.[38]

Compound **50** displayed fragmentation 431 → 269, and was characterized as apigenin-*O*-hexoside.[34]

Isorhamnetin-*O*-hexoside (compound **51**) showed $[M + HCOOH - H]^-$ ion at *m/z* 493 and MS^3 base peak at *m/z* 315 (by loss of 46 + 162 Da).[4]

Other Compounds

Compound **1**, with $[M - H]^-$ ion at *m/z* 683, displayed fragment ions at *m/z* 503, 341, and 179, which were consistent with the losses of hexoside moieties. The presence of hexoses was confirmed by the presence of fragment ions at *m/z* 143, 131, 119, 113, 89, and 71, typical of oligosaccharides.[39] Other oligosaccharide derivatives (**8**, **11**, **25**, **30**, and **71**) were also identified based on their fragmentation behavior.

Quinic acid (compound **4**) showed $[M - H]^-$ at *m/z* 191 and typical fragment ions at *m/z* 173, 127, 111 and 85.[28] Compound **2** with $[M - H]^-$ ion at *m/z* 533, exhibited fragment ion at *m/z* 191 after MS^2 fragmentation. Thus, it was identified as a derivative of quinic acid.

Malic acid (compound **3**) was characterized based on previous publication.[28]

Compound **14** with $[M + HCOOH - H]^-$ at *m/z* 399 suffered a loss of 208 Da (46 + 162 Da) at MS^2. Based on further fragmentation data (shown in *Table 1*), **14** was characterized as scopoletin-*O*-hexoside.[40]

Drovomifoliol-*O*-glucoside (roseoside) with $[M + HCOOH - H]^-$ ion at *m/z* 431 was assigned to compound **19**.[41] Furthermore, compounds **21** and **23**, with $[M + HCOOH - H]^-$ at *m/z* 433, were characterized as isomers of dihydrovomifoliol-*O*-glucoside. Compound **36**, with $[M - H]^-$ ion at *m/z* 523, gave origin to a fragment ion at *m/z* 361 (by loss of 162 Da) and was characterized as secoisolariciresinol-*O*-hexoside.[42]

Compound 60 displayed $[M - H]^-$ ion at *m/z* 519 and gave origin to MS^2 base peak at 315 by loss of 204 Da. By loss of 15 Da, it displayed typical ellagic acid fragment ions. This fragmentation behavior was consistent with that of methyl-ellagic acid-*O*-acetylhexoside.[31]

Quantitative Analysis of Phenolic Compounds

In total, 15 main polyphenols were quantified by the HPLC-DAD method (*Table 2*). It was not possible to quantify all identified compounds due to their low UV-absorption and because some of them were present in trace amounts.

Table 2. Determination of individual polyphenols from target plant species by the HPLC-DAD method (mg/g DE)

No.	Compound	*Melanoselinum decipiens*	*Monizia edulis*	
		Madeira	Madeira	P. Santo
Hydroxycinnamic acids				
9	3-*O*-Caffeoylquinic acid	–	0.47 ± 0.02	0.49 ± 0.01
12	Sinapic acid-*O*-hexoside	0.19 ± 0.01	–	–
17	5-*O*-Caffeoylquinic acid	13.30 ± 0.40	11.58 ± 0.25	10.02 ± 0.30
22	Caffeoylquinic	0.16 ± 0.01	0.17 ± 0.01	0.20 ± 0.01
26	Coumaroylquinic acid	0.16 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
27	Feruloylquinic acid	0.74 ± 0.01	0.64 ± 0.02	0.35 ± 0.01
48	1,5-*O*-Dicaffeoylquinic acid	–	–	1.57 ± 0.04
	Total	14.54 ± 0.42	12.98 ± 0.28	12.83 ± 0.34
Flavonols				
32	Quercetin-*O*-(pentosyl)hexoside	1.37 ± 0.03	0.33 ± 0.01	0.82 ± 0.01
39	Quercetin-*O*-hexoside	0.79 ± 0.01	0.76 ± 0.02	1.88 ± 0.04
47	Quercetin-*O*-(malonyl)hexoside	2.64 ± 0.08	0.96 ± 0.02	1.11 ± 0.04
53	Quercetin-*O*-(acetyl)hexoside	–	0.12 ± 0.01	0.22 ± 0.01
	Total	4.80 ± 0.09	2.17 ± 0.06	4.03 ± 0.09
Flavones				
40	Luteolin-*O*-hexoside	0.19 ± 0.01	–	–
56	Luteolin-*O*-(diacetyl)hexoside	0.83 ± 0.02	–	–
58	Luteolin-*O*-(acetyl)hexoside	0.16 ± 0.01	0.12 ± 0.01	0.09 ± 0.002
	Total	1.18 ± 0.03	0.12 ± 0.01	0.09 ± 0.002
Flavanones				
13	Naringenin-6,8-di-*C*-glucoside	–	0.49 ± 0.01	–
	Total		0.49 ± 0.01	
	TIPC	19.52 ± 0.49	15.76 ± 0.34	16.95 ± 0.38

TIPC, total individual phenolic content.

Quantification of individual phenolic compounds varied among the analyzed samples, with levels ranging between 15.76 and 19.52 mg/g (*Table 2*) with *M. decipiens* extract being the richest one. Statistical significant differences ($P < 0.05$) were found between total individual phenolic content (TIPC) of *M. edulis* populations. Hydroxycinnamic acids were the dominant phenolic group (> 76% of TIPC), followed by *O*-glycosylated flavonoids (flavonols, flavones, and flavanones type). 5-CQA was the main component in all target plants (ranging between 61 and 76% of TIPC). Quercetin-*O*-(malonyl)hexoside, luteolin diacetyl, and quercetin-*O*-hexoside were the most abundant flavonoids in *M. decipiens*. In case of *M. edulis*, a different trend was observed: 5-CQA > quercetin-*O*-(malonyl)hexoside > quercetin-*O*-hexoside (Madeira) and 5-CQA > quercetin-*O*-hexoside > quercetin-*O*-(malonyl)-hexoside (Porto Santo).

A previous chemical characterization of Apiaceae species,[27] revealed lower amounts of 3-*O*-CQA (**9**), quercetin-*O*-(pentosyl)hexoside (**32**), quercetin-*O*-hexoside (**39**), and 1,5-di-*O*-CQA (**48**) on centella leaf extracts. *Martins et al.*[43] found TIPC of 42 mg/g dry extracts (DE) and 2.2 mg/g DE for anise and coriander seeds, respectively; although 5-*O*-CQA (**17**) and luteolin-*O*-hexoside (**40**) were present in lower quantities than in present work.

Principal Component Analysis

PCA of 15 compounds in two Apiaceae species was performed, and as shown in *Fig. 2*, the distribution plots were divided into three groups.

The PCA score scatter plot of the two-first principal components (which explains 100% of the total variability) is shown in *Fig. 2a*. The loadings of each compound (variable) that contribute to explain the differentiation between the plant species and collection area is shown in *Fig. 2b*.

PC1 that explained 69% of the total variability shows target plants discrimination based on their species, where *M. edulis* (Madeira and Porto Santo) are projected in PC1 negative and *M. decipiens* is on PC1 positive. Taking in account the loading plot (*Fig. 2b*), the compounds that contribute to these results were: sinapic acid-*O*-hexoside (**12**), luteolin-*O*-hexoside (**40**), and luteolin-*O*-diacetylhexoside (**56**). However, PC2 (that explained 31% of the total variability) separated

M. edulis samples based on subspecies (or collection area): subsp. *isambertoi* (Madeira) samples are below PC2 axis while subsp. *santosii* (Porto Santo) is positioned in PC2 positive. In case of *M. edulis* samples, the obtained results support their taxonomical separation into two distinct taxonomic groups as suggested by *Fernandes* and *Carvalho*.[18] According to *Fig. 2b*, polyphenols responsible for the obtained results are naringenin-6,8-di-*C*-glucoside (**13**), quercetin-*O*-pentosylhexoside (**32**), and 1,5-*O*-dicaffeoylquinic acid (**48**). Based on the statistical analysis, these compounds can be used as potential geographic markers.

Previously, flavonoids and phenolic acids have been used as chemotaxonomic markers for other Apiaceae species. For example, chrysoeriol-*O*-(pentosyl) hexoside and luteolin-*O*-pentoside are markers for different *Torilis* species.[44] (*R*)-3′-O-β-D-Glucopyranosylrosmarinic acid is used as chemotaxonomic marker for the subfamily Saniculoideae,[9] while luteolin-7-*O*-glycosides are useful to discriminate *Soranthus* and *Ferula* species.[45]

Compounds with flavonol-like structures (rutin, isoquercitrin, isorhamnetin-3-glycoside, isorhamnetin-3-rutinoside, and quercetin) are chemotaxonomically important in plants of the genus *Peucedanum* (Apiaceae).[46]

Total Phenolic and Flavonoid Contents and In Vitro Antioxidant Activity Assays

The amounts of total phenolics and total flavonoids varied in the different analyzed extracts and ranged from 34.10 to 42.63 mg GAE/g DE and from 10.33 to 19.66 RUE mg/g DE, respectively (*Fig. 3a*). *M. decipiens* showed the highest contents, followed by *M. edulis* Porto Santo > *M. edulis* Madeira. Only for total flavonoid content (TFC) there were significant differences between *M. edulis* samples ($P < 0.05$). This variation can be expected for plant extracts due to the presence of other constituents and/or the presence of different types of polyphenols. Also, the absolute numerical value of TIPC was, as it generally is, lower than those determined by colorimetric methods (*Fig. 3a*). This difference shows well that colorimetric assays are not specific to polyphenols. Despite their shortcomings, colorimetric assays for measurement of phenolic and flavonoids contents are still present in many publications and are useful to establish comparison with other available data.

Recently,[47] TPC and TFC of seven Indian Apiaceae spices were determined. Using those for comparison, *M. decipiens* showed higher TPC than tested plants (< 38.83 mg GAE/g DE); while caraway and coriander had higher amounts than *M. edulis*. In case of TFC results, coriander, cumin, and carom presented higher contents (> 27.45 mg RU/g DE) than our targeted species. In another study,[48] coriander and parsley (leaves and seeds) showed lower TPC (6.2 – 9.2 mg GAE/g DE) than reported in present work. *Hinneburg et al.*[49] also found lower amounts of TPC in parsley, aniseed, and fennel (20.8 – 30.3 mg GAE/g DE); only cumin was richer than *M. edulis* extracts (37.4 mg GAE/g DE). TPC varied in different parts of fennel (8.61 – 65.85 mg GAE/g DE).[50] Only shoots had higher values than our targeted species, while leaves and inflorescences showed comparable contents with *M. edulis*.

In all antioxidant assays, a good scavenging activity was shown for all species with a wide range from 0.035 to 1.09 mmol TE/g DE (*Fig. 3b*). The higher values obtained by ABTS method could not only be related to differences in the sensitivity of these methods but also it measures both hydrophilic and lipophilic antioxidants. Based on the obtained results, target species may also prevent the formation of other biologically important oxidative species resultant from the reaction of NO and SO, like peroxynitrite and hydroxyl radical.[51]

In general, *M. decipiens* was found to be the most potent radical scavenger toward DPPH, NO, and SO, followed by *M. edulis* Porto Santo > *M. edulis* Madeira. Only in NO assay, there were significant differences ($P < 0.05$) between *M. edulis* populations. These results were also supported by quantitative determination of antioxidant compounds using the HPLC-DAD quantitative analysis. For ABTS, a different trend was observed (*M. decipiens* > *M. edulis* Madeira > *M. edulis* Porto Santo). This variation in the observed antioxidant effects may be related with the distribution of polyphenols on different samples.

An explanation for reported differences of *M. edulis* samples may be because these samples were collected in different geographical locations (Madeira and Porto Santo Islands), which according to *Fernandes* and *Carvalho*[18] correspond to different subspecies. Porto Santo population (subsp. *santosii*) were grown on a wild environment (at sea level) and was subjected to harsh environmental conditions, such as high UV levels from the sunlight and dryness. In the wild environment, plant species are more subjected to stress factors which induce intense synthesis of phenolic compounds as a response to abiotic stress in order to prevent oxidative damage of the plant cellular structures. Madeira counterpart (subsp. *isambertoi*) was collected in Madeira Botanical Garden where the growth conditions are 'controlled', with less sun exposure, higher altitude (ca. 300 m), regular watering, *etc.*

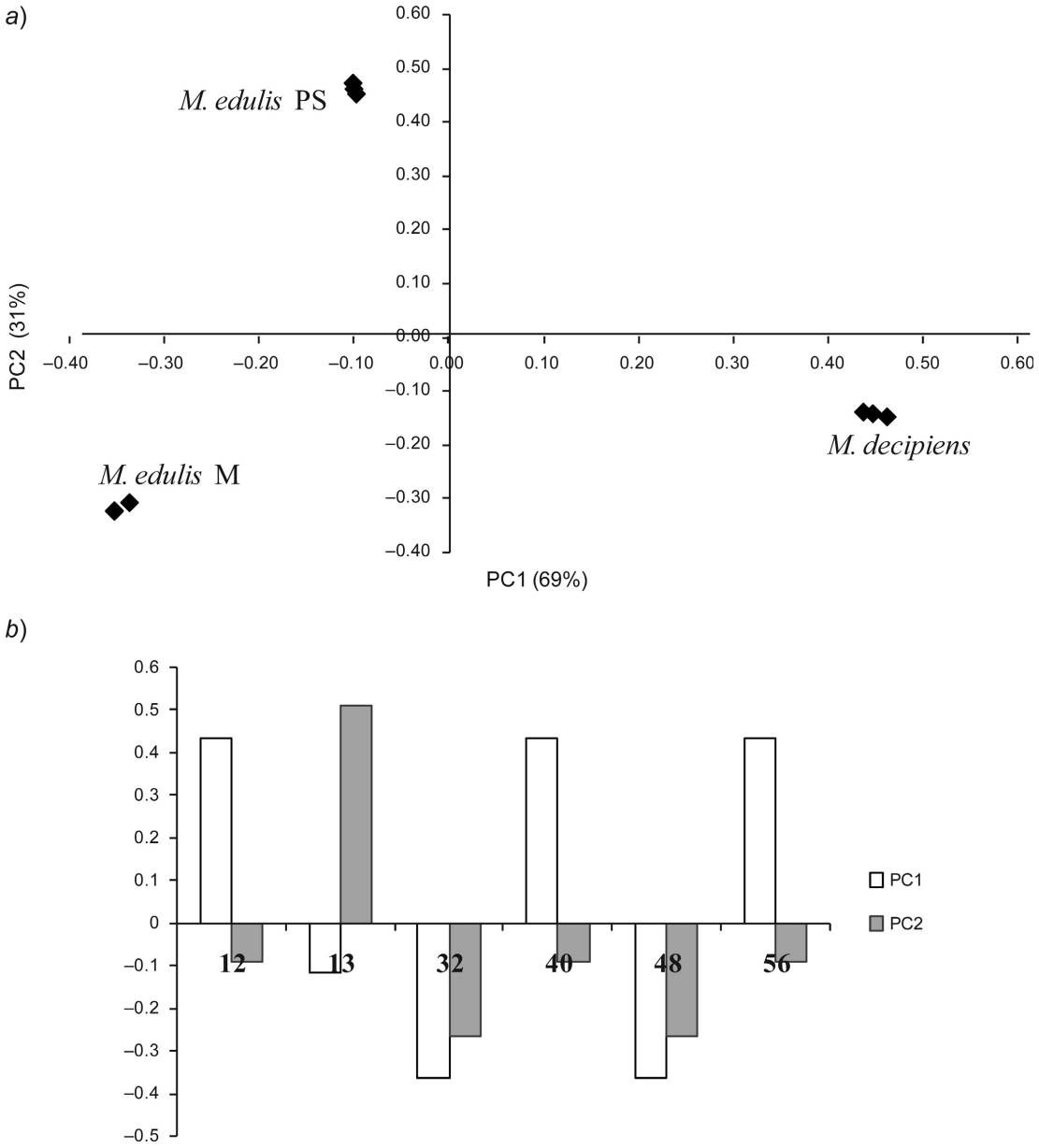

Figure 2. *a*) PC1× PC2 of scores scatter plot between target plant species and collection area (M: Madeira; PS: Porto Santo), *b*) Loadings of the most significant polyphenols that contribute for differentiation of species and collection area (the number identification is shown in *Table 1*).

These environmental factors are well-known to affect the phytochemical composition of plants.[52]

Nevertheless, the observed trend for the antioxidant activities of the different extracts correlated well with the TIPC values ($R^2 \geq 0.906$) (*Table 3*). In general, hydroxycinnamic acids were better correlated than flavonoids in all assays. Individual components correlation with antioxidant activities was also determined (*Table 3*): coumaroylquinic acid (**26**), quercetin-*O*-(pentosyl)hexoside (**32**), and quercetin-*O*-(malonyl)hexoside (**47**) are the most important contributors to the obtained data. Antioxidant activity is directly related to the particular structure of polyphenols. In fact, alterations in the arrangement of the OH groups and degree of substitution by glycosylation decrease the antioxidant activity. Moreover, interactions established between components of a matrix can lead to synergistic/antagonist effects.[53]

Conclusions

In this work, the phytochemical profile of two endemic plants from Madeira archipelago was established for the first time. 5-*O*-Caffeoylquinic acid, quercetin-*O*-(malonyl)hexoside, luteolin diacetyl, and quercetin-*O*-hexoside were the dominant phenolics in both

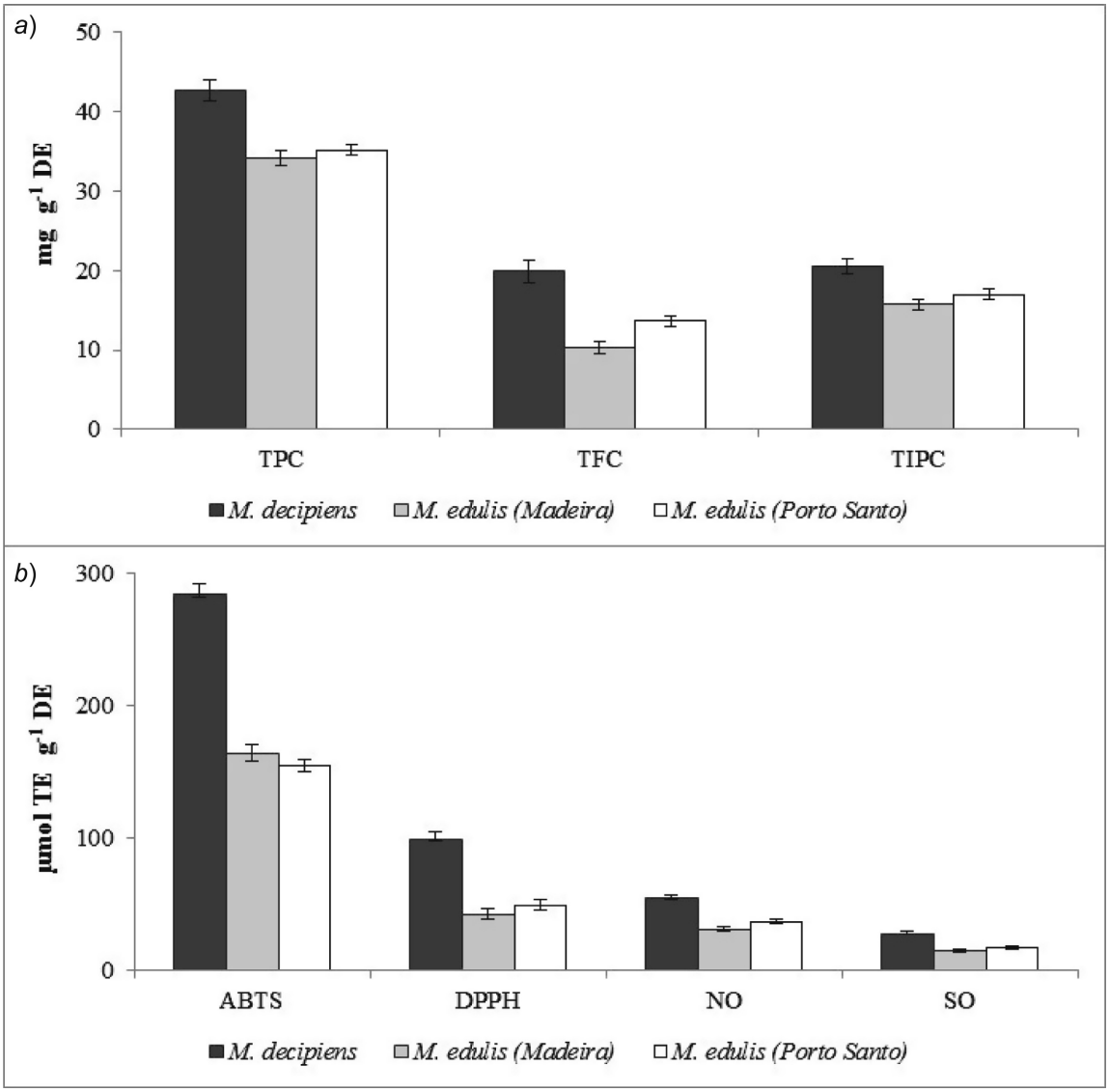

Figure 3. *a*) Total phenolic and flavonoid contents and *b*) *in vitro* antioxidant activities (NO, SO, ABTS, and DPPH) of targeted plant species.

species. A comparison study was made for *M. edulis* populations collected in two different geographical locations. Samples from Porto Santo showed a higher phenolic content and slightly different phytochemical composition than Madeira counterpart; naringenin-6,8-di-*C*-glucoside, quercetin 3-*O*-pentosylhexoside, and 1,5-*O*-dicaffeoylquinic acid can be used as geographical markers or, if not most importantly, as taxonomic markers. A natural follow-up of this work will be the cultivation of *M. edulis* subsp. *santosii* in the control conditions of a Botanical Garden, from seeds collected in the wild. Finally, evaluation of antioxidant activity revealed that *M. decipiens* was the most active compared to *M. edulis* samples, which is in agreement with the higher phenolic composition. Taking into account the obtained data and relative abundance, *M. decipiens* could be a good candidate as novel spice/additive for seasoning foods.

Experimental Section

Chemicals and Standards

The following reagents were purchased from *Panreac* (Barcelona, Spain): *Folin–Ciocalteu*'s phenol reagent (FCR), NaCl, KCl, gallic acid (> 98%), and MeCOOK. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH, > 95%), and 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were obtained from *Fluka* (Lisbon, Portugal). *N*-(1-Naphthyl)ethylenediamine dihydrochloride (≥ 98%), phenazine methosulfate (PMS, ≥ 90%), sulfanilamide (≥ 99%), β-nicotinamide adenine dinucleotide (NADH,

Table 3. Correlation coefficients (R^2) of ABTS, DPPH, NO, and SO assays with individual phenolic compounds determined in targeted Apiaceae species

Compounds	ABTS	DPPH	NO	SO
TIPC	0.906	0.983	0.965	0.979
Hydroxycinnamic acids	0.999	0.963	0.903	0.969
Flavonoids	0.765	0.896	0.959	0.886
5-*O*-Caffeoylquinic acid	0.828	0.673	0.551	0.687
Caffeoylquinic	0.548	0.369	0.253	0.383
Coumaroylquinic acid	0.995	0.987	0.944	0.990
Feruloylquinic acid	0.554	0.375	0.259	0.390
Quercetin-*O*-hexoside	0.289	0.142	0.167	0.253
Quercetin-*O*-(malonyl)hexoside	0.978	0.998	0.975	0.999
Quercetin-*O*-(pentosyl)hexoside	0.719	0.864	0.937	0.854
Luteolin-*O*-(acetyl)hexoside	0.866	0.722	0.605	0.736

$\geq$ 94%), caffeic acid (> 98%), $K_2S_2O_8$, and HCOOH from *Sigma–Aldrich* (St. Louis, MO, USA). Al_2O_3 . 6 H_2O, Na_2HPO_4, and quercetin dihydrate (99%) were acquired from *Riedel-de Haën* (Hanover, Germany). KH_2PO_4 and sodium nitroprussiate (99%) were acquired from *Merck* (Darmstadt, Germany), and nitroblue tetrazolium chloride (NBT, 90%) from *Acros Organics*. *o*-H_3PO_4 (85%) was obtained from *BDH AnalaR*, and hesperidin (> 98%) and apigenin (99%) from *Extrasynthese* (Genay, France). 1,5-*O*-Dicaffeoylquinic (99.2%), 3,4-*O*-dicaffeoylquinic (99.0%), 3,5-*O*-dicaffeoylquinic (99.2%), and 4,5-*O*-dicaffeoylquinic (99.3%) acids were purchased from *Biopurify Phytochemicals Ltd.* (Chengdu, China). LC-MS^n grade MeCN (*LabScan*; Dublin, Ireland) and ultrapure H_2O (*Milli-Q* water purification system, Millipore, USA) were used for analysis.

Collection Area and Sample Information

Samples were collected at three different geographical locations of Madeira archipelago during April – June 2014: *M. decipiens* (Ribeira das Cales, Madeira Island), *M. edulis* subsp. *isambertoi* (Madeira Botanical Garden, Madeira Island), and *M. edulis* subsp. *santosii* (Ilhéu de Cima, Porto Santo Island). Identification of the plant samples was carried out by specialists from Madeira Botanical Garden. Vouchers were deposited with the Madeira Botanical Garden Herbarium: MADJ 13186 (*M. decipiens*), MADJ 14103 (*M. edulis* subsp. *isambertoi*), and MADJ 12984 (*M. edulis* subsp. *santosii*). Due to their rarity, only two subspecies of *M. edulis* were included in this study.

Sample Preparation

For analysis, leaves were lyophilized to dryness (*Alpha 1-2 LD plus*; *Christ,* Germany), ground to powder, and stored at −20 °C. Dried material (1 g) was extracted with 25 ml of methanol (25 ml) using a sonicator *Bandelin Sonorex* (Germany) at 35 Hz and 200 W for 60 min (r.t.). Then, chlorophylls were removed by adsorption on activated charcoal, and extracts were filtered and concentrated to dryness under reduced pressure in a rotary evaporator (*Büchi Rotavapor R-114*; USA) at 40 °C. The resulting dry extracts (DE) were stored at 4 °C until further analysis.

Chromatographic Conditions

The HPLC analysis was carried out on a *Dionex ultimate 3000* series instrument coupled to a binary pump, a diode-array detector (DAD), an autosampler, and a column compartment (kept at 20 °C). Separation was performed on a *Phenomenex Gemini C_{18}* column (5 μm, 250 × 3.0 mm i.d.) using a mobile phase composed by MeCN (*A*) and HCOOH/H_2O (0.1%, *v/v*) at a flow rate of 0.4 ml/min. The following gradient program was used: 25% *A* (10 min), 25% *A* (20 min), 50% *A* (40 min), 100% *A* (42 – 47 min), and 20% *A* (49 – 55 min). Spectral data for all peaks were accumulated in the range of 210 – 400 nm. Plant extracts were filtered (0.45 μm) and injected (5 μl). For HPLC-DAD/ESI-MS^n analysis, a *Bruker Esquire model 6000* ion trap mass spectrometer (Bremen, Germany) with an ESI source was used. The MS^n analysis worked in negative mode and scan range was set at *m/z* 100 – 1000 with speed of 13 000 Da/s. The conditions of ESI were as follows: drying and nebulizer gas (N_2) flow rate and pressure, 10 ml/min and 50 psi; capillary temp., 325 °C; capillary voltage, 4.5 keV; collision gas (He) pressure and energy, 1×10^{-5} mbar and 40 eV; and fragmenter, 1.0 eV. Esquire control software was used for the data acquisition and data Analysis for processing.

Quantitative Analysis of Polyphenols

Caffeic acid, quercetin, apigenin, and hesperidin were used for hydroxycinnamic, flavonols, flavones, and flavanones quantification, resp., according to previous publication.[54] Calibration curves (5 – 100 mg/l) were prepared by diluting the stock solns. (1000 mg/l in MeOH) with initial mobile phase. Quantification was carried out by plotting peak area *vs.* concentration ($R^2 \geq 0.967$ in all cases).

Determination of Total Phenolic and Flavonoid Contents and In Vitro *Antioxidant Assays*

Total Phenolic Content (TPC). The TPC was determined by the *Folin–Ciocalteu* method[54]: aliquots (50 μl,

5 mg/ml of DE dissolved in MeOH) were mixed FCR (1.25 ml, diluted 1:10) and Na_2CO_3 soln. (1 ml, 7.5%). After 30 min in darkness and r.t., the absorbance was measured at 765 nm in a *Perkin–Elmer V–vis Lambda 2* spectrophotometer. The amounts of total phenolics were expressed as milligram gallic acid equiv. (GAE) per gram DE.

Total Flavonoid Content (TFC). The TFC was determined as follows[54]: aliquot (0.5 ml, 2.5 mg/ml) was mixed with MeOH (1.5 ml), distilled H_2O (2.8 ml), MeCOOK (0.1 ml, 1 mol/l), $AlCl_3 \cdot 6\ H_2O$ (0.1 ml, 10% in MeOH). The absorbance was measured at 415 nm after 30 min of reaction. The final results were expressed as milligram of rutin equiv. (RUE) per gram DE.

ABTS Radical Scavenging Activity

For each determination, an aliquot (40 μl, 5 mg/ml) was added to 1.96 ml of $ABTS^{\bullet+}$ soln. (1.96 ml, diluted with phosphate-buffered saline (PBS) absorbance 0.700 ± 0.021).[54] The reduction of absorbance at 734 nm was measured during 6 min, and the results were expressed as milimole Trolox equiv. (TE) per gram DE.

DPPH Radical Scavenging Activity

The DPPH assay followed a previously reported method[54]: aliquot (100 μl, 5 mg/ml) was added to DPPH radical soln. (3.5 ml, 0.06 mmol/l). Absorbance at 516 nm was measured after 30 min of reaction. The results were expressed as mmol TE/g DE.

Nitric Oxide (NO) Scavenging Activity

The antiradical activity was determined from a described procedure[55]: sodium nitroprusside (50 μl, 20 mM) was mixed with sample (50 μl, 5 mg/ml) for 60 min, at r.t., under light. All solns. were prepared in 0.1M phosphate buffer (pH 7.4). After incubation, *Greiss* reagent (50 μl, 1% sulfanilamide and 0.1% naphthylethylenediamine in 2% H_3PO_4) was added to each well. After 10 min, absorbance was read at 550 nm (*Victor*^{3} microtiter reader *Perkin–Elmer*, Germany) and results were expressed as mmol TE/g DE.

Superoxide Radical (SO) Scavenging Activity

Superoxide radicals were generated by the NADH/PMS system as described previously[56]: sample (25 μl, 5 mg/ml) was mixed with soln. (200 μl, 0.1 mM EDTA, 62 μM NBT, and 98 μM NADH). The reaction was initiated with the addition of PMS (25 μl, 33 μM containing 0.1 mM EDTA) to each well. All solutions were prepared in 0.1M phosphate buffer (pH 7.4). The absorbance was read at 550 nm (Victor^3 microtiter reader; *Perkin–Elmer*, Germany) and results were expressed as mmol TE/g DE.

Statistical Analysis

All samples were assayed in triplicate and the results were given as the means ± standard deviations. Data was analyzed by a one-way ANOVA using SPSS for Windows, and IBM SPSS Statistics 20 (*SPSS, Inc.*, USA). A value of $P < 0.05$ was considered statistically significant. PCA was applied to the concentration of individual polyphenols, determined by the HPLC-DAD method.

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