

http://www.aimspress.com/journal/Molecular

AIMS Molecular Science, 4(3): 288-300.

DOI: 10.3934/molsci.2017.3.288

Received 4 May 2017, Accepted 7 August 2017, Published 14 August 2017

Research article

Phytochemical comparison with quantitative analysis between two flower phenotypes of *Mentha aquatica* L.: pink-violet and white

Alessandro Venditti^{1,*}, Claudio Frezza², Diana Celona¹, Fabio Sciubba¹, Sebastiano Foddai², Maurizio Delfini¹, Mauro Serafini², and Armandodoriano Bianco¹

- ¹ Dipartimento di Chimica, Sapienza Università di Roma, P.le Aldo Moro, 5-00185 Roma, Italy
- ² Dipartimento Biologia Ambientale, Sapienza Università di Roma, P.le Aldo Moro, 5-00185 Roma, Italy
- * Correspondence: Email: alessandro.venditti@uniroma1.it, alessandro.venditti@gmail.com; Tel: +39 0649913229; Fax: +39 0649913841.

Abstract: In this work, the first phytochemical comparison performed on the polar fractions of two different phenotypes of *Mentha aquatica* L. having pink-violet and white flowers, is reported. This comparison was conducted through both a classical phytochemical analysis and a metabolomic one by means of spectroscopic and spectrometric approaches. Twenty-seven compounds were identified in both phenotypes. Most of them were evidenced in both. Anyway, all these compounds possess chemotaxonomic, pharmacological and nutritional value. In particular, one compound represents a new phytochemical for the species and two other compounds are new phytochemicals for the genus, instead. The results obtained allowed us to ascertain, from a phytochemical standpoint, that also this species can be used as possible medicinal plant like all the other species of this genus and as a source of important nutritional factors due to the presence of compounds with high nutritional properties.

Keywords: *Mentha aquatica* L.; pink-violet flowers; white flowers; phytochemical analysis; phytochemical comparison; quantitative analysis

1. Introduction

1.1. Botanical description

Mentha aquatica L. (water mint) is a perennial herbaceous plant belonging to the Lamiaceae

family. Its name derives from the union of the greek term indicating the genus (*Mentha*) and of the latin word referring to the species habitat (*aquatica*). The species is characterized by an ascending stem which is fully hairy and branched. The leaves are short and opposite two by two with an oblong oval shape. The flowers are tiny, densely crowded forming a terminal hemispherical inflorescence, odorous and hermaphrodite with a color ranging from pink to violet. These bloom from June to October. Lastly, the fruit is formed by four oval nuculae with a warty surface. The phenotype with white flowers present the same morphologic characters [1] (Figure 1).



Figure 1. Mentha aquatica L. with pink-violet (left) and white (right) flowers

This is a typical European-Asiatic species even if with some extensions to Northern Africa and America. In Italy, it can be found everywhere along the national territory where it grows along hydrous places like rivers, lakes and swamps but also in meadows and woods till the altitude of 1200 m a.s.l. As well as other *Mentha* species, *M. aquatica* hybridizes with other *Mentha* species generating several known hybrids such as *Mentha* × *piperita*, *Mentha* × *suavis* and *Mentha* × *smithiana* [2].

1.2. Ethno-pharmacological uses of the genus

In literature, there is no specific use about this species but, in general, the plants of this genus have been employed in many different fields and, in some cases, are still used. In particular, these uses were: pharmaceutics as a flavor corrective of some drugs [3] and as herbal medicine for their analgesic, antigenotoxic, spasmolytic, antibacterial and astringent properties [4-6]; cosmetics as a component of fragrances [7] creams and soaps [3]; nutrition as a condiment [8], in the preparation of confectioneries and salads [9,10] and as a flavoring of beverages and sweets [3].

1.3. Aims and objectives of the work

The main objective of this work was to study the total metabolite content of the two plant samples in order to perform a direct comparison between the two phenotypes under this aspect and highlight all the eventual similarities and differences. Another scope was to observe if any connection between the ethnic uses of the species in general and its phytochemical composition exists. Moreover, in literature, there are no previous studies about the phytochemical pattern comparison of the two phenotypes and even about the phenotype with white flowers, itself. These have focused, instead, only on the essential oil composition [11,12] and flavonoids [13,14].

2. Materials and Method

2.1. Plant materials

Both studied samples were collected on the edge of the Turano Lake, province of Rieti, Central Italy (geographic coordinates 42°12'30"N; 12°57'26"E) in July 2015. The botanical identification of the species was performed by one of us (A. V.) using available literature [1,2].

The two samples were protected from direct light and exsiccated at room temperature before analysis. The voucher specimens of the studied samples are stored in our laboratory for further references and registered under the accession numbers MAVF12072015 for *M. aquatica* with pink-violet flowers and MAWF12072015 for that with white flowers.

2.2. Chemicals

Throughout the work, such chemicals were employed: ethanol 96% for the extraction of the metabolites; n-butanol, distilled water, methanol, n-hexane and ethyl acetate as pure solvents or in mixtures among them all at different concentrations to be used as mobile phase for the column chromatographic separations 40 - 63size: on silica gel having um particle 3-(trimethylsilyl)-propionic-2,2,3,3-d4 acid sodium salt for the quantitative analysis; sulfuric acid 2N for the development of TLCs; CD3OD, D2O and methanol having RS purity grade for the identification of the metabolites by NMR Spectroscopy (NMR) and Mass Spectrometry (MS), respectively. The natural solvents, having RPE analytical grade, if not otherwise specified, the deuterated solvents, 3-(trimethylsilyl)-propionic-2,2,3,3-d4 acid sodium salt and methanol having RS purity grade were purchased from "Sigma Aldrich" while silica gel was from "Fluka Analytical".

2.3. Instruments

NMR spectra were recorded on a Varian Mercury 300 MHz instrument or on a Bruker AVANCE III 400 MHz instrument. The chemical shifts were expressed using the internal solvent signal (m5) at 3.31 ppm as reference for the spectra recorded in CD_3OD while the signal of HDO(s) at 4.79 ppm was set as reference for spectra in D_2O .

MS spectra were, instead, performed on a Q-TOF MICRO spectrometer (Waters, Manchester, UK) equipped with an ESI source operating in the negative and/or positive ion mode. The flow rate of the sample infusion was $10~\mu L/min$ with 100 acquisitions per spectrum. Data were analysed using the MassLynx software developed by Waters.

2.4. Quantitative analysis

The quantitative analysis was performed solubilizing a precise amount of the total dried crude extracts of the two separate *M. aquatica* phenotypes with 600 µL of D₂O containing 3-(trimethylsilyl)-propionic-2,2,3,3-d4 acid sodium salt 2 mM used as chemical shift and concentration reference. All spectra were recorded at 298 °K on the Bruker AVANCE III spectrometer operating at the proton frequency of 400 MHz and equipped with a Bruker multinuclear z-gradient inverse probehead. ¹H spectra were acquired employing the presat pulse sequence for

solvent suppression with 128 transients, a spectral width of 6000 Hz and 64 K data points for an acquisition time of 5.45 s. The recycle delay was set to 6.55 s in order to achieve complete resonance relaxation between successive scansions. Bidimensional ¹H-¹H TOCSY spectra were acquired in order to univocally assign the resonances. They were acquired with a spectral width of 6000 Hz in both dimensions, a data matrix of 8K × 256 points, mixing time of 110 ms and relaxation delay of 2.0 s. Resonance assignments were carried out on the basis of bidimensional TOCSY experiments and confirmed by literature data. The signals clearly identifiable without overlapping with neighbouring resonances were integrated for each sample and quantification was performed by comparison of the signal integral with the reference one. The quantities were expressed in mg/g of extract and reported in Table 1. Spectra are available as supplementary materials.

2.5. Extraction of the metabolites

The two plant samples (7.5 g for *M. aquatica* with pink-violet flowers, 1.390 g for *M. aquatica* with white flowers) consisting each on all the exsiccated aerial parts were put separately in two different flasks according to their belonging. They were covered in ethanol 96% and left closed for 48 hours to let metabolites come into solution. At this point the two solutions were filtered and concentrated under reduced pressure at a temperature of about 50 degrees C. These operations were repeated two more times in order to have an exhaustive extraction of the plant materials and all the different concentrations were done in the same separated containers. During the first concentration of each sample, pH was checked in the two solutions in order to verify that these were not too acid (meaning not below 5.5). In fact, this could lead to unpleasant degradation reactions like hydrolysis of the glycosidic compounds. Once all the ethanol was eliminated from both solutions, the two water suspensions were frozen and then lyophilized obtaining in the end, two dried crude extracts weighing 1.60 and 0.34 g for *M. aquatica* with pink-violet flowers and for that with white flowers respectively.

2.6. General procedure for the phytochemical analysis

A sample of the two total crude extracts was separately subjected to several experiments by means of both mono- and bidimensional NMR and MS analysis in positive and negative ionization modes, in order to have a first general overview of the present metabolites. After this, the two separated extracts for their own complete weights underwent several chromatographic separations. This led to the isolation and identification of several constituents, most of them already observed in the initial total analysis. The complete schemes of the initial identifications, the details of the chromatographic separations and the identified compounds for each plant are described in the following specific paragraphs.

2.6.1. *Mentha aquatica* L. with pink-violet flowers

The NMR and MS experiments on the total crude extract of M. aquatica with pink-violet flowers brought the direct identification of twenty-two metabolites: rosmarinic acid (5) [15], choline (10), acetic acid (11), caffeic acid (12), formic acid (14) [16], γ -aminobutyric acid (GABA) (15) [17], lactic acid (16) [16], quinic acid (18) [18], salicylic acid (19) [19], succinic acid (20), fructose (21), glucose (22), sucrose (23), xilose (24), alanine (25), aspartic acid (26), glycine (27), isoleucine (28),

leucine (29), phenylalanine (31), threonine (32), valine (34) [16].

Two chromatographic separations were performed. The first one was conducted on 2.5 g of the crude extract using a correspective amount of silica gel of 75.0 g (ratio 1:30 w/w) and a mixture of *n*-BuOH saturated with distilled water (82:18 v/v) as eluting system. The size of the column was 60 cm high and 2.5 cm internal diameter. The flow rate was 1.0–1.2 mL/min. The column was placed on an automatic collector and tubes were changed every 20 minutes. Eight compounds were identified from this separation: rosmarinic acid (5) [15] as almost pure compound from the assembly of fractions 22–23 for the total weight of 6.5 mg; again rosmarinic acid (5) with glucose (22) [16] as a mixture in ratio 3:1 from the assembly of fractions 45–48 for the total weight of 9.3 mg; quinic acid (17) [18], fructose (21), glucose (22), sucrose (23), xilose (24), alanine (25), threonine (32) and valine (34) [16] all in one only mixture in ratio 3:6:10:1:2:2:2 from the column wash in methanol for the total volume of 200 mL and the total weight of 112.8 mg.

The second chromatographic separation was performed on the assembly of fraction 3-17 deriving from the first one for the total weight of 400.2 mg using a correspective amount of silica gel of 12.0 g (ratio 1:30 w/w) and a mixture of n-hexane and ethyl acetate as mobile phase. The size of the column was 30 cm high and 1.5 cm internal diameter. The flow rate was 0.5-0.7 mL/min. The column was placed on an automatic collector and tubes were changed every 10 minutes. The initial concentration between the solvents was 98:2 (v/v) but during the chromatographic run, this was gradually modified to raise the polarity up to 95:5 (v/v), 9:1 (v/v), 8:2 (v/v), 7:3 (v/v), 6:4 (v/v), 4:6 (v/v), 3:7 (v/v), 2:8 (v/v), 0:100 (v/v) in order to allow the elution of the most polar compounds more strongly retained by silica gel. From this chromatographic separation five more compounds were evidenced: 1-O-linoleoyl-2-O-enadecanoyl-3-O-palmitoleoyl-sn-glycerol (1),1-O-linoleoyl-2-O-palmitoleoyl- sn-glycerol (4) [20] and oleanolic acid (6) [15] as a mixture in ratio 2:4:5 from the assembly of fractions 33–36 for the total weight of 7.2 mg; again oleanolic acid (6) in mixture with corosolic acid (7) [21] in ratio 4:1 from the assembly of fractions 56-91 for the total weight of 258.4 mg; again corosolic acid (7) but in mixture with asiatic acid (8) [21] in ratio 1:1 from the column washing in ethyl-acetate for the total volume of 150 mL and for the total weight of 87.4 mg.

2.6.2. *Mentha aquatica* L. with white flowers

The NMR and MS experiments on M. aquatica with white flowers crude dried extract allowed the direct identification of twenty-one metabolites: rosmarinic acid (5), trigonelline (9), acetic acid (11), citric acid (13), formic acid (14) [16], γ -aminobutyric acid (GABA) (15), lactic acid (16), malic acid (17) [16], salicylic acid (19), fructose (21), glucose (22), sucrose (23), xilose (24), alanine (25), glycine (27), isoleucine (28), leucine (29), phenylalanine (31), threonine (32), tryptophan (33), valine (34).

One chromatographic separation was performed. This was conducted on the total extract using a correspective amount of silica gel of 56.0 g (ratio 1:40 w/w) and a mixture of *n*-BuOH saturated with distilled water (82:18 v/v) as eluting system. The size of the column was 60 cm high and 2.5 cm internal volume. The flow rate was 1.2–1.0 mL/min. The column was placed on an automatic collector and tubes were changed every 15 minutes. From this chromatographic separation five more constituents were isolated and identified: 1-*O*-oleoyl-2-*O*-enadecanoyl-3-*O*-palmitoleoyl-*sn*-glycerol (2) [20] as almost pure compound from the assembly of fractions 1–2 for the total weight of 3.8 mg;

again 1,3-*O*-di-oleoyl-2-*O*-eicosanoyl-*sn*-glycerol (**3**) [20] as almost pure compound from fraction 3 for the total weight of 2.4 mg; oleanolic acid (**6**) in mixture with corosolic acid (**7**) and asiatic acid (**8**) in ratio 3:1:1 from the assembly of fractions 4–6 for the total weight of 15.7 mg; rosmarinic acid (**5**) as almost pure compound from the assembly of fractions 7–10 for the total weight of 4.4 mg; fructose (**21**), glucose (**22**), sucrose (**24**) and lysine (**30**) in ratio 10:10:20:2 from the column wash in methanol for the total volume of 100 mL and the total weight of 49.4 mg.

3. Results

3.1. Phytochemical analysis

The phytochemical analysis of the two phenotypes of *Mentha aquatica* L. showed the presence of twenty-seven compound each. Most of them are in common between the two phenotypes while some are peculiar of one only.

In particular, for *M. aquatica* with pink-violet flowers these were:

1-*O*-linoleoyl-2-*O*-enadecanoyl-3-*O*-palmitoleoyl-*sn*-glycerol (1),

1-*O*-linoleoyl-2-*O*-palmitoleoyl-*sn*-glycerol (**4**), rosmarinic acid (**5**), oleanolic acid (**6**), corosolic acid (**7**), asiatic acid (**8**), choline (**10**), acetic acid (**11**), caffeic acid (**12**), formic acid (**14**), γ-aminobutyric acid (GABA) (**15**), lactic acid (**16**), quinic acid (**18**), salicylic acid (**19**), succinic acid (**20**), fructose (**21**), glucose (**22**), sucrose (**23**), xylose (**24**), alanine (**25**), aspartic acid (**26**), glycine (**27**), isoleucine (**28**), leucine (**29**), phenylalanine (**31**), threonine (**32**) and valine (**34**).

Instead, for *M. aquatica* with white flowers these were:

1-O-oleoyl-2-O-enadecanoyl-3-O-palmitoleoyl-sn-glycerol (2),

1,3-*O*-dioleoyl-2-*O*-eicosanoyl-*sn*-glycerol (**3**), rosmarinic acid (**5**), oleanolic acid (**6**), corosolic acid (**7**), asiatic acid (**8**), trigonelline (**9**), acetic acid (**11**), citric acid (**13**), formic acid (**14**), γ-aminobutyric acid (GABA) (**15**), lactic acid (**16**), malic acid (**17**), salicylic acid (**19**), fructose (**21**), glucose (**22**), sucrose (**23**), xylose (**24**), alanine (**25**), glycine (**27**), isoleucine (**28**), leucine (**29**), lysine (**30**), phenylalanine (**31**), threonine (**32**), tryptophan (**33**) and valine (**34**) (Figures 2, 3, 4).

Figure 2. Structures of all glycerides and triterpenoids identified in both *M. aquatica* L. flowers phenotypes.

Figure 3. Structures of all organic and phenolic acids and nitrogen containing compounds identified in both *M. aquatica* L. flowers phenotypes.

Figure 4. Structures of all saccharides and amino acids identified in both *M. aquatica* L. flowers phenotypes.

3.2. Quantitative analysis

The final results of the quantitative analysis conducted on the two separate samples for three replicates are reported in Table 1.

Table 1. Quantitative analysis performed on both *M. aquatica* flowers phenotypes and comparison of the compositions between the two flower phenotypes.

Compounds	Mentha aquatica with	Mentha
	pink-violet flowers	aquatica with
		white flowers
	Quantity revealed	Quantity
	(mg/g)	revealed (mg/g)
1-O-linoleoyl-2-O-enadecanoyl-3-O-palmitoleoyl-sn-glycerol	X	-
(1)		
1-O-oleoyl-2-O-enadecanoyl-3-O-palmitoleoyl-sn-glycerol (2)	-	X
1,3-O-dioleoyl-2-O-eicosanoyl-sn-glycerol (3)	-	X
1-O-linoleoyl-2-O-palmitoleoyl-sn-glycerol (4)	X	-
rosmarinic acid (5)	143.15 ± 4.29	92.44 ± 2.77
oleanolic acid (6)	X	X

1' '1/7'	V	V
corosolic acid (7)	X	X
asiatic acid (8)	X	X
trigonelline (9)	-	not estimated
choline (10)	3.94 ± 0.12	-
acetic acid (11)	1.86 ± 0.09	1.62 ± 0.08
caffeic acid (12)	X	-
citric acid (13)	-	not estimated
formic acid (14)	0.98 ± 0.05	0.45 ± 0.02
(GABA) (15)	7.68 ± 0.23	12.64 ± 0.38
lactic acid (16)	15.34 ± 0.46	11.26 ± 0.34
malic acid (17)	-	not estimated
quinic acid (18)	X	-
salicylic acid (19)	3.35 ± 0.10	9.67 ± 0.29
succinic acid (20)	X	-
fructose (21)	61.35 ± 1.84	46.50 ± 1.40
glucose (22)	150.82 ± 4.52	218.74 ± 6.56
sucrose (23)	269.55 ± 8.09	488.87 ± 14.67
xylose (24)	not estimated	not estimated
alanine (25)	8.22 ± 0.25	12.78 ± 0.38
aspartic acid (26)	19.83 ± 0.59	-
glycine (27)	30.89 ± 0.93	not estimated
isoleucine (28)	4.34 ± 0.13	9.76 ± 0.29
leucine (29)	4.03 ± 0.12	11.95 ± 0.36
lysine (30)	-	X
phenylalanine (31)	not estimated	not estimated
threonine (32)	9.01 ± 0.27	not estimated
tryptophan (33)	-	not estimated
valine (34)	4.98 ± 0.15	9.10 ± 0.27

Legend: X = present but not quantified; - = absent; not estimated = below the quantifiable value

4. Discussion

The isolated compounds belong to eight different classes of natural metabolites: acylglycerols (compounds 1–4), pentacyclic triterpenes (compounds 6–8), polyphenolic acids (compound 5, 12, 19), organic acids (compounds 11, 13, 14, 16–18 and 20), saccharides (compounds 21–24), alkaloids (compound 9), quaternary ammonium salts (compound 10), and amino acids (15 and compounds 25–34) (Figures 2–4).

Beside the pentacyclic triterpenes, the polyphenolic acids and the alkaloid, all the other components are primary metabolites, necessary for the survival of the plants themselves.

Anyway, the presence of all these compounds is important under three different aspects: chemotaxonomy, pharmacology, nutrition.

4.1. Chemotaxonomic implications of isolated compounds

For what concerns the chemotaxonomy, rosmarinic acid (5) is not a new phytochemical for the genus or even for the species [22]. However, it is quite common in the Lamiaceae family [15,23] and is considered as a chemosystematic marker in the Nepetoideae subfamily [24]. Oleanolic acid (6) represents, instead, a new compound for the species even if its presence has been already reported in the genus [25,26] and in the family [15,27]. Lastly, to the best of our knowledge, corosolic acid (7) and asiatic acid (8) are totally new phytochemicals for the genus but not for the Lamiaceae family [28,29].

4.2. Pharmacological implications of isolated compounds

Under the pharmacological standpoint, a lot of these compounds perform medicinal activities. Rosmarinic acid (5) possesses anti-viral, anti-bacterial, anti-oxidant, anti-tumor, anti-allergic, anti-thrombotic and anti-carcinogenic effects [30]. Oleanolic acid (6) shows hepato-protective, anti-inflammatory, analgesic, cardiotonic, sedative, antiulcer and anti-cancer properties [31]. Corosolic acid (7) owns a strong cytotoxic activity [32]. Asiatic acid (8) is an anti-hypertensive, anti-oxidant, anti-diabetic, anti-hyperlipidemic, hepato-protective, anti-inflammatory, anti-cancer, anxiolytic and anti-depressant compound [33]. Trigonelline (9) has neuroprotective, sedative, anti-bacterial, anti-viral, anti-tumor, hypoglycemic and hypolipidemic activities [34]. Choline (10) can treat liver disorders, hepatitis, glaucoma, atherosclerosis, Alzheimer's disease and bipolar disorder [35-38]. Acetic acid (11) exhibits strong anti-bacterial effects against skin infections [39]. Caffeic acid (12) presents anti-oxidant, anti-inflammatory and cytotoxic properties [40,41]. Formic acid (14) is used to cure warts [42]. GABA (15) exerts relaxing, anti-anxiety and anti-convulsive effects [43,44]. Salicylic acid (19) has a powerful anti-inflammatory activity [45].

4.3. Nutritional value of isolated compounds

As for the nutritional implications of the identified compounds, a lot of them have nutritional properties, too. Above all, the presence of the glycerides (compounds 1-4) with prevalently unsaturated fatty acid chains in the structures, is important since unsaturated fatty acids have been proved to be healthier than the saturated ones. In fact, these can lower the levels of total cholesterol in blood [46] and poly-unsaturated fatty acids, also present, are able to fight cardiac arrhythmias. Also the organic acids (compounds from 11–20) exhibit a strong nutritional value. Acetic acid (11) is essential for all the living organisms representing a relevant intermediate (when bounded to coenzyme A) in the carbohydrate and fatty acid metabolism. It is also an approved food additive (E260) in many countries. Citric acid (13) is an intermediate of the Krebs Cycle, a dietary supplement and a food additive (E330), especially for acidifying processes. Formic acid (14) is used as preservative and an anti-bacterial agent in livestock food reducing the loss of its nutritional value [47]. Lactic acid (16) is also a food additive (E270), a preservative and a flavoring agent. Malic acid (17) is a food additive (E296) and a poor sweetener. Quinic acid (18) has strong astringent properties. Succinic acid (20) is an acidity regulator used in food and beverage industry [48] and also an excipient in pharmaceutical products. The sugars (compounds 21–24) present nutritional value at their own time only according to their nature. They are extremely essential to provide energy for the metabolism in order to perform its normal functions. In controlled quantity, they are promoters of several health-beneficial effects, too. Also the amino acids (compounds from 25–34) are important from the nutritional point of view. In particular, isoleucine (28), leucine (29), lysine (30), phenylalanine (31), threonine (32) tryptophan (33) and valine (34) are essential amino acids meaning that they must be supplied through alimentation.

4.4. Phytochemical comparison

The direct comparison between the phytochemical results obtained from the two phenotypes of M. aquatica may be seen in Table 1. This allowed us to make several important considerations. Primarily, the two samples brought different results although belonging to the same species and collected together in the same place and at the same time (the same phenological development). Small but important differences could be detected. Actually, almost the 75% of the compounds reported from each species were in common (20 compounds out of 27). One difference was observed in the specific structures of the identified glycerides and in particular, only in M. aquatica with pink-violet flowers a diacylglycerol was recognized. Another difference was, from a qualitative point of view, in the organic acid content since the number of them in both phenotypes was almost equal but caffeic (12) succinic (20) and quinic (18) acids were recognized only in the sample with white flowers while citric (13) and malic (17) acids were recognized only in the sample with pink-violet flowers. The last difference, again only in a quality sense, was evidenced for what concerns the amino acid content. The plus number was, this time, from the white flowers phenotype side which showed a major number of different free amino acids and a major presence of essential amino acids in respect with the pink-violet phenotype. In pharmacological and nutritional sense, these differences don't seem to correspond to relevant displacements about the total values of one phenotype in respect to the other. In fact, the lack of any pharmacological and nutritional property in one phenotype due to the absence of a particular compound is well compensated by the presence of another particular compound with a similar property in that one and in the other phenotype.

5. Conclusion

The phytochemical analysis performed on the separate crude extracts of the two phenotypes of *M. aquatica* L., provided, then, a direct and specific rationale for the possible future employment of also this species in the ethno-pharmacological and nutraceutical fields just like it happens with many other vegetal entities belonging to the *Mentha* genus. The phytochemical comparison demonstrated the similar value of the two phenotypes in this sense not placing one over or below the other. Lastly, the quantitative analysis highlighted some differences in the two phenotypes but in no significant manner to suppose the presence of two chemotypes.

Conflict of Interest

The authors declare no conflict of interest.

References

- 1. Pignatti S (1982) *Flora d'Italia*, Eds., Bologna: Edagricole, 496.
- 2. Conti F, Abbate G, Alessandrini A, et al. (2005) *An annotated Checklist of the Italian vascular flora*, Rome: Palombi.
- 3. Lanzara P (1997) Piante medicinali, Torriana: Orsa Maggiore Editore.
- Sylianco CYL, Blanco F R, Lim CM (1987) Mutagenicity, clastogenicity and antimutagenicity of medicinal plant tablets produced by the NSTA pilot plant IV, Yerba buena tablets. *Philipp J Sci* 115: 299-305.
- 5. Tyler VE (1993) *The honest herbal*, 3Eds, Binghamton: Pharmaceutical Products Press.
- 6. Bisset NG (1994) Herbal Drugs, Stuttgart: Medpharm Scientific Publishers.
- 7. Hendriks H (1998) Pharmaceutical aspects of some *Mentha* herbs and their essential oils. *Perfum Flavor* 23: 15-23.
- 8. Başer KHC, Kürkcüoglu M, Tarimicilar G, et al. (1999) Essential oils of *Mentha* species from northern Turkey. *J Essent Oil Res* 11: 579-588.
- 9. El-Gohary AE, El-Sherbeny SE, Ghazal GMEM, et al. (2014) Evaluation of essential oil and monoterpenes of peppermint (*Mentha piperita* L.) under humic acid with foliar nutrition. *J Mater Environ Sci* 5: 1885-1890.
- 10. Guarrera PM, Savo V (2016) Wild food plants used in traditional vegetable mixtures in Italy. *J Ethnopharmacol* 185: 202-234.
- 11. Senatore F, D'Alessio A, Formisano C, et al. (2005) Chemical composition and antibacterial activity of the essential oil of a 1,8-Cineole chemotype of *Mentha aquatica* L. growing Wild in Turkey. *J Essent Oil Bear Plants* 8: 148-153.
- 12. Esmaeili A, Rustaiyan A, Masoudi S, et al. (2006) Composition of the Essential Oils of *Mentha aquatica* L. and *Nepeta meyeri* Benth. from Iran. *J Essent Oil Res* 18: 263-265.
- 13. Voirin B, Bayet C, Faurec O, et al. (1999) Free flavonoid aglycones as markers of parentage in *Mentha aquatica*, *M. citrata*, *M. spicata* and *M. piperita*. *Phytochem* 50: 1189-1193.
- 14. Olsen HT, Stafford GI, Van Staden J, et al. (2008) Isolation of the MAO-inhibitor naringenin from *Mentha aquatica* L. *J Ethnopharmacol* 117: 500-502.
- 15. Venditti A, Frezza C, Riccardelli M, et al. (2016) Unusual molecular pattern in Ajugoideae subfamily: the case of *Ajuga genevensis* L. from Dolomites. *Nat Prod Res* 30: 1098-1102.
- 16. Sciubba F, Capuani G, Di Cocco ME, et al. (2014) Nuclear magnetic resonance analysis of water soluble metabolites allows the geographic discrimination of pistachios (*Pistacia vera*). *Food Res Int* 62: 66-73.
- 17. Rothman DL., Petroff OAC, Behar KL, et al. (1993) Localized ¹H NMR measurements of y-aminobutyric acid in human brain in vivo. *Proc Nat Acad Sci USA* 90: 5662-5666.
- 18. Pauli GF, Poetsch F, Nahrstedt A (1988) Structure assignment of natural quinic acid derivatives using proton nuclear magnetic resonance techniques. *Phytochem Analysis* 9: 177-185.
- 19. Jadrijevi-Mladar Takaĉ M, Vikiĉ Topiĉ D (2004) FT-IR and NMR spectroscopic studies of salicylic acid derivatives. II. Comparison of 2-hydroxy- and 2,4- and 2,5-dihydroxy derivatives. *Acta Pharm* 54: 177-191.
- 20. Sciubba F, Di Cocco ME, Gianferri R, et al. (2014) Metabolic profile of different Italian cultivars of hazelnut (*Corylus avellana*) by nuclear magnetic resonance spectroscopy. *Nat Prod Res* 28: 1075-1081.

- 21. Aguirre C, Delporte C, Backhouse N, et al. (2006) Topical anti-inflammatory activity of 2a-hydroxy pentacyclic triterpene acids from the leaves of *Ugni molinae*. *Bioorg Med Chem Lett* 14: 5673-5677.
- 22. Shekarchi M, Hajimehdipoor H, Saeidnia S, et al. (2012) Comparative study of rosmarinic acid content in some plants of Labiatae family. *Pharmacogn Mag* 8: 37-41.
- 23. Bai N, He K, Roller M, et al. (2010) Flavonoids and phenolic compounds from *Rosmarinus officinalis*. *J Agric Food Chem* 58: 5363-5367.
- 24. Pedersen JA (2000) Distribution and taxonomic implications of some phenolics in the family Lamiaceae determined by ESR spectroscopy. *Biochem Syst Ecol* 28: 229-253.
- 25. Karasawa D, Shimizu S (1980) Triterpene acids in callus tissues from *Mentha arvensis* var. *piperascens* Mal. *Agr Biol Chem* 44: 1203-1205.
- 26. Suga T, Hirata T, Yamamoto Y (1980) Lipid constituents of callus tissues of *Mentha spicata*. *Agr Biol Chem* 44: 1817-1820.
- 27. Kokdil G, Topcu G, Goren A C, et al. (2002) Steroids and terpenoids from *Ajuga relicta*. *Zeitschrift für Naturforschung B* 57: 957-960.
- 28. Aladedunye FA, Okorie DA, Ighodaro OM (2008) Anti-inflammatory and antioxidant activities and constituents of *Platostoma africanum* P. Beauv. *Nat Prod Res* 22: 1067-1073.
- 29. Zielińska S, Matkowski A (2014) Phytochemistry and bioactivity of aromatic and medicinal plants from the genus *Agastache* (Lamiaceae). *Phytochem Rev* 13: 391-416.
- 30. Al-Dhabi NA., Arasu MV, Park CH, et al. (2014) Recent studies on rosmarinic acid and its biological and pharmacological activities. *Exclie J* 13: 1192-1195.
- 31. Liu J (1995) Pharmacology of oleanolic acid and ursolic acid. J Ethnopharmacol 49: 57-68.
- 32. Ahn K, Hahm M S, Park E J, et al. (1998) Corosolic acid isolated from the fruit of *Crataegus pinnatifida* var. *psilosa* is a protein kinase C inhibitor as well as a cytotoxic agent. *Planta Med* 64: 468-470.
- 33. Bunbupha S, Prachaney P, Kukongviriyapan U, et al. (2015) Asiatic acid alleviates cardiovascular remodelling in rats with L-NAME-induced hypertension. *Clin Exp Pharmacol P* 42: 1189-1197.
- 34. Zhou J, Chan L, Zhou S (2012) Trigonelline: a plant alkaloid with therapeutic potential for diabetes and central nervous system disease. *Curr Med Chem* 19: 3523-3531.
- 35. Stoll AL, Sachs GS, Cohen BM, et al. (1996) Choline in the treatment of rapid-cycling bipolar disorder: clinical and neurochemical findings in lithium-treated patients. *Biol Psychiatry* 40: 382-388.
- 36. Chan KC, So KF, Wu EX (2009) Proton magnetic resonance spectroscopy revealed choline reduction in the visual cortex in an experimental model of chronic glaucoma. *Expe Eye Res* 88: 65-70.
- 37. Zeisel SH, da Costa KA (2009) Choline: an essential nutrient for public health. *Nutr Rev* 67: 615-623.
- 38. Van Beek AH, Claassen JA (2010) The cerebrovascular role of the cholinergic neural system in Alzheimer's disease. *Behav Brain Res* 221: 537-542.
- 39. Nagoba BS, Selkar SP, Wadher BJ, et al. (2013) Acetic acid treatment of pseudomonal wound infections a review. *J infect public health* 6: 410-415.
- 40. Matsuno T (1992) Isolation and characterization of the tumoricidal substances from Brazilian propolis. *Honeybee Sci* 13: 49-54.

- 41. Olthof M R, Hollman PC, Katan MB (2001) Chlorogenic acid and caffeic acid are absorbed in humans. *J Nutr* 131: 66-71.
- 42. Bhat RM, Vidya K, Kamath G, et al. (2001) Topical formic acid puncture technique for the treatment of common warts. *Int J Dermatol* 40: 415-419.
- 43. Chapouthier G, Venault P (2001) A pharmacological link between epilepsy and anxiety?. *Trends Pharmacol Sci* 22: 491-493.
- 44. Foster AC, Kemp JA (2006) Glutamate- and GABA-based CNS therapeutics. *Curr Opin Pharmacol* 6: 7-17.
- 45. Madan RK, Levitt J (2014) A review of toxicity from topical salicylic acid preparations. *J Am Acad Dermatol* 70: 788-792.
- 46. Catapano A L, Reiner Z, De Backer G, et al. (2011) ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Atherosclerosis* 217:S1-44.
- 47. Hietala J, Vuori A, Johnsson P, et al. (2016) Formic Acid, In: *Ullmann's Encyclopedia of Industrial Chemistry*. Wiley, 1-22.
- 48. Zeikus JG, Jain MK, Elankovan P (1999) Biotechnology of succinic acid production and markets for derived industrial products. *Appl Microbiol Biot* 51: 545.



© 2017 Alessandro Venditti et al., licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License

(http://creativecommons.org/licenses/by/4.0)