Lipophilic and hydrophilic antioxidants, lipid peroxidation inhibition and radical scavenging activity of two Lamiaceae food plants

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Running title: Lipophilic and hydrophilic antioxidants of Lamiaceae

ABSTRACT

Medicinal and aromatic plants are highly prized all over the world. According to local cuisine and pharmacopoeias, they used to be important as dietary supplements, providing bioactive compounds. Herein we describe lipophilic (fatty acids, tocopherols and carotenoids) and hydrophilic (ascorbic acid, sugars and phenolic compounds) antioxidants, lipid peroxidation inhibition and free radical scavenging activity in aerial parts of two Lamiaceae species (*Mentha pulegium* and *Thymus pulegioides*). *M. pulegium* gave the highest antioxidant properties (EC_{50} < 0.56 mg/ml), which is in agreement with its highest content in tocopherols, mainly α-tocopherol (69.54 mg/100 g), ascorbic acid (7.90 mg/100 g), reducing sugars (7.99 g/100 g) and phenolics. The presence of these lipophilic and hydrophilic antioxidants could explain its use as antiseptic, anti-inflammatory and as food preservative and special sauce. *M. pulegium* revealed the highest content of fat, α-linolenic (omega-3) and linoleic (omega-6) fatty acids, while *T. pulegioides* revealed the highest content of carbohydrates (89.35 g/100 g). This could explain its use to improve the nutrition value of rye flour broth or potato based soups.

Keywords: *Lamiaceae*; *Mentha pulegium*/*Thymus pulegioides*; Lipophilic/hydrophilic antioxidants; Lipid peroxidation inhibition; Radical scavenging activity

1 Introduction

Even though wild edible species were very important in traditional rural societies current research still appears to be focused on the popular or commonly used species, some of which may have already been fully or partially domesticated. Therefore, it is vital that more research is conducted on potentially exploitable wild species. This would promote their increased utilization thereby simultaneously contributing to conserving their genetic resources [1]. Plants are good sources of natural preparations containing effective bioactive compounds, including lipophilic antioxidants such as tocopherols, carotenoids and unsaturated fatty acids, and hydrophilic antioxidants such as polyphenols and reducing sugars, which can be used for different applications, particularly as food additives and health promoting ingredients in the formulations of functional foods and nutraceuticals [2]. In our continuous study to find new natural antioxidant sources, we focused on two wild Lamiaceae species widely used in two regions of Portugal, Alentejo and Trás-os-Montes.

Pennyroyal (*Mentha pulegium* L., *port.* poejo, manjerico-do-rio) is an emblematic flavour of the gastronomy from Alentejo (Southern Portugal) [3] and also particularly appreciated in Trás-os-Montes (Northeastern Portugal) [4,5]. Very popular all over the country because of the famous liqueur prepared with inflorescences (licor de poejo), the traditional use of the species concerns food and pharmaceutical applications, without a clear frontier between these two purposes. Aerial parts and inflorescences are gathered during summer, dried in shadow and kept at home for seasoning and preparing homemade remedies (infusions, syrups, elixir) recommended for indigestion, stomachache, headache, respiratory system and cholesterol [5,6]. The liqueur and the flower infusion are drunk both for pleasure and for their digestive and carminative properties [4,5]. In Alentejo, a kind of "pesto", locally known as "piso", is prepared with fresh plant material, salt, garlic and olive oil, the mix being preserved for future use along the year, when the plant is not

61 available [3,6,7]. Different recipes of "piso" flavour a very typical cuisine based on fish,
62 bread and different kinds of goat or sheep cheese. Besides, in Alentejo, pennyroyal is
63 usually cultivated nearby the windows to repel flies and mosquitoes in summer [6].
64 Large thyme, also known as broad-leaved thyme (*Thymus pulegioides* L., *port.* Pojinha) is
65 a species from the meadows of the north of Portugal, occurring rarely in the southern
66 region. In Trás-os Montes large thyme is highly prized in folk therapy [4]. The species has
67 been traditionally used for its antiseptic and anti–inflammatory properties in the treatment
68 of cold, cough, sinusitis, bronchitis, pneumonia and tuberculosis [4,8]. Carminative and
69 emmenagogue effects have also been reported [4]. Medicinal infusions and tisanes are
70 prepared with the aerial part of the plant, gathered in June and hung in branches to dry.
71 Dried flower heads were often used for seasoning insipid and famine food during
72 starvation periods. Several informants claimed that, besides the nice taste, large thyme also
73 improved the nutritional value of such food, as well as other aromatic herbs, traditionally
74 gathered from the wild [4,5].
75 Most of the described uses remain undocumented and unstudied from an ethnobotanical
76 and pharmacological perspective. The present work evaluates lipophilic (fatty acids,
77 tocopherols and carotenoids) and hydrophilic (ascorbic acid, sugars and phenolic
78 compounds) antioxidants in aerial parts of two Lamiaceae species (*Mentha pulegium* and
79 *Thymus pulegioides*). Furthermore, it describes their lipid peroxidation inhibition and free
80 radical scavenging activity.
81

82 2 Materials and methods

83 2.1 Plant material

84 The aerial parts of the two species (inflorescences and leafy flowering stems about 20cm
85 long) were gathered along 2009 summer, in the Natural Park of Montesinho territory, Trás-

os-Montes, North-eastern Portugal, according local folk criteria of use and plant growth patterns. Morphological key characters from the Flora Iberica were used for plant identification. Voucher specimens are kept in the herbarium of *Escola Superior Agrária de Bragança* (BRESA). Each sample was lyophilized (Ly-8-FM-ULE, Snijders, HOLLAND) and kept in the best conditions for subsequent use.

2.2 Standards and reagents

Acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, ascorbic acid, tocopherols and sugars standards, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid and (+)-catechin. Racemic tocol, 50 mg/ml, was purchased from Matreya (PA, USA). DPPH (2,2-diphenyl-1-picrylhydrazyl) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.3. Nutritional value

The samples were analysed for macronutrients composition (moisture, fat, protein and ash) using AOAC procedures [9]. The crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the crude protein content (N × 6.25) of the samples was estimated by the macro-Kjeldahl method; the ash content was determined by incineration at 600±15 ºC. Total carbohydrates were calculated by difference. Reducing sugars were determined by DNS (dinitrosalicylic acid) method.

Total energy was calculated according to the following equation: Energy (kcal) = 4 × (g protein +g carbohydrates) + 9 × (g fat).

2.4. Lipophilic compounds

Fatty acids were determined by GC/FID (Gas chromatography/Flame ionization detector) as described previously by the same authors [10]. The equipment was a DANI model GC 1000 with a split/splitless injector, a FID and a Macherey-Nagel column (30 m × 0.32 mm ID × 0.25 µm d_f). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 10ºC/min ramp to 240 ºC and held for 11 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

Carotenoids were determined according to Barros et al. [11]. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 mL) = -0.0458 × A_{663} + 0.204 × A_{645} + 0.372 × A_{505} - 0.0806 × A_{453}; β-carotene (mg/100 mL) = 0.216 × A_{663} – 1.220 × A_{645} - 0.304 × A_{505} + 0.452 × A_{453}. The results were expressed as mg of carotenoids per 100 g of dry weight (dw).

Tocopherols content was determined by HPLC (high-performance liquid chromatography)/fluorescence following a procedure described by us [11], using tocol as internal standard. The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a

Polyamide II (250 × 4.6 mm) normal-phase column from YMC Waters operating at 30ºC. The mobile phase used was a mixture of *n*-hexane and ethyl acetate (7:3, v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method. Tocopherol contents in the samples are expressed in mg per 100 g of dry weight (dw).

2.5. Hydrophilic compounds

Ascorbic acid was determined according to Barros et al. [11]. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.006-0.1 mg/mL; $y = 3.0062x + 0.007$; $R^2 = 0.9999$), and the results were expressed as mg per 100 g of dry weight (dw).

Free sugars were determined by HPLC/RI (Refraction index detector) as described by Heleno et al. [10], using melezitose as internal standard. The equipment described above was coupled to a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1 mL/min. Sugar identification was made by comparing the relative retention times of sample peaks with standards. Quantification was made by internal normalization of the chromatographic peak area and the results are expressed in g per 100 g of dry weight (dw).

2.6 Lipid peroxidation inhibition and radical scavenging activity assays

A fine dried powder (20 mesh; ~1g) was extracted by stirring with 30 mL of methanol at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then

160 extracted with one additional 30 mL portion of methanol. The combined methanolic
161 extracts were evaporated at 35ºC under reduced pressure (rotary evaporator Büchi R-210),
162 re-dissolved in methanol at a concentration of 10 mg/mL, and stored at 4 ºC for further use.
163 Total phenolics and flavonoids were estimated based on procedures described by the
164 authors [11]. Gallic acid (0.05-0.8 mM; $y = 1.9799x + 0.0299$; $R^2 = 0.9997$) and (+)-
165 catechin (0.0156-1.0 mM; $y = 0.9186x - 0.0003$; $R^2 = 0.9999$) were used to calculate the
166 standard curves. The results were expressed as mg of gallic acid equivalents (GAE) and
167 mg of (+)-chatequin equivalents (CE), respectively for phenolics and flavonoids, per g of
168 extract. Flavanols were estimated based on the procedure described by Mazza et al. [12].
169 Quercetin (0.2-3.2 mM; $y = 0.1962x - 0.0636$; $R^2 = 0.9986$) was used to calculate the
170 standard curve, and the results were expressed as mg of quercetin equivalents (QE) per g of
171 extract.
172 The antioxidant activity was evaluated by inhibition of β-carotene bleaching in the
173 presence of linoleic acid radicals, inhibition of lipid peroxidation in brain homogenates by
174 TBARS (thiobarbituric acid reactive substances), reducing power and DPPH radical-
175 scavenging activity assays, following procedures described previously by the authors [11].
176 β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene
177 content after 2h of assay/initial β-carotene content) × 100. The TBARS formation
178 inhibition (%) was calculated using the following formula: Inhibition (%) = [(A – B)/A] x
179 100%, where A and B were the absorbance of the control and the compound solution,
180 respectively. The radical scavenging activity (RSA) was calculated as a percentage of
181 DPPH discolouration using the equation: % RSA = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is
182 the absorbance of the solution when the sample extract has been added at a particular level,
183 and A_{DPPH} is the absorbance of the DPPH solution. The extract concentrations providing
184 50% of antioxidant activity (EC_{50}) were calculated from the graphs of antioxidant activity

185 percentages against extract concentrations (for each assay). Trolox was used as standard.

186

187 **2.7 Statistical analysis**

188 All the assays were carried out in triplicate. The results were expressed as mean values and
189 standard deviation (SD), and were analyzed using one-way analysis of variance (ANOVA)
190 followed by Tukey's HSD Test with $\alpha = 0.05$ (SPSS v. 16.0 program).

191

192 ## 3 Results and discussion

193 In former times, both species were highly consumed and frequently gathered from the
194 wild. Nowadays *M. pulegium* (pennyroyal) is still a very popular species but *T. pulegioides*
195 (large thyme) is less used because special skills are needed to find it feral. As edibles it is
196 important to know and to characterize its nutritional value. The macronutrients profile and
197 estimation of energy content are shown in **Table 1**. Pennyroyal revealed the highest
198 content of moisture, fat, protein, ash and energy, while large thyme revealed the highest
199 content of carbohydrates. These compounds were the most abundant macronutrients.
200 The results of fatty acid composition, SFA (Saturated Fatty Acids), MUFA
201 (Monounsaturated Fatty Acids) and PUFA (Polyunsaturated Fatty Acids) are shown in
202 **Table 2**. The most abundant fatty acids were α-linolenic (C18:3), linoleic (C18:2) and
203 palmitic (C16:0) acids (**Figure 2**). Eighteen more fatty acids were identified and
204 quantified, being PUFA the main group. The omega-3 (including α-linolenic acid) and
205 omega-6 (including linoleic acid) fatty acids are associated with several beneficial health
206 effects in inflammatory diseases, hypertension, heart disease, prostate and breast cancers,
207 among others [13,14]. Furthermore, the ratios PUFA/SFA were higher than 0.45 and the
208 ratios n-6/n-3 fatty acids were lower than 4.0, which are recommended for the human diet,

209 and contribute to the decrease of total amount of fat in blood (cholesterol), reducing the
210 risk of cancer, cardiovascular, inflammatory and autoimmune diseases [15].
211 Non-enzymatic antioxidants including lipophilic (carotene and α-tocopherol) and
212 hydrophilic (ascorbic acid) compounds may play an important role in the cellular response
213 to oxidative stress by reducing certain ROS, and retard the progress of many chronic
214 diseases as well as the lipid oxidative rancidity in food, cosmetics and pharmaceutical
215 materials [16]. The studied plants revealed the presence of those antioxidant molecules
216 (**Table 3**); pennyroyal showed the highest levels of tocopherols, particularly α-tocopherol
217 (69.54 mg/100 g dw), and ascorbic acid (7.90 mg/100 g), while large thyme gave the
218 highest levels of carotenoids (2.04 mg/100 g).
219 Other hydrophilic molecules such as sugars were determined and the results are given in
220 **Table 4**. Pennyroyal revealed the highest content of total sugars, and in particular fructose,
221 glucose, sucrose and trehalose, while large thyme revealed the highest content of raffinose.
222 Fructose, glucose, trehalose, raffinose and sucrose were detected in both samples; sucrose
223 was the most abundant sugar. Sucrose and threalose are non-reducing sugars and,
224 therefore, total sugars obtained by HPLC/RI (**Table 4**) were higher than reducing sugars,
225 measured by DNS colorimetric assay (**Table 1**). Sugars were a small part of carbohydrates
226 due to the presence of polysaccharides such as starch and cellulose.
227 The pharmacological effect of polyphenols (a diverse class of natural products suggested to
228 be the key bioactives present in plant foods), are attributed to their antioxidant (e.g. ROS
229 scavenging activity), indirect antioxidant (e.g. enzyme inhibition), anti-inflammatory as
230 well as gene expression-modifying effects [17]. The amount of phenolics found in wild
231 pennyroyal methanolic extract (331.69 mg GAE/g) was higher than the amount found in
232 other Portuguese sample bought in a traditional market (ethanolic extract 71.7 mg/g;
233 aqueous extract 57.9 mg/g) [18]. Otherwise the amount found in large thyme methanolic

234 extract (210.49 mg/g) was lower than the amount found in ethanolic extract from Italian
235 material (435.1 mg/g) [17]. These molecules were also found in *Thymus pulegioides*
236 chemotypes from Lithuania [2] and in Algerian samples [19]. As it can be observed in
237 **Table 4**, flavanols represent an important group of flavonoids in both spices.
238 Pennyroyal and large thyme methanolic extracts showed antioxidant properties measured
239 by four different assays targeted to lipid peroxidation inhibition and radical scavenging
240 activity evaluation (**Table 4**). Pennyroyal gave the highest antioxidant activity (EC_{50}
241 values < 0.56 mg/ml), may be due to its major content in hydrophilic antioxidants
242 (phenolics, flavonoids, ascorbic acid and reducing sugars) but also in lipophilic
243 antioxidants such as tocopherols. Other authors also correlated the antioxidant activity
244 found in *Thymus pulegioides* from Lithuania [2] and in *Mentha pulegium* from Spain [20]
245 with the concentration of polyphenolic compounds such as phenolic acids and flavonoids.
246 The antioxidant activity of large thyme ethanolic extract from Italy [17] and of pennyroyal
247 methanolic extract from Spain [21] was also reported. A pennyroyal sample bought in a
248 Portuguese traditional market gave higher DPPH radical scavenging activity (EC_{50} value
249 for ethanolic extract: 24.9 μg/ml), but lower β-carotene bleaching inhibition capacity (165
250 μg/ml) [18] than our sample (**Table 4**).
251
252 The studied plants could have some potential in food industry because of its flavouring
253 properties and nutritional composition (including omega-3 and omega-6 fatty acids, and
254 sugars), but also in pharmaceutical industry due to its biological and medicinal benefits
255 (demonstrated by their *in vitro* lipid peroxidation inhibition and radical scavenging
256 properties). The presence of lipophilic (carotenoids and tocopherols) and hydrophilic
257 (ascorbic acid, phenolics and flavonoids) antioxidants, could be related to their traditional
258 uses as antiseptic and anti-inflammatory (activities related to oxidative stress) [4,8,22].

Furthermore, the presence of those compounds could explain the use of pennyroyal as food preservative. The nutritional value of large thyme characterized by high levels of carbohydrates could also explain its use to improve the nutrition value of some food usually eaten long ago during famine periods, such as potato based soups seasoned with the leaves and a broth prepared with the top leaves and flowers boiled in water and then thickened with a tablespoon of rye [4,5,18].

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Conflict of interest statement

The authors have declared no conflict of interest.

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Table 1. Nutritional value of two Lamiaceae (mean ± SD; n=3). In each row, different letters mean significant differences ($p<0.05$).

	Mentha pulegium	*Thymus pulegioides*
Moisture (g/100 g fw)	59.47 ± 9.22 a	47.66 ± 12.60 a
Fat (g/100 g dw)	2.22 ± 0.22 a	0.18 ± 0.02 b
Proteins (g/100 g dw)	7.12 ± 0.49 a	5.53 ± 1.40 b
Ash (g/100 g dw)	5.92 ± 0.09 a	4.94 ± 0.62 b
Carbohydrates (g/100 g dw)	84.74 ± 0.59 b	89.35 ± 1.54 a
Energy (Kcal/100 g dw)	387.44 ± 0.53 a	381.14 ± 1.76 b
Reducing Sugars (g/100 g dw)	7.99 ± 1.72 a	2.11 ± 0.15 b

Table 2. Composition in fatty acids (percentages) of two Lamiaceae (mean ± SD; n=3). In each row different letters mean significant differences ($p<0.05$).

	Mentha pulegium	*Thymus pulegioides*
C6:0	0.79 ± 0.04	0.28 ± 0.05
C8:0	1.03 ± 0.06	nd
C12:0	1.75 ± 0.20	0.24 ± 0.01
C14:0	3.42 ± 0.07	5.70 ± 0.74
C14:1	0.32 ± 0.02	1.42 ± 0.16
C15:0	0.14 ± 0.04	0.30 ± 0.00
C16:0	14.82 ± 0.09	16.70 ± 0.22
C16:1	0.11 ± 0.01	nd
C17:0	0.52 ± 0.07	nd
C18:0	4.96 ± 0.03	3.39 ± 0.05
C18:1n9	5.77 ± 0.20	11.40 ± 0.10
C18:2n6	16.27 ± 0.33	12.98 ± 0.52
C18:3n3	37.00 ± 0.35	36.69 ± 0.25
C20:0	2.36 ± 0.07	1.37 ± 0.08
C20:1	0.63 ± 0.02	nd
C20:2	0.15 ± 0.01	nd
C20:3n3 and C21:0	0.20 ± 0.04	nd
C22:0	0.90 ± 0.07	nd
C22:2	1.93 ± 0.09	nd
C23:0	5.46 ± 0.05	8.04 ± 0.41
C24:0	1.47 ± 0.27	1.50 ± 0.18
SFA	37.62 ± 0.83 a	37.52 ± 0.82 a
MUFA	6.82 ± 0.19 b	12.81 ± 0.05 a
PUFA	55.48 ± 0.53 a	49.67 ± 0.77 b
PUFA/SFA	1.48 ± 0.05 a	1.32 ± 0.05 b
n-6/n-3	0.44 ± 0.00 a	0.35 ± 0.01 b

C6:0 (caproic acid); C8:0 (caprylic acid); C12:0 (lauric acid); C14:0 (myristic acid); C14:1 (myristoleic acid); C15:0 (pentadecanoic acid); C16:0 (palmitic acid); C16:1 (palmitoleic acid); C17:0 (heptadecanoic acid); C18:0 (stearic acid); C18:1n9 (oleic acid); C18:2n6 (linoleic acid); C18:3n3 (α-linolenic acid); C20:0 (arachidic acid); C20:1 (eicosenoic acid); C20:2 (*cis*-11,14-eicosadienoic acid); C20:3n3 and C21:0 (*cis*-11, 14, 17-eicosatrienoic acid and heneicosanoic acid); C22:0 (behenic acid); C22:2 (*cis*-13,16-docosadienoic acid); C23:0 (tricosanoic acid); C24:0 (lignoceric acid); SFA (Saturated Fatty Acids); MUFA (Monounsaturated Fatty Acids); PUFA (Polyunsaturated Fatty Acids); nd- not detected.

Table 3. Composition in carotenoids and vitamins of two Lamiaceae (mean ± SD; n=3). In each row different letters mean significant differences ($p<0.05$).

	Mentha pulegium	*Thymus pulegioides*
Carotenoids (mg/100 g dw)	0.42 ± 0.00 b	2.04 ± 0.04 c
α-tocopherol	69.54 ± 11.44 a	12.63 ± 0.82 b
β-tocopherol	1.84 ± 0.26 a	0.08 ± 0.00 b
γ-tocopherol	9.84 ± 1.54 a	0.77 ± 0.08 b
δ-tocopherol	8.48 ± 1.55 a	nd
Total tocopherols (mg/100 g dw)	89.70 ± 14.79 a	13.48 ± 0.91 b
Ascorbic acid (mg/100 g of dw)	7.90 ± 0.17 a	5.95 ± 0.11 b

nd- not detected

Table 4. Composition in sugars of two Lamiaceae (mean ± SD; n=3). In each row, different letters mean significant differences ($p<0.05$).

	Mentha pulegium	*Thymus pulegioides*
Fructose	2.39 ± 0.11 a	0.22 ± 0.00 b
Glucose	3.37 ± 0.22 a	0.33 ± 0.03 b
Sucrose	4.62 ± 0.28 a	1.06 ± 0.02 b
Trehalose	0.61 ± 0.05 a	0.24 ± 0.04 b
Raffinose	0.29 ± 0.05 b	0.55 ± 0.04 a
Total Sugars (g/100 g dw)	11.29 ± 0.61 a	2.39 ± 0.13 b

Table 5. Extraction yields, composition in phenolics and flavonoids, and antioxidant activity EC_{50} values of two Lamiaceae (mean ± SD; n=3). In each row different letters mean significant differences ($p<0.05$).

	Mentha pulegium	*Thymus pulegioides*
η (%)	54.62 ± 4.26 a	24.61 ± 0.60 b
Phenolics (mg GAE/g extract)	331.69 ± 19.63 a	210.49 ± 21.16 b
Flavonoids (mg CE/g extract)	139.85 ± 1.27 a	128.24 ± 6.00 b
Flavanols (mg QE/g extract)	128.57 ± 0.62 a	126.74 ± 0.59 a
β-carotene bleaching inhibition (mg/mL)	0.01 ± 0.00 b	0.03 ± 0.00 a
TBARS inhibition (mg/mL)	0.08 ± 0.00 b	0.22 ± 0.01 a
Reducing power (mg/mL)	0.12 ± 0.01 b	0.49 ± 0.03 a
DPPH scavenging activity (mg/mL)	0.56 ± 0.05 b	0.68 ± 0.03 a

Figure 1. Aerial parts with inflorescences of *Mentha pulegium*, a culinary herb, folk remedy and insecticide, and *Thymus pulegioides*, widely reputed as antiseptic and anti–inflammatory. These two Lamiaceae are tradicionally used in Portugal.

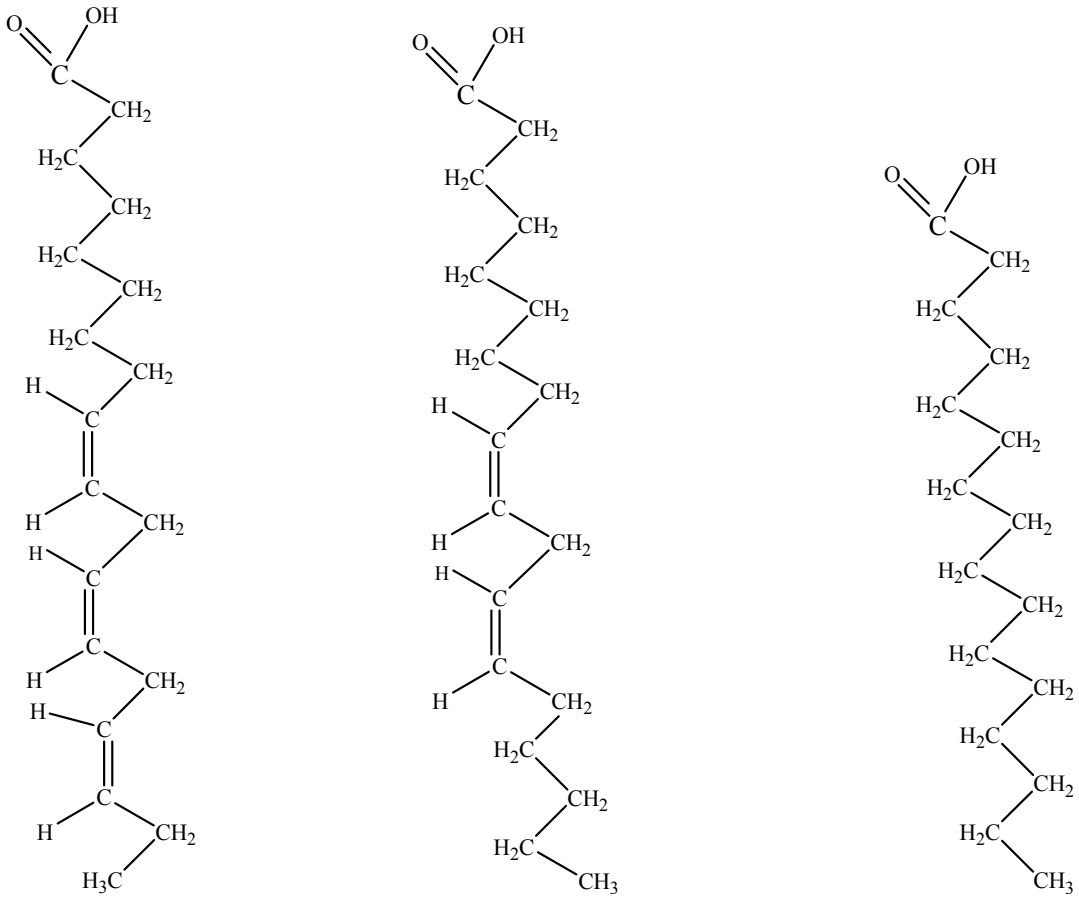

Figure 2. Chemical structures of α-linolenic, linoleic and palmitic acids.