From famine plants to tasty and fragrant spices: Three Lamiaceae of general dietary relevance in traditional cuisine of Trás-os-Montes (Portugal)

LILLIAN BARROS, ANA MARIA CARVALHO, ISABEL C.F.R. FERREIRA*

Centro de Investigação de Montanha (CIMO), ESA, Instituto Politécnico de Bragança, *Campus* de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.

* Author to whom correspondence should be addressed (e-mail: iferreira@ipb.pt telephone +351-273-303219; fax +351-273-325405).

Abstract

The chemical composition and nutritional value of three Lamiaceae often used as spices in Portuguese traditional cuisine: Ground ivy (*Glechoma hederaceae* L.), oregano (*Origanum vulgare* subsp. *virens* (Hoffmanns. & Link) Ietswaart) and mastic thyme (*Thymus mastichina* L.) were determined. Chemical composition evaluation included moisture, total fat content, crude protein, ash, carbohydrates, and nutritional value determination. The macronutrient profile revealed that these spices are rich sources of carbohydrates and that an edible portion of 100 g assures, on average, 161 Kcal. The composition in individual sugars was determined by high performance liquid chromatography coupled to a refraction index detector (HPLC/RID), being this methodology completely validated. All the compounds were separated in a period of time of 15 min; the method used proved to be sensitive, reproducible and accurate. Fructose, glucose, sucrose and raffinose were the most abundant sugars. The analysis of fatty acid composition, performed by gas chromatography coupled to a flame ionization detector (GC/FID), allowed the quantification of twenty two fatty acids. Polyunsaturated fatty acids and, in particular, α-linolenic and linoleic acids, were predominant.

Keywords: Lamiaceae, Sugars profile, Fatty acids profile, HPLC validation

1. Introduction

Trás-os-Montes, a province from the north of Portugal, is very diverse both in ecological and ethnographical conditions. Within its most north-eastern region many wild plants are usually gathered from the scrubland, preserved and consumed in daily diets and also used as important ingredients in traditional cuisine since a long time ago. Some of these wild botanicals have been locally considered famine food, particularly during the last five decades of the twentieth century. Throughout historical periods of starvation they were the tastiest ingredient of very poor, insufficient and monotonous daily meals. Wild edible plants were the main source of nourishment for the rural families. Although this prejudice, mainly perceived by elders, the use of wild spices has always been significant in the regional and traditional cuisine, specifically to preserve food, such as olives, sausages and pickles. Recently, their importance has increased because there is a great interest in every species with natural flavors suitable for enhancing the taste and smell of food. Thus the most appreciated wild plants are semi-domesticated, cultivated in homegardens, and present in every homesteads (Carvalho, 2005).

Ethnobotanical surveys conducted in the region (Carvalho, 2005) have documented a great number of use-reports (151) and species (30 per cent of those cited) used as spices and related with the processes of food preservation. Ground ivy (*Glechoma hederaceae* L.), oregano (*Origanum vulgare* subsp. *virens (*Hoffmanns. & Link) Ietswaart) and mastic thyme (*Thymus mastichina* L.) are some of the most popular plants used as food additives in this Portuguese region. They are widespread Mediterranean perennial herbs also considered as medicinal plants (Carvalho, 2005; Ivanova, Gerova, Chervenkov, & Yankova, 2005; Kumarasamy et al., 2007; Pardo de Santayana et al., 2007), though it has also been reported some other common uses (Carvalho, 2005). Their leaves and inflorescences are dried and stored and therefore used all year round as spices, to flavour several traditional recipes and to preserve food (**Table 1**). It may be that their use in traditional cuisine is not only culinary but medicinal too, to increase the digestibility of the cooked food as also stated by Bonet and Vallès (2002).

There are some reports about the presence of immunomodualtory nutrients, including vitamins and minerals in these spices (Leonard, Hardin, & Leklem, 2001). They also contain several bioactive phytochemicals such as flavonoids (Justesen & Knuthsen, 2001; Lin, Mukhopadhyay, Robbins & Harnly, 2007) and essential oils (Miguel et al., 2004; Zheng et al., 2009). Nevertheless, we could not find reports on their macronutrients composition, including the sugars and fatty acids profiles.

Sugars are the basic building blocks of polymeric carbohydrates which are important as short-term energy-storage compounds and also as major structural compounds in plant and bacterial cell walls and in the extracellular matrix. Small carbohydrates such as glucose and fructose occupy key roles in energy metabolism and supply carbon skeletons for the synthesis of other compounds (Zubay, 2006). Fatty acids are building blocks of most lipids which have important roles in human body, either as sources of metabolic energy or as structural and functional components of biomembranes. Oleic acid is the most common unsaturated fatty acid in mammals, but two other unsaturated fatty acids, linoleic and linolenic acids, are not synthesised by mammals and therefore important dietary requirements. Like vitamins, these two fatty acids are required for growth and good health, and hence are called essential fatty acids. Plants are able to synthesise linoleic and linolenic acids and are the source of these fatty acids in our diet (Zubay, 2006).

All this information is very important since most foods consumed in developing countries are deficient in essential nutrients, and these spices may be rich in nutrients but, because of lack of information about them, their uses as food supplements are limited. In the present study we report the chemical composition of three Lamiaceae often used as spices in Portuguese traditional cuisine. On the basis of the samples composition (contents of moisture, proteins, fat, carbohydrate and ash), an estimation of their nutritional role was performed. Fatty acids were obtained by GC/FID and sugars by HPLC/RID, after a complete validation of the analytical methodology.

2. Materials and methods

2.1. Standards and reagents

Acetonitrile 99.9% pure, of HPLC grade was purchased from Lab-Scan (Lisbon, Portugal). All the other reagents were of analytical grade purity: methanol and diethyl ether were supplied by Lab-Scan (Lisbon, Portugal); toluene from Riedel-de-Haën; sulphuric acid from Fluka (St. Gallen, Switzerland). The fatty acids methyl ester (FAME) reference standard mixture 37 (fatty acids C4 to C24; standard 47885-U) was from Supelco (Bellefonte, PA, USA) and purchased from Sigma (St. Louis, MO, USA), as well as other individual fatty acid isomers and the sugar standards. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

2.2. Samples

Samples of leaves and steams of *Glechoma hederaceae* (ground ivy) and flowering inflorescences of *Origanum vulgare* subsp. *virens* (oregano) and *Thymus mastichina* (thyme) were gathered in Trás-os-Montes, North-eastern Portugal. The selected sites and gathering practices took into account local consumers' criteria for the seasoning use of these species and the optimal growth stage and gathering period of each species. The plant material was collected early in the morning, in half shade sites at meadows' edges: ground ivy and thyme in July 2008; oregano in September 2008. Morphological key characters from Franco (1984) were used for plant identification. Voucher specimens are deposited in the Herbarium of the Escola Superior Agrária de Bragança. The material was lyophilized (Ly-8-FM-ULE, Snijders, Holand) and kept in the best conditions (-20ºC, ~30 days) for subsequent use.

2.3. Nutritional value

The samples were analysed for chemical composition (protein, fat, carbohydrates and ash) using the AOAC procedures (1995). The crude protein content (N × 6.25) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at (600±15) ºC; reducing sugars were determined by DNS (dinitrosalicylic acid) method. Total carbohydrates were calculated by difference: Total carbohydrates = 100 – (moisture + protein + fat + ash), where moisture, protein, fat and ash, stand for their masses respectively, expressed in units of 1 g. Total energy was calculated according to the following equations: Energy (Kcal) = 4 × (protein + carbohydrate) + 9 × (lipid), where protein and carbohydrate stand for their masses, respectively, expressed per gram.

2.4. Sugars profiles

Preparation of standard solutions. Individual solutions (~10 mg/ml) of L(+)-arabinose, D(−)-fructose, L-fucose, D(+)-galactose, D(+)-glucose anhydrous, lactose 1-hydrate, maltose 1-hydrate, maltulose monohydrate, D(+)-mannitol, D(+)-mannose, D(+)-melezitose, D(+)-melibiose monohydrate, D(+)- raffinose pentahydrate, L(+)-rhamnose monohydrate, D(+)-sucrose, D(+)-trehalose, D(+)-turanose and D(+)-xylose were prepared in water and stored at −20 ºC. A stock standard mixture with fructose, glucose, sucrose and raffinose was prepared in water with the final concentration of 30 mg/ml. Melezitose was used as internal standard (IS), being prepared a stock solution at 25 mg/ml in water, kept at −20 ºC.

Extraction procedure. Dried sample powder (1.0 g) was spiked with the IS (5 mg/ml), and was extracted with 40 ml of 80% aqueous ethanol at 80 ºC for 30 min. The resulting suspension was centrifuged at 15,000 *g* for 10 min. The supernatant was concentrated at 60 ºC under reduced pressure and defatted three times with 10 ml of ethyl ether, successively. After concentration at 40 ºC, the solid residues were dissolved in water to a final volume of 5 ml, filtered through a 0.22 µm disposable LC filter disk, transferred into an injection vial and analysed by HPLC.

HPLC analysis. The HPLC equipment consisted of an integrated system with a Smartline pump 1000, a degasser system Smartline manager 5000, a Smartline 2300 RI detector (Knauer, Germany), and an AS-2057 auto-sampler (Jasco, Japan). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with an Eurospher 100-5 NH_2 column (4.6 mm × 250 mm, 5 mm, Knauer) operating at 35 ºC

(7971R Grace oven). The mobile phase used was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1 ml/min, and the injection volume was 20 µl. The compounds were identified by chromatographic comparisons with authentic standards. The results are expressed in g/100 g of fresh weight, calculated by internal normalization of the chromatographic peak area.

The linearity and sensitivity of the HPLC analysis was determined and the method was validated by the instrumental precision, repeatability and accuracy, using *Origanum vulgare*.

2.5. Fatty acids profiles

Fatty acids were determined by gas chromatography with flame ionization detection (GC/FID)/capillary column as described previously by the authors (Barros, Venturini, Baptista, Estevinho, & Ferreira, 2008), and after the following trans-esterification procedure: fatty acids were methylated with 5 ml of methanol:sulphuric acid:toluene 2:1:1 (v:v), during at least 12 h in a bath at 50 ºC and 160 rpm; then 3 ml of deionized water were added, to obtain phase separation; the FAME were recovered with 3 ml of diethyl ether by shaking in vortex , and the upper phase was passed through a micro-column of sodium sulphate anhydrous, in order to eliminate the water; the sample was recovered in a vial with Teflon, and before injection the sample was filtered with 0.2 µm nylon filter from Milipore. The fatty acid profile was analyzed with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (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 10 ºC/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. For each analysis 1 µl of the sample was injected in GC. Fatty acid identification was made by comparing the relative retention times from samples with FAME peaks (standards). The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

2.6. Statistical analysis

For each species three samples were analysed and also all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD) or standard errors (SE). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 16.0 software.

3. Results and discussion

3.1. Nutritional value

The macronutrients profile and estimated energetic value (expressed on fresh weight basis) obtained for the three Lamiaceae are shown in **Table 2**. Ground ivy revealed the highest moisture content (73 g/100 g), while oregano showed the lowest moisture contents (52 g/100 g). Carbohydrates were the most abundant macronutrients in all the samples and ranged from 21 g/100 g in ground ivy to 40 g/100 g in oregano. Reducing sugars are only a small part of carbohydrates content since wild plants are rich in polysaccharides such as starch and cellulose. Cellulose is a structural polysaccharide found as the major component of cell walls in plants; it is the most abundant of organic compounds constituting approximately 50% all the carbon found in plants (Zubay, 2006). Starch is the major polysaccharide used of energy storage in plant cells, occurring both as unbranched amylase and as branched amylopectin (Zubay, 2006).

In Trás-os-Montes, seasoning and preserving food are still common procedures that influence the traditional cuisine and are fundamental to many regional recipes. All the three Lamiaceae studied are often used for flavour traditional delectable soups and summer salads. Specifically, the fresh leaves of ground-ivy are added, at the last minute, to potato, onion and chopped kale-based soup and to bean-based or chickpea-based soups. They are also used in stewed beans prepared with vegetables and sausages. In former times, a restorative bouillon was prepared with boiling water and a tablespoon of rye flour and enriched with the leaves of ground-ivy. It was claimed that this bouillon can satisfy the nutritional needs of breast-feeding women and the plant can provide a balanced set of nutrients for nourishing newborn babies whose mothers could not breastfeed and for recovering young children who have been ill (Carvalho, 2005). Oregano and mastic thyme inflorescences are mainly used dried for seasoning. Both species are use to preserve and give flavour to the watery sauce where freshly harvested olives are kept for several months. Oregano and garlic are the most important ingredients of the bouillon prepared for manufacturing traditional sausages ('alheiras'). Dried leaves and inflorescences of mastic thyme are used for cooking instead of salt to prevent hypertension (Carvalho, 2005; Pardo de Santayana et al, 2007).

Proteins and fat were the less abundant macronutrients; proteins varied between 1.3 g/100 g in ground ivy and 2.2 g/100 g in oregano. Fat was found in 3.8, 2.8 and 1.2% for mastic thyme, oregano and ground ivy, respectively. On the basis of the proximate analysis, it can be calculated that a fresh portion of 100 g of these herbs assures, on average, 162 kcal. The highest values are guaranteed by oregano, while ground ivy gave the lowest energy contribution (**Table 2**). Ash content was more abundant in ground ivy (3.5 g/100 g), while the lowest values were found in mastic thyme (2.7 g/100 g).

3.2. Sugars profiles

The analytical characteristics of the method for sugars analysis included evaluation of linearity and determination of limits of detection and quantification (**Table 3**). For each compound, a 7-level calibration curve was constructed using the peak-area ratio between the sugars and IS *versus* concentration ratio between the standards and IS. The average of triplicate determinations for each level was used. Melezitose was used as IS because it was not detected in the analyzed samples. An internal standard should be similar to the substance to be quantified, have a proximate, but different, retention time to the substance, not react with the substance or other components present in the matrices, and not be present in the sample (Snyder et al., 1997); melezitose presented all these characteristics. The method validation was performed using glucose, fructose, sucrose and raffinose because these sugars were the main sugars present in the analysed samples.

The correlation coefficients were always higher than 0.999 for all the compounds (**Table 3**). The limits of detection (LOD), calculated as the concentration corresponding to three times the calibration error divided by the slope, ranged from 0.05 to 0.09 mg/ml. The limits of quantification (LOQ) were calculated using the concentration corresponding to ten times the calibration error divided by the slope, and ranged from 0.18 mg/ml to 0.30 mg/ml.

In order to evaluate the instrumental precision, the sample (*Oreganum vulgare*) extract was injected six times. The chromatographic method proved to be precise (CV% between

1.72% and 3.39%, **Table 4**). Repeatability was evaluated by applying the whole extraction procedure 6 times to the same sample. All the obtained values were low (CV% ranging from 0.82% to 3.74%, **Table 4**). The accuracy of the method was evaluated by the standard addition procedure (% of recovery) with three addition levels (0.375 mg/ml, 1.5 mg/ml and 6 mg/ml, each one in duplicate). The standard mixture was added to the sample, and all the extraction procedure was carried out. The results demonstrate good recovery for the compounds under study (ranging from 94% and 100%).

In what concerns sugar composition (**Table 5**), fructose, glucose and sucrose were detected in all the samples. For oregano (0.58 g/100 g) and mastic thyme (0.97 g/100 g) glucose was the most abundant sugar (**Figure 1**), while raffinose predominates in ground ivy (0.42 g/100g). Mastic thyme revealed the highest sugar contents (1.44 g/100 g), while ground ivy revealed the lowest levels (1.04 g/100g). Despite the reports of sucrose as the most important sugar in plants, this compound was not the most abundant sugar in the analysed species. Nevertheless, some percentage of the sucrose present in the samples could have been hydrolyzed into their monosaccharide's constituents, contributing to an increase in glucose and fructose levels (**Table 5**).

Total sugars (**Table 5**) were higher than reducing sugars (**Table 2**), which is explained by the presence in the samples of non-reducing sugars such as sucrose (O-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside) and raffinose (O-α-D-galactopyranosyl-(1→6),O-α-D-glucopyranosyl-(1→2)β-D-fructofuranoside).

3.3. Fatty acids profiles

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of the studied species are shown in **Table 6**. In ground ivy, the major fatty acid found was oleic acid (C18:1; approximately 35%), followed by α-linolenic acid (C18:3), palmitic acid (C16:0) and linoleic acid (C18:2). Oleic acid is a non-essential monounsaturated fatty acid included in the omega-9 family and it was reported that lead to a reduction of the risk of coronary artery disease in subjects with permanently activated endothelium by improving vascular inflammatory response postprandially (Pacheco et al., 2008).

For oregano the most abundant fatty acid was α-linolenic acid (approximately 62%), followed by linoleic acid, oleic acid and palmitic acid. α-Linolenic acid is an essential fatty acid and it is precursor of the omega-3 fatty acids series in humans, related to a decrease in total amount of fat in blood (cholesterol), and a reducing of the risk of cardiovascular diseases (Connor, 2000). Nevertheless, it should be pointed out that this pathway has low conversion percentages of dietary α-linolenic acid to eicosapentaenoic acid (EPA) and especially to docosahexaenoic acid (DHA). Linoleic acid is also an essential fatty acid and originates the omega-6 fatty acids series. Omega-3 and -6 fatty acids are biosynthetic precursors of eicosanoids involved in several metabolic functions (Zubay, 2006).

In mastic thyme α-linolenic acid (approximately 46%) also predominates and was followed by linoleic, palmitic and oleic acids. In this sample tricosanoic acid (C23:0) was found in non-negligible percentage (~ 9%) (**Figure 2**).

Besides the five main fatty acids already described, seventeen more were identified and quantified. PUFA were the main group of fatty acids in all the samples (**Table 5**). UFA predominate over SFA and ranged from 71% to 91%. *Trans* isomers of unsaturated fatty acids were not detected in the studied spices.

A deficient intake of essential fatty acids can be responsible for many problems, such as dermatitis, imunosupression and cardiac dysfunctions (Burtis, Ashwood & Tietz, 1996) and therefore the studied spices could be used as a dietary source of these compounds. Particularly, α-linolenic and linoleic acids are precursors of omega-3 and omega-6 fatty acids often related to an increase in HDL cholesterol and decrease in LDL cholesterol, triacylglycerol, lipid oxidation, and LDL susceptibility to oxidation (Connor, 2000). The sugars identified in the samples, namely glucose and fructose, occupy key roles in energy metabolism and supply carbon skeletons for the synthesis of other compounds. The method optimized for the analysis of free sugars proved to be sensitive, reproducible and accurate, being all the compounds separated in a short period of 15 min.

The chemical composition and nutritional value determined for these species is in agreement with empirical uses and procedures of traditional cuisine of Trás-os-Montes. Our results confirm the nutritional value of *Glechoma hederacea* although the lowest energy contribution; the fatty acid profile of the studied species could be related to eventual effectiveness in reducing cholesterol levels and avoiding cardiovascular diseases, which is according to the general opinion about the benefits of using these species in local diets; the above mentioned properties and the highest sugar contents are enough reasons for cooking with *Thymus mastichina* instead of salt to prevent hypertension. As far as we know, nothing has been reported on macronutrients composition of these three Lamiaceae: ground ivy, oregano and mastic thyme.

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Table 1. Edible uses in Trás-os-Montes (Bragança, Vinhais, Miranda do Douro) of the studied Lamiaceae.

Species	English name	Local name	Edible uses
Glechoma hederacea	Ground ivy	Malvela, malbela	Condiment/spices flavouring and seasoning traditional dishes. Soups and stews. Restorative bouillon
Origanum vulgare	Oregano	Oregão, mangerico do monte	Condiment/spices flavouring and seasoning traditional dishes and sausages. Summer salads. To preserve olives
Thymus mastichina	Mastic thyme	Bela-luz, sal-puro, salpurro, tomilho-branco	Condiment/spices flavouring and seasoning traditional dishes and salads. To preserve olives. Used instead of salt.

Table 2. Macronutrients composition (g/100 g) and energetic value (Kcal/100 g) of the three Lamiaceae in a fresh weight basis (mean ± SD; n=3). In each row, different letters mean significant differences (p<0.05).

	Glechoma hederacea	*Origanum vulgare*	*Thymus mastichina*
Moisture	73.01 ± 8.05 a	51.82 ± 5.11 c	54.67 ± 7.03 b
Ash	3.47 ± 0.01 a	2.87 ± 0.07 b	2.67 ± 0.08 c
Fat	1.18 ± 0.23 c	2.81 ± 0.33 b	3.80 ± 0.10 a
Proteins	1.34 ± 0.00 b	2.28 ± 0.03 a	2.22 ± 0.05 a
Carbohydrates	21.00 ± 0.17 c	40.22 ± 0.28 a	36.64 ± 0.08 b
Reducing sugars	0.16 ± 0.01 c	0.68 ± 0.17 b	1.20 ± 0.03 a
Energy	99.96 ± 0.80 c	195.31 ± 0.96 a	189.65 ± 0.44 b

Table 3. Analytical characteristics of the sugars analysis method.

	R_t (retention time)		Correlation coefficient (r^2)	Linearity range (mg/ml)	Limit	
	min	CV, %(n=10)			LOD (mg/ml)	LOQ (mg/ml)
Fructose	5.97	0.27	0.9999	0.2 – 24	0.05	0.18
Glucose	6.36	0.26	0.9999	0.3 – 24	0.08	0.25
Sucrose	7.41	0.33	0.9999	0.2 – 24	0.06	0.21
Melezitose (IS)	9.79	0.41	-	-	-	-
Raffinose	10.75	0.36	0.9991	0.3 – 24	0.09	0.30

Table 4. Method validation parameters obtained using *Origanum vulgare*.

	Precision CV, % (n=6)	Repeatability CV, % (n=6)	Accuracy (Recovery, %)
Fructose	1.72	3.74	99
Glucose	3.39	1.59	94
Sucrose	2.79	0.82	100
Raffinose	2.90	1.68	96

Table 5. Sugars composition (g/100 g of fresh weight) of the three Lamiaceae (mean ± SD; n=3). In each row, different letters mean significant differences (p<0.05).

	Glechoma hederacea	*Origanum vulgare*	*Thymus mastichina*
Fructose	0.15 ± 0.01 c	0.19 ± 0.01 b	0.45 ± 0.01 a
Glucose	0.08 ± 0.02 c	0.58 ± 0.01 b	0.97 ± 0.11 a
Sucrose	0.40 ± 0.06 a	0.30 ± 0.00 b	0.02 ± 0.00 c
Raffinose	0.42 ± 0.03 a	0.05 ± 0.00 b	*nd*
Total sugars	1.04 ± 0.07 b	1.12 ± 0.02 b	1.44 ± 0.11 a

nd- not detected.

Table 6. Fatty acids composition of the three Lamiaceae. The results are expressed as mean ± SD (n=3). In each column different letters mean significant differences ($p<0.05$).

	Glechoma hederacea	*Origanum vulgare*	*Thymus mastichina*
C6:0	0.11 ± 0.00	0.02 ± 0.00	0.05 ± 0.00
C8:0	0.24 ± 0.04	nd	0.21 ± 0.00
C10:0	0.43 ± 0.02	nd	nd
C12:0	0.52 ± 0.01	0.02 ± 0.00	0.12 ± 0.01
C14:0	1.17 ± 0.01	0.09 ± 0.01	1.32 ± 0.02
C14:1	0.36 ± 0.04	0.03 ± 0.00	0.16 ± 0.00
C15:0	0.11 ± 0.01	0.03 ± 0.00	0.09 ± 0.00
C16:0	12.23 ± 0.23	4.95 ± 0.10	10.22 ± 0.20
C16:1	0.33 ± 0.01	0.08 ± 0.00	0.38 ± 0.08
C17:0	0.67 ± 0.04	0.09 ± 0.01	0.38 ± 0.09
C18:0	4.53 ± 0.41	1.98 ± 0.00	2.35 ± 0.22
C18:1n9c	35.12 ± 0.27	5.08 ± 0.01	9.82 ± 0.18
C18:2n6c	8.15 ± 0.08	23.22 ± 0.14	11.83 ± 0.06
C18:3n3	27.87 ± 0.20	62.34 ± 0.04	45.65 ± 0.55
C20:0	2.23 ± 0.14	0.34 ± 0.02	1.77 ± 0.18
C20:1c	0.35 ± 0.02	0.08 ± 0.01	0.34 ± 0.07
C20:2c	0.43 ± 0.00	0.04 ± 0.00	1.16 ± 0.08
C20:3n3+C21:0	0.45 ± 0.04	0.14 ± 0.01	0.15 ± 0.02
C22:0	1.84 ± 0.06	0.37 ± 0.03	0.87 ± 0.00
C22:2c	0.14 ± 0.03	0.06 ± 0.00	1.20 ± 0.15
C23:0	1.66 ± 0.09	0.59 ± 0.00	9.33 ± 0.34
C24:0	1.04 ± 0.03	0.46 ± 0.02	2.63 ± 0.05
Total SFA	26.79 ±0.53 b	8.92 ± 0.15 c	29.32 ± 0.08 a
Total MUFA	36.16 ± 0.23 a	5.27 ± 0.01 c	10.69 ± 0.32 b
Total PUFA	37.05 ± 0.29 c	85.80 ± 0.17 a	59.99 ± 0.40 b

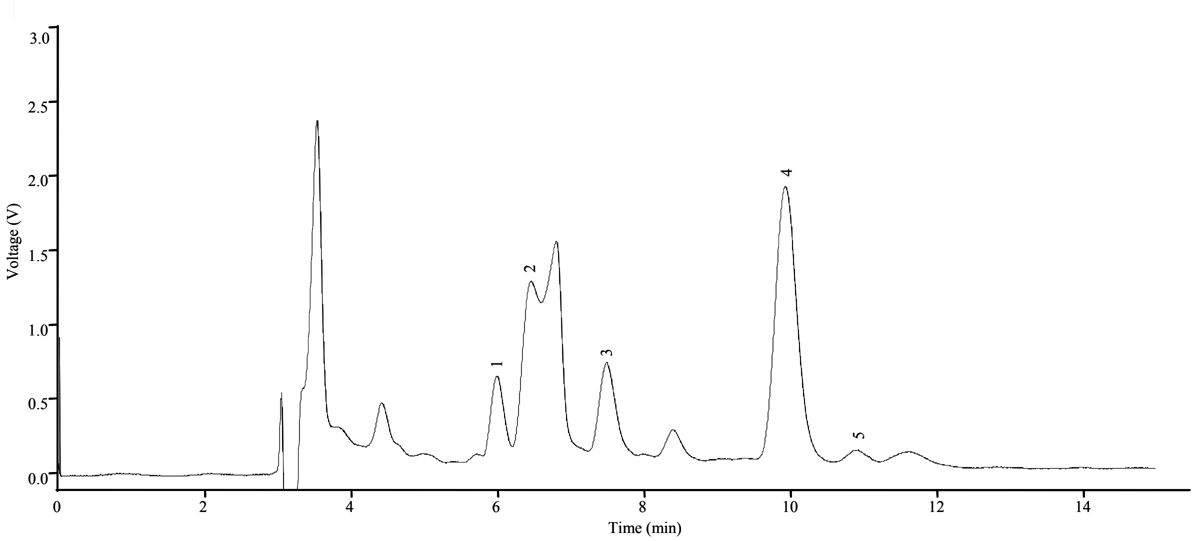

Figure 1. Individual sugar chromatogram of *Origanum vulgare* (oregano). 1- fructose; 2- glucose; 3- sucrose; 4-IS (melezitose); 5- raffinose.

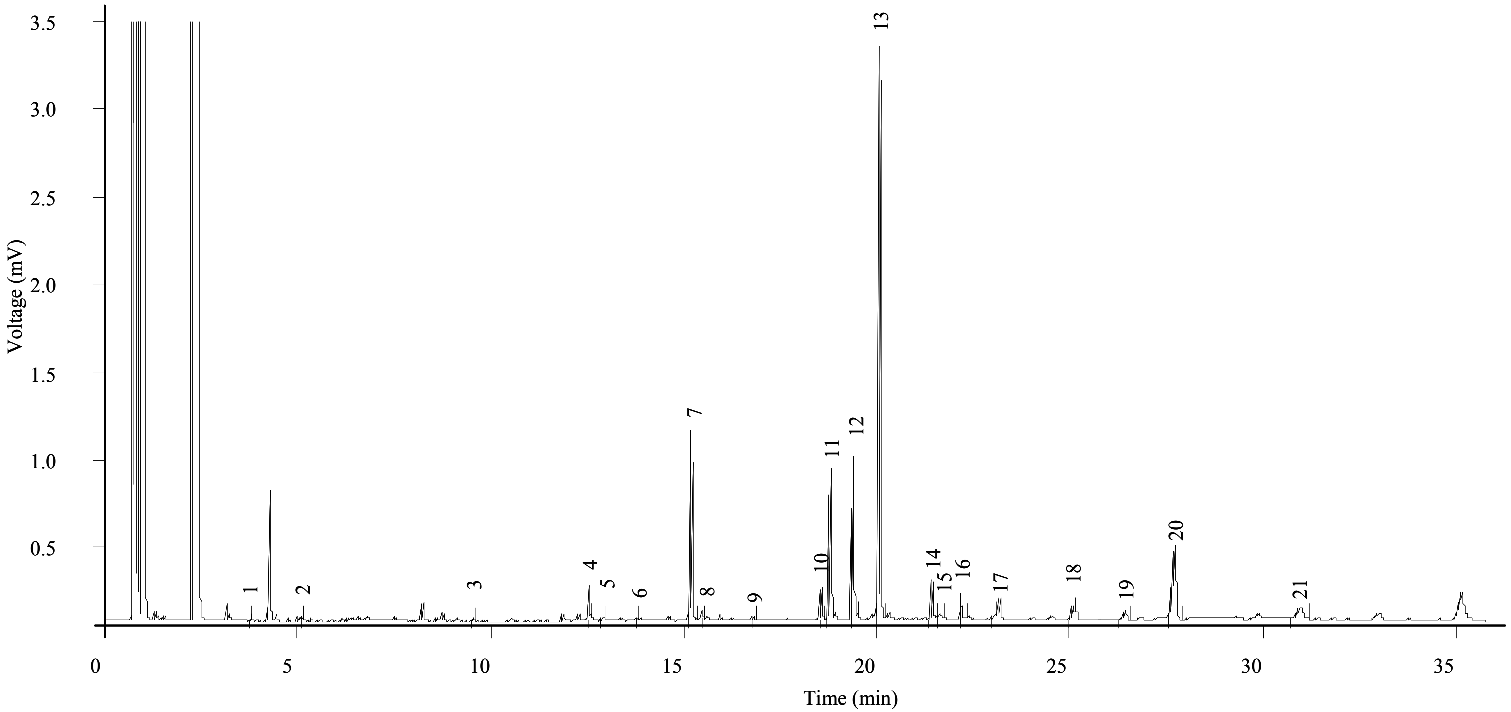

Figure 2. Individual fatty acids chromatogram of *Thymus mastichina*. 1- caproic acid (C6:0); 2- caprylic acid (C8:0); 3- lauric acid (C12:0); 4- myristic acid (C14:0); 5- myristoleic acid (C14:1); 6- pentadecanoic acid (C15:0); 7- palmitic acid (C16:0); 8- palmitoleic acid (C16:1); 9- heptadecanoic acid (C17:0); 10- stearic acid (C18:0); 11- oleic acid (C18:1n9c); 12- linoleic acid (C18:2n6c); 13- α-linolenic acid (C18:3n3); 14- arachidic acid (C20:0); 15- Heneicosanoic acid (C20:1c); 16- *cis*-11,14-eicosadienoic acid (C20:2c); 17- *cis*-11,14,17-eicosatrienoic acid + heneicosanoic acid (C20:3n3+C21:0); 18- behenic acid (C22:0); 19- *cis*-13,16-docosadienoic acid (C22:2c); 20- tricosanoic acid (C23:0); 21- lignoceric acid (C24:0).