Topical anti-inflammatory plant species: Bioactivity of *Bryonia dioica*, *Tamus communis* and *Lonicera peryclimenum* fruits

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ABSTRACT

The practice of rubbing different plant material juices or extracts into the skin to relieve pain and rheumatic symptoms is deeply rooted in folk medicine and has been used for a long time. Several common species, usually available in agroecosystems of the Iberian Peninsula, were/are used for topical medicinal preparations as reported in recent ethnobotanical surveys. Based on these studies, the fruits of three relevant species (*Bryonia dioica* or white-bryony, *Lonicera periclymenum* or common honeysuckle and *Tamus communis* or black-bryony) were gathered and different analyses and assays were performed in order to characterize their phytochemical composition and to find biologically active compounds for pharmaceutical application. Black-bryony ripened fruits revealed the highest antioxidant properties which are in agreement to its highest concentration in phenolics, flavonoids, ascorbic acid, tocopherols and lycopene. The studied fruits revealed interesting antioxidant properties and bioactive phytochemicals that could provide scientific evidence for their folk uses as anti-inflammatory species.

Keywords: Medicinal fruits; Topical anti-inflammatory; Antioxidant properties; Bioactive compounds; Portuguese ethnobotany.

1. Introduction

Inflammatory diseases are accompanied by the chronic release of cytokines and reactive oxygen (ROS) and nitrogen (RNS) species, which may be involved in increased tissue injury. Much evidence has shown that the production of reactive species such as superoxide anion radical, hydrogen peroxide, hydroxyl radical and peroxynitrite occurs at the site of inflammation and contributes to tissue damage (Conner and Grisham, 1996; Nardi *et al.*, 2007).

Under normal circumstances, ROS and RNS are detoxified by an efficient antioxidant system that includes enzymes such as superoxide dismutase, catalase and glutathione peroxidises (Halliwell, 1994; Roome *et al.*, 2008). In acute and chronic inflammation, high concentrations of those species are produced, which generate an oxidative imbalance and decrease the capacity of the endogenous antioxidant enzymes to remove them, contributing to tissue damage (Nardi *et al.*, 2007) and a variety of chronic inflammatory diseases such as arthritis and atherosclerosis as well as other ailments *viz.* cancer, diabetes, hepatitis, neurodegeneration and early aging (Halliwell, 1994; Roome *et al.*, 2008). Therefore, considering the involvement of oxidative stress in inflammation, topical antioxidants might bring health benefits (Casagrande *et al.*, 2006). Particularly, antioxidants from natural products present novel possibilities for the treatment and prevention of oxidative stress-mediated inflammatory diseases.

Plants contain many constituents with local physical impact on body tissues and the topical use of herbal remedies is among the most noticeable in the simplest traditions of healthcare (Marc *et al.*, 2008). Recent ethnobotanical surveys conducted in the Iberian Peninsula (Neves, 2009; Benitez *et al.*, 2010; Carvalho, 2010; Gonzalez *et al.*, 2010)

have recorded interesting uses of very common plant materials applied as topical homemade remedies.

Three of the most cited and topically used wild species (Carvalho, 2010) are *Bryonia dioica* Jacq. (white-bryony; *port.* norça), *Lonicera periclymenum* L. (common honeysuckle; *port.* madressilva) and *Tamus communis* L. (black-bryony; *port.* budanha), perennial, deciduous climbers often found in woodland, in hedgerows or scrubland and, sometimes, occurring also in orchards and homegardens. These three species are generally and popularly considered toxic to humans (Carvalho, 2010). Besides the medicinal use and the very specific edible use of bryonies sprouts as greens in the Iberian Peninsula (Martins *et al.*, 2011), every people interviewed were conscious of their poisonous effects, in particularly of the fruits and of the underground organs (tuberous rootstalks) (Carvalho, 2010). It seems that the noxious effects are mainly due to triterpene glucosides and calcium oxalate crystals that are found mainly in the fruit (Castroviejo *et al.*, 2005)

Traditional practices include mainly different liquid dosage forms such as plant juices, tinctures and related products (alcoholic or hydroalcoholic solutions prepared from botanicals). Freshly harvest plant parts (leaves, flowers and mainly fruits) are frequently applied directly or macerated in water, alcohol or brandy. Their use is claimed to have local anti-inflammatory effects on minor wounds and lesions and on the management and relief of inflammations affecting joins, muscles and other subcutaneous tissues.

Although these topical remedies have been used for hundreds of years and liquid herbal preparations do appear to be quite efficient for the extraction of a wide variety of compounds found in medicinal plants, a number of studies have highlighted the importance of the ratio (the correct choice of the alcohol percentage to maximize the

quality of the preparation) and little is known about the chemical composition of plant juices or their possible reactions in aqueous and in alcoholic media.

In the present work, the bioactive phytochemicals of fruits of the three previously mentioned species were characterized. Furthermore, the evaluation of their antioxidant properties could support folk uses as topical anti-inflammatory species.

2. Materials and methods

2.1. Samples

Both immature fruits (hard green fruits in early summer) and ripened fruits (fleshy and soft red fruits in late autumn) of the three selected species were gathered in Bragança, Trás-os-Montes, North-eastern Portugal. The samples for analysis were collected and prepared according to the main medicinal topical applications as described by informants from this Portuguese region (Carvalho, 2010) (**Table 1**). All the vegetal material was gathered at random from several plants inside a selected area which has been visited twice considering the two different stages of fruit maturity. Fruits of *Bryonia dioica* and *Tamus communis* were collected in July (immature fruits) and September (ripened fruits); the fruits of *Lonicera periclymenum* were collected in July and September of 2010.

Local traditional practitioners usually rubbed the juice of fresh immature fruits on body surfaces to relieve pains and minor wounds; the ripened fruits were used in the form of alcoholic extracts for arthritis and rheumatic complains; vapors (steam) from the hot decoction of common honeysuckle' fruits were recommended for asthma crisis (Neves, 2009; Carvalho, 2010; Gonzalez *et al.*, 2010).

Morphological key characters from the Flora Iberica were used for plant identification. Voucher specimens (ETBO33, ETBO36, ETBO53) are deposited in the herbarium of the Escola Superior Agrária de Bragança (BRESA). Each sample was lyophilized (Ly-8-FM-ULE, Snijders, Holland) and stored in the deep-freezer at -20ºC for subsequent analysis.

2.2. Standards and Reagents

Acetonitrile 99.9%, *n*-hexane 95%, and ethyl acetate 99.8% were of HPLC grade (Lab-Scan, Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO), as also other individual fatty acid isomers, ascorbic acid, tocopherols (α, β, δ, and γ-tocopherols), and sugars (D(-)-fructose, D(+)-glucose anhydrous, D(+)-melezitose hydrate, D(+)-raffinose pentahydrate, D(+)-sucrose, and D(+)-trehalose) 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 (Chalfont, PA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (Pure Water Systems, Brea, CA).

2.3. In vitro evaluation of antioxidant properties

2.3.1. Preparation of the methanolic extracts

A fine dried powder (20 mesh; ~1 g) 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 extracted with one additional 30 mL portion of methanol (this procedure was followed according to the extraction conditions optimized by us in a previous report (Barros *et al.*, 2010a)). The combined methanolic extracts were evaporated at 40ºC under reduced pressure, re-dissolved in methanol at a concentration of 20 mg/mL, and stored at 4 ºC for further use. *In vitro* assays which have already been described by the authors (Barros *et al.*, 2010b) were applied to evaluate the antioxidant activity of all the samples. Different concentrations of the extracts were used to find EC_{50} values.

2.3.2. DPPH radical-scavenging activity

This methodology was performed using an ELX800 Microplate Reader (BioTek Instruments, Inc., Winooski, VT). The reaction mixture in each of the 96-wells consisted of one of the different concentrations of the extracts (30 μL) and aqueous methanolic solution (80:20, v/v, 270 μL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: % RSA = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration. Trolox was used as standard.

2.3.3. Reducing power

This methodology was performed using the Microplate Reader described above. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1%, w/v, 0.5 mL). The mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10%, w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionised water (0.8 mL) and ferric chloride (0.1%, w/v, 0.16 mL), and the absorbance was measured at 690 nm. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 690 nm against extract concentration. Trolox was used as standard.

2.3.4. Inhibition of β-carotene bleaching

A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two millilitres of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40ºC under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a 200-2004 spectrophotometer (Analytikjena, Jena, Germany). β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2h of assay/initial β-carotene content) × 100. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration. Trolox was used as standard.

2.3.5. Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

Brains were obtained from pig (*Sus scrofa*) of body weight ~150 Kg, dissected and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000g for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the different concentrations of the extracts (0.2 mL) in the presence of $FeSO_4$ (10 μM; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at 37ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28%, w/v, 0.5 mL), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 mL), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000*g* (Centorion K24OR- 2003 refrigerated centrifuge) for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A – B)/A] x 100%, where A and B were the absorbance of the control and the compound solution, respectively. The extract concentration providing 50% lipid peroxidation inhibition (EC_{50}) was calculated from the graph of TBARS inhibition percentage against extract concentration. Trolox was used as standard.

2.4. Bioactive compounds

2.4.1. Phenolics

For total phenolics estimation an aliquot of the methanolic extract solution (1 mL) was mixed with *Folin-Ciocalteu* reagent (5 mL, previously diluted with water 1:10, v/v) and

sodium carbonate (75 g/L, 4 mL). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm (Wolfe *et al.*, 2003). Gallic acid was used to calculate the standard curve (9.4×10^{-3}-0.15 mg/mL), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

2.4.2. Flavonoids

For total flavonoids content determination, an aliquot (0.5 mL) of the methanolic extract solution was mixed with distilled water (2 mL) and subsequently with $NaNO_2$ solution (5%, 0.15 mL). After 6 min, $AlCl_3$ solution (10%, 0.15 mL) was added and allowed to stand further 6 min, thereafter, NaOH solution (4%, 2 mL) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 mL. Then the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink colour was measured at 510 nm (Jia *et al.*, 1999). (+)-Catechin was used to calculate the standard curve (4.5×10^{-3}-0.29 mg/mL) and the results were expressed as mg of (+)-catechin equivalents (CE) per g of extract.

2.4.3. Ascorbic acid

A fine dried powder (20 mesh; 150 mg) was extracted with metaphosphoric acid (1%, 10 mL) for 45 min at room temperature and filtered through Whatman Nº 4 filter paper. The filtrate (1 mL) was mixed with 2,6-dichloroindophenol (9 mL) and the absorbance was measured within 30 min at 515 nm (Klein and Perry, 1982). Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (6.0×10^{-3}-0.10 mg/mL), and the results were expressed as mg of ascorbic acid per 100 g

of dry weight.

2.4.4. Tocopherols

Tocopherols content was determined following a procedure previously described by the authors (Martins *et al.*, 2011). BHT solution in *n*-hexane (10 mg/mL; 100 μL) and IS solution in *n*-hexane (tocol; 50 μg/mL; 400 μL) were added to the sample prior to the extraction procedure. The samples (~500 mg) were homogenized with methanol (4 mL) by vortex mixing (1 min). Subsequently, *n*-hexane (4 mL) was added and again vortex mixed for 1 min. After that, saturated NaCl aqueous solution (2 mL) was added, the mixture was homogenized (1 min), centrifuged (5 min, 4,000*g*) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with *n*-hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 2 mL of *n*-hexane, dehydrated with anhydrous sodium sulphate, filtered through 0.2 μm nylon filters from Whatman and transferred into a dark injection vial. The equipment consisted of an integrated system with a Smartline 1000 pump (Knauer, Berlin, Germany), a Smartline manager 5000 degasser, an AS-2057 auto-sampler (Jasco, Easton, MD) and an FP-2020 fluorescence detector (Jasco, Easton, MD) programmed for excitation at 290 nm and emission at 330 nm. The column used was a normal-phase 250 mm × 4.6 mm i.d., 5 μm, Polyamide II, with a 10 mm × 4 mm i.d. guard column of the same material (YMC Waters, Dinslaken, Germany), operating at 30 ºC. The mobile phase used was a mixture of *n*-hexane and ethyl acetate (70:30, 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.

2.4.5. Pigments

A fine dried powder (150 mg) was vigorously shaken with 10 mL of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm (Nagata and Yamashita, 1992). Content of β-carotene was calculated according to the following equation: β-carotene (mg/100 mL) = $0.216 \times A_{663} – 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$; Lycopene (mg/100 mL) = $– 0.0458 \times A_{663} + 0.204 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$; Chlorophyll a (mg/100 mL) = $0.999 \times A_{663} - 0.0989 \times A_{645}$; Chlorophyll b (mg/100 mL) = $- 0.328 \times A_{663} + 1.77 \times A_{645}$, and further expressed in mg per 100 g of dry weight.

2.4.6. Sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) as previously described by the authors (Martins *et al.*, 2011). Dried sample powder (1.0 g) was spiked with the melezitose as internal standard (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 (rotary evaporator Büchi R-210) 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 and filtered through 0.2 µm nylon filters from Whatman. The equipment described

above was connected to a Smartline 2300 RI detector. Data were analysed using Clarity DataApex 2.4 Software. The column used was a 250 mm × 4.6 mm i.d., 5 µm, Eurospher 100-5 NH_2 with a 5 mm × 4mm i.d. guard column of the same material (Knauer, Berlin, Germany), operating at 30 ºC in a 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.

2.4.7. Fatty Acids

Fat was extracted with petroleum ether in a Soxhlet apparatus. Fatty acids were determined by gas chromatography with flame ionization detection (GC-FID) as described previously by the authors (Martins *et al.*, 2011), and after the following transesterification procedure: fatty acids (obtained after Soxhlet extraction) were methylated with 5 mL of methanol:sulphuric acid:toluene 2:1:1 (v/v/v), during at least 12 h in a water bath at 50 ºC and 160 rpm; then 3 mL of deionised 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 dehydrated with sodium sulphate anhydrous; finally the sample was filtered with 0.2 µm nylon filter from Whatman. The equipment was a DANI model GC 1000 with a split/splitless injector, and a FID. The column used was a 30 m × 0.32 mm i.d., 0.25 µm, 50% cyanopropyl-methyl-50% phenylmethylpolysiloxane (Macherey-Nagel, Düren, Germany). The FID temperature was 260 ºC. The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30ºC/min ramp to 125 ºC, 5ºC/min ramp to

160 ºC, 20ºC/min ramp to 180 ºC, 3ºC/min ramp to 200 ºC, 20ºC/min ramp to 220 ºC and held for 15 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 DataApex 1.7 software and expressed in relative percentage of each fatty acid.

2.5. Statistical analysis

For each species, three samples were analysed and the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD), and. were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$ (different letters mean significant differences; the letter a is attributed to the highest value). This treatment was carried out using SPSS v. 16.0 program.

3. Results and discussion

3.1. In vitro evaluation of antioxidant properties

Four different assays were used for the *in vitro* evaluation of the antioxidant properties of immature and ripened fruits used in Portuguese and Spanish folk medicine as described in **Table 1**. The extracts were prepared using methanol to mimetize the ethnopharmacological use of the fruits: maceration in alcohol or brandy.

The results of scavenging activity on DPPH radicals, reducing power, inhibition of β-carotene bleaching, and inhibition of lipid peroxidation in brain tissue homogenates are shown in **Figure 1**, and antioxidant activity EC_{50} values as also phenolic and flavonoids contents are given in **Table 2.** Black-bryony ripened fruits proved to have the most

promissory antioxidant activity (the lowest EC_{50} values, ranging from 0.29 to 1.26 mg/mL), with the highest phenolic (119.78 mg GAE/g extract) and flavonoids (52.69 mg CE/g extract; **Table 2**) contents. Otherwise, white-bryony immature fruits revealed the lowest antioxidant properties (the highest EC_{50} values, ranging from 1.07 to 18.01 mg/mL) which are compatible to its lower phenolics (33.37 mg GAE/g extract) and flavonoids (10.32 mg CE/g extract; **Table 2**) content. Both species and maturity stage of fruits seemed to have influence in the antioxidant properties. Nevertheless, all the samples revealed antioxidant activity, which might be related to its traditional use as topical anti-inflammatory, as previously explained (Nardi *et al.*, 2007; Roome *et al.*, 2008).

As far as we know this is the first study dealing with fruits of white (*Bryonia dioica*) and black (*Tamus communis*) bryonies, and common honeysuckle (*Lonicera periclymenum*). The antioxidant properties of bryonies edible parts (shoots) were already reported by our research group (Martins *et al.*, 2001). Other authors reported antioxidant activity of *Lonicera japonica* under cultivation (the pharmaceutical name in Chinese Pharmacopoeia is *Flos lonicerae* and is used for acute infections of respiratory tract, the mammary glands and appendicitis) (Liu *et al.*, 2008; Tsai *et al.*, 2008). The latter authors reported 45.2 ± 1.65% of DPPH radical scavenging activity at 1 mg of methanolic extract/ml, a higher activity than the observed in *Lonicera periclymenum* fruits herein studied (**Figure 1**). Moreover, the samples obtained in Taiwan (Tsai *et al.*, 2008) and in China (Liu *et al.*, 2008) revealed lower phenolics content, but higher flavonoids content than fruits of *Lonicera periclymenum* herein studied.

3.2. Bioactive compounds

Vitamins (tocopherols and ascorbic acid) and pigments (carotenoids and chlorophylls) contents of the immature and ripened fruits are given in **Table 3**.

Ascorbic acid was the most abundant vitamin in all the studied samples; Black-bryony ripened fruits gave the highest levels (292.62 mg/100 g dry weight), and revealed higher contents than the corresponding immature sample.

Among tocopherols, all the isoforms were found being α-tocopherol the major compound in all the samples. Once more, black-bryony ripened fruits presented the highest content of tocopherols (176.61 mg/ 100 g of dry weight), with the highest levels of α, β, and δ-tocopherols. **Figure 2** presents the profile obtained in that sample. The lowest levels of ascorbic acid and tocopherols were found in white-bryony and common honeysuckle immature fruits, respectively.

Oxygen-derived species such as superoxide radical, hydrogen peroxide, hypochlorous acid and possibly hydroxyl radical are generated at sites of inflammation and tissue injury and may aggravate the damage taking place. There is considerable current interest in the possibility that therapeutic agents are metabolized to toxic free radicals. Therefore, it is important to consider free radicals as potential mediators of the side-effects of a wide range of drugs and food additives. Ascorbic acid decreased the pro-oxidant effects of an anti-inflammatory drug, phenylbutazone (Evans *et al.*, 1992). Furthermore, anti-inflammatory properties of the two major forms of vitamin E, α-tocopherol and γ-tocopherol had been reported (Reiter *et al.*, 2007). The studied samples proved to be important sources of either ascorbic acid or tocopherols that as antioxidants could bring benefits in inflammatory processes.

Carotenoids and chlorophylls were also found in all the fruits, but carotenoids predominated in ripened over immature fruits, while the opposite occurred with

chlorophylls content. Common honeysuckle ripened fruits revealed the highest concentration of β-carotene (195.68 mg/100 g dry weight). Lycopene and chlorophylls were found in very low amounts, giving black-bryony ripened fruits the highest concentration of lycopene (1.85 mg/100 g), and white-bryony immature fruits the highest content in chlorophylls a (2.43 mg/100 g) and b (1.14 mg/100 g).

In relation to sugar composition (**Table 4**) common honeysuckle ripened fruits gave the highest total sugars content (28.15 g/100 g dry weight), with the highest levels of fructose (10.19 g/100 g) and glucose (11.91 g/100 g). Immature fruits of the same sample showed the highest levels of trehalose (2.21 g/100 g) and raffinose (1.08 g/100 g). Ripened fruits gave higher total sugars, fructose and glucose content than immature fruits, as it can be observed in the example of common honeysuckle (**Figure 3**). Nevertheless, the amounts of trehalose (disaccharide) and raffinose (trisaccharide) decreased in the ripened fruits. It should be highlighted that the anti-inflammatory actions of some sugars such as 2-deoxyglucose, was already reported (Goth, 1968).

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) are shown in **Table 5**. Twenty three fatty acids were identified and quantified. The major fatty acid found in white-bryony and common honeysuckle was linoleic acid (C18:2n6), while oleic acid (C18:1n9) predominated in black-bryony, contributing to the prevalence of PUFA in the first two species and MUFA in the last species. Palmitic acid (C16:0) was also a main fatty acid in all the studied fruits. Overall, white-bryony immature fruits revealed the highest PUFA levels, including linoleic acid. Some n-3

fatty acids were also found in the studied samples. It was reported that n-3, or omega-3 PUFA exhibit anti-inflammatory properties in many inflammatory diseases (Gil, 2002). Traditional medicinal uses, whose knowledge and practices have been orally transmitted over the centuries, are important approaches for discovering therapeutic molecules and compounds. This study provides novel information about phytochemical composition of wild fruits traditionally used for medicinal purposes, useful to researchers in phytopharmacology, phytotherapy and phytotoxicology. Black-bryony ripened fruits revealed the highest antioxidant properties which are in agreement to its highest concentration in phenolics, flavonoids, ascorbic acid, tocopherols and lycopene. Otherwise, white-bryony immature fruits gave the lowest antioxidant potential and hydrophilic antioxidants (phenolics, flavonoids, ascorbic acid and sugars) concentration. Overall, the studied fruits revealed interesting antioxidant properties and bioactive phytochemicals such as phenolics, flavonoids, vitamins, carotenoids, sugars, and fatty acids that could provide scientific evidence for some folk uses as anti-inflammatory species.

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Table 1. Medicinal uses of the studied fruits reported in ethnobotanical surveys recently carried out in the Iberian Peninsula.

Scientific name (Botanical family)	English name	Local name	Therapeutic indication	Preparation	References
Bryonia dioica Jacq. (Cucurbitaceae)	White-bryony	Norça, norça-branca, nóscora	Bruises, rheumatic pains, arthritis, minor wounds and lesions	Maceration in alcohol or brandy The juice of crushed fruits for rubbing into the skin	Gallego and Gallego, 2008 Carvalho, 2010 González et al.., 2010
Tamus communis L. (Dioscoreaceae)	Black -bryony	Norça-preta, budanha	Bruises, goat, minor wounds, rheumatic pains	Maceration in alcohol or brandy The juice of crushed fruits Crushed fruits mixed with lard to produce a sort of paste	Gallego and Gallego, 2008 Carvalho, 2010 González et al., 2010
Lonicera periclymenum L. (Caprifoliaceae)	Common honeysuckle	Madressilva	Asthma and respiratory affections Rheumatic pains, arthritis, minor wounds	Inhalants from the hot decoction of the fruits Maceration in alcohol	Neves et al., 2009 Carvalho, 2010

Caution: In spite of their medicinal use, these fruits are considered toxic for humans and domestic animals, therefore their traditional use is only recommended for external applications. Moreover, in cases of broken skin and individuals with certain contact sensitivities their use should be avoid or extracts must be applied at low doses.

Table 2. Extraction yields, antioxidant activity (EC_{50} values, mg/mL) and composition in phenolics and flavonoids (mean ± SD; n=9) of the studied fruits. In each row different letters mean significant differences (p<0.05).

	White-bryony Immature fruits	White-bryony Ripened fruits	Black-bryony Immature fruits	Black-bryony Ripened fruits	Common honeysuckle Immature fruits	Common honeysuckle Ripened fruits
η (%)	25.32 ± 1.23	24.56 ± 0.87	41.82 ± 2.01	26.74 ± 0.55	79.37 ± 3.25	68.15 ± 1.98
DPPH scavenging activity	18.01 ± 0.64 a	1.21 ± 0.02 dc	1.26 ± 0.12 dc	0.91 ± 0.01 d	1.44 ± 0.06 c	2.97 ± 0.13 b
Reducing power	1.07 ± 0.01 a	0.40 ± 0.00 e	0.57 ± 0.01 d	0.21 ± 0.01 f	0.76 ± 0.01 c	1.05 ± 0.01 b
β-carotene bleaching inhibition	1.87 ± 0.25 a	0.58 ± 0.09 d	0.79 ± 0.04 c	0.21 ± 0.01 e	1.48 ± 0.14 b	1.74 ± 0.09 a
TBARS inhibition	5.18 ± 0.46 a	0.22 ± 0.02 cd	0.29 ± 0.01 cbd	0.15 ± 0.03 d	0.43 ± 0.02 cb	0.49 ± 0.03 b
Phenolics (mg GAE/g extract)	33.37 ± 6.50 e	150.12 ± 1.38 b	119.78 ± 15.70 c	445.96 ± 31.41 a	71.47 ± 1.88 d	55.47 ± 0.37 d
Flavonoids (mg CE/g extract)	10.32 ± 0.49 e	15.77 ± 2.34 d	14.54 ± 1.78 d	52.69 ± 3.51 a	28.15 ± 0.83 b	23.33 ± 0.63 c

EC_{50} values for the standard trolox: 43 μg/ml (DPPH scavenging activity); 30 μg/ml (Reducing power); 3 μg/ml (β-carotene bleaching inhibition) and 4 μg/ml (TBARS inhibition).

Table 3. Composition in vitamins and pigments (mg/100 g dry weight) of the studied fruits (mean ± SD; n=9). In each row different letters mean significant differences (p<0.05).

	White-bryony Immature fruits	White-bryony Ripened fruits	Black-bryony Immature fruits	Black-bryony Ripened fruits	Common honeysuckle Immature fruits	Common honeysuckle Ripened fruits
α-tocopherol	30.11 ± 1.53 d	96.14 ± 9.41 b	59.27 ± 6.97 c	160.21 ± 9.83 a	6.35 ± 0.00 e	9.37 ± 0.30 e
β-tocopherol	0.71 ± 0.02 d	3.55 ± 0.16 b	1.09 ± 0.14 c	5.69 ± 0.04 a	0.25 ± 0.00 e	0.25 ± 0.02 e
γ-tocopherol	21.63 ± 1.05 a	10.44 ± 0.69 b	5.05 ± 0.75 d	7.16 ± 0.14 c	1.42 ± 0.02 f	3.35 ± 0.10 e
δ-tocopherol	1.01 ± 0.16 b	0.87 ± 0.01 cb	0.85 ± 0.17 cb	1.55 ± 0.20 a	0.54 ± 0.00 cd	0.25 ± 0.02 d
Total tocopherols	53.46 ± 2.75 c	111.00 ± 10.26 b	66.26 ± 8.01 c	176.61 ± 10.13 a	8.56 ± 0.02 d	13.22 ± 0.24 d
Ascorbic acid	63.61 ± 6.21 f	217.10 ± 7.36 b	96.08 ± 4.57 e	292.62 ± 1.84 a	141.40 ± 0.03 d	150.94 ± 0.10 c
β-carotene	69.82 ± 0.13 d	155.28 ± 6.50 b	0.24 ± 0.00 e	139.92 ± 7.81 c	6.68 ± 0.65 e	195.68 ± 6.30 a
Lycopene	0.01 ± 0.00 e	1.80 ± 0.01 b	0.02 ± 0.00 e	1.85 ± 0.01 a	0.30 ± 0.02 d	0.41 ± 0.03 c
Chlorophyll a	2.43 ± 0.00 a	0.11 ± 0.00 e	0.55 ± 0.00 c	0.14 ± 0.00 e	0.94 ± 0.03 b	0.38 ± 0.05 d
Chlorophyll b	1.14 ± 0.00 a	0.18 ± 0.00 d	0.20 ± 0.00 d	0.23 ± 0.00 d	0.81 ± 0.02 b	0.43 ± 0.08 c

Table 4. Composition in sugars (g/100 g of dry weight) of the studied fruits (mean ± SD; n=9). In each row, different letters mean significant differences (p<0.05).

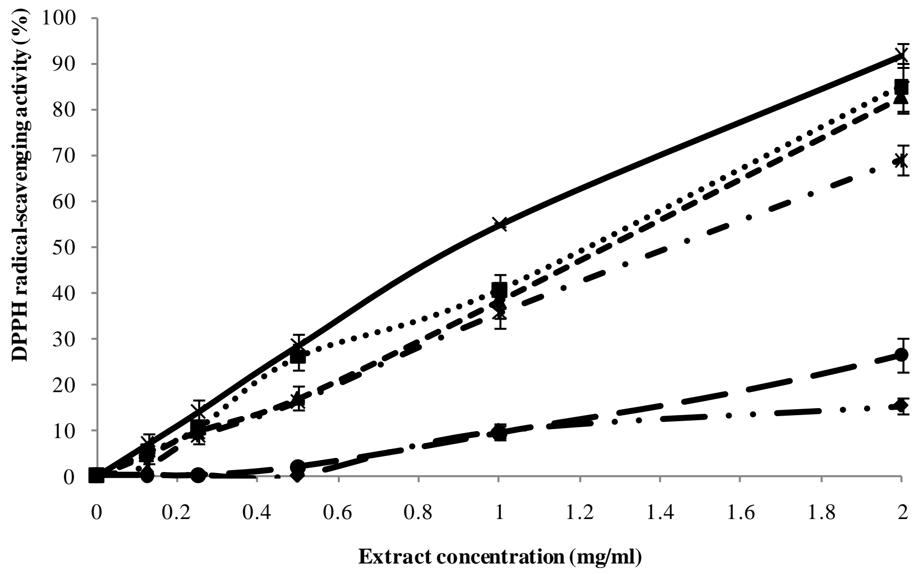
	White-bryony Immature fruits	White-bryony Ripened fruits	Black-bryony Immature fruits	Black-bryony Ripened fruits	Common honeysuckle Immature fruits	Common honeysuckle Ripened fruits
Fructose	2.26 ± 0.09 d	3.24 ± 0.50 c	0.89 ± 0.10 e	6.44 ± 0.01 b	2.22 ± 0.05 d	10.19 ± 0.02 a
Glucose	2.48 ± 0.02 d	2.95 ± 0.34 dc	2.86 ± 0.10 dc	6.26 ± 0.01 b	3.40 ± 0.39 c	11.91 ± 0.12 a
Sucrose	0.46 ± 0.02 d	2.19 ± 0.40 c	4.56 ± 0.20 a	2.58 ± 0.11 c	2.39 ± 0.11 c	3.81 ± 0.02 b
Trehalose	0.59 ± 0.02 c	0.35 ± 0.09 d	0.27 ± 0.02 d	0.30 ± 0.03 d	2.21 ± 0.06 a	1.76 ± 0.02 b
Raffinose	0.12 ± 0.02 c	nd	nd	nd	1.08 ± 0.09 a	0.48 ± 0.02 b
Total sugars	5.92 ± 0.13 e	8.72 ± 1.32 d	8.59 ± 0.41 d	15.57 ± 0.10 b	11.30 ± 0.69 c	28.15 ± 0.13 a

nd- not detected

Table 5. Composition (percentage) in fatty acids of the studied fruits (mean ± SD; n=3). In each column different letters mean significant differences (p<0.05).

	White-bryony Immature fruits	White-bryony Ripened fruits	Black-bryony Immature fruits	Black-bryony Ripened fruits	Common honeysuckle Immature fruits	Common honeysuckle Ripened fruits
C6:0	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.01 ± 0.00	0.05 ± 0.00	0.01 ± 0.00
C8:0	0.01 ± 0.00	0.03 ± 0.00	0.06 ± 0.02	0.02 ± 0.00	0.03 ± 0.01	0.01 ± 0.00
C10:0	0.01 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.00	0.04 ± 0.00	0.01 ± 0.00
C12:0	0.02 ± 0.00	0.10 ± 0.01	0.10 ± 0.02	0.12 ± 0.02	0.28 ± 0.00	0.08 ± 0.00
C14:0	0.06 ± 0.00	0.23 ± 0.00	0.35 ± 0.07	0.29 ± 0.02	0.36 ± 0.01	0.17 ± 0.01
C14:1	nd	nd	0.06 ± 0.01	nd	0.08 ± 0.01	0.02 ± 0.00
C15:0	0.05 ± 0.01	0.09 ± 0.00	0.21 ± 0.03	0.11 ± 0.00	0.08 ± 0.00	0.03 ± 0.00
C16:0	7.80 ± 0.04	11.60 ± 0.31	13.15 ± 1.11	13.54 ± 0.58	8.35 ± 0.00	6.11 ± 0.01
C16:1	0.09 ± 0.01	0.11 ± 0.01	0.34 ± 0.07	0.08 ± 0.01	0.18 ± 0.00	0.17 ± 0.00
C17:0	0.10 ± 0.00	0.16 ± 0.01	0.24 ± 0.08	0.18 ± 0.02	0.20 ± 0.00	0.08 ± 0.01
C18:0	3.27 ± 0.05	2.85 ± 0.09	3.33 ± 0.81	2.30 ± 0.16	2.59 ± 0.11	2.40 ± 0.01
C18:1n9	17.07 ± 0.13	28.47 ± 2.62	56.40 ± 0.58	51.40 ± 2.19	17.40 ± 0.28	31.82 ± 0.09
C18:2n6	66.83 ± 0.35	50.49 ± 3.30	21.01 ± 1.51	24.65 ± 0.58	44.96 ± 0.33	54.64 ± 0.37
C18:3n3	3.04 ± 0.09	2.92 ± 0.06	2.14 ± 0.01	3.96 ± 0.83	9.38 ± 0.05	1.43 ± 0.03
C20:0	0.19 ± 0.03	0.39 ± 0.03	0.57 ± 0.04	0.53 ± 0.04	0.36 ± 0.01	0.15 ± 0.00
C20:1	0.11 ± 0.01	0.30 ± 0.05	0.56 ± 0.04	0.47 ± 0.01	0.07 ± 0.00	0.12 ± 0.01
C20:2	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.07 ± 0.00	0.02 ± 0.00
C20:3n6	0.29 ± 0.03	0.01 ± 0.00	nd	nd	nd	nd
C20:3n3+C21:0	0.25 ± 0.09	0.21 ± 0.01	0.06 ± 0.00	0.07 ± 0.01	0.12 ± 0.01	nd
C20:5n3	0.39 ± 0.07	0.26 ± 0.03	0.04 ± 0.00	0.01 ± 0.00	nd	nd
C22:0	0.13 ± 0.01	0.70 ± 0.05	0.60 ± 0.03	0.87 ± 0.15	1.30 ± 0.08	0.23 ± 0.01
C23:0	0.01 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	8.04 ± 0.29	1.40 ± 0.19
C24:0	0.25 ± 0.01	0.97 ± 0.06	0.65 ± 0.03	1.29 ± 0.11	6.07 ± 0.17	1.10 ± 0.09
Total SFA	11.91 ± 0.11 d	17.21 ± 0.54 c	19.36 ± 2.00 b	19.33 ± 0.72 b	27.74 ± 0.63 a	11.78 ± 0.30 d
Total MUFA	17.26 ± 0.15 e	28.88 ± 2.67 d	57.35 ± 0.55 a	51.96 ± 2.17 b	17.73 ± 0.28 e	32.13 ± 0.19 c
Total PUFA	70.82 ± 0.25 a	53.91 ± 3.21 b	23.28 ± 1.44 d	28.71 ± 1.45 c	54.53 ± 0.35 b	56.10 ± 0.09 b
Total fat (g/100 g dry weight)	1.52 ± 0.04 e	1.90 ± 0.14 e	3.26 ± 0.05 d	11.54 ± 0.49 c	16.49 ± 0.18 b	23.04 ± 0.26 a

nd- not detected.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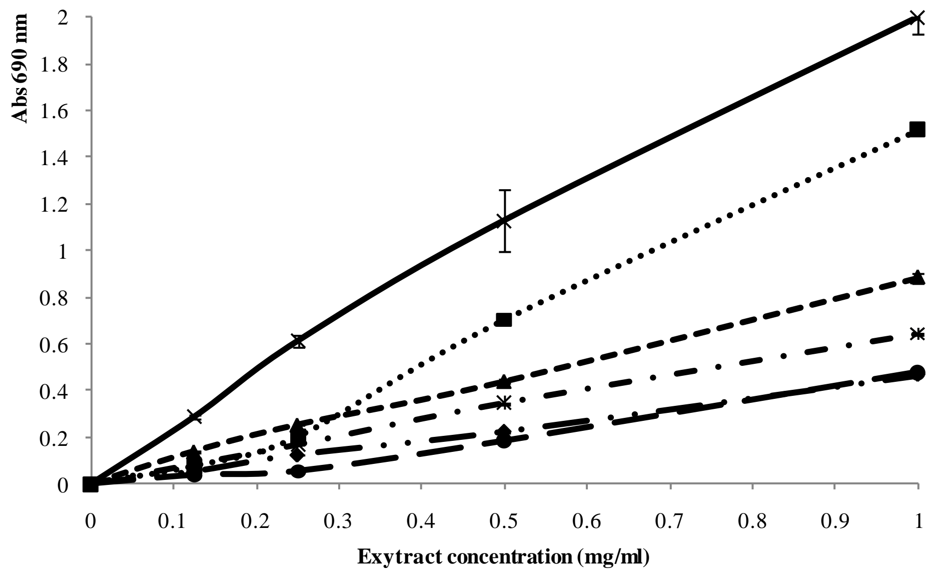
2
Extract concentration (mg/ml)


Abs 690 nm
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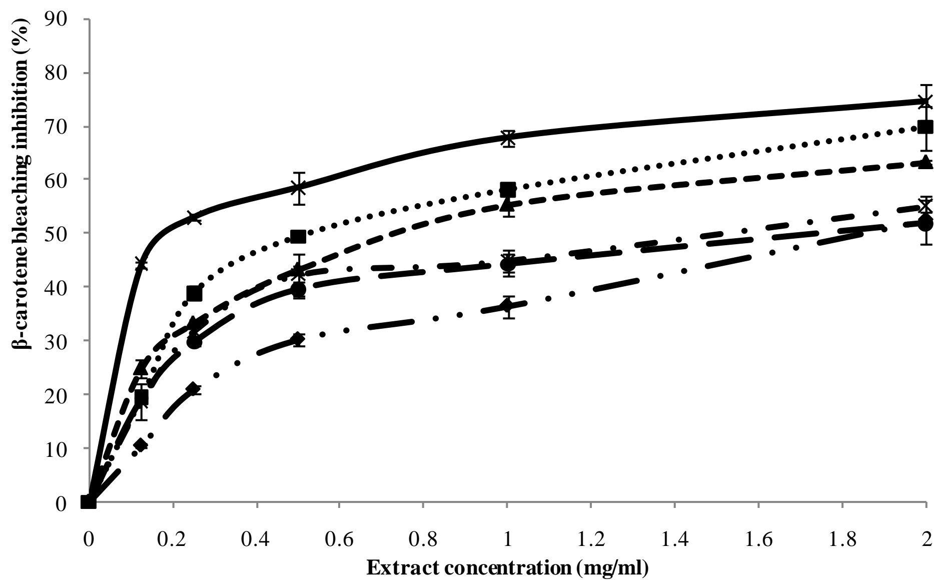

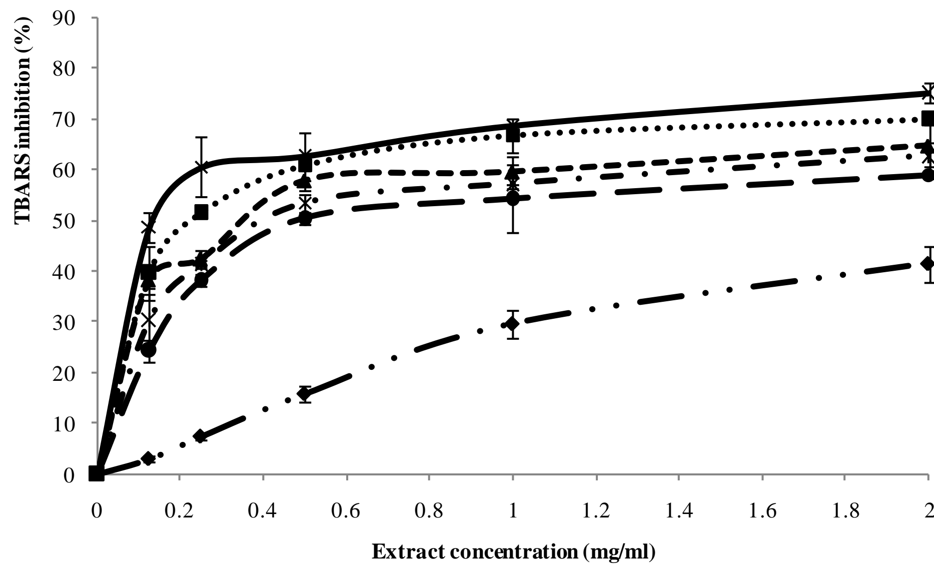

Figure 1. Antioxidant activity of white-bryony immature fruits ( ); white-bryony ripened fruits ( ); black-bryony immature fruits ( ); black-bryony ripened fruits ( ); common honeysuckle immature fruits ( ); common honeysuckle ripened fruits ( ).

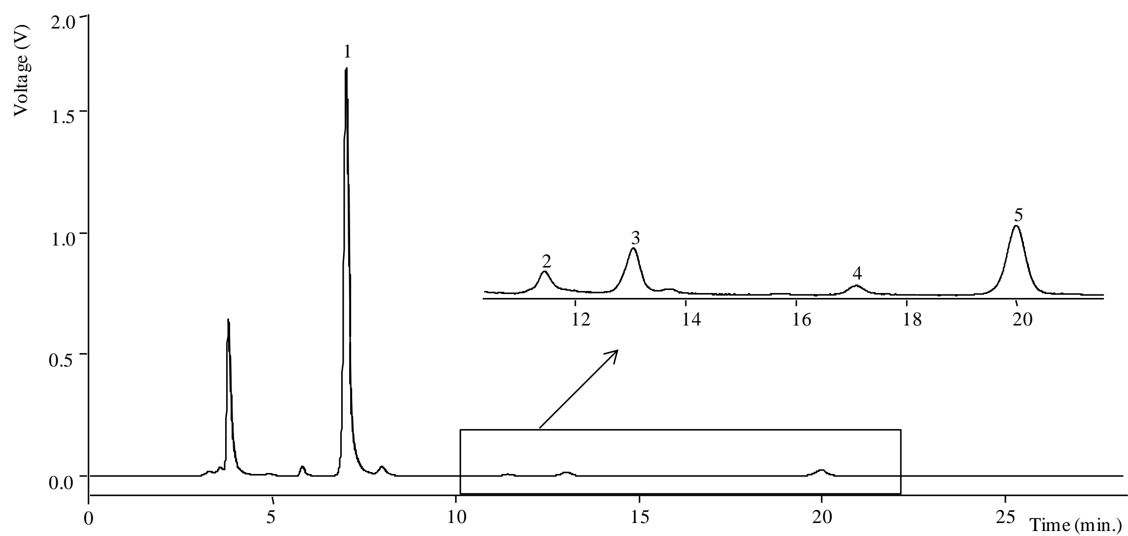

Figure 2. Individual tocopherols chromatograms of black-bryony ripened fruits: 1- α-tocopherol; 2-β-tocopherol; 3- γ-tocopherol; 4- δ-tocopherol; 5-tocol (IS).

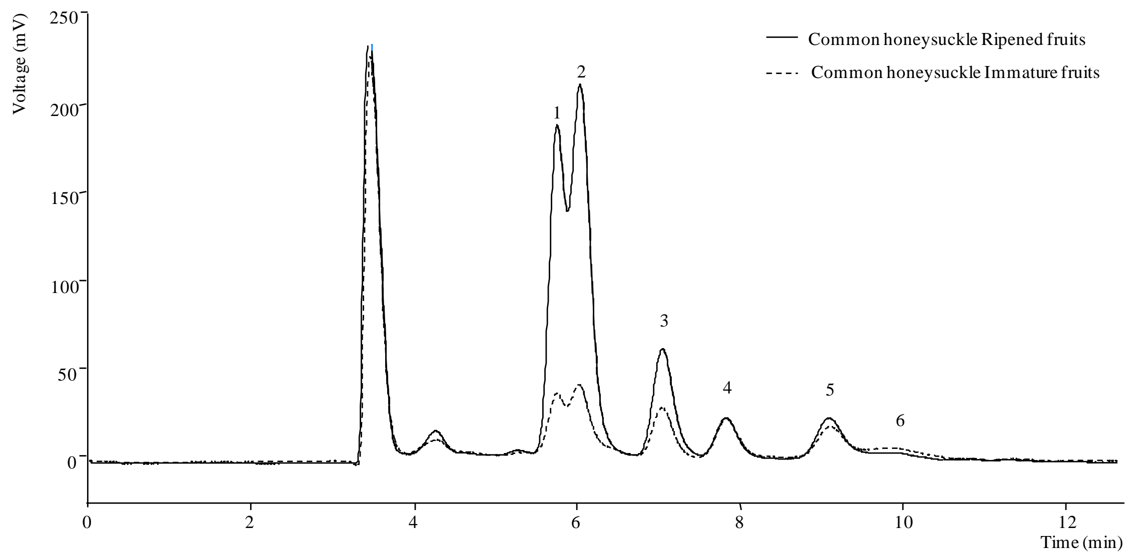

Figure 3. Individual sugars chromatogram of common honeysuckle fruits: 1- fructose; 2- glucose; 3- sucrose; 4- trehalose; 5- melezitose (IS); 6- raffinose.