Analytical tools used to distinguish chemical profiles of plants widely consumed as infusions and dietary supplements: artichoke, milk thistle and borututu

Carla Pereira, Lillian Barros, 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).

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Abstract

Artichoke, borututu and milk thistle are three medicinal plants widely consumed as infusions or included in dietary supplements (*e.g.*, pills and syrups). Despite their high consumption, studies on nutritional value and primary metabolites are scarce, being only reported the composition in secondary metabolites such as phenolic compounds. Therefore, their nutritional value was assessed and analytical tools (liquid and gas chromatography coupled to different detectors) were used to distinguish the chemical profiles namely in hydrophilic (sugars and organic acids) and lipophilic (fatty acids and tocopherols) compounds. Chromatographic techniques are important analytical tools used in the identification and quantification of several molecules, also being a standard requirement to distinguish different profiles. Borututu gave the highest energetic value with the highest content of carbohydrates and fat, sucrose and total sugars, shikimic and citric acids, α-, β-, δ- and total tocopherols. Artichoke had the highest ash and protein contents, oxalic acid, SFA (mainly palmitic acid acid), and γ-tocopherol, as also the best n6/n3 ratio. Milk thistle showed the highest levels of fructose and glucose, quinic acid and total organic acids, PUFA, mainly linoleic acid, and the best PUFA/SFA ratio. The hydrophilic compounds identified in the studied plants, mostly sugars, are the responsible for the energetic contribution of their widely consumed infusions. Otherwise, the bioactivity of lipophilic compounds namely, unsaturated fatty acids and tocopherols, is lost in those preparations but can be recovered in dietary supplements based on the plants. As far as we know this is the first report on detailed composition of molecules with nutritional features.

Keywords: Chromatography; Medicinal plants; Nutritional Value; Hydrophilic compounds; Lipophilic compounds.

Introduction

Medicinal plants are the oldest form of healthcare known to humanity and have been used in all cultures for thousands of years for the treatment or prevention of various human diseases (Barnes et al. 2007). These botanicals are known as natural sources of different chemical compounds that may have therapeutic purposes but also nutritional properties such as vitamins, minerals and fiber, among others (Chirinos et al. 2013). Nowadays, medicinal plants are widely consumed as boiling water extracts (herbal infusions) (Liu 2003) and as dietary supplements (such as pills, syrups and capsules, among others), accounting for 15-20% of the whole European botanical market (Bilia 2013). Examples of plants consumed in the mentioned forms are *Cynara scolymus* L. (artichoke), *Silybum marianum* (L.) Gaertn (milk thistle) and *Cochlospermum angolensis* Welw. (borututu), which are medicinally used for antioxidant and hepatoprotective activities (Pereira et al. 2013a).

Borututu bark infusion is used for the treatment of hepatic diseases and for the prophylaxis of malaria in Angola (Poppendieck 1981; Presber et al. 1991; Silva et al. 2011), the flowers and leaves extracts of milk thistle are used in the treatment of liver, spleen and gallbladder disorders (Rainone 2005) and artichoke leaves are used for their cholagogue, choleretic and choliokinetic actions, and also for treatment of dyspepsia and as anti-diabetics (Koubaa et al. 1999).

Nevertheless and despite the high consumption of those plants, the studies on nutritional value and primary metabolites are scarce, being only reported their phytochemical composition in non-nutrients such as phenolic compounds (Kukić et al. 2008; Wang et al. 2010; Abu-Reidah et al. 2013; Ferreres et al. 2013).

Profiling of primary metabolites (hydrophilic and lipophilic) is of utmost importance because they are directly involved in practically all biochemical processes, such as

normal growth, development and reproduction (Schiesel et al. 2010). Although the mentioned plants are consumed with medical/functional purposes and incorporated in different dietary supplements (borututu and milk thistle are directly used in the pills), artichoke and milk thistle can also be eaten (Lattanzio et al. 2009; Vaknin et al. 2008), so it becomes important to know their nutritional/energetic contribution.

In previous works, artichoke revealed high levels of proteins, minerals, vitamin C and fibre, with low amount of lipids (Lattanzio et al. 2009). Nevertheless, as far as we know nothing was reported on milk thistle and borututu nutritional value.

Given the importance of the consumption of these plants either as infusions or as dietary supplements, analytical tools (liquid and gas chromatography) were used to distinguish their chemical profiles namely, nutritional value, hydrophilic (sugars and organic acids) and lipophilic (fatty acids and tocopherols) compounds.

Material and Methods

Samples

Cynara scolymus L. (artichoke), *Silybum marianum* (L.) Gaertn (milk thistle) and *Cochlospermum angolensis* Welw. (borututu) were obtained from an herbalist shop in Bragança (Portugal), as dry material (leaves, plant and bark, respectively). All the samples were reduced to powder and submitted to determination of nutritional value, hydrophilic and lipophilic compounds.

Standards and reagents

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Fisher Scientific (Lisbon, Portugal). Fatty acids methyl ester (FAME) reference

standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also were other individual fatty acid isomers, L-ascorbic acid, tocopherol, sugar and organic acid standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (Pleasant Gap, PA, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

Nutritional value

The samples were analyzed for chemical composition (protein, fat, carbohydrates and ash) using the AOAC procedures (AOAC 1995). The samples crude protein content (N×6.25) was estimated by the macro-Kjeldahl method; the crude fat was determined using a Soxhlet apparatus by extracting a known weight of sample with petroleum ether; the ash content was determined by incineration at 600±15 °C. Total carbohydrates were calculated by difference and total energy was calculated according to the following equations: Energy (kcal)=4×(g protein+g carbohydrates)+9×(g fat).

Hydrophilic compounds

Sugars. Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI), after an extraction procedure previously described by the authors (Reis et al. 2013) using melezitose as internal standard (IS). 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 an RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH2 column (4.6×250 mm, 5 mm, Knauer) operating at 30 ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow

rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight.

Organic acids. Organic acids were determined following a procedure previously described by the authors (Pereira et al. 2013b). The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Corporation). Separation was achieved on a SphereClone (Phenomenex) reverse phase C18 column (5 μm, 250 mm × 4.6 mm i.d.) thermostatted at 35 ºC. The elution was performed with sulphuric acid 3.6 mM using a flow rate of 0.8 mL/min. Detection was carried out in a PDA, using 215 nm and 245 nm (for ascorbic acid) as preferred wavelengths. The organic acids found were quantified by comparison of the area of their peaks recorded at 215 nm or 245 nm with calibration curves obtained from commercial standards of each compound. For quantitative analysis, calibration curves were prepared from different standard compounds: oxalic acid ($y=1x10^7x+96178$; $R^2=0.999$); quinic acid ($y=601768x+8853.2$; $R^2=1$); malic acid ($y=952269x+17803$; $R^2=1$); citric acid ($y=1x10^6+4170.6$; $R^2=1$); fumaric acid ($y=172760x+52193$; $R^2=0.999$); shikimic acid ($y=8x10^7+55079$; $R^2=0.999$). The results were expressed in g per 100 g of dry weight.

Lipophilic compounds

Fatty acids. Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Reis et al. 2013). The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID at 260 ºC) and a Macherey–Nagel column (30 m × 0.32 mm i.d. × 0.25 μm df). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min,

then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/ min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/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 the CSW 1.7 Software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

Tocopherols. Tocopherols were determined following a procedure previously described by the authors (Reis et al. 2013). Analysis was performed by HPLC (equipment described above), 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 mm × 4.6 mm i.d.) normal-phase column from YMC Waters 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, and the injection volume was 20 µL. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g of dry weight.

Statistical analysis

For all the experiments three samples (n=3) were analyzed and all the assays were carried out in triplicate. The results are expressed as mean values ± standard deviation (SD). The differences between the different samples were analyzed using one-way

analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test with $\alpha = 0.05$, coupled with Welch's statistic. This treatment was carried out using SPSS v. 18.0 program.

Results and Discussion

Nutritional value

The nutritional value and energetic composition of artichoke, milk thistle and borututu are shown in Table 1. Among the three studied plants, artichoke had the highest ash (24.48 g/100 g) and protein (11.78 g/100 g) contents with the lowest fat and carbohydrate levels, which is in accordance with a previous study that reported the high content of proteins and low amount of fat in this plant (Lattanzio et al. 2009). On the other hand, borututu possessed the highest carbohydrate (85.39 g/100 g) and fat (2.48 g/100 g) amounts with, consequently, the highest energetic contribution (384.18 g/100 g), and revealed the lowest protein content. Overall, all the samples contained carbohydrates and fat as the major and the minor component, respectively.

Hydrophilic compounds

The results obtained for hydrophilic compounds are presented in Table 2. Milk thistle showed the highest contents of fructose (2.16 g/100 g) and glucose (0.97 g/100 g), whereas borututu revealed the highest sucrose (1.06 g/100 g) and total sugars amount (4.13 g/100 g), with the contribution of trehalose that was only detected in this plant (0.98 g/100 g) (Figure 1A). Fructose was the only sugar found in artichoke (2.01 g/100 g). Carbohydrates are the most abundant organic molecules found in nature and all organisms synthesize and metabolize these compounds. Glucose (present in borututu and milk thistle) is a common monosaccharide that is oxidized to form carbon dioxide

and water, providing energy for important cellular processes (protein synthesis, movement and transport). Furthermore, cell surface glycans are involved in many physiologically important functions, acting as signaling, recognition and adhesion molecules due to their structural variability and complexity (Sharon and Lis 1993). Regarding organic acids, artichoke revealed the highest levels of oxalic acid (1.95 g/100 g), milk thistle showed the highest content in quinic acid (2.76 g/100 g) and borututu presented the highest amounts of shikimic (0.01 g/100 g) and citric (0.57 g/100 g) acids. Malic and fumaric acids contents were similar in artichoke and milk thistle and the latest was also present in similar amount in borututu. The highest total organic acids content was detected in milk thistle (5.37 g/100 g) and the individual profile can be observed in Figure 1B.

Organic acids are widely present in fruits, wine and beverages and some of them have many applications in food industry: citric acid, for example, is largely used as food additives in many kinds of beverages, soft drinks and wines. A moderate amount of organic acids can promote appetite, help digestion, and is beneficial to human health (Cameron and Campbell 1974). These compounds are very important at the cellular level for several biochemical pathways, such as energy production, formation of precursors for amino-acid biosynthesis and at the whole plant level in modulating adaptation to the environment (López-Bucio et al. 2000).

Being infusions the most common forms of consumption of artichoke, borututu and milk thistle, hydrophilic compounds like organic acids and sugars are the main responsible molecules for their energetic contribution.

Lipophilic compounds

The results for fatty acids composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), ratios of PUFA/SFA and n-6/n-3, and the tocopherols content of the studied herbals are shown in Table 3. Artichoke revealed the highest levels of SFA (87.88%) with the contribution of palmitic acid (C16:0; 47.16%) as the main fatty acid, followed by behenic acid (C22:0; 9.28%), stearic acid (C18:0; 8.61%), arachidic acid (C20:0; 7.54%) and lignoceric acid (C24:0; 6.81%). Its chromatographic profile is provided on Figure 2A. Borututu also showed prevalence of SFA (48.23%) due to the main contribution of palmitic acid (C16:0; 25.98%) and arachidic acid (C20:0; 6.67%), but with considerable percentages of PUFA (30.93%) with the contribution of linoleic acid (C18:2n6; 24.11%) and α-linolenic acid (C18:3n3; 6.98%), and MUFA (20.84%) mainly oleic acid (C18:1n9; 19.96%). Lastly, milk thistle gave the highest percentages of PUFA (45.32%) followed by significant amounts of SFA (34.98%) and MUFA (19.70%) with the main contribution of linoleic acid (C18:2n6; 42.34%), palmitic acid (C16:0; 19.94%) and oleic acid (C18:1n9; 18.52%). Essential fatty acids (mostly n3 and n6 fatty acids) play important nutritional roles in growth, reproduction and good health, for the prevention of cardiovascular diseases (Simopoulos and Ordovas 2004; von Schacky and Harris 2006) and maintenance of the homeostasis of our bodies (Psota et al. 2006). These PUFA are derived biosynthetically from linoleic acid (18:2n6) and α–linolenic acids (18:3n3, ALA), respectively, which are present in the three studied plants. These essential dietary components can be synthesized in plants, but not in animal tissues (Din et al. 2004; Siddiqui et al. 2008), thus identifying new sources for omega-3 PUFAs within medicinal plants is of great importance and could be included in the diet.

For "good nutritional quality", including health beneficial effects, PUFA/SFA ratio should be higher than 0.45, while n-6/n-3 fatty acids ratio should be lower than 4.0

(Guil et al. 1996). As observed in Table 3, borututu was the only species that presented both ratios within the cited values (0.64 and 3.65, respectively). Artichoke showed the best n6/n-3 ratio (2.01), wile milk thistle revealed the best PUFA/SFA ratio (1.30).

The four vitamers of tocopherols were detected in borututu, being β-tocopherol the major compound in this species (597.06 mg/100 g) with the highest levels of all the isoforms except for the γ-tocopherol that was present in higher levels is artichoke (12.39 mg/100 g). δ-tocopherol was neither detected in artichoke nor in milk thistle and the latest also did not reveal β-tocopherol. Thus, the total tocopherols highest content was found in borututu (646.07 mg/100 g); the individual profile can be observed in Figure 2B. Tocopherols have a confirmed superb antioxidant activity in different food systems (Ko et al. 2010) being considered the most powerful natural antioxidants. Their most important function in biological membranes is that they act as recyclable chain reaction terminators of PUFA free radicals generated by lipid oxidation (Schneider 2005; Fryer 1992), but these compounds have also been suggested to play a major role in the maintenance and protection of the photosynthetic machinery in the plant (Collakova and DellaPena 2003). Lipophilic compounds are, therefore, important constituents of the studied plants but, when consumed in infusions, they are not available. Nevertheless, dietary supplements that contain the whole plant material, namely pills, are a good alternative for the consumption of the concerned compounds.

In conclusion, by using chromatographic techniques, important analytical tools used in the identification and quantification of different molecules, it was possible to distinguish different chemical profiles of the three studied species. Borututu possessed the highest energetic contribution with the highest content of carbohydrates and fat, sucrose and total sugars, shikimic and citric acids, α-, β-, δ- and total tocopherols.

Artichoke had the highest ash and protein contents, oxalic acid, SFA with the main contribution of palmitic acid, γ-tocopherol and the best n6/n3 ratio. Milk thistle showed the highest levels of fructose and glucose, quinic acid and total organic acids, PUFA due to the presence of linoleic acid, and the best PUFA/SFA ratio. The hydrophilic compounds identified in the studied plants, mostly sugars, are the most involved in the energetic contribution of their widely consumed infusions. Otherwise, the significant bioactive properties of lipophilic compounds such as unsaturated fatty acids or tocopherols, are lost in those preparations but can be remained in dietary supplements based on the entire plants. As far as we know this is the first report on the detailed composition of borututu, artichoke and milk thistle in molecules with nutritional features.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

Conflict of Interest

Carla Pereira declares that she has no conflict of interest.

Lillian Barros declares that she has no conflict of interest.

Isabel C.F.R. Ferreira declares that she has no conflict of interest.

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Table 1. Nutritional value and energetic value of artichoke, borututu and milk thistle (mean ± SD).

	Artichoke	Borututu	Milk thistle
Ash (g/100 g dw)	24.48 ± 0.57[a]	7.05 ± 0.07[b]	6.93 ± 0.32[b]
Proteins (g/100 g dw)	11.78 ± 0.26[a]	5.08 ± 0.01[c]	8.87 ± 0.07[b]
Fat (g/100 g dw)	1.02 ± 0.03[c]	2.48 ± 0.04[a]	1.46 ± 0.01[b]
Carbohydrates (g/100 g dw)	62.72 ± 0.38[c]	85.39 ± 0.02[a]	82.74 ± 0.26[b]
Energy (kcal/100 g dw)	307.14 ± 1.51[c]	384.18 ± 0.35[a]	379.56 ± 0.94[b]

Table 2. Hydrophilic compounds in artichoke, borututu and milk thistle (mean ± SD).

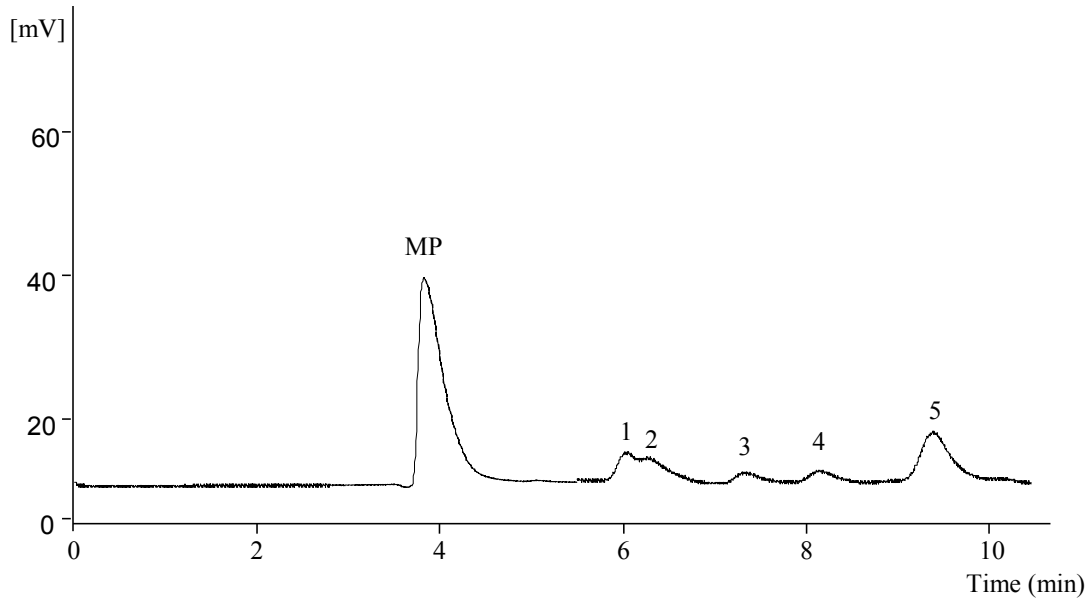
	Artichoke	Borututu	Milk thistle
Fructose (g/100 g dw)	2.01 ± 0.13[b]	1.30 ± 0.07[c]	2.16 ± 0.04[a]
Glucose (g/100 g dw)	nd	0.79 ± 0.02[b]	0.97 ± 0.07[a]
Sucrose (g/100 g dw)	nd	1.06 ± 0.13[a]	0.47 ± 0.08[b]
Trehalose (g/100 g dw)	nd	0.98 ± 0.05	nd
Total sugars (g/100 g dw)	2.01 ± 0.13[c]	4.13 ± 0.18[a]	3.61 ± 0.12[b]
Oxalic acid (g/100 g dw)	1.95 ± 0.09[a]	0.70 ± 0.04[c]	1.39 ± 0.05[b]
Quinic acid (g/100 g dw)	1.32 ± 0.04[b]	nd	2.76 ± 0.17[a]
Malic acid (g/100 g dw)	1.03 ± 0.07[a]	0.63 ± 0.03[b]	0.96 ± 0.05[a]
Shikimic acid (g/100 g dw)	nd	0.01 ± 0.00[a]	0.01 ± 0.00[b]
Citric acid (g/100 g dw)	0.33 ± 0.02[b]	0.57 ± 0.03[a]	0.24 ± 0.02[c]
Fumaric acid (g/100 g dw)	0.004 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
Total organic acids (g/100 g dw)	4.63 ± 0.23[b]	1.92 ± 0.10[c]	5.37 ± 0.19[a]

nd- not detected. In each row different letters mean significant differences (p<0.05).

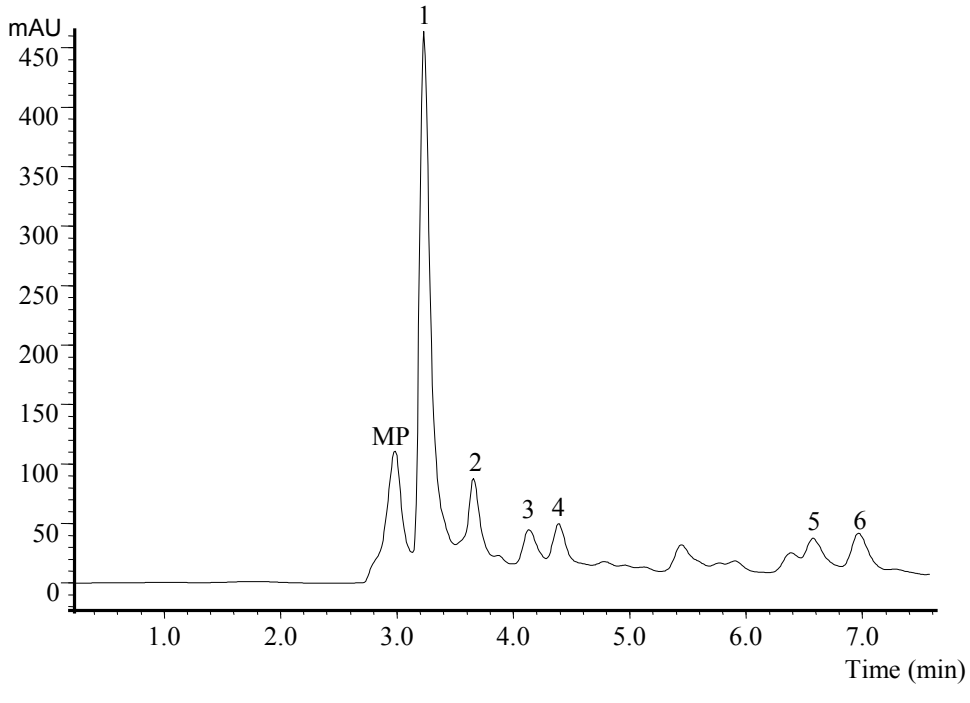
Table 3. Lipophilic compounds in artichoke, borututu and milk thistle (mean ± SD).

	Artichoke	Borututu	Milk thistle
C6:0	0.39 ± 0.01	0.06 ± 0.02	0.61 ± 0.00
C8:0	0.25 ± 0.01	0.29 ± 0.03	0.16 ± 0.00
C10:0	0.21 ± 0.03	0.26 ± 0.04	0.17 ± 0.00
C11:0	nd	0.07 ± 0.01	nd
C12:0	0.46 ± 0.01	2.05 ± 0.02	0.21 ± 0.00
C13:0	0.18 ± 0.00	0.16 ± 0.00	0.14 ± 0.00
C14:0	2.85 ± 0.60	4.69 ± 0.14	0.58 ± 0.00
C14:1	0.72 ± 0.08	0.12 ± 0.01	0.16 ± 0.00
C15:0	1.51 ± 0.05	0.82 ± 0.01	0.43 ± 0.00
C15:1	0.13 ± 0.01	0.13 ± 0.01	0.04 ± 0.00
C16:0	47.16 ± 0.46	25.98 ± 0.10	19.94 ± 0.00
C16:1	nd	0.62 ± 0.00	0.35 ± 0.00
C17:0	1.88 ± 0.01	1.34 ± 0.04	0.46 ± 0.00
C18:0	8.61 ± 0.20	2.91 ± 0.10	5.81 ± 0.00
C18:1n9	2.12 ± 0.24	19.96 ± 0.03	18.52 ± 0.00
C18:2n6	5.86 ± 1.11	24.11 ± 0.06	42.34 ± 0.00
C18:3n6	0.17 ± 0.02	nd	nd
C18:3n3	2.31 ± 0.50	6.98 ± 0.12	2.72 ± 0.00
C20:0	7.54 ± 0.29	6.67 ± 0.27	2.87 ± 0.00
C20:1	nd	nd	0.62 ± 0.00
C20:2	0.12 ± 0.02	0.22 ± 0.02	0.06 ± 0.00
C20:3n3+C21:0	0.69 ± 0.05	0.23 ± 0.01	0.21 ± 0.00
C22:0	9.28 ± 1.34	1.26 ± 0.00	2.03 ± 0.00
C23:0	0.76 ± 0.15	0.38 ± 0.04	0.22 ± 0.00
C24:0	6.81 ± 1.03	1.30 ± 0.01	1.35 ± 0.00
Total SFA	87.88 ± 1.85^{a}	48.23 ± 0.17^{b}	34.98 ± 1.37^{c}
Total MUFA	2.97 ± 0.33^{b}	20.84 ± 0.03^{a}	19.70 ± 0.01^{a}
Total PUFA	9.15 ± 1.52^{c}	30.93 ± 0.20^{b}	45.32 ± 1.39^{a}
PUFA/SFA	0.10 ± 0.01^{c}	0.65 ± 0.01^{b}	1.30 ± 0.09^{a}
n-6/n-3	2.01 ± 0.06^{c}	3.65 ± 0.05^{b}	14.45 ± 0.60^{a}
α-tocopherol (mg/100 g dw)	0.07 ± 0.00^{c}	3.67 ± 0.19^{a}	0.42 ± 0.01^{b}
β-tocopherol (mg/100 g dw)	0.87 ± 0.03^{b}	597.06 ± 13.98^{a}	nd
γ-tocopherol (mg/100 g dw)	12.30 ± 0.34^{a}	1.97 ± 0.13^{b}	0.88 ± 0.01^{c}
δ-tocopherol (mg/100 g dw)	nd	43.36 ± 1.61	nd
Total tocopherols (mg/100 g dw)	13.25 ± 0.31^{b}	646.07 ± 15.90^{a}	1.30 ± 0.00^{b}

SFA- Saturated fatty acids; MUFA- Monounsaturated fatty acids; PUFA- Polyunsaturated fatty acids. Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Undecanoic acid (C11:0); Lauric acid (C12:0); Tridecanoic acid (C13:0); Myristic acid (C14:0); Myristoleic acid (C14:1); Pentadecanoic acid (C15:0); *cis*-10-Pentadecenoic acid (C15:1); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9); Linoleic acid (C18:2n6); γ-Linolenic acid (C18:3n6); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosenoic acid (C20:1); *cis*-11,14-Eicosadienoic acid (C20:2); *cis*-11,14,17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3 + C21:0); Behenic acid (C22:0); Tricosanoic acid (C23:0); Lignoceric acid (C24:0). nd - not detected. The results are expressed in percentage. In each row different letters mean significant differences ($p<0.05$).

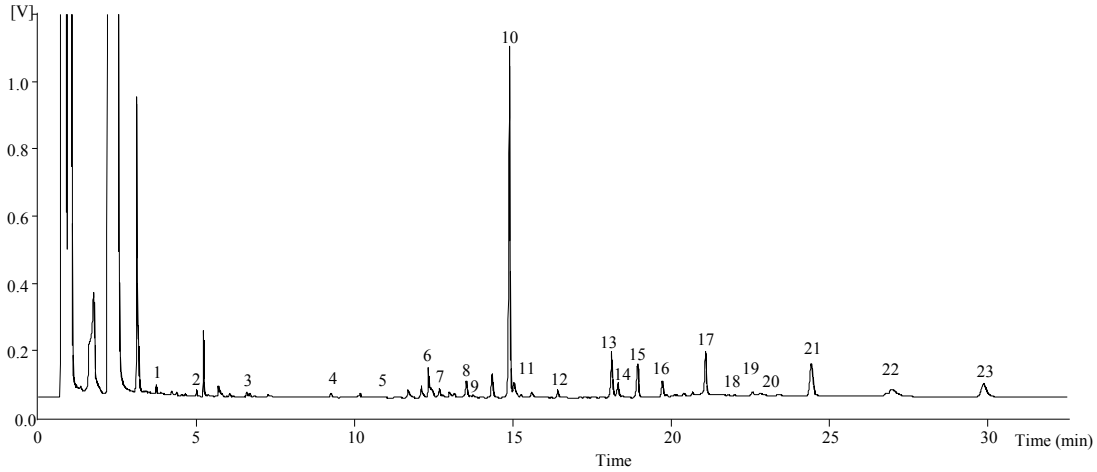


1A

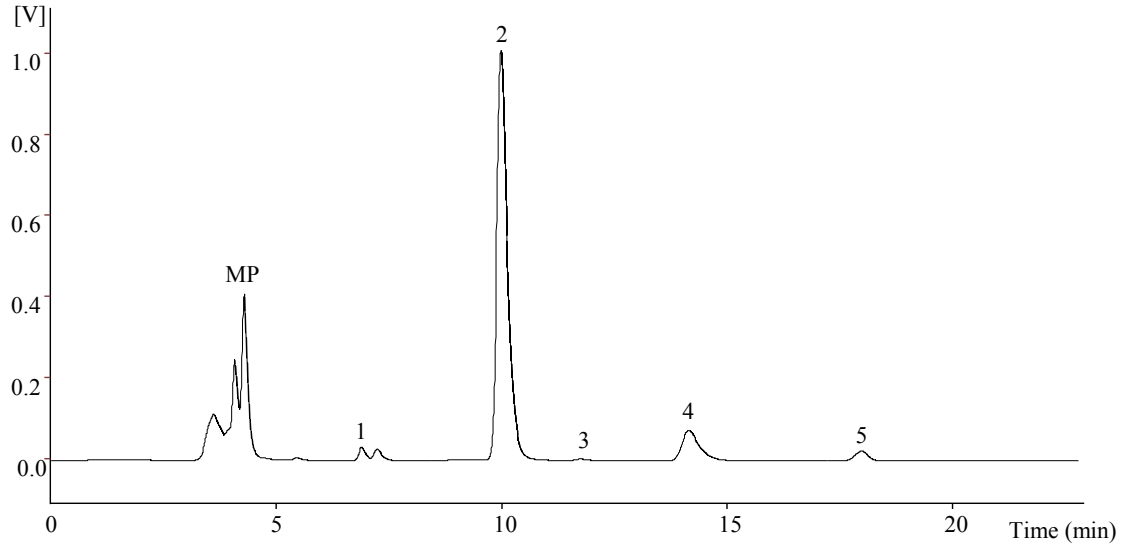


1B

Figure 1. **1A**. Individual sugars chromatogram of borututu: 1- fructose; 2- glucose; 3- sucrose; 4-trehalose; 5- melezitose (IS). **1B**. Individual organic acids chromatogram of Milk thistle: 1- oxalic acid; 2- quinic acid; 3- malic acid; 4- shikimic acid; 5- citric acid; 6- fumaric acid. MP- Mobile phase.



2A



2B

Figure 2. 2A. Individual fatty acids chromatogram of artichoke: 1- C6:0; 2- C8:0; 3- C10:0; 4- C12:0; 5- C13:0; 6- C14:0; 7- C14:1; 8- C15:0; 9- C15:1; 10- C16:0; 11- C16:1; 12- C17:0; 13- C18:0; 14- C18:1n9; 15- C18:2n6; 16- C18:3n3; 17- C20:0; 18- C20:1; 19- C20:2; 20- C20:3n3+C21:0; 21- C22:0; 22- C23:0; 23- C24:0. **2B**. Individual tocopherols chromatogram of borututu: 1-α-tocopherol; 2-β-tocopherol; 3- γ-tocopherol; 4- δ-tocopherol; 5-tocol (IS). MP- Mobile phase.