Chemical characterization of *Ginkgo biloba* L. and antioxidant properties of its extracts and dietary supplements

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ABSTRACT

Ginkgo biloba L. is the most commercialized medicinal plant worldwide, being its consumption related to prevention, and even decrease of the progression of degenerative neurological diseases. Considering the correlation between oxidative stress and the mentioned diseases, the antioxidant activity of different dietary supplements (syrup and several pills) was evaluated and compared to the leaves infusion, aqueous and methanolic extracts. Furthermore, *G. biloba* was chemically characterized in nutritional and bioactive components namely, fatty acids, sugars, organic acids, tocopherols, phenolics and flavonoids. Palmitic, α-linolenic and oleic acids were the main fatty acids found; fructose was the most abundant sugar; quinic acid was the most abundant organic acid and α-tocopherol was, by far, the most abundant vitamer. Dietary supplements showed higher antioxidant activity than *G. biloba* infusion and extracts due to their higher phenolics and flavonoids concentration. The pills with the highest concentration of plant extract (100 mg) allow the intake of the highest antioxidants concentration.

Keywords: *Ginkgo biloba*; Chemical characterization; Antioxidant activity; Extracts; Dietary supplements

1. Introduction

Ginkgo biloba L. (Ginkgoaceae) is an ancient tree growing in China for centuries; however, it was only during the last couple of decades that its true value was recognized, being considered sacred for its health-promoting properties (Smith et al., 1996; Singh et al., 2008).

The medical interest in western *G. biloba* has increased since the 1980s, due to its potent action on cardiovascular system and cerebral vascular activity. In recent decades, in Western countries, the concentrated extracts of the leaves have been marketed as herbal medicines due to the presence of bioactive components (*e.g.*, terpenoids, polyphenols, organic acids, carbohydrates, essential fatty acids, inorganic salts and amino acids), and to the capacity to increase microcirculation in brain (with the supply of oxygen and nutrients) and in body extremities (Beek, 2002; Singh et al., 2008). Thus, it can be useful in the improvement of symptoms of poor memory, impaired mental concentration, particularly in the elderly, for whom this function is sometimes lowered. It also has positive effects in certain situations such as tinnitus (ringing) and hearing capacity altered, bringing also cardiovascular protection due to the ability to prevent platelet aggregation and thrombus formation. Furthermore, due to the antioxidant properties it has been used in Alzheimer's patients (Smith et al., 1996; Diamond et al., 2000; Beek and Montoro, 2009).

Brain is particularly prone to damage by reactive oxygen species (ROS) and reactive nitrogen species (RNS) due to five main reasons: the high oxygen required by this organ; the abundance of redox-active metals; the relative deficit in antioxidant systems; the presence of great amount of oxidizable polyunsaturated fatty acids and catecholamines; and the fact that neurons are post-mitotic cells with relatively restricted

replacement by progenitor cells during lifetime (Mangialasche et al., 2009; Wang and Michaelis, 2010).

Thus, a variety of mechanisms of neuronal degeneration in Alzheimer diseases has been proposed, including formation of free radicals, oxidative stress, mitochondrial dysfunction, inflammatory processes, genetic factors, environmental impact factors, apoptosis, among others (Zhi-you and Yong, 2007). Different studies reported that neuronal earliest changes and pathological features of this disease are related to oxidative damage (with a very high input of oxidative stress), mainly in the development of neuritic abnormalities. In fact, paired helical filaments are more often found in neurites with membrane abnormalities, which is indicative of extensive lipid peroxidation (Zhu et al., 2004; Zhi-you and Yong, 2007). Moreover, crosslinking of proteins by oxidative processes may lead to the resistance of the lesions to intracellular and extracellular removal even though when they are extensively ubiquitinated; this resistance of neurofibrillary tangles to proteolysis might play an important role in the progression of the degenerative disease (Zhu et al., 2004). In this context, the use of polyphenols may be useful, since they increase the cellular stress response and improve mitochondrial respiration, thus allowing the neuron to counteract free radical-induced damage and produce the ATP necessary to maintain the normal membrane potential (Mancuso et al., 2012).

Taking into account the described relation between antioxidants and Alzheimer's disease, and considering the use of *G. biloba* in the mentioned pathology, the antioxidant activity of different dietary supplements (syrup and several pills) was evaluated and compared to the leaves infusion, aqueous and methanolic extracts. Furthermore, *G. biloba* was chemically characterized in nutritional and bioactive components.

2. Materials and methods

2.1. Samples

Ginkgo biloba dry leaves and dietary supplements (syrup and different pills based on leaves standardized extract with 24% glycosides and 6% terpenes) (**Table 1**) were obtained from an herbalist shop and a pharmacy, respectively, located in Bragança, Portugal.

2.2. Standards and Reagents

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Fisher Scientific (Lisbon, Portugal). The 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, tocopherols (α-, β-, γ-, and δ-isoforms), sugars (D(-)-fructose, D(+)-sucrose, D(+)-glucose, D(+)-trehalose and D(+)-raffinose pentahydrate), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid and (+)-catechin standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.3. Chemical characterization of Ginkgo biloba

2.3.1. Macronutrients. G. biloba dry leaves were analysed for chemical composition (moisture, proteins, fat, carbohydrates and ash) using the AOAC procedures (AOAC, 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. Total carbohydrates were calculated by difference.

2.3.2. Free 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 (Rafael et al., 2011) using melezitoze 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 a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 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 (dw).

2.3.3. Organic acids. Organic acids were determined following a procedure previously described by the authors (Pereira et al., 2013a). The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Coperation). Separation was achieved on a SphereClone (Phenomenex) reverse phase C_{18} 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 with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight (dw).

2.3.4. Fatty acids. Fatty acids were determined by gas–liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Rafael et al., 2011). 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 x 0.32 mm ID x 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 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.

2.3.5. Tocopherols. Tocopherols were determined following a procedure previously described by the authors (Rafael et al., 2011). 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 x 4.6 mm) normal-phase column from YMC Waters operating at 30ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (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 (dw).

2.4. Evaluation of antioxidant properties of Ginkgo biloba extracts and dietary supplements

2.4.1. Samples preparation. The samples were prepared as indicated in the label: For infusion preparation, 2 g of powdered dry leaves were added to 200 mL of boiling distilled water, left to stand at room temperature for 10 min, and then filtered under reduced pressure, frozen, lyophilized and redissolved in distilled water at a final concentration of 20 mg/mL; for dietary supplements, one pill was dissolved in distilled water. Information regarding the plant weight in each pill, destilled water volumes and final concentrations of the prepared solutions is provided in **Table 1**. Methanolic and aqueous extracts were also prepared from the dry leaves, stirring 1 g with 30 mL methanol or water, respectively, at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residues were then extracted with one additional 30 mL portion of the corresponding solvent. The combined extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland) and re-dissolved in the corresponding solvent at 20 mg/mL.

Several dilutions of all the prepared solutions were used in the antioxidant activity assays.

2.4.2. Phenolics and flavonoids content. Total phenolics were estimated by Folin-Ciocalteu colorimetric assay according to procedures previously described (Guimarães et al., 2009) and the results were expressed as mg of gallic acid equivalents (GAE) per g of sample.

Total flavonoids were determined by a colorimetric assay using aluminum trichloride, following procedures previously reported (Barros et al., 2010); the results were expressed as mg of (+)-catechin equivalents (CE) per g of sample.

2.4.3. DPPH radical-scavenging activity. This methodology was performed using an ELX800 microplate Reader (Bio-Tek Instruments, Inc; Winooski, USA). The reaction mixture on 96 well plate consisted in the sample solutions (30 µL) and methanolic solution (270 µL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 30 min in the dark, and the absorption was measured at 515 nm (Barros et al., 2010). The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: $\%RSA=[(A_{DPPH}-A_S)/A_{DPPH}]\times100$, where A_S is the absorbance of the solution containing the sample, and A_{DPPH} is the absorbance of the DPPH solution. The results were expressed in EC_{50} values (sample concentration providing 50% of radical scavenging activity). Trolox was used as positive control.

2.4.4. Reducing power. The sample solutions (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 °C 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 plate, the same with deionised water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm in the Microplate Reader mentioned above (Barros

et al., 2010). The results were expressed in EC_{50} values (sample concentration providing 0.5 of absorbance). Trolox was used as positive control.

2.4.5. Inhibition of β-carotene bleaching. A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two milliliters of this solution were pipetted into a round-bottom flask. The chloroform was removed at 40 °C under vacuum and 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 test tubes containing sample solutions (0.2 mL). The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm (Barros et al., 2010). β-Carotene bleaching inhibition was measured by the formula: β-carotene absorbance after 2h/initial absorbance) × 100. The results were expressed in EC_{50} values (sample concentration providing 50% of antioxidant activity). Trolox was used as positive control.

2.4.6. TBARS assay. Porcine (*Sus scrofa*) brains were obtained from official slaughtered animals, dissected, and homogenized with Polytron in an ice cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 w/v brain tissue homogenate which was centrifuged at 3000*g* for10 min. An aliquot (100 µL) of the supernatant was incubated with the sample solutions (200 µL) in the presence of $FeSO_4$ (10 mM; 100 µL) and ascorbic acid (0.1mM; 100 µl) at 37 °C for 1h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 500 µL), followed by thiobarbituric acid (TBA, 2%, w/v, 380 µL), and the mixture was then heated at 80 °C for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the color intensity of the

malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm (Barros et al., 2010). The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A−B)/A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively. The results were expressed in EC_{50} values (sample concentration providing 50% of lipid peroxidation inhibition). Trolox was used as positive control.

2.5. Statistical analysis

For each formulation, three samples were used and all the assays were carried out in triplicate. The results were expressed as mean values and standard deviation (SD). 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. 18.0 program.

3. Results and Discussion

3.1. Chemical characterization of Ginkgo biloba

The nutritional value of *G. biloba* leaves was evaluated, and the results are given in **Table 2**. Carbohydrates, calculated by difference, were the most abundant macronutrients (72.98 g/100 g dw). Otherwise, fat was the macronutrient present in lower amount, which confers a more healthy character to this medicinal plant (4.75 g/100 g dw). The levels of proteins and ash were 12.27 and 10.01 g/100 g dw, respectively.

Chemical composition in fatty acids, sugars, organic acids and tocopherols was also accessed and the results are shown in **Table 3.**

Palmitic (C16:0), α-linolenic (C18:3n3) and oleic (C18:1n9) acids were the most abundant fatty acids (35.90, 18.03 and 11.18%, respectively). The latter is nowadays considered as the preferred fatty acid for edible purposes, because it combines a hypocholesterolemic effect and a high oxidative stability (Mensink and Katan, 1989). In general, saturated fatty acids appeared in higher concentrations (59.15%), followed by polyunsaturated (28.85%) and lastly monounsaturated fatty acids (12%).

The sugars found were fructose, glucose and sucrose, being fructose (1.42 g/100 g dw) the main one and sucrose the least abundant sugar (0.23 g/100 g dw).

Oxalic, quinic, malic and shikimic acids were also identified and quantified, being quinic acid the most abundant organic acid (2.26 g/100 g dw). This acid is a very useful and versatile chiral pool starting material for natural product synthesis and many groups have developed elegant syntheses based on stereoselective reactions of quinic acid derivatives (Murray et al., 2004). Shikimic acid is also present in high quantity (2.24 g/100 g dw); it is used as key starting material for the synthesis of the neuramidase inhibitor GS4104 for treatment of antiviral infections (Krämer et al., 2003). On the other hand, malic acid was the organic acid found in lower quantity.

Regarding tocopherols, the isoforms α-, β-, γ- and δ- tocopherol were all detected. α-Tocopherol was, by far, the most abundant vitamer (124.88 mg/100 g dw in a total of 126.23 mg/100 g dw). Considering its antioxidant potential and various functions at the molecular level, this vitamer reduces the risk of cardiovascular diseases (eliminating reactive oxygen species, inhibiting lipid peroxidation and attenuating inflammatory reactions) and neurodegenerative disorders, particularly in Alzheimer's disease (Burton, 1994; Berman and Brodaty, 2004; Kontush and Schekatolina, 2004).

As far as we know, the present study is pioneer regarding chemical characterization of *G. biloba* in nutritional molecules, which is important considering that it is widely consumed as infusion and incorporated in dietary supplements.

3.2. Antioxidant properties of Ginkgo biloba extracts and dietary supplements

The antioxidant properties of different dietary supplements based on *G. biloba* (syrup and pills) and of extracts prepared from the leaves (infusion, methanolic and aqueous extracts) were compared. Dietary supplements showed higher antioxidant activity than extracts prepared from the dry leaves (**Table 4**). Among dietary supplements, pills gave higher antioxidant activity than syrup. Furthermore, it was observed an increase of antioxidant properties with the increase of *G. biloba* extract concentration in the pills. In fact, P3 (with 100 mg *G. biloba* standardized extract) was better than P2 (with 60 mg *G. biloba* standardized extract), and this one better than P1 (with 40 mg *G. biloba* standardized extract), for all the tested assays. The same behavior was observed for bioactive components namely, phenolics and flavonoids; the samples with highest antioxidant activity also gave the highest contents of the mentioned compounds, which pointed out for an involvement of phenolics and flavonoids in the observed activity.

Our research group reported opposing results in a study with *Cynara scolymus* L. (artichoke), *Silybum marianum* (L.) Gaertn (milk thistle) and *Cochlospermum angolensis* Welw. (borututu), in which infusions showed higher antioxidant activity than dietary supplements (Pereira et al., 2013b). This could be attributed to the very low amounts of phenolics found in those dietary supplements (3.35 to 30.70 mg GAE/g) in comparison with the ones obtained in the present study for *G. biloba* syrup and pills (396.98 to 553.41 mg GAE/g).

Regarding the extracts prepared from the dry leaves, methanolic extract (LME) showed higher DPPH radical scavenging activity, reducing power and lipid peroxidation inhibition, measured by β-carotene bleaching and TBARS inhibition. It also gave higher phenolics and flavonoids content than infusion or aqueous extract; a decrease in bioactive compounds was observed in infusion in comparison with aqueous extracts probably related to a degradation caused by heat. According to other authors, phenolic compounds are unstable and easily became non-antioxidative under heating and in the presence of antioxidants; thus, heat could destroy the structures of polyphenols and cause a decrease in their antioxidant activity (Yen and Hung, 2000).

Overall, dietary supplements containing plant extracts are complex mixtures whose therapeutic effect is often attributed to the cumulative effects of several components with bioactive properties. Thus, it is important to have an overview of all the elements present to evaluate product quality at nutraceutical and nutritional level. Particularly in *G. biloba* plant, several bioactive compounds were identified and quantified, such as tocopherols, mainly α-tocopherol, and phenolic compounds (potent antioxidants with an active role in relation to reducing the risk of atherosclerosis and attenuating neurological damage in patients with Alzheimer's disease). *G. biloba* pills (mainly P3, the dietary supplement with the highest concentration of plant extract) allow the intake of the highest antioxidants concentration.

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Table 1. Information about the studied *Ginkgo biloba* samples.

Code	Sample	Composition	Mode of consumption (minimal dose/day)	Concentration of the prepared solution
LI	Plant	Dry leaves	Infusion	20 mg/mL
LME		Methanolic extract from dry leaves	-	20 mg/mL
LAE		Aqueous extract from dry leaves	-	20 mg/mL
S	Syrup	40 mg of *Ginkgo biloba* standardized extract[a] /mL	1 mL diluted in a glass of water (200 mL); 2 or 3 times	200 µg/mL
P1	Pills	40 mg of *Ginkgo biloba* standardized extract[a]/pill	1 pill dissolved in ½ glass of water (100 mL); 2 or 3 times	400 µg/mL
P2		60 mg of *Ginkgo biloba* standardized extract[a]/pill	1 pill dissolved in a glass of water (200 mL); 2 times	300 µg/mL
P3		100 mg of *Ginkgo biloba* standardized extract[a]/pill	1 pill dissolved in a large glass of water (350 mL); 1 time	286 µg/mL

[a]Corresponds to *G. biloba* leaves extract with 24% glycosides and 6% terpenes (information available in the label).
Therapeutic indications: Antioxidant properties, antiasthmatic, scavenge radicals, wound healing and neuroprotective properties as well as it improves mental capacities in Alzheimer's patients.

Table 2. Nutritional value of *Ginkgo biloba* dry leaves.

Parameter	Amount
Ash	10.01 ± 0.06 g/100 g dw
Proteins	12.27 ± 0.24 g/100 g dw
Fat	4.75 ± 0.22 g/100 g dw
Carbohydrates	72.98 ± 0.20 g/100 g dw

dw- dry weight

Table 3. Individual compounds present in *Ginkgo biloba* dry leaves.

Fatty acids (relative percentage)	
C6:0	0.24 ± 0.01
C8:0	0.27 ± 0.04
C10:0	0.24 ± 0.01
C12:0	0.61 ± 0.09
C14:0	6.13 ± 0.47
C15:0	0.68 ± 0.03
C16:0	35.90 ± 0.97
C16:1	0.82 ± 0.12
C17:0	1.28 ± 0.02
C18:0	4.17 ± 0.24
C18:1n9	11.18 ± 1.23
C18:2n6c	10.53 ± 0.09
C18:3n3	18.03 ± 0.06
C20:0	2.70 ± 0.05
C20:3n6	0.11 ± 0.07
C20:3n3+C21:0	0.19 ± 0.08
C22:0	2.19 ± 0.01
C23:0	0.92 ± 0.00
C24:0	3.82 ± 0.04
SFA	59.15 ± 1.39
MUFA	12.00 ± 1.35
PUFA	28.85 ± 0.04

Free sugars (g/100 g dw)	
Fructose	1.42 ± 0.05
Glucose	0.78 ± 0.01
Sucrose	0.23 ± 0.02
Total	2.43 ± 0.04
Organic acids (g/100 g dw)	
Oxalic acid	0.90 ± 0.00
Quinic acid	2.26 ± 0.09
Malic acid	0.58 ± 0.01
Shikimic acid	2.24 ± 0.01
Total	5.98 ± 0.10
Tocopherols (mg/100 g dw)	
α-Tocopherol	124.88 ± 0.37
β-Tocopherol	0.36 ± 0.03
γ-Tocopherol	0.72 ± 0.06
δ-Tocopherol	0.28 ± 0.01
Total	126.23 ± 0.47

SFA- Saturated fatty acids; MUFA- Monounsaturated fatty acids; PUFA- Polyunsaturated fatty acids; dw- dry weight.

Table 4. Phenolics, flavonoids and antioxidant properties of *Ginkgo biloba* extracts and dietary supplements.

	LI	LME	LAE	S	P1	P2	P3
Phenolics (mg GAE/g)	37.71 ± 0.04^{g}	129.5 ± 5.30^{e}	61.58 ± 0.53^{f}	461.45 ± 5.75^{c}	396.98 ± 4.84^{d}	501.86 ± 4.24^{b}	553.41 ± 1.19^{a}
Flavonoids (mg CE/g)	1.53 ± 0.01^{f}	14.87 ± 0.84^{d}	1.39 ± 0.32^{f}	5.89 ± 0.90^{e}	28.30 ± 1.61^{c}	47.89 ± 0.55^{b}	53.21 ± 0.59^{a}
DPPH scavenging activity (EC_{50}, mg/mL)	1.52 ± 0.17^{a}	0.74 ± 0.04^{b}	1.58 ± 0.05^{a}	0.14 ± 0.00^{cd}	0.18 ± 0.01^{c}	0.11 ± 0.01^{cd}	0.07 ± 0.01^{d}
Reducing power (EC_{50}, mg/mL)	0.83 ± 0.02^{a}	0.36 ± 0.01^{c}	0.73 ± 0.00^{b}	0.14 ± 0.00^{d}	0.13 ± 0.00^{e}	0.08 ± 0.00^{f}	0.06 ± 0.00^{g}
β-carotene bleaching inhibition (EC_{50}, mg/mL)	4.71 ± 0.35^{a}	4.47 ± 0.08^{b}	4.85 ± 0.10^{a}	0.47 ± 0.05^{c}	0.56 ± 0.07^{c}	0.43 ± 0.03^{c}	0.22 ± 0.02^{d}
TBARS inhibition (EC_{50}, mg/mL)	1.29 ± 0.18^{a}	0.13 ± 0.01^{cd}	0.82 ± 0.09^{b}	0.24 ± 0.06^{c}	0.12 ± 0.01^{de}	0.03 ± 0.00^{de}	0.02 ± 0.01^{e}

LI- infusion prepared from dry leaves; LME- methanolic extract prepared from dry leaves; LAE- aqueous extract prepared from dry leaves; S- syrup; P- pills. EC_{50} values correspond to the sample concentration achieving 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. GAE- gallic acid equivalents; CE- catechin equivalents. In each row different letters mean significant differences ($p<0.05$).