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Analytical Methods

Chlorogenic acids and related compounds in medicinal plants and infusions

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ABSTRACT

The consumption of plant infusions for prevention and treatment of health disorders is a worldwide practise. Various pharmacological activities inherent to medicinal plants have been attributed to their phenolic composition, including chlorogenic acids (CGA). Studies have shown potential beneficial properties of CGA to humans such as antioxidant, hepatoprotective, hypoglycaemic. In the present study, the CGA composition of 14 dried medicinal plants was determined by HPLC-UV and LC-DAD–ESI-MS. The plants with the highest CGA contents were *Ilex paraguariensis*, *Bacharis genistelloides*, *Pimpinella anisum*, *Achyrochine satureioides*, *Camellia sinensis*, *Melissa officinalis* and *Cymbopogon citratus*, with 84.7 mg/100 g–9.7 g/100 g, dry weight. Plant infusions were prepared (at 0.5%) in order to evaluate the actual consumption of CGA through these beverages. Total CGA contents in the infusions were similar to those in the methanolic extracts and indicated that a satisfactory extraction occurs during the preparation of infusions. These CGA-rich plants deserve attention regarding the pharmacological properties attributed to CGA.

1. Introduction

For centuries, plants have been widely used as food and for medicinal purposes in both Western and Eastern cultures. In the last few years, interest in plant medicines has increased worldwide. Because of the immense flora existing all over the world along with cultural aspects, the use of plants in the form of crude extracts, infusions or plasters has been revived as a usual practise to treat common infections. The World Health Organization estimates that about 80% of the developing countries inhabitants rely on the traditional medicine for their primary health care needs, and that most of these therapies involve the use of plant extracts or their active components (WHO, 2000). Not only in developing countries but all over the world the use of medicinal plants has been playing a significant role in maintaining human health and improving the quality of human life. For example, teas made from the leaves of *Camellia sinensis* have been for centuries commonly consumed all over the world. Recently, epidemiological and preclinical studies have indicated that drinking green and black teas may lower the risk of development of cancer and cardiovascular diseases. Additional beneficial effects of tea drinking such as anti-inflammatory and anti-obesity have also been reported (Nishitani & Sagesaka, 2004).

Various beneficial health properties inherent to *C. sinensis* and other plants have been attributed to their phenolic composition. Phenolic compounds occur in nature as mixtures of esters, ethers, or free acids (Shahrzad & Bitsch, 1996). A major class of phenolic compounds is the hydroxycinnamic acids, which are found in almost every existing plant. Caffeic, ferulic and *p*-coumaric acids are *trans*-cinnamic acids that occur naturally in their free forms or as a family of mono or diesters with (−)-quinic acid, collectively known as chlorogenic acids (CGA). CGA are antioxidant components produced by plants in response to environmental stress conditions such as infections by microbial pathogens, mechanical wounding, and excessive UV or visible light levels (Farah & Donangelo, 2006). The main classes of CGA found in nature are the caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), and, less commonly, feruloylquinic acids (FQAs), each group with at least three isomers (Fig. 1) (Clifford & Ramirez-Martinez, 1990). Potentially beneficial properties to humans such as antioxidant, hypoglycaemic, antiviral and hepatoprotective (Farah & Donangelo, 2006) activities have been also attributed to CGA in *in vitro*, *in vivo* and epidemiological studies. Their lactones (CGL), which are formed during heating by dehydration from the quinic acid moiety and formation of an intramolecular ester bond (Farah, De Paulis, Moreira, Trugo, & Martin, 2006) have also shown biological effects such as inhibition of adenosine transport and affinity with μ-opioide receptor (De Paulis et al., 2004), and hypoglycaemic activity (Shearer et al., 2003).

Despite CGA potentially beneficial effects in humans, data on their content and distribution in plants, foods and beverages is scarce. Moreover, most of the existing data either include CGA within total phenolic contents or just measure the content of 5-CQA, which is the most abundant CGA in nature. Additionally,

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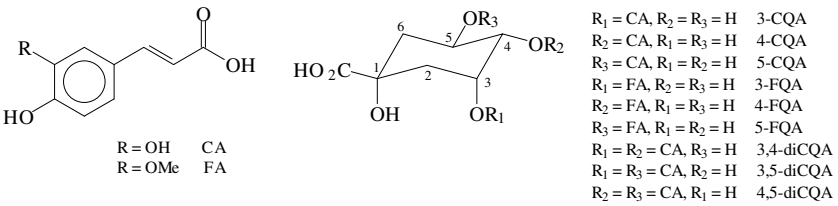

Fig. 1. Chemical Structures of caffeoylquinic, feruloylquinic and dicaffeoylquinic acids.

the variety of methods employed for CGA analysis increases the difficulty of data comparison in the literature.

In this study, medicinal plants commonly consumed in South America were selected according to their popular use and their CGA composition was determined. Subsequently, in order to evaluate the actual consumption of CGA and related compounds through plant infusions, homemade-type infusions were prepared with the evaluated plants carrying the highest CGA content.

2. Material and methods

2.1. Samples

The following 14 samples of dried medicinal plants popularly used in South America were obtained from reliable commercial sources in Rio de Janeiro, Brazil, for screening of CGA contents: leaves of *Ilex paraguariensis* (green and toasted maté); *Baccharis genistelloides* (“carqueja”), *Camellia sinensis* (green and black fermented tea); *Melissa officinalis* (lemon balm); *Cymbopogon citratus* (lemon grass); *Cydonia oblonga* (quince); *Maytenus ilicifolia* (“espinheira santa”); *Annona muricata* (“graviola”); *Ginkgo biloba* (Maidenhair or Ginkgo); *Peumus boldus* (boldo) and *Syzygium cumini* (jambolan); seeds of *Pimpinella anisum* (anise); flowers of *Achyrocline satureioides* (“macela”) and peels of *Erythrina velutina* (“mulungú”).

Three commercial brands of each of the following plants: leaves of *I. paraguariensis, B. genistelloides*, *C. sinensis, C. citratus* and *M. officinalis*; seeds of *P. anisum* and dried flowers of *A. satureioides*, from different states in Brazil (Rio de Janeiro, São Paulo, Paraná and Santa Catarina) were used for infusions preparation followed by analyses of CGA, CGL and phenolic acids.

2.2. Moisture content

In order to express the amount of CGA and related compounds on dry weight basis, the moisture content of the plants was determined according to the AOAC method (AOAC, 2000).

2.3. Phenolic compounds extraction

(a) *Methanolic extracts* – Methanolic extractions were performed for screening of CGA content. Dried medicinal plants were macered by mortar and pistil and ground in an electric mill to pass a 0.75 mm sieve. Samples were extracted in duplicate (extraction variation coefficient <5%) with an aqueous solution of 40% methanol, according to a modification of the method of Trugo and Macrae, described in details by Farah, De Paulis, Trugo, and Martin (2005).

(b) *Infusions* – Infusions at 0.5% were prepared from the selected plants in the following way: 190 mL of 95 °C water was added to 1 g of each dried plant, corresponding to approximately one tea bag, and let rest for 15 min, according to most manufacturer’s instructions. Each infusion was filtered through paper (Whatman No. 1) and the residue was washed with warm water. For precipitation of proteins and other high molecular weight compounds, Carrez solutions were used as in Farah et al. (2005). The volume was made up to 200 mL and the mixture was agitated, let rest for 10 min and filtered. The clarified infusion was used directly for chromatography.

2.4. Standards

5-Caffeoylquinic acid (5-CQA), caffeic acid, syringic acid, *p*-coumaric acid, gallic acid, sinapinic acid, ferulic acid and vanillic acid were purchased from Sigma–Aldrich (St Louis, MO). A mixture of 3-CQA, 4-CQA and 5-CQA was prepared from 5-CQA, applying the isomerization method of Trugo and Macrae, also described in Farah et al. (2005). For diCQA, a mixture of 3,4-diCQA; 3,5-diCQA and 4,5-diCQA from Roth (Germany) was used. In the present investigation, the authors used IUPAC rules for numbering of CGA. When citing other authors, their numbering has been changed for consistency.

2.5. HPLC and LC–MS analyses

Plant extracts and infusions were analysed by a HPLC-UV system as described in details by Farah et al. (2005), using UV at 325 nm for CGA, CGA lactones, caffeic acid, ferulic acid, sinapinic acid and *p*-coumaric acid, and at 280 nm for gallic acid, syringic acid and vanilic acid. Peaks identity were confirmed by LC-DAD–ESI-MS (liquid chromatography with diode array detection and electrospray ionisation mass spectrometry), using peak mass (Farah et al., 2006) and UV spectra. The detection and quantification limits for CGA and related compounds under the conditions used in this investigation were 1.70 and 5.00 μg/mL, respectively. Data are presented as mean ± standard deviation (SD).

2.6. Statistical analyses

Aqueous and methanolic extractions results were statistically tested for correlations with the GraphPad Prism® software, version 4.0 (San Diego, California, USA), using pared *t*-test method and considered significant when $p \leqslant 0.05$.

3. Results and discussion

3.1. Analyses of methanolic extracts

A total of nine CGA compounds were identified and quantified in the different investigated medicinal plants: 3-CQA, 4-CQA, 5-CQA, 3-FQA, 4-FQA, 5-FQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA. Their contents are depicted in Table 1. Additionally, we observed the presence of peaks with *m/z* and retention times compatible with less common CGA compounds, more specifically, caffeoylferuloylquinic acid (CFQA – *m/z* 529), *p*-coumaroylquinic acid (*p*-CoQA – *m/z* 337), and the following 1,5-γ-quinolactones: caffeoylquinic lactone (CQL – *m/z* 335), caffeoylferuloylquinic lactone (CFQL – *m/z* 511), dicaffeoylquinic lactone (diCQL – *m/z* 497), feruloylquinic lactone (FQL – *m/z* 349) and *p*-coumaroylquinic lactone (*p*-CoQL – *m/z* 319).

Table 1
Chlorogenic acids content in methanolic extracts of dried medicinal plants[a]

Samples	Moisture (%)	3-CQA[b]	4-CQA[b]	5-CQA[b]	3-FQA[b]	4-FQA[b]	5-FQA[b]	3,4-diCQA[b]	3,5 -diCQA[b]	4,5-diCQA[b]	CA[b]	GA[b]
Green *I. paraguariensis*	7.6	2386.5 ± 115.7	1337.9 ± 21.8	1599.6 ± 77.3	83.1 ± 0.7	47.4 ± 2.2	28.6 ± 1.1	549.7 ± 25.5	2332.9 ± 115.2	1364.9 ± 24.1	15.0 ± 0.7	Nd[c]
Toasted *I. paraguariensis*	5.8	316.3 ± 16.6	442.7 ± 0.6	670.9 ± 35.3	18.8 ± 0.6	23.5 ± 1.2	27.4 ± 1.6	81.9 ± 1.6	145.0 ± 3.3	242.8 ± 21.8	3.0 ± 0.2	Nd[c]
B. genistelloides	8.3	229.0 ± 9.9	180.1 ± 1.7	362.8 ± 1.0	13.1 ± 0.6	5.65 ± 0.2	4.4 ± 0.2	120.3 ± 5.9	301.2 ± 3.2	194.0 ± 2.9	7.4 ± 0.4	Nd[c]
P. anisum	7.6	16.1 ± 0.7	24.1 ± 0.9	87.1 ± 4.8	2.4 ± 0.1	2.02 ± 0.1	3.84 ± 0.3	18.3 ± 0.8	18.5 ± 0.8	19.2 ± 0.4	Nd[c]	Nd[c]
A. satureioides	9.6	6.6 ± 0.3	9.7 ± 0.5	33.6 ± 1.2	Nd[c]	Nd[c]	Tr[c]	57.1 ± 3.2	30.9 ± 0.3	24.2 ± 0.7	3.4 ± 0.1	Nd[c]
Black *C. sinensis*	7.0	26.5 ± 1.4	63.5 ± 0.1	49.5 ± 1.7	Tr[c]	Tr[c]	Nd[c]	Tr[c]	Tr[c]	Tr[c]	Nd[c]	36.2 ± 1.1
Green *C. sinensis*	7.0	36.6 ± 1.2	81.8 ± 4.5	22.4 ± 0.2	Tr[c]	Tr[c]	Tr[c]	Tr[c]	Tr[c]	Tr[c]	Nd[c]	19.1 ± 0.6
M. officinalis	9.5	10.6 ± 0.3	3.5 ± 0.2	17.0 ± 0.8	Nd[c]	Nd[c]	Nd[c]	16.8 ± 0.8	Tr[c]	45.5 ± 1.5	39.3 ± 0.9	Nd[c]
C. citrates	8.7	4.8 ± 0.2	3.0 ± 0.1	44.9 ± 0.3	17.3 ± 0.1	3.8 ± 0.1	0.9 ± 0.1	1.7 ± 0.1	4.7 ± 0.2	3.5 ± 0.1	Nd[c]	Nd[c]
C. oblonga	13.6	15.2 ± 0.1	15.1 ± 0.6	33.1 ± 1.8	Tr[c]	Tr[c]	Tr[c]	3.1 ± 0.1	2.1 ± 0.1	11.0 ± 0.2	0.4 ± 0.1	Nd[c]
M. ilicifolia	10.0	7.1 ± 0.3	12.7 ± 0.4	60.6 ± 2.2	Nd[c]	Nd[c]	Tr[c]	Nd[c]	Nd[c]	Nd[c]	1.3 ± 0.1	Nd[c]
E. velutina	10.7	1.3 ± 0.1	1.2 ± 0.1	5.5 ± 0.2	Tr[c]	Tr[c]	Tr[c]	3.0 ± 0.1	Tr[c]	Tr[c]	Nd[c]	Nd[c]
A. muricata	10.4	3.6 ± 0.1	0.5 ± 0.1	3.3 ± 0.2	Tr[c]	Nd[c]	Nd[c]	Tr[c]	Nd[c]	Nd[c]	Nd[c]	48.6 ± 1.5
G. biloba	10.2	Nd[c]	Nd[c]	4.0 ± 0.2	Nd[c]	Nd[c]	Nd[c]	Tr[c]	Tr[c]	Tr[c]	1.0 ± 0.1	Nd[c]
P. boldo	10.2	Tr[c]	Tr[c]	1.8 ± 0.1	Tr[c]	Tr[c]	Tr[c]	Tr[c]	Tr[c]	Tr[c]	2.7 ± 0.1	Nd[c]
S. cumini	11.0	Tr[c]	Tr[c]	0.6 ± 0.1	Nd[c]	Nd[c]	Nd[c]	Tr[c]	Tr[c]	Tr[c]	0.9 ± 0.1	7.0 ± 0.4

[a] Results are shown as the means of extractins in duplicate ±standard deviation, expressed in mg/100 g of dry weight plant.
[b] CQA = caffeoylquinic acid; FQA = feruloylquinic acid; diCQA = dicaffeoylquinic acid; CA = caffeic acid; GA = gallic acid.
[c] Nd = not detected (under detection limit of 1.70 μg/mL); Tr = trace amount (above detection limit but below quantification limit of 5.00 μg/mL).

Total CGA contents varied considerably in the investigated plant material, from 0.6 mg/100 g (*S. cumini*) to 9.7 g/100 g (green *I. paraguariensis*), on dry weight basis (dwb). In general, CQA and diCQA isomers were the most prevalent and abundant CGA compounds, although diCQA isomers were not identified in *M. ilicifolia*. The contribution of each of these two classes for total CGA content varied according to the type of plant. The contents of CQA isomers varied from 0.6 mg/100 g dwb (*S. cumini*) to 5.3 g/100 g dwb (green *I. paraguariensis*), with higher contents observed in *I. paraguariensis*, *B. genistelloides*, *P. anisum* and *C. sinensis* (55–100% of total CGA).

The contents of diCQA isomers varied from 3.0 mg/100 g (*P. anisum*) to 4.2 g/100 g (green *I. paraguariensis*), dwb. Amongst the plants with high diCQA contents, *I. paraguariensis*, *B. genistelloides* and *A. satureioides* stood out (24–69% of total CGA), which explains the previous isolation of diCQA isomers from these plants (Filip, Lopez, Giberti, Coussio, & Ferraro, 2001; Robinson, Cordeiro, et al., 1996; Robinson, Reinecke, Abdel-Malek, Jia, & Chow, 1996). FQA isomers were only identified in five plants, and their contents varied from 2.6 mg/100 g (*P. anisum*) to 159 mg/100 g, dwb (green *I. paraguariensis*) (2–4% of total CGA).

Regarding individual compounds, 5-CQA was the only CGA identified in all investigated plants and also the most abundant in the majority of them, which indicates the importance of 5-CQA to plant metabolism in nature (Farah & Donangelo, 2006). 5-CQA contents ranged from 0.6 mg/100 g dwb (*S. cumini*) to 1.6 g/100 g dwb (green *I. paraguariensis*). A detailed discussion on the individual CGA isomers composition for each evaluated plant follows.

3.1.1. I. Paraguariensis

It is known that extracts made from green and toasted leaves of *I. paraguariensis* (green and toasted maté) are excellent sources of CGA (Clifford & Ramirez-Martinez, 1990). In the present study, nine CGA compounds were identified in this plant, being 3-FQA and 4-FQA apparently identified for the first time in both green and toasted *I. paraguariensis* leaves. Also, until present, 5-FQA had apparently not yet been identified in toasted leaves of this plant. The contents of the main CQA and diCQA isomers obtained in the present investigation (Table 1) are in accordance with Clifford and Ramirez-Martinez (1990) and with Filip et al. (2001). To our knowledge, high contents of CGA such as those obtained for green *I. paraguariensis* have not been reported for any other food or plant material, except for green (raw) seeds of *Coffea canephora* cv. Conillon, commonly produced in Brazil (9.5 g/100 g, dwb) (Farah & Donangelo, 2006). In *I. paraguariensis*, the contribution of CQA isomers to total CGA content was predominant, corresponding, on average, to 55% and 73% of total CGA content for green and toasted leaves, respectively. However, the individual isomers distribution was different in green compared to toasted leaves. In green leaves, 3-CQA was the predominant CQA isomer (about 45% of the total CQA), being the most abundant CQA amongst all presently investigated plants. In toasted *I. paraguariensis*, 5-CQA was the predominant CQA (about 47% of the total CQA). The predominance of 3-CQA and 5-CQA isomers in green and toasted *I. paraguariensis*, respectively, is in accordance with reports from Clifford and Ramirez-Martinez (1990). Since green and toasted *I. paraguariensis* leaves were not from the same source, they cannot be compared. Regarding diCQA, 3,5-diCQA was the major isomer in green *I. paraguariensis* (about 55% of total diCQA), whilst 4,5-diCQA was the most prevalent diCQA in toasted *I. paraguariensis* (about 52% of total diCQA). The content of the main FQA isomers also varied in green compared to toasted *I. paraguariensis* leaves, being 3-FQA and 5-FQA the most abundant FQA in green and toasted leaves, respectively. Additionally, we observed in green and toasted leaves the presence of peaks

with *m/z* compatible with CFQA, *p*-CoQA, CQL, CFQL and diCQL, excluding diCQL in the toasted leaves. From these lactones, only 3-caffeoylquinic-1,5-lactone (3-CQL) and 4-caffeoylquinic-1,5-lactone (4-CQL) were quantified in toasted *I. paraguariensis* (107.7 ± 5.1 mg/100 g and 75.0 ± 3.3 mg/100 g dwb, respectively). Only trace amounts of the remaining lactones were observed. The presence of 3-CQL has been previously reported in *I. paraguariensis* by Hauschild (1935), but not confirmed by Clifford and Ramirez-Martinez (1990), who believed such peak to be an artefact associated with a large 3-CQA content based in previous reports.

B. genistelloides, *P. anisum*, *A. satureioides*, green and black *C. sinensis*, *M. officinalis* and *C. citratus* also showed expressive CGA contents compared to other plants investigated in this study as well as to additional plants considered as good CGA sources in the literature such as *Lavandula officinalis*, *Hyssopus officinalis* (Zgorka & Glowniak, 2001) and *Bidens pilosa* (Chiang et al., 2004).

3.1.2. B. genistelloides

Nine CGA compounds were identified in *B. genistelloides* (Table 1). 5-CQA and 3,5-diCQA were the most abundant CGA compounds (26% and 21% of total CGA, respectively). Additionally, we observed the presence of peaks with *m/z* compatible with CFQA and *p*-CoQA compounds. Results on CGA composition in *B. genistelloides* are, to our knowledge, unavailable in the literature, although 4,5-diCQA and 3,5-diCQA have been isolated from this plant by Robinson et al. (1996), Robinson, Cordeiro, et al. (1996).

3.1.3. P. anisum

Nine CGA isomers were identified in the seeds of *P. anisum* (Table 1). From these compounds, 5-CQA was the only one previously identified and reported in phytochemical studies (Hänsel, Sticher, & Steinegger, 1999). CQA class was prevalent in *P. anisum* (66% of total CGA), being 5-CQA the major CGA compound (69% of total CQA and 45% of total CGA). Additionally, peaks with m/z compatible with CFQA and *p*-CoCA were also identified in *P. anisum*.

3.1.4. A. satureioides

From the seven CGA compounds identified in *A. satureioides* (Table 1), only 3-CQA and 5-CQA have been previously identified by a phytochemical study (Broussalis, Ferraro, Gurni, & Coussio, 1988) and isolated by Robinson et al. (1996). However, the presence of diCQA compounds was predominant in *A. satureioides* extract, corresponding to approximately 69% of total CGA. A peak with *m/z* compatible with a CFQA isomer was also identified in *A. satureioides*.

3.1.5. C. sinensis

Whilst nine CGA compounds were identified in green *C. sinensis*, eight CGA were identified in black (fermented) *C. sinensis*. Due the low amounts of FQA and diCQA isomers as well as coelution with other peaks, these compounds were only identified by LC–MS and not quantified. The contents of CQA compounds are shown in Table 1. From these compounds, to our knowledge, only 3-CQA and 5-CQA have been previously identified in *C. sinensis* (Nishitani & Sagesaka, 2004). Surprisingly, 4-CQA, which apparently was identified for the first time, in the present investigation gave the highest contribution to the total CQA content observed in this plant (about 58% and 45% of CQA in green and black *C. sinensis*, respectively). The identification of FQA isomers in aqueous and ethanolic extracts of *C. sinensis* have been reported by Bastos et al. (2007), who observed peaks with *m/z* compatible with FQA using ESI-MS. On the other hand, diCQA have not been identified in the referred study. This difference in compounds identification may derive from various factors such as distinct chromatographic methods and origins of the plants. Additionally, we observed peaks with *m/z* compatible with *p*-CoCA in the green and black extracts, confirming data from Nishitani and Sagesaka (2004), peaks compatible with CQL, FQL, diCQL and *p*-CoQL compounds in green *C. sinensis* extract and peaks compatible with CQL and diCQL compounds in black *C. sinensis* extract. These lactones were probably formed during processing of the plants.

3.1.6. M. officinalis

Six CGA compounds were identified in the leaves of *M. officinalis* (Table 1). To our knowledge, the occurrence of such compounds in this plant have not been previously reported, even though Zgorka and Glowniak (2001) have attempted to investigate the presence of 5-CQA in *M. officinalis* without success. In the present investigation, diCQA were the most abundant CGA compounds (about 67% of total CGA). Additionally, peaks with *m/z* compatible with CFQA, CQL and CFQL compounds were identified in *M. officinalis* extracts.

3.1.7. C. citratus

Nine CGA compounds were identified in the leaves of *C. citratus* (Table 1). The most abundant was 5-CQA, corresponding to approximately 53% of total CGA. This content is in alignment with the literature (Cheel, Theoduloz, Rodriguez, & Schmeda-Hirschmann, 2005). To our knowledge, the other eight CGA identified in the present investigation have not been previously reported. Additionally, peaks with *m/z* compatible with CFQA and *p*-CoQL compounds were identified in *C. citratus* extract.

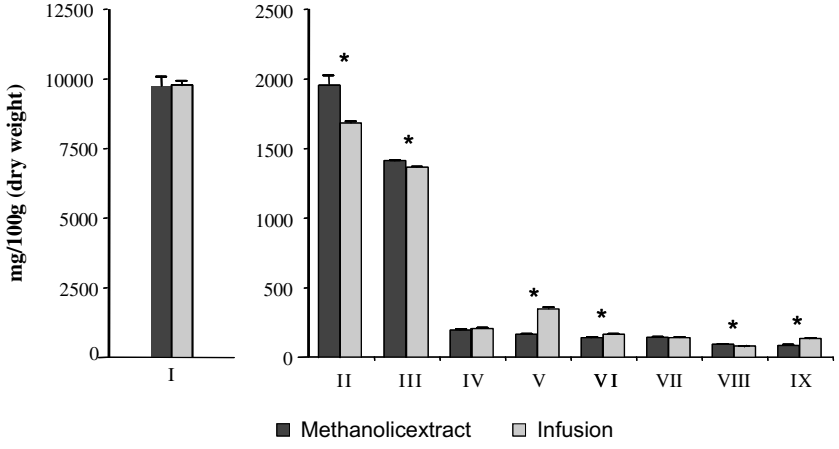

Fig. 2. Total CGA contents in methanolic extracts and infusions of medicinal plants (mg/100 g, dry weight basis). I – green *I. paraguariensis*; II – toasted *I. paraguariensis*; III – *B. genistelloides*; IV – *P. anisum*; V – *A. satureioides*; VI – black *C. sinensis*; VII – green *C. sinensis*; VIII – *M. officinalis*; IX – *C. citratus*. *Significant differences ($p \leqslant 0.05$).

Table 2
Chlorogenic acids contents in plant infusions commonly consumed in South America[a]

Samples	3-CQA[b]	4-CQA[b]	5-CQA[b]	3-FQA[b]	4-FQA[b]	5-FQA[b]	3,4-diCQA[b]	3,5 -diCQA[b]	4,5-diCQA[b]	CA[b]	GA[b]
Green *I. paraguariensis* brand A	21.73 ± 1.32	10.73 ± 0.48	14.75 ± 0.57	0.48 ± 0.02	0.27 ± 0.01	0.06 ± 0.00	5.37 ± 0.12	29.23 ± 0.07	15.19 ± 0.34	0.19 ± 0.01	Nd[c]
Green *I. paraguariensis* brand B	24.71 ± 0.11	10.23 ± 0.10	14.39 ± 0.25	0.65 ± 0.03	Tr[c]	0.56 ± 0.01	5.06 ± 0.09	26.88 ± 0.27	13.12 ± 0.23	0.20 ± 0.01	Nd[c]
Green *I. paraguariensis* brand C	20.96 ± 1.12	9.48 ± 0.50	14.90 ± 0.90	0.56 ± 0.02	0.04 ± 0.00	0.35 ± 0.00	3.58 ± 0.08	18.58 ± 0.40	10.19 ± 0.25	0.11 ± 0.00	Nd[c]
Toasted *I. paraguariensis* brand A	4.33 ± 0.04	5.55 ± 0.09	7.51 ± 0.19	0.25 ± 0.00	0.26 ± 0.01	0.33 ± 0.01	1.51 ± 0.00	2.10 ± 0.01	3.29 ± 0.15	0.15 ± 0.00	Nd[c]
Toasted *I. paraguariensis* brand B	3.10 ± 0.02	3.84 ± 0.02	5.85 ± 0.17	0.18 ± 0.00	0.21 ± 0.01	0.34 ± 0.00	1.20 ± 0.01	1.41 ± 0.05	2.59 ± 0.03	0.12 ± 0.00	Nd[c]
Toasted *I. paraguariensis* brand C	1.88 ± 0.01	2.41 ± 0.02	3.78 ± 0.17	0.09 ± 0.00	0.17 ± 0.01	0.23 ± 0.00	0.44 ± 0.00	0.63 ± 0.00	0.97 ± 0.02	0.02 ± 0.00	Nd[c]
B. genistelloides brand A	1.96 ± 0.02	1.57 ± 0.04	3.23 ± 0.04	0.09 ± 0.00	Tr[c]	0.09 ± 0.00	1.20 ± 0.05	3.20 ± 0.05	2.28 ± 0.06	0.10 ± 0.00	Nd[c]
B. genistelloides brand B	0.89 ± 0.03	0.87 ± 0.02	2.08 ± 0.02	0.07 ± 0.00	Tr[c]	0.10 ± 0.00	0.73 ± 0.03	1.89 ± 0.01	1.38 ± 0.02	0.10 ± 0.00	Nd[c]
B. genistelloides brand C	0.12 ± 0.01	0.27 ± 0.01	1.08 ± 0.03	0.09 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.70 ± 0.01	1.59 ± 0.04	0.56 ± 0.01	0.05 ± 0.00	Nd[c]
A. satureioides brand A	0.12 ± 0.01	0.14 ± 0.00	0.69 ± 0.03	Nd[c]	Nd[c]	Tr[c]	0.94 ± 0.02	1.06 ± 0.03	0.53 ± 0.01	0.08 ± 0.00	Nd[c]
A. satureioides brand B	0.11 ± 0.00	0.13 ± 0.01	0.52 ± 0.02	Nd[c]	Nd[c]	Tr[c]	0.75 ± 0.03	1.12 ± 0.03	0.39 ± 0.01	0.09 ± 0.00	Nd[c]
A. satureioides brand C	0.05 ± 0.00	0.03 ± 0.00	0.16 ± 0.01	Nd[c]	Nd[c]	Tr[c]	0.14 ± 0.00	0.57 ± 0.02	0.27 ± 0.02	0.07 ± 0.00	Nd[c]
P. anisum brand A	0.22 ± 0.01	0.15 ± 0.01	0.71 ± 0.01	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.10 ± 0.00	1.07 ± 0.00	0.29 ± 0.00	Nd[c]	Nd[c]
P. anisum brand B	0.24 ± 0.01	0.14 ± 0.01	0.92 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.12 ± 0.00	Tr[c]	0.91 ± 0.02	Tr[c]	Nd[c]	Nd[c]
P. anisum brand C	0.20 ± 0.01	0.23 ± 0.01	0.96 ± 0.02	0.02 ± 0.00	0.02 ± 0.00	0.12 ± 0.01	0.07 ± 0.00	0.21 ± 0.00	0.23 ± 0.00	Nd[c]	Nd[c]
Black *C. sinensis* brand A	0.68 ± 0.03	0.98 ± 0.04	0.72 ± 0.03	Tr[c]	Tr[c]	Nd[c]	Nd[c]	Nd[c]	Nd[c]	Nd[c]	0.40 ± 0.01
Black *C. sinensis* brand B	0.43 ± 0.02	0.85 ± 0.03	0.36 ± 0.02	Tr[c]	Tr[c]	Nd[c]	Nd[c]	Nd[c]	Nd[c]	Nd[c]	0.74 ± 0.01
Black *C. sinensis* brand C	0.33 ± 0.00	0.76 ± 0.02	0.24 ± 0.01	Tr[c]	Tr[c]	Nd[c]	Nd[c]	Nd[c]	Nd[c]	Nd[c]	0.55 ± 0.01
Green *C. sinensis* brand A	0.36 ± 0.00	0.84 ± 0.02	0.37 ± 0.01	Tr[c]	Tr[c]	Tr[c]	Nd[c]	Nd[c]	Nd[c]	Nd[c]	0.11 ± 0.01
Green *C. sinensis* brand B	0.36 ± 0.02	0.80 ± 0.04	0.26 ± 0.00	Tr[c]	Tr[c]	Tr[c]	Nd[c]	Nd[c]	Nd[c]	Nd[c]	0.18 ± 0.00
Green *C. sinensis* brand C	0.10 ± 0.00	0.10 ± 0.00	0.34 ± 0.00	Tr[c]	Tr[c]	Tr[c]	Nd[c]	Nd[c]	Nd[c]	Nd[c]	0.22 ± 0.01
C. citratus brand A	0.04 ± 0.00	0.04 ± 0.00	0.52 ± 0.02	0.15 ± 0.00	Tr[c]	0.03 ± 0.00	0.30 ± 0.01	0.24 ± 0.01	0.02 ± 0.00	Nd[c]	Nd[c]
C. citratus brand B	0.02 ± 0.00	0.02 ± 0.00	0.41 ± 0.02	0.07 ± 0.00	Tr[c]	0.04 ± 0.00	0.21 ± 0.01	0.08 ± 0.00	0.01 ± 0.00	Nd[c]	Nd[c]
C. citratus brand C	0.02 ± 0.00	0.02 ± 0.00	0.30 ± 0.00	0.13 ± 0.00	Tr[c]	0.04 ± 0.00	Tr[c]	0.08 ± 0.00	0.03 ± 0.00	Nd[c]	Nd[c]
M. officinalis brand A	1.08 ± 0.03	0.77 ± 0.02	0.98 ± 0.00	0.03 ± 0.00	Tr[c]	Tr[c]	0.35 ± 0.01	2.15 ± 0.09	0.72 ± 0.02	0.01 ± 0.00	Nd[c]
M. officinalis brand B	0.03 ± 0.00	0.01 ± 0.00	0.11 ± 0.00	Nd[c]	Nd[c]	Nd[c]	0.07 ± 0.00	Tr[c]	0.57 ± 0.00	0.14 ± 0.00	Nd[c]
M. officinalis brand C	Tr[c]	Tr[c]	0.02 ± 0.00	Nd[c]	Nd[c]	Nd[c]	0.01 ± 0.00	0.01 ± 0.00	Nd[c]	0.16 ± 0.00	Nd[c]

[a] Results are shown as the means of extractions in duplicates ±standard deviation, expressed in mg per cup (200 mL).
[b] CQA = caffeoylquinic acid; FQA = feruloylquinic acid; diCQA = dicaffeoylquinic acid; CA = caffeic acid; GA = gallic acid.
[c] Nd = not detected (under detection limit of 1.70 μg/mL); Tr = trace amount (above detection limit but below quantification limit of 5.00 μg/mL).

All other seven investigated plants showed total CGA contents lower than 80 mg/100 g (dwb) (Table 1). Nine CGA compounds were identified in leaves of *C. oblonga*. To our knowledge, from these compounds, only 3-CQA, 4-CQA, 5-CQA and 3,5-diCQA have been previously identified in *C. oblonga* fruit jams (Silva, Andrade, Martins, Seabra, & Ferreira, 2006), with no reports for the leaves. This is also apparently the first time that CGA compounds are quantified in *M. ilicifolia*, *A. muricata*, *G. biloba*, *P. boldus* and *S. cumini* leaves, and in peels of *E. velutina*. Amongst them, *M. ilicifolia* stood out for having the highest CGA contents (80 mg/100 g, dwb).

The presence of phenolic acids (caffeic, syringic, *p*-coumaric, gallic, sinapinic, ferulic and vanillic) was also investigated in the plant extracts. However, only caffeic and gallic acids were identified. Caffeic acid was identified in almost all investigated extracts (Table 1), which highlights its importance to plant metabolism (Farah & Donangelo, 2006). In addition, potential health properties to humans have been reported, such as antioxidant (Yen, Duh, & Su, 2005), antiviral (Chiang, Chiang, Chang, Ng, & Lin, 2002) and antidepressive (Takeda, Tsuji, Inazu, Egashira, & Matsumiya, 2002). Caffeic acid contents ranged from 0.4 mg/100 g, dwb (*C. oblonga*) to 39.3 mg/100 g, dwb (*M. officinalis*). The contents of

caffeic acid found in *M. officinalis* and *I. paraguariensis* were similar to those reported in the literature (Filip et al., 2001; Zgorka & Glowniak, 2001; respectively). Small amounts of gallic acid were observed in *C. sinensis* (green and black), *A. muricata* and *S. cumini* extracts. The presence of gallic acid in green and black *C. sinensis* extracts was consistent with the typically high amounts of epigallocatechins in this plant (Nishitani & Sagesaka, 2004). Although in the present investigation caffeic and gallic acids were the only phenolic acids detected in *G. biloba* leaves, other phenolic acids such as *p*-coumaric acid have been previously observed in these leaves, with variable contents according to the harvest season (van Beek, 2002).

In order to evaluate the actual consumption of CGA and related compounds in *I. paraguariensis*, *B. genistelloides*, *P. anisum*, *A. satureioides*, *C. sinensis*, *M. officinalis* and *C. citratus* – the main sources of CGA amongst the investigated plants – through plant infusions and considering that the CGA content in plants may vary not only according to genetics but also according to climate, soil, agricultural practises and methods of extraction (Farah et al., 2006, Farah & Donangelo, 2006), infusions were prepared with three commercial samples of each of these seven plants from different origins and their CGA contents were determined.

3.2. Chlorogenic acids and related compounds in medicinal plant infusions

The infusions showed similar CGA profiles with small variations in the distribution of individual isomers. Even though significant differences were observed between both methanolic and aqueous extractions in most of the plants (6 amongst 9), these differences were small and indicate that a satisfactory extraction occurs during infusions preparation (Fig. 2). The contents of individual CGA compounds expressed as mg/200 mL are depicted in Table 2.

Most commercial brands of the same medicinal plants showed similar distribution of CGA compounds. However, a significant variation in the contents of individual isomers was observed for some of the plants, such as *C. sinensis* and *M. officinalis* (Table 2). Total CGA contents varied from 0.04 mg (*M. officinalis* – lemon grass) to 97.8 mg/200 mL (green *I. paraguariensis* – green maté). Data on the CGA content in beverages and other food material are rare in the literature, except for coffee, one of the best sources of CGA. However, comparing the CGA data from plant infusions with those of coffee brews is unfair because coffee brews are prepared with 6–20% of solid material whilst the plant infusions were prepared at 0.5%, which was the amount suggested or packed by most manufacturers for one cup (200 mL) serve. Nevertheless, even though coffee brews are much more concentrated, their CGA content may be comparable to that observed in green *I. paraguariensis* infusion (about 91 mg/200 mL). A cup (200 mL) of medium roasted *C. canephora* cv. Conillon at 10% contains about 140 mg of CGA, whilst a cup prepared from dark roasted beans contains 8 mg of CGA (unpublished data). It is also possible to say that the total CGA content observed in *I. paraguariensis* is comparable to that of total phenolic compounds in red wine (about 130 mg/200 mL) (Greenrod, Stockley, Burcham, Abbey, & Fenech, 2005) and that 5-CQA content in green *I. paraguariensis* (about 15 mg/200 mL) is similar to that previously observed in cherry juice (17 mg/200 mL) (Shahrzad & Bitsch, 1996), being both beverages known as good sources of phenolic compounds (Greenrod et al., 2005; Shahrzad & Bitsch, 1996).

The peaks with *m*/z compatible with CFQA, *p*-CoQA and CGL compounds that were identified in the methanolic extracts were also observed in the infusions. 3-CQL and 4-CQL were identified and quantified only in the infusions of toasted *I. paraguariensis*. 3-CQL contents in the infusions were 0.82 ± 0.04 mg, 0.80 ± 0.02 mg and 0.61 ± 0.03 mg/200 mL, whilst 4-CQL contents were 0.77 ± 0.03 mg, 0.64 ± 0.02 mg and 0.61 ± 0.02 mg /200 mL.

The presence of phenolic acids was also investigated in the infusions and, as with the methanolic extracts, only caffeic and gallic acids were identified. Caffeic acid was found in green and toasted *I. paraguariensis*, *B. genistelloides*, *A. satureioides* and *M. officinalis* infusions (Table 2). In various plant infusions such as in *M. officinalis*, caffeic acid contents showed significant variation amongst the investigated brands. Gallic acid was only identified in green and black *C. sinensis* infusions (Table 2).

In general, the highest CGA contents were observed in plants in which properties such as antioxidant (Bastos et al., 2007; Cheel et al., 2005), hepatoprotective (Anderson & Fogh, 2001) and antiviral (Yam, Shah, & Hamilton-Miller, 1997), have been observed in *in vitro*, animal and/or epidemiological studies. Interestingly, these properties have been previously associated with the presence of CGA in some of the investigated plants and in other food material such as coffee, propolis, artichoke and *Plantago major* extracts (Basnet, Matsushige, Hase, Kadota, & Namba, 1996; Chiang et al., 2002; Farah & Donangelo, 2006; Gebhardt & Fausel, 1997). Therefore, we suggest a thorough investigation of the presently studied plants with expressive CGA contents for antioxidant, hepatoprotective and antiviral properties.

In conclusion, in the present work, CGA and related compounds were identified and quantified in different medicinal plants. The highest CGA contents were observed in *I. paraguariensis*, *B. genistelloides*, *P. anisum*, *A. satureioides*, *C. sinensis*, *M. officinalis* and *C. citratus* infusions. However, factors such as climate, seasons, soil and agricultural practises should be considered when comparing the chemical composition of these plants. *I. paraguariensis* and *B. genistelloides* were good sources of both CQA and diCQA compounds, whilst *C. sinensis* contained more CQA and *A. satureioides* more diCQA compounds. CGA contents in the infusions were very similar to those in the methanolic extracts indicating that a satisfactory extraction of CGA occurs during home preparation of infusions. These CGA-rich plants commonly used as energy drink or as plant infusions deserve attention regarding the pharmacological properties attributed to CGA in the literature. However, we cannot assert that all plants with high CGA content are good sources of CGA compounds to humans because the influence of food matrix on CGA bioavailability is still unknown. In the same way, it would not be correct to say that the lower contents observed in some of the investigated plants are not important to humans, since the metabolism and requirements of these compounds seem to vary amongst individuals, with no dietary recommendations established for them.

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