Phytochemical evaluation and antioxidant and toxicity activities of compounded teas indicated for weight loss

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ABSTRACT

The use of teas that contain antioxidant substances may produce a protective effect on the body and aid in the process of weight loss. This study aims to evaluate the antioxidant activity, secondary metabolite content, and toxicity in three commercially available slimming teas in Brazil. Preliminary phytochemical analysis followed traditional colorimetric techniques. The content of polyphenols, flavonoids, condensed tannins and alkaloids were quantified by UV-Vis spectrophotometry. In order to evaluate the antioxidant capacity, the DPPH radical capture method was used. The evaluation of cytotoxicity occurred in *Artemia salina* and the genotoxicity test in *Allium cepa*. Metabolite assays confirmed their positivity in the preliminary phytochemical tests. In the preliminary phytochemical analysis, condensed tannins, flavonoids, catechins, steroids, saponins, and alkaloids were identified. The assays of the secondary metabolites presented a considerable number of polyphenols and flavonoids and a lower concentration of condensed tannins and alkaloids. The DPPH radical capture method showed higher antioxidant activity for the samples obtained by maceration and infusion. The teas showed no cytotoxicity, although a high number of cellular abnormalities was observed, which indicates genotoxicity. Additionally, the samples did not show toxicity to *A. salina*. Further tests are necessary to confirm the safety of using slimming teas.
Key words: Anorectics, Antioxidants, Cytotoxicity, Genotoxicity, Phenolic compounds.

INTRODUCTION

Medicinal plants have been traditionally used in many cultures. Despite the widespread use of these plants, most of their actions have not been scientifically proved, hence some may lead to intoxication or even death. Thus, understanding the characteristics of each plant through scientific, pre-clinical or clinical tests is necessary so that people may be able to use them as medicine (Veiga Júnior et al. 2005). Studies have shown that the consumption of antioxidant substances may produce protective action against oxidative processes, which occur naturally in the body or when endogenous antioxidant production is no longer sufficient (Degáspari and Waszczynskyj 2004).

Obesity is one of the main reasons why people search for teas as an alternative to lose weight, especially due to their low cost and few side effects (Silveira et al. 2008). Nowadays, the use of medicinal herbs for weight loss is becoming more and more frequent as a result of their easy availability. Therefore, by considering the extensive use of weight loss teas as well as the lack of studies involving the association of plants present in these products, this study proposes to evaluate the antioxidant activity, secondary metabolite content and toxicity of three slimming teas marketed in southern Brazil.

MATERIALS AND METHODS

Plant materials

Three different brands of compound weight loss teas were used. The teas are commercially available and very popular in the central region of Rio Grande do Sul State, Brazil. The composition of these samples, as labeled in their packages, are

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TABLE 1 - Scientific and Brazilian folk names of the drugs of the weight loss teas.

Samples	Composition		
	Scientific name	**Brazilian folk name**	**Part of the plant present in the tea**
Sample 1	*Centella asiatica* (L.) Urb.	Centela	Leaves and stalks
	Fucus vesiculosus (L.)	Cavalo Marinho	Algae
	Camellia sinensis (L.) Kuntze	Chá verde	Leaves
	Cordia ecalyculata Vell.	Café bugre	Leaves and stalks
	Cassia angustifolia Vahl.	Sene	Leaves
	Mentha piperita L.	Hortelã	Leaves
	Baccharis triptera Mart.	Carqueja	Leaves and stalks
	Stevia rebaudiana (Bertoni) Bertoni	Stevia	Leaves
	Equisetum arvense L.	Cavalinha	Aerial parts
Sample 2	*Camellia sinensis* (L.) Kuntze	Chá verde	Leaves and stalks
	Camellia sinensis (L.) Kuntze	Chá branco	Leaves and stalks
	Baccharis genistelloides (Lam). Pers.	Carqueja Doce	Leaves
	Stevia rebaudiana (Bertoni) Bertoni	Stevia	Leaves
	Matricaria recutita L.	Camomila Mista	Floral chapters
	Hibiscus sabdariffa Linn.	Hibiscos	Flowers
	Lippia citriodora (Lam.) Kunth	Erva Cidreira	Leaves and stalks
	Mentha arvensis L.	Hortelã	Leaves and stalks
Sample 3	*Camellia sinensis* (L.) Kuntze	Chá Verde	Leaves and stalks
	Matricaria recutita L.	Camomila	Leaves
	Jasminum officinale L.	Jasmim	Flowers
	Melissa officinalis L.	Melissa	Leaves and stalks
	Foeniculum vulgare Mill.	Funcho	Fruits
	Cymbopogon citratus (DC.) Stapf	Capim Cidró	Leaves
	Mentha piperita L.	Hortelã	Leaves
	Stevia rebaudiana (Bertoni) Bertoni	Estévia	Leaves
	Hibiscus rosa-sinensis L.	Hibísco	Flowers

described in Table 1. The samples were prepared as described in the package (decoction or infusion) and by maceration. Each sample was prepared by both methods once, which originated six samples. After extraction, all samples were filtered and dried in a rotary evaporator.

Assays

Preliminary phytochemical analyzes were performed for the extracts obtained by maceration and decoction/infusion of all samples. Cyanogenic heterosides, condensed and hydrolysable tannins, anthocyanins, anthocyanidins, flavonols, catechins, steroids, triterpenoids, saponins, resins, and alkaloids were searched for according to the methodology proposed by Matos (2009).

Total polyphenol content was evaluated by the colorimetric method described by Gindri et al. (2014) using the Folin Ciocalteau 2 *N* reagent and spectrophotometer reading at 730 nm. Samples were tested at a concentration of 0.100 mg/ml. Total polyphenol content was calculated by preparing an analytical curve using gallic acid as reference in a concentration range of 10 to 30 µg/ml ($y = 0.0489x + 0.0059$, $R^2 = 0.9986$). The flavonoid assay was performed according to the colorimetric method in which a 2% aluminum chloride solution and spectrophotometer reading at 420 nm were used. The samples were tested at a concentration of 0.100 mg/ml. A quercetin analytical curve in the range of 2 to 18 µg/ml ($y = 0.0487x - 0.0365$, $R^2 = 0.9989$) was used to calculate the flavonoid content. The determination of condensed tannins used 8% hydrochloric acid and 1% vanillin solutions. The reading was performed at 500 nm. Samples were tested at a concentration of 30 mg/ml. The assay

was performed in triplicate and an analytical curve with the standard catechin was used in the concentration of 30 µg/ml (y = 0.0193x + 0.0177, R^2 = 0.9896) to determine condensed tannin content (Gindri et al. 2014). Alkaloid determination followed a technique described by Oliveira et al. (2006) using Dragendorff Reagent. The mixture of nitric acid and thiourea was used as a blank and the reading was performed at 435 nm. The entire assay was performed in triplicate and the bismuth nitrate analytical curve was used in the concentration of 10 to 90 µg/ml (y=2.2783x+0.0361, R^2= 0.9994). The samples were tested at a concentration of 10 mg/ml.

In order to evaluate antioxidant capacity, the colorimetric method of DPPH (2,2-diphenyl-1-picryl-hydrazyl) was used. The samples were tested at concentrations of 500 to 7,8 µg/ml. The inhibition percentage of DPPH radicals calculated by Equation 1 is described below.

$$\% \text{ inhibition } = 100 - \frac{[(\text{Abs of sample} - \text{Abs of blank}) \text{ X } 100]}{\text{Abs of control}}$$

Equation 1. Calculation of inhibition percentage where: Abs of the Sample: absorbance of the tea extracts with the addition of DPPH solution; Abs of the blank: absorbance of the tea extracts without the addition of DPPH solution; Abs of control: absorbance of the DPPH solution.

After calculations, a graph with the inhibition percentage versus the extract concentration was constructed and the equation of the line calculated, which was used to calculate the IC_{50} index (concentration of the sample able to inhibit 50% of oxidative activity of the DPPH radical) (Mensor et al. 2001).

For cytotoxicity in *Artemia salina*, eggs were hatched between 22-29 ºC in artificial saline according to a method described by Silva et al. (2010). After 24 h, ten nauplii were transferred to Petri dishes containing artificial saline and tea extracts. The count of live and dead nauplii was performed after 24 h. Only artificial saline was used as negative control. As a positive control, sodium lauryl sulfate at concentrations of 100, 10, and 1µg/ml. The samples were tested at concentrations of 1000, 500, and 100 µg/ml.

For genotoxicity in *Allium cepa* rootlets, 14 groups of 5 bulbs were initially placed to root in distilled water. After this period, the groups were submitted to the following treatments: T1= negative control (NC) in distilled water; T2-T13 = Samples 1, 2, and 3 obtained by infusion/decoction and maceration process of the teas; T14 = Positive Control in glyphosate 2%. Groups were submitted to the treatments for 24 h. Bulbs were then transferred to the ethanol-acetic acid solution (3:1) for 24 h. After this period, bulbs were collected and removed from this solution and kept in 70% alcohol. To evaluate the antiproliferative potential, rootlets were collected, hydrolyzed in 1*N* hydrochloric acid, and washed in distilled water. After that, the meristematic region of the rootlet was selected and the slides prepared by the crushing technique (Guerra and Souza 2002). A 2% acetic orcein solution was used as dye. The slides were examined by observing the cell cycle phases (interphase, prophase, metaphase, anaphase, telophase) in an optical microscope. The samples were tested at concentrations of 1000 and 100 µg/ml.

One-way ANOVA was applied in order to evaluate any significant difference between the samples. The averages were compared using the Tukey test considering a significance level of p=0.05. In the *A. salina* assay, the samples were evaluated through the Probitos analysis. All statistical tests were performed using the software Statistica® (Statsoft, version 7.0).

RESULTS AND DISCUSSION

Acronyms were defined for the samples as well as for the different extraction types for a better understanding. The acronyms and yield of each fraction are shown in Table 2.

The teas studied are composed of an association of several traditional medicinal plants, some with their uses and chemical composition already defined. In order to facilitate the reading and understanding of the results, a bibliographic review of the pharmacological activities and chemical composition of the plants that compose the slimming teas was carried out (Table 3).

The antioxidant activity is the most frequently observed. Oxidative stress is commonly associated with changes in serum glucose and lipid concentrations (Barreiros et al. 2006). Thus, antioxidant activity already described for the species may be correlated with the weight-loss action of the teas. Additionally, the presence of plants with diuretic properties, which help eliminate fluids, is observed. This causes tea drinkers to expel excess fluids from their bodies, often reducing their weight. Furthermore, the laxative action evidenced in some plants leads tea drinkers to eliminate more fecal matter on a more frequent basis, which may also aid in weight loss.

Preliminary phytochemical analysis detected condensed tannins, catechins, steroids and triterpenoids, and saponins in all samples as seen in Table 4. Anthocyanins, anthocyanidins, and flavonoids were only observed in Samples 1 and

TABLE 2 - Yields obtained for the samples submitted to different types of extraction.

Sample	Types of extraction and acronyms	Yield (%)
Sample 1	Maceration - A1M	16.6 %
	Infusion - A1I	15.4 %
Sample 2	Maceration - A2M	9.8 %
	Infusion - A2I	12.5 %
Sample 3	Maceration - A3M	18.3 %
	Infusion - A3I	12.4 %

TABLE 3 - Pharmacological properties and chemical constituents of the plant species that make up the slimming teas.

Plants	Pharmacological properties	Chemical constituents
Baccharis genistelloides (Sample 2)	Analgesic, antidiabetic, antifungal, anti-inflammatory, antileukemic, antimicrobial, antioxidant, antiviral, spasmodic (Karam et al. 2013)	Flavonoids, flavones, isoflavones, anthocyanidines, condensed tannins (Karam et al. 2013)
Camellia sinensis (Samples 1, 2 e 3)	Antiulcerous, astringent, antioxidant, antibacterial and antiviral (Souza et al. 2011), antioxidant (Schmitz et al. 2005), weight reducer (Abeso 2011), hypocholesterolemiant (Batista et al, 2009)	Flavonoids, essential oils (Souza et al. 2011), tannins (Robbers et al. 1997), catechins (Matsubara and Rodrigues-Awaya, 2006)
Cassia angustifólia (Sample 1)	Laxative (Robbers et al. 1997; Rocha et al. 2006; Barnes et al., 2012)	Flavonoids, anthraquinones (Robbers et al. 1997), carbohydrates (Barnes et al. 2012)
Centella asiática (Sample 1)	Anti-inflammatory, healing, (Souza et al. 2011), antibacterial (Barnes et al. 2012)	Triterpenes, saponins (Souza et al. 2011), flavonoids (Barnes et al. 2012)
Cordia ecalyculata (Sample 1)	Tonic, diuretic, appetite-reductor, antiviral (Cardozzo et al. 2008)	Saponins, alkaloids, cardiotonic heterosides, phenolic compounds, flavonoids, coumarins, tannins (Cardozzo et al. 2008)
Cymbopogon citratus (Samples 1and 3)	Analgesic, antispasmodic and anxiolytic (Souza et al. 2011)	Essential oils (Souza et al. 2011), catechins (Matsubara and Rodrigues-Awaya 2006)
Equisetum arvense (Sample 1)	Diuretic, anti-inflammatory, digestive, hypotensive, healing, antioxidant, hypoglycemic (Mello et al. 2013)	Flavonoids, alkaloids, saponins and tannins (Mello et al. 2013)
Foeniculum vulgare (Sample 3)	Carminative and antispasmodic (Souza et al. 2011).	Essential oils (Souza et al. 2011)
Fucus vesiculosus (Sample 1)	Reduces cholesterol metabolism, antiviral, weight loss, hypothyroidism treatment (Paiva 2011), anticoagulant (Barnes et al. 2012)	Polysaccharides, mucilage, polyphenols, vitamins and pro-vitamins A and D, mineral salts (Paiva 2011; Barnes et al. 2012)
Hibiscus rosa-sinensis (Sample 3)	Antitumoral, antidiabetic, antioxidant, diuretic (Borba 2010)	Flavonoids, polyphenols, carotene, niacin, riboflavin, anthocyanin (Borba 2010)
Hibiscus sabdariffa (Sample 2)	Soothing, antioxidant, antiseptic, astringent, digestive, diuretic, antihypertensive, treatment of obesity, laxative (Freitas et al. 2013)	Saponins, phenols, condensed tannins, terpenes, alkaloids, flavonoids, hydrolysable tannins, steroids, resins (Freitas et al. 2013)
Jasminum officinale (Sample 3)	Antioxidant, antimicrobial, anticholinesterase (Alpiavezza et al. 2012)	Flavonoids, tannins, alkaloids, saponins, mucilage, anthracenes, steroids, essential oils, triterpenes (Alpiavezza et al. 2012)
Lippia citriodora (Sample 2)	Digestive, antidepressant, sedative, antiseptic (Souza et al. 2011)	Essential oil (Souza et al. 2011)

TABLE 3 - Continuation

Plants	Pharmacological properties	Chemical constituents
Matricaria recutita (Samples 2 and 3)	Anti-inflammatory, soothing, anxiolytic, antiallergic, antispasmodic, cicatrization (Robbers et al. 1997; Lucca et al. 2010; Souza et al . 2011), antibacterial, antifungal antispasmodic, antiulcerogenic (Barnes et al. 2012; Souza et al. 2011)	Essential oil (Lucca et al. 2010; Souza et al. 2011), flavonoids, terpenes (Sartori et al. 2003; Souza et al. 2011), coumarins (Barnes et al. 2012), catechins (Matsubara and Rodrigues-Awaya 2006). Mucilage (Souza et al. 2011)
Melissa officinalis (Sample 3)	Sedative, spasmolytic, antibacterial, antifungal, carminative, hypotensive, soothing, antiviral (Barnes et al. 2012; Souza et al. 2011).	Essential oil (Barnes et al. 2012; Souza et al. 2011).
Mentha sp. (Samples 1 and 3)	Carminative (Robbers et al. 1997), analgesic, anti-inflammatory, antiseptic, anesthetic (Souza et al. 2011)	Essential oil (Robbers et al. 1997; Souza et al. 2011), catechins (Matsubara and Rodrigues-Awaya 2006)
Stevia rebaudiana (Samples 1, 2 and 3)	Hypoglycemic (Cecílio et al., 2008), cardiotonic, antacid (Jarma et al. 2010)	Diterpenes glycosides (Cecílio et al. 2008)

2 and alkaloids in the three samples obtained by maceration.

According to the literature review (Table 3), no plant was described to possess cyanogenic heterosides, reinforcing the data obtained in this study as observed in Table III. The absence of these compounds in slimming teas can be considered beneficial since their presence is an indicative of hydrocyanidric acid presence. This acid has an uncertain effect on the body; however, it is known that cyanide ions absorbed in small proportions react with a cysteine derivative to form thiocyanates and thiosulphates. Moreover, thiocyanate elevation in the blood is related to the appearance of neuropathies (Fracaro 2004).

All samples showed positive results for catechins. Their presence in *C. sinensis* and *Mentha sp,* which were components of Samples 1, 2, and 3 and *M. recutita* and *C. citratus*, which were only present in Sample 3, has been described (Matsubara and Rodrigues-awaya 2006), confirming the results obtained in this study. The catechins belong to a colorless and water-soluble compound group of polyphenols that contribute to astringency and bitterness (Matsubara and Rodrigues-awaya 2006). Catechin is one of the main therapeutic chemical components of the species *C. sinsensis* and it is a potent antioxidant (Schmitz et al. 2005) that may aid in the pharmacological effects promised in teas.

The presence of steroids detected in

TABLE 4 - Preliminary phytochemical analysis of slimming teas.

	Sample 1		Sample 2		Sample 3	
Phytochemicals groups	**A1M**	**A1I**	**A2M**	**A2I**	**A3M**	**A3I**
Cyanogenic heterosides	-	-	-	-	-	-
Condensed tannins	+	+	+	+	+	+
Hydrolysable tannins	-	-	-	-	-	-
Anthocyanins	-	-	-	-	-	-
Anthocyanidins	-	-	-	-	-	-
Flavonols	+	+	+	+	-	-
Catechins	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Triterpenoids	-	-	-	-	-	-
Saponins	+	+	+	+	+	+
Resins	-	-	-	-	-	-
Alkaloids	+	-	+	-	+	-

M: maceration; I: infusion/decoction.

teas may have occurred due to the presence of *J. officinale* in Sample 3 and *H. Sabdariffa* in Sample 2 (Alpiavezza et al. 2012; Freitas et al. 2013). According to Matos (2009), persistent and abundant foam (collar) indicates the presence of saponins (saponin heterosides). All samples showed a positive result for saponins in preliminary phytochemical analysis (Table III). Saponins are found in several species, although their presence must be highlighted in *C. ecalyculata* (Cardozzo et al. 2008), *C. asiatica* (Souza et al. 2011), and *E. arvense* (Mello and Budel 2013) in Sample 1, *H. sabdariffa* (Freitas et al. 2013) in Sample 2, and *J. officinale* (Alpiavezza et al. 2012) in Sample 3, respectively.

Saponins are glycosides of steroids or polycyclic terpenes that when in aqueous solution form persistent and abundant foam that present a lipophilic part, called aglycone or sapogeny, and another hydrophilic part consisting of sugars in their structure. Studies have shown that the use of saponins in nutrition reduces serum cholesterol levels (Simões et al. 2010), which may favor the action promised by slimming teas. In addition, saponins have antiviral, anti-inflammatory, expectorant, and diuretic properties (Simões et al. 2010).

In order to provide more information about the studied samples, total polyphenols, flavonoids, condensed tannins, and alkaloids were quantified in the tea samples. These results are described in Table IV. Polyphenols are heterogeneous compounds, which have antioxidant properties, are present in considerable amounts in the vast majority of food and medicinal plants (Simões et al. 2010; Vargas et al, 2008). In this study, in addition to the positive results for groups belonging to this class of metabolites in preliminary phytochemical analysis (Table 4), these compounds were also quantified in all samples as observed in Table 5.

The samples obtained by the maceration process presented higher concentrations of polyphenols (Table 5). The A1M sample (79.83 ± 9.39 mg of galic acid equivalents/g sample) presented a value which was two times higher than the values obtained for the second and third samples with higher polyphenols content, A3M (38.59 ± 3.33 mg/g) and A2M (34.84 ± 5.4 mg/g), hence presenting statistical difference for these last samples (Table 5).

Several studies have confirmed the presence of phenolic compounds in the chemical composition of different plant species, among which *H. rosa sinensis* L. (Borba 2010), *B. genistelloides* Lamark (Karam et al. 2013), *H. sabdariffa* L. (Freitas et al. 2013), *F. vesiculosus* (Paiva 2011; Barnes et al. 2012), and *C. ecalyculata* should be highlighted (Cardozzo et al. 2008). These plant species are part of the composition of the weightloss teas studied (Table 1), hence reaffirming the presence of these compounds in the samples tested.

The technique of extraction by maceration enabled a greater extraction of polyphenols than infusion and decoction. In this study, 50% hydroalcoholic solvent was used in maceration, and just distilled water for the infusion and decoction processes. Moreover, the stability of phenolic compounds during dehydration and extraction may be affected by chemical and enzymatic degradations and compound volatilization. However, thermal decomposition has been pointed out as the leading factor to polyphenol content reduction (Andreo and Jorge 2006), which explains the lower polyphenol content in the samples extracted by infusion and decoction as seen in Table 5.

During the thermal decomposition process, polyphenols may react with other components and prevent their extraction (Moure et al. 2001). Thus, the total phenolic content decreases as the temperature increases (Conde et al. 1998), similarly to the concentration of condensed tannins under the same

TABLE 5 - Content of polyphenols, flavonoid, condensed tannins and alkaloids obtained in the samples of slimming teas.

Sample	Polyphenols (mg GAE/g ext ± SD)	Flavonoid (mg QE/g ext ± SD)	Condensed tannins (mg CE/g ext ± SD)	Alkaloids (mg AE/g ext ± SD)
A1M	79.83 ± 9.39 ^{a}	19.72 ± 0.47 ^{c}	0.041 ± 0.005$^{c, d}$	25.14 ±1.49
A1I	51.78 ± 1.29 ^{b}	13.70 ± 0.37 ^{e}	0.041 ± 0.006$^{c, d}$	67.28 ± 2.46 a
A2M	34.84 ± 5.74 ^{c,d}	34.68 ±0.36 ^{a}	0.106 ± 0.010^{b}	3.14 ± 1.06
A2I	32.04 ± 2.05 ^{c,d}	16.40 ± 0.15 ^{d}	0.259 ± 0.012^{a}	16.72 ± 1.68
A3M	38.59 ± 3.33 ^{b,d}	23.83 ± 1.29 ^{b}	0.030 ± 0.003^{d}	9.17 ± 0.44
A3I	40.84 ± 5.66 ^{b,c}	15.14 ± 0.44 ^{d,e}	0.061 ± 0.008^{c}	25.14 ±1.49

mg GAE/g extract: milligrams of gallic acid equivalents per gram of extract. SD: Standard deviation; mg QE/g am: mg of quercetin equivalents per g of extract; mg CE/g am: mg of catechin equivalents per g of extract; mg AE/g am: mg equivalents of alkaloids per g extract; Different letters represent significant differences according to Tukey test ($p < 0.05$).

conditions (Cadahía et al. 1998). Along these lines, it is clear that extraction under mild temperatures is desirable when the aim is extracting degradable compounds, such as polyphenols.

One of the most important classes of phenolic compounds are flavonoids, which are widely distributed in plants (Simões et al. 2010). Their presence is found in innumerous plant species, including those present in the weight loss teas (Table 4) and detected in Samples 1 and 2 as flavonols (Table 4), which are a subclass of flavonoids. These compounds were quantified in the slimming teas (Table 5) and the sample that presented the highest value in the flavonoid quantification was A2M (34.68 ± 0.36 mg quercetin equivalents/g extract), which presented a significantly higher value than the second and third samples A3M (23.83 ± 1.29 mg/g extract) and A1M (19.72 ± 0.47 mg/g extract), respectively

Flavonoids are compounds with several functions in plants, including visible and ultraviolet (UV) protection, protection against insects, antioxidants, control of the action of plant hormones, enzyme inhibitors, and allopathic agents. Some of their pharmacological activities include antitumor, antispasmodic, anti-inflammatory, antimicrobial, antimutagenic, antioxidant, antiviral actions, among others (Simões et al. 2010). Flavonoid presence is found in several plant species and was described in many plants that are present in the slimming teas such as: *C. ecalyculata* (Cardozzo et al. 2008); *C. angustifolia* (Rocha et al. 2006; Barnes et al. 2012) and *C. asiatica* (Barnes et al. 2012; Souza et al. 2011) present in Sample 1; *B. genistelloides* (Karam et al. 2013) and *H. sabdariffa* (Freitas et al. 2013) present in Sample 2; *J. officinale* (Alpiavezza et al. 2012) and *H. rosa-sinensis* (Borba 2010) present in Sample 3; *M. recutita* (Sartori et al. 2003; Barnes et al. 2012; Souza et al. 2011) present in the Samples 2 and 3. *C. sinensis* (Souza et al. 2011) present in all samples.

Condensed tannins were identified in all slimming teas (Table 4) and presented/showed a higher concentration in those samples obtained by the infusion process (Table 5). Sample A2I (0.259 ± 0.012 mg E.A.G./g sample) presented a significant value in relation to the other samples. The presence of condensed tannins in plant species is described for *C. ecalyculata* (Cardozzo et al. 2008), *C. asiatica* (Souza et al. 2011) and *E. arvense* (Mello and Buddel 2013), species present in Sample 1; *B. genistelloides* (Karam *et al*., 2013) and *H. sabdariffa* (Freitas et al. 2013), components of Sample 2, and *J. officinale* (Alpiavezza et al. 2012), present in Sample 3.

Tannins are phenolic compounds that have the ability to precipitate proteins, which confers astringency in many fruits and plant products (Monteiro et al. 2005). Regarding their chemical structure, tannins are classified into two groups: hydrolysable tannins and condensed tannins. Condensed tannins are distributed in woody vegetables, and hydrolysable tannins are distributed in herbaceous and woody dicotyledonous vegetables. These metabolites are used in medicine to treat various diseases, including diarrhea, hypertension, rheumatism, hemorrhages, wounds, and burns. Additionally, they have antioxidant activity, free radical scavenger and anti-inflammatory properties (Simões et al. 2010).

Alkaloids were detected just in the samples obtained by maceration (Table 4), although they were quantified in all samples as observed in Table 5. Sample 1 obtained the highest concentration of these compounds, followed by Sample 3 (Table 5). Notably, alkaloid positivity in the tested samples is the description of alkaloid presence for plant species *E. arvense L*. (Sample 1 - Mello and Budel 2013), *H. sabdariffa* (Sample 2 - Freitas et al. 2013), and *J. officinale* (Sample 3 - Alpiavezza et al. 2012) as seen in Table 3. Studies have shown several pharmacological activities related to alkaloids. Aporophinoid alkaloids, for example, are compounds with cytotoxic and antitumor potential (Silva et al. 2007). Indole alkaloids, in turn, are characterized as partial agonists at the adrenergic, serotonergic, cholinergic, dopaminergic, and noradrenergic receptors (Oliveira, et al 2009). These activities are related to teas since *H. sabdariffa* (Sample 2) has pharmacological action, such as a soothing agent (Freitas et al. 2013), and *J. officinale* (Sample 3) with anticholinesterase activity (Alpiavezza et al. 2012) (Table 3).

The antioxidant activity of the teas was evaluated by DPPH radical assay. The results of CI_{50} are shown in Table 6, which represents the concentration of the sample that is able to inhibit 50% of the DPPH oxidizing radical. Thus, the lower the CI_{50} value, the better the antioxidant capacity presented by the plant.

The sample that presented the best antioxidant capacity was Sample 1 obtained by maceration (36.67 ± 1.96 µg/ml) and infusion (42.77 ± 1.40 µg/ml). These results are confirmed by the high values obtained for this tea in the phenolic content assay (79.83 mg/g for the extract obtained by maceration and 51.78 mg/g for the extract obtained by infusion as observed in Table 5. However, Sample 1 had lower values than the other samples for flavonoids in the extracts obtained by maceration and infusion (19.72 and 13.70 mg/g, respectively - Table 5). This indicates that the plant may present other phenolic compounds that are responsible for the antioxidant action of the samples. Sample A2M had a lower antioxidant capacity (59.74 µg/ml) compared

TABLE 6 - Results obtained in DPPH radical capture test, expressed as 50% inhibitory concentration of DPPH (CI_{50}) ± standard deviation (SD).

Sample	CI_{50} ± SD (µg/ml)
A1M	36.67± 1.96[d]
A1I	42.77 ± 1.40[c,d]
A2M	59.74± 5.61
A2I	48.75 ± 3.29[c]
A3M	51.02 ± 4.12
A3I	138.07± 2.84[a]

Different letters represent significant differences according to Tukey test ($p < 0.05$).

to Sample 1 and statistically equal compared to A3M (Table 6). In addition, the antioxidant capacity of Sample 2 obtained by maceration is lower than the one obtained by decoction, which does not match the results obtained in the assays. In the polyphenol assay, the samples extracted by maceration and decoction presented lower values than the other samples (34.84 mg/g and 32.04 mg/g, respectively). On the other hand, Sample A2M had the best value in flavonoid quantification (34.68 mg/g), which was obtained by the extraction of maceration. This may suggest that flavonoids are the main class of phenolic compounds in this sample.

Sample 3 extracted by maceration presented similar antioxidant capacity to Sample 2 extracted with the same technique (51.02 and 59.74 µg/ml, respectively). Similarly, both samples did not present significant differences in phenolic content (34.84 and 38.59 µg/ml, respectively - Table 5). However, flavonoid quantification in Sample 2 obtained by maceration presented a significantly higher value than those of Samples 3 and 1, which were extracted with the same technique (34.68, 23.83 and 19.72 µg/ml, respectively). In Sample 3, it was also possible to observe that when extracted by decoction, the antioxidant capacity was extremely low (138.07 µg/ml), which is the lowest value for all the samples tested. This reinforces the idea that the phenolic compound groups responsible for this activity may have been degraded during the extractive process. The use of the DPPH method demonstrated the excellent antioxidant capacity of the samples (Table 6), which is especially evident in Sample 1 obtained by maceration (36.67 ± 1.96 µg/ml) and infusion (42.77 ± 1.40 µg/ml). Such activity may be related to flavonoids present in the plants that make up slimming teas and metabolites that have an excellent antioxidant capacity (Mensor et al. 2001). Similar to the results obtained by Morais (2009), the samples in this study showed varied results due to the type of plants present in the teas and type of extraction. Additionally, these authors also observed that maceration presented a better antioxidant capacity result. This kind of extraction is done at room temperature and does not lead to the depletion of raw material, hence favoring a greater extraction of phenolic compounds and antioxidant capacity (Miyake 2016).

The cytotoxicity and genotoxicity of the samples were evaluated with the assay of *A. salina* and *A. cepa* (Tables 7 and 8, respectively).

From *A. salina*, it is possible to detect bioactive compounds and good correlation with cytotoxic activity (Amarante et al. 2011). Low cytotoxicity activity was evidenced for the slimming tea samples when compared to the positive control sodium lauryl sulfate (Table 7). With the results of the *A. salina* test (Table 7), all the samples obtained LC_{50} values (lethal concentration for 50% of the nauplii) at least 10 times higher than the positive control LSS. As a result, the teas can be considered non-cytotoxic.

In the experiment with *A. cepa* (Table 8), inhibition or stimulation of the mitotic index as well as a high number of abnormalities were evaluated. The most evident abnormalities were anaphases with disorganized chromosomes and chromosomal bridges. The samples obtained by infusion A1I (100 µg/ml and 1000 µg/ml - 0.28% and 0.23%, respectively), A2I (1000 µg/ml - 0.30%), A3I (100 µg/ml, and 1000 µg/ml - 0.27% and 0.21%, respectively) obtained a mitotic index lower than the water mitotic index. Thus, the samples studied showed to have inhibition potential for cellular mitosis. The samples obtained by maceration A1M (100 µg/ml and 1000 µg/ml - 0.42% and 0.61%, respectively), A2M (100 µg/ml and 1000 µg/ml - 0.44% and 0.99%, respectively), A3M (100 µg/ml and 1000 µg/ml - 0.65% and 1.14%, respectively), and A2I (100 µg/ml - 0.41%) obtained mitotic index higher than that of water, stimulating the mitosis of the tested plant. These results reaffirm kind of the different metabolites extracted by different extraction methods. According to Rossato et al. (2010) and Fachinetto et al. (2007), *P. sagittalis* and *A. saturioides* are plants that may inhibit mitosis, while the aqueous extracts of boldo species, such as *P. barbatus* and *P. amboinicus*, may stimulate increases in mitotic index. Additionally, the same plant can stimulate and inhibit mitotic index depending on the concentration tested (Gindri et al. 2015). Evaluation of the chromosomal alterations is only possible if the sample is in constant cell division, which enables the identification of the toxic effects and alterations during the cellular cycle (Bagatini et al. 2007). Samples extracted by the infusion method,

except for 100 µg/ml A2I, induced abnormalities in the *A. cepa* cells. This does not express a positive result, as these samples showed more abnormalities than the positive control glyphosate 2%, which is a widely known herbicide which may cause cellular abnormalities. Infusions may present a more toxic profile with mutagenic effects, which explain the results obtained (Bagatini et al. 2007).

Samples obtained by maceration showed significantly lower values. Such a difference in these values can be explained by the different extraction methods performed a different kind of metabolites (Gindri et al. 2015).

Finally, the presence of condensed tannins, flavonols, catechins, saponins, steroids, and alkaloids was identified in the slimming teas. Additionally, a considerable number of polyphenols and flavonoids as well as a lower concentration of condensed tannins and alkaloids were also evidenced. Higher concentrations of these metabolites were observed in the samples obtained by maceration. The antioxidant capacity of the products was more pronounced in the extracts from Sample 1, which was obtained by maceration and infusion, and the maceration method presented higher activity in all samples. The samples did not show cytotoxicity in *A. salina*, although a high number of cellular abnormalities was evidenced in *A. cepa* cells. Also, the reduction of the mitotic index of the test plant was observed, indicating genotoxicity.

CONCLUSION

This study demonstrated the presence of important metabolites in the tested slimming teas and that they show antioxidant activity and low cytotoxicity, although they present high genotoxicity. However, further genotoxicity assays as well as in vivo anorectic activity assays are relevant in order to confirm their efficacy and verify their safety for consumption.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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