Methylxanthines in 100 Brazilian herbs and infusions: determination and consumption

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ABSTRACT

The contents of caffeine, theophylline and theobromine were studied in 100 plants commercialized in Brazil to investigate new sources of these compounds, and evaluate the consumption of these compounds when these plants are used as infusions (teas). Therefore, two extraction methods were performed, one used an ethanol solution and one used an aqueous infusion process. The extracts were analyzed by ultra-high-performance liquid chromatography. White, green, and black tea (Camellia sinensis) and yerba mate (Ilex paraguariensis) can be considered as potential sources of both caffeine and theobromine. Boldo (Peumus boldus) was a source of theobromine, and small-leaved lime (Tilia cordata), stonebreaker (Phylantus niruri), cow's foot (Bauhinia forficata) and calendula (Calendula officinalis) presented the highest contents of theophylline. This study highlighted herbs not yet investigated for these compounds. The results obtained may be useful in future studies to evaluate the use of these plants as sources of methylxanthines in foods or drugs.

Keywords: Caffeine; Plants; Tea; Theophylline; Theobromine

INTRODUCTION

Methylxanthines are heterocyclic compounds derived from xanthine, characterized by the combination of an imidazole ring and pyrimidinedione, also known as purine alkaloids. These compounds are naturally present in products such as cocoa, coffee, teas and some medicinal herbs. Beverages, such as cola and guarana based soft drinks, also contain methylxanthines. To date, seven methylxanthines have been identified; caffeine, theobromine and theophylline are the most common types (Brunetto et al., 2007; Onãtibia-Astibia et al., 2016; Zacharis et al., 2013).

There are several pharmacological properties that relate methylxanthines to the prevention and/or treatment of respiratory problems such as apnea (Armanian et al., 2015; Mosca et al., 2014), asthma (Makino et al., 2007), and acute or chronic cough (Onãtibia-Astibia et al., 2016). When administered with agomelatine and mianserin (antidepressants), caffeine presented a synergistic effect on the antidepressant activity (Poleszak et al., 2016). Other actions of caffeine include reduced incidence of dyspraxia in children (Doyle et al., 2014), suppressed evolution of glioblastoma (Cheng et al., 2016), and protection against neurodegeneration caused by Parkinson's disease (Xu et al., 2016).

Regarding the stimulant function of caffeine, it stimulates the central nervous system and cardiac activities (Seifert et al., 2011; Trabulo et al., 2011), increasing the physical anbd mental performance, improving the sense of humor and memory (Bizzotto et al., 2013; Malinauskas et al., 2007; Pohler, 2010). Paech et al. (2016) administered caffeine to adults who were kept awake for 50 hours; the results showed that the individuals who received caffeine presented a lower reduction of performance when compared with the control group; however, they did not observe any reduction in the sensation of sleep and tiredness.

On the other hand, when administered in doses above 400 mg/day (considering an adult of 70 kg), caffeine may cause anxiety, insomnia (Chaudhary et al., 2016), tachycardia and tremor, among other undesirable symptoms (Reissig et al., 2009). At the dose of 1,000 mg/day and above, it causes body toxicity, and from 5,000 to 10,000 mg/day, it may be lethal (Seifert et al., 2011).

Theobromine and theophylline have antioxidant activity, presenting positive influence to suppress oxidative stress. Both compounds been used medically to promote diuresis, relaxation of bronchial muscle and excitation of the nervous and circulatory systems (Wu, et al, 2019). It has been shown that theobromine seems to be toxic to some animal models, including pets, however, a large number of clinical trials proved that for humans, there is no toxic effect (Martínez-Pinilla, et al 2015). van Zyl, et al, (2008) have even concluded that the use of theobromine, instead of other drugs including caffeine, to treat respiratory diseases generates higher diffusion in lung structures.

Several plant matrixes are used in Brazilian popular medicine, especially in the form of infusion, popularly known as tea, to help in the treatment of several diseases (Bieski et al., 2015; Dutra et al., 2016; Leitão et al., 2014). The Brazilian Ministry of Health, through its 'National Policy on Integrative and Complementary Practices of SUS', has encouraged the use of herbs as one form of access to alternative and complementary medications (Brasil, 2006).

Due to the different applications, effects of methylxanthines and the expressive consumption of plant infusions in Brazil, it is extremely important to know the content of methylxanthines in these plants. Even though there are several studies that quantified xanthines, we did not find a study comparing a large variety of plants used in the preparation of infusion using the same method of extraction, making it difficult, therefore, to have comparative studies among plants. The objective of this study was to quantify theobromine, theophylline and caffeine in herbs commercialized in Brazil, searching for new sources of methylxanthines, and to estimate the consumption of these compounds when these plants are used for the preparation of infusions.

MATERIAL AND METHODS

Samples and reagents
The samples of the plants were acquired in public markets and street fairs in the cities of Campinas and Americana, in the state of São Paulo, Brazil, each from three different suppliers.

Caffeine, theophylline and theobromine standards were obtained from Sigma Aldrich (St. Louis, USA). The water used in the experiments was ultra-purified in Milli-Q® system from Millipore (Kankakee, USA). Formic acid was obtained from Merck (São Paulo, Brazil), chromatographic grade acetonitrile from JT Backer (Goiânia, Brazil) and ethanol P.A. from Synth (Diadema, Brazil). All solutions were filtered through PVDF membranes of 0.22 μm porosity from Millipore (Kankakee, USA).

Sample preparation
About 40 g of sample from the three suppliers were homogenized and milled in a hammer mill (Marconi TE 600, Piracicaba, Brazil), on a 200 mesh porosity sieve. The extraction of methylxanthines was made through two methods: one with ethanol: water solution and one with a hot aqueous infusion, simulating the traditional popular preparation.

The extraction with the ethanol solution was performed according to Meinhart et al., 2017. About 0.5 g of milled sample was weighed and 15 mL of extractive solution (74% water:26% ethanol) were added to hermetically sealed tubes. The tubes were placed in a water bath at 60°C and shaken at 240 rpm (rotations per minute) for 22 minutes. After that, the volume was measured, the extracts were filtered and then injected. Only the samples that presented the concentration of methylxanthines above the quantification limit were submitted to the infusion extraction.

For the aqueous extraction, it was used a form of preparation usually used by Brazilian consumers and also described by da Silveira et al., 2014 as the best preparation for the extraction of phenolic compounds. For this purpose, 300 ml of boiling water (amount equivalent to a mug) were added to 2 g of the sample (amount equivalent to a commercial sachet); the solution remained in infusion for 16 minutes. Then, the mixture was cooled, the volume was measured, and the extracts were filtered and injected.

Methylxanthine analysis method
An ultra-efficient liquid chromatography (UPLC) Acquity® (Waters, Morganville, USA) equipment was used with a diode arrangement detector. The column was a C18 Kinetex®, 100 mm long, 2.1 mm internal diameter, and 1.7 μm particle size (Phenomenex, Torrance, USA), with mobile phases and gradient as described by Meinhart et al., 2017. The compounds were identified by comparison with standards through the retention time, DAD absorption spectrum at 274 nm, and by co-chromatography. The method was validated and data was analyzed with the software Statistica 7.0 (Statsoft, Tulsa, USA).

Evaluation of consumption and comparison with other foods and beverages
From the results obtained in the aqueous infusions, the content of methylxanthines was estimated in home measures to estimate how much is consumed. In addition, the contents were correlated with other foods and beverages reported in the literature.

RESULTS AND DISCUSSION

Analytical method validation
The limits of detection and quantification (evaluated as three and six times the signal-noise intensity) were adequate,

with the quantification limit smaller than 0.06 mg L^{-1}. The precision values (within the day and between days) were lower than 6.6% for all levels (limit of quantification, intermediate concentration and maximum concentration of the curve). The linear range was adequate and did not present lack of adjustment, with F values lower than the critical F (3.49), with 95% confidence. The parameters observe the limits issued by IUPAC (2002), showing that it is recommended for quantitative analyses with analytical safety. Fig 1 show HPLC analysis results of standard, water-extracted, and ethanol-extracted samples of *Ilex paraguariensis* sample.

Theobromine

Table 1 shows the content of methylxanthines determined in samples. Out of the 100 samples, 61 presented at least one of the methylxanthines analyzed in this study; the samples that did not result in quantifiable concentrations are presented at the end of the Table 2.

Regarding the content of theobromine extracted by ethanol as a ethanol solution, 29 samples presented concentration between 1.45 mg kg^{-1} [pennyroyal, (Mentha pylegium)] and 895.79 mg kg^{-1} [yerba mate (Ilex paraguariensis)]; of these, 24 samples showed contents below 66.55 mg kg^{-1} and only five presented values above 200.00 mg kg^{-1}: boldo (Peumus boldus), black, white and green tea (Camellia sinensis) and yerba mate (Ilex paraguariensis). Several studies reported the identification and quantification of theobromine in Camellia sinensis (Wang et al., 2011; Zielinski et al., 2016) and yerba mate (Meinhart et al., 2010; Urfer et al., 2002); however, the authors did not find in the literature studies that correlated boldo with theobromine.

When the extraction was performed with water and infusion process, only 26 samples presented quantifiable concentration of theobromine, since the values were between 1.49 mg kg^{-1} for arnica (Arnicae flos) and 837.95 mg kg^{-1} for yerba mate. Only the samples of Camellia sinensis (black, green and white tea) and yerba mate presented concentrations above 200 mg kg^{-1}. The extraction methods presented a small difference (between 0.7% and 7%) for black tea, white tea and yerba mate; however, the quantification of theobromine in boldo and green tea through infusion extraction was 55.86 mg kg^{-1} and 612.49 mg kg^{-1}, respectively, that is, 293% and 34% lower than when using extraction with ethanol.

These results show a low content of theobromine in the samples and the potential of boldo, black, white and green tea and yerba mate as an accessible sources of theobromine.

Theophylline

Only 11 samples presented quantifiable concentrations of this compound. These results showed that theophylline was the least abundant compound in the samples. The content of this compound in the samples extracted with ethanol were between 0.69 mg kg^{-1} for ginseng (Panax ginseng) and 47.65 mg kg^{-1} for small-leaved lime (Tilia cordata). Stonebreaker (Phylantus niruri) and cow's foot (Bauhinia forficata) also presented high contents of theophylline, 38.42 mg kg^{-1} and 30.71 mg kg^{-1}, respectively. This study presents unpublished results in terms of determination of the content of theophylline in small-leaved lime, stonebreaker and cow's foot, and, consequently, the identification of these plants as potential sources of this compound in the 100 analyzed plants.

Unlike theobromine, all samples with quantifiable concentrations of theophylline through ethanol extraction also presented contents of this compound when the extraction was performed by infusion; fennel (Foeniculum vulgare) was the sample with the lowest content of theophylline (1.65 mg kg^{-1}) and small-leaved lime presented the highest content (50.46 mg kg^{-1}) through aqueous infusion.

Only four samples presented a difference below 10% when comparing the extraction methods: small-leaved lime [Tilia cordata (5.5%)], stonebreaker [Phylantus niruri (9.0%)], calendula [Canlendula officinallis(2.2%)] and mint [Mentha sp (8.9%)]. Husks of jatoba (Hymenaea courabril) presented 250% more theophylline through the ethanol extraction versus infusion method, and ginseng presented 72% more theophylline through the infusion method. These results confirm that the extraction method is specific for each matrix and, for this reason, individual studies are required to optimize the extraction of xanthine compounds.

Caffeine

Caffeine was the most frequent methylxanthine in the analyzed samples, with 55% of the plants presenting quantifiable concentrations. The levels ranged from 0.65 mg kg^{-1} for ginseng to 9,544.87 mg kg^{-1} for black tea. However, 30 samples presented a content below 10.00 mg kg^{-1}, 15 samples presented concentrations between 10.10 mg kg^{-1} and 30.00 mg kg^{-1}, 6 samples between 30.10 mg kg^{-1} and 100.00 mg kg^{-1}, and only 4 samples were potential sources of caffeine: black tea [Camellia sinensis (9,544.84 mg kg^{-1})], green tea [Camellia sinensis (5,190.72 mg kg^{-1})], yerba mate [Ilex paraguariensis (5,132.43 mg kg^{-1})], and white tea [Camellia sinensis (2,654.83 mg kg^{-1})].Several studies identified and quantified caffeine in Camellia sinensis (Lin et al., 1998; Mizukami et al., 2007; Samanidou et al., 2012; Wang et al., 2011; Yang et al., 2007; Zielinski et al., 2016), and in yerba mate (Bassani et al., 2014; Murakami et al., 2013; Urfer et al., 2002).
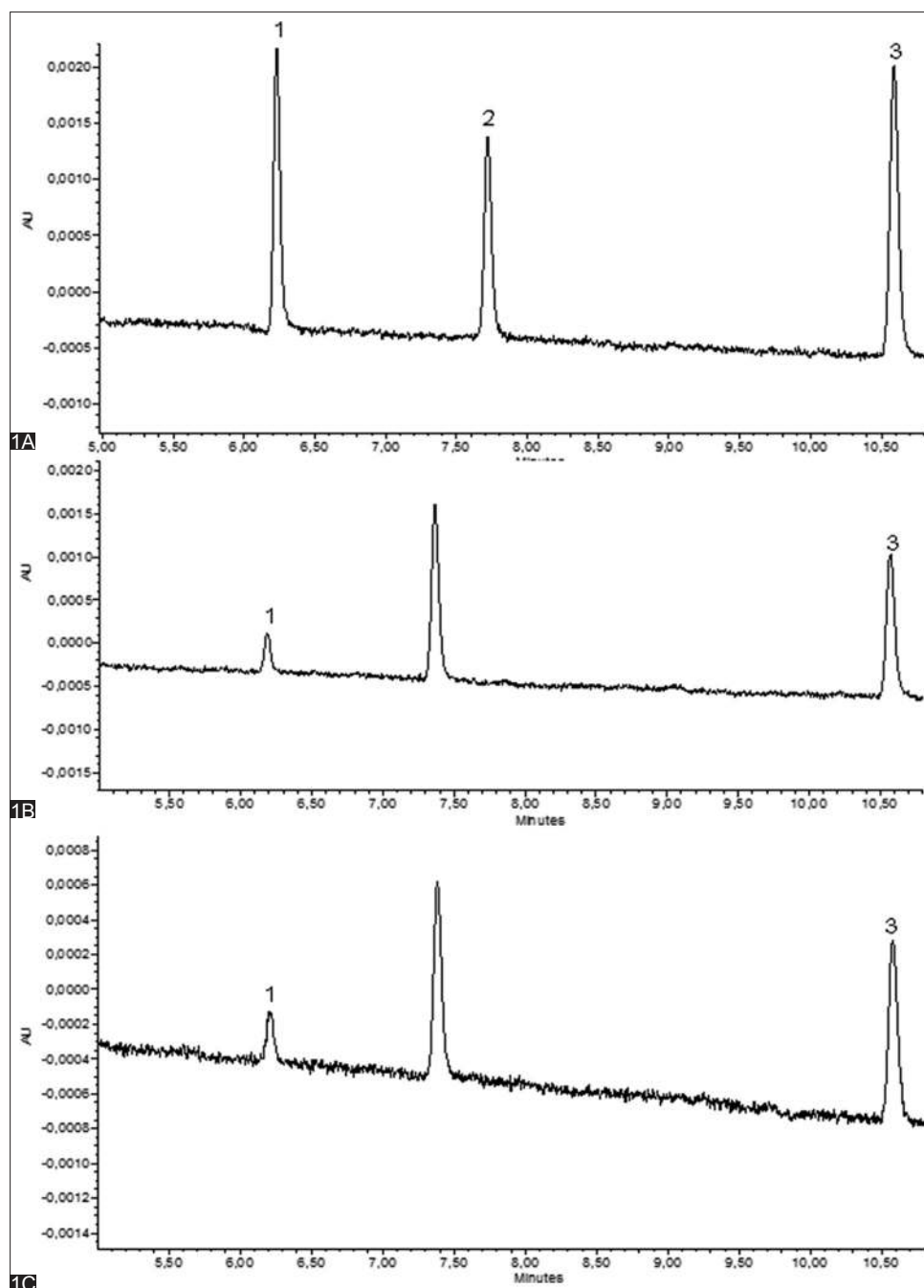
Fig 1. HPLC analysis results of standards. (1A), etanol-extracted (1B) and water-extracted (1C) of *Ilex paraguariensis* sample. Compounds: 1: Theobromine; 2: Theophylline; 3: Caffeine

The aqueous infusion process extracted caffeine from 49 samples at quantifiable levels, ranging from 2.28 mg kg^{-1} for jalapa (Operculina alata) to 6,979.90 mg kg^{-1} for black tea (Camellia sinensis). Cats claw(Acacia plumosa), cuphea (Cuphea ingrata), black sage (Cordia verbenácea), buckthorn (Rhamnus purshiana), greater burdock (Arctium lappa), and maidenhair (Adiantum capillus-veneris), unlike the result from the ethanol extraction, did not present a quantifiable content of the compound in the aqueous extraction. As in the ethanol extraction, white tea (2,751.53 mg kg^{-1}), green tea (3,740.88 mg kg^{-1}), yerba

mate (4,800.95 mg kg^{-1}), and black tea (6,979.90 mg kg^{-1}) were the samples with the highest contents of caffeine in the aqueous infusion.

In the comparison between the extraction methods, the difference between them does not have a trend, that is, it is characteristic for each sample, ranging between 0.3% (winters bark) and 177% (boldo). The difference between the extraction methods for the main sources of caffeine in this study was 3% for white tea, 7% for yerba mate, 37% for black tea, and 39% for green tea.

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Table 1: Content of methylxanthines (mg kg⁻¹) in herbs and infusions

Scientific name[a]	Popular name	Parts of the plant	Extraction with ethanol solution (Mean±SD)			Aqueous extraction (Mean±SD)		
			Theobromine	Theophylline	Caffeine	Theobromine	Theophylline	Caffeine
Acacia plumosa	Cat's claw	Roots	-	-	3.46±0.11	-	-	-
Adiantum capillus-veneris	Maidenhair	Leaves	-	-	8.12±0.09	-	-	4.20±0.25
Agerathum conyzoides	Billygoat-weed	Flowers, Leaves, and Thalli	38.21±0.76	9.40±0.50	-	26.63±1.68	11.04±0.10	-
Angelica officinalis	Garden angelica	Roots	-	-	0.96±0.07	-	-	4.94±0.09
Anona muricata	Soursop	Leaves	-	-	4.94±0.15	-	-	5.93±0.06
Arctium lappa	Greater burdock	Leaves and Thalli	2.39±0.14	-	6.24±0.20	-	-	-
Aristolochia brasiliensis	Rooster flower	Stem	-	-	4.00±0.08	-	-	4.52±0.20
Arnicae flos	Arnica	Leaves and Thalli	1.64±0.05	-	7.33±0.34	1.49±0.05	-	7.80±0.17
Artemisia vulgaris	Mugwort	Leaves and Thalli	30.17±0.95	-	4.00±0.19	33.44±0.53	-	3.64±0.19
Bauhinia forticata	Cow's foot	Leaves	66.55±1.41	30.71±1.91	-	58.29±2.20	34.36±1.57	-
Boehmeria caudata	Assa-peixe	Leaves and Thalli	17.37±0.18	-	13.59±0.47	19.37±1.01	-	12.79±0.44
Calendula officinalis	Calendula	Flowers	-	21.44±1.56	4.56±0.32	19.37±1.01	21.93±0.72	4.65±0.43
Camellia sinensis	White tea	Leaves	662.05±5.21	-	2,654.83±16.91	678.04±32.61	-	2,751.53±52.48
Camellia sinensis	Green tea	Leaves	820.96±63.79	-	5,190.72±419.40	612.49±67.19	-	3,740.88±491.48
Camellia sinensis	Black tea	Leaves	275.90±3.25	-	9,544.87±354.73	277.72±7.17	-	6,979.90±740.66
Cecropia palmata	Pumpwood	Leaves	-	-	10.68±0.63	-	-	9.79±0.30
Cinchonae calisaya	Cinchona	Bark	-	-	2.94±0.09	-	-	7.53±0.17
Citrus aurantium	Orange	Leaves	44.29±0.55	-	-	48.95±1.58	-	-
Cordia verbenácea	Black sage	Leaves and Thalli	-	-	3.87±0.12	-	-	-
Cuphea ingrata	Cuphea	Leaves and Thalli	-	-	3.56±0.19	-	-	-
Cymbopogon citratus	Lemongrass herb	Leaves	2.65±0.06	-	18.43±0.36	-	-	13.79±0.17
Cynara scolymus	Artichoke	Leaves	-	-	16.45±0.99	-	-	14.06±0.73
Drimys winteri	Winter's bark	Bark	-	-	24.92±0.64	-	-	24.84±1.52
Equisetum arvensis	Horsetail	Leaves and Thalli	-	-	58.04±1.15	-	-	79.00±2.75
Erytroxylon catuaba	Catuaba	Bark	-	-	12.35±0.67	-	-	14.52±0.13
Eucaliptus globulos	Eucalyptus	Leaves and Thalli	-	-	20.74±0.57	-	-	20.02±0.77
Foeniculum vulgare	Fennel	Seeds	-	1.84±0.07	19.72±0.31	-	1.65±0.02	14.76±1.23
Galinsoga parviflora	Gallant soldier	Stem	14.35±0.27	10.65±0.38	2.75±0.10	25.57±0.66	14.43±0.34	2.99±0.04
Gardenia jasminoides	Gardenia	Flowers	-	-	91.97±18.66	-	-	134.78±2.42
Hymenaea courabril	Jatoba	Bark	-	6.19±0.28	-	-	1.77±0.03	-
Ilex paraguariensis	Yerba mate	Leaves and Thalli	895.79±38.82	-	5,132.43±355.24	837.95±13.60	-	4,800.95±75.14
Illicium verum	Star anise	Fruit	-	-	64.64±1.45	14.21±0.83	-	78.00±2.26
Jacaranda caroba	Caroba	Leaves and Thalli	14.82±0.08	-	8.73±0.27	-	-	9.46±1.20
Juniperus communis	Juniper	Seeds	-	-	5.93±0.16	-	-	4.10±0.26
Lavandula officinalis	Green lavender	Flowers	3.67±0.16	-	4.14±0.05	3.35±0.20	-	4.07±0.21
Matricaria chamomilla	Chamomile	Flowers	3.11±0.07	2.38±0.16	3.04±0.09	3.57±0.23	1.72±0.04	2.96±0.07
Maytenus ilicifolia	Espinheira santa	Leaves and Thalli	4.52±0.47	-	11.42±1.12	4.58±0.14	-	12.55±0.22
Melissa officinalis	Lemon balm	Leaves and Thalli	-	-	1.69±0.09	-	-	4.89±0.39
Mentha pylegium	Pennyroyal	Leaves and Thalli	1.45±0.03	-	-	-	-	-
Mentha sp	Mint	Leaves	2.89±0.15	2.90±0.07	2.84±0.14	1.50±0.15	3.15±0.18	3.08±0.06

(Contd...)

Table 1: (Continued)

Scientific name[a]	Popular name	Parts of the plant	Extraction with ethanol solution (Mean±SD)			Aqueous extraction (Mean±SD)		
			Theobromine	Theophylline	Caffeine	Theobromine	Theophylline	Caffeine
Mentha pylegium	Pennyroyal	Leaves and thalli	1.45±0.03	-	-	-	-	-
Mentha sp	Mint	Leaves	2.89±0.15	2.90±0.07	2.84±0.14	1.50±0.15	3.15±0.18	3.08±0.06
Mikania glomerata	Guaco	Leaves and thalli	24.55±1.29	-	41.98±3.03	20.11±1.68	-	32.09±1.64
Myrcia sphaerocarpa	Pedra-ume-caá	Leaves	8.14±0.26	-	3.29±0.12	7.15±0.21	-	4.03±0.07
Occimum basilicum	Basil	Leaves and thalli	3.30±0.11	-	4.38±0.16	7.43±0.29	-	4.97±0.12
Operculina alata	Jalapa	Tubercle	-	-	3.37±0.25	-	-	2.28±0.01
Panax ginseng	Ginseng	Roots	-	0.69±0.03	0.64±0.04	-	2.47±0.07	3.39±0.11
Petiveria tetrandra	Guinea	Leaves and thalli	4.27±0.06	-	-	5.17±0.17	-	-
Peumus boldus	Boldo	Leaves	219.78±14.61	-	7.06±0.10	55.86±1.14	-	2.54±0.02
Phylantus niruri	Stonebreaker	Leaves and thalli	40.93±0.54	38.42±0.77	7.43±0.16	35.49±0.95	35.24±0.94	8.86±0.54
Pimpinella anisum	Anise	Flowers	-	-	17.25±0.46	-	-	21.43±1.16
Rhamnus purshiana	Buckthorn	Bark	-	-	4.97±0.09	-	-	-
Rosmarinus officinalis	Rosemary	Leaves	-	-	15.20±0.45	-	-	6.88±0.38
Ruta graveolens	Rue	Leaves and thalli	-	-	10.52±0.48	-	-	12.79±0.47
Salvia officinalis	Sage	Leaves and thalli	-	-	2.47±0.04	-	-	2.40±0.05
Sambucus nigra	Sabugueiro	Flores	2.76±0.09	-	8.22±0.31	9.25±0.17	-	14.26±0.28
Sida cordifolia	Flannel weed	Leaves	13.46±0.16	-	28.68±0.29	2.86±0.06	-	34.63±1.39
Solanum paniculatum	Jurubeba	Leaves and thalli	-	-	15.89±1.57	-	-	6.96±0.15
Tabebuia impetiginosa	Pau D'Arco	Bark	-	-	2.38±0.02	-	-	-
Taraxacum officinale	Dandelion	Leaves and thalli	2.26±0.20	-	17.66±0.72	2.30±0.18	-	16.70±3.25
Tilia cordata	Small-leaved lime	Leaves	12.23±0.37	47.65±0.99	49.00±0.37	14.21±0.11	50.46±1.81	46.09±2.23
Valeriana officinalis	Valerian	Roots	-	-	35.21±1.50	-	-	35.63±2.25
Verbena officinalis	Common vervain	Leaves	-	-	4.96±0.31	-	-	4.75±0.20

[a]The identification of scientific names was according to the Brazilian Pharmacopeia (Brasil, 2010), and when not available, the name informed by the suppliers was used. – not detected.

Table 2: Plants with non-detected amounts in the extraction with ethanol solution

Scientific name[a]	Popular name	Parts of the plant
Anadenanthera colubrina	*Angico*	Bark
Artemisia absinthum	Wormwood	Leaves and thalli
Baccharis trimera	Carqueja	Leaves and thalli
Bidens Pilosa	Black-jack	Leaves and thalli
Bixa Orellana	Achiote	Seeds
Casearia sylvestris	*Guaçatonga*	Leaves and thalli
Chenopodium ambrosioides	Wormseed	Leaves, thalli, and seeds
Chododendron platyphyllum	*Abútua*	Stem
Cinnamomum cassia	Cinnamon	Bark
Cirsium vulgare	Common thistle	Leaves and thalli
Cordia salicifolia	*Porangabae*	Leaves and thalli
Cordia sellowiana	*Chá de bugre*	Leaves
Costus spicatus	Spiked spiralflag ginger	Leaves
Echinodorus grandiflorus	*Chapéu de couro*	Leaves and thalli
Fevillea trilobata	*Andiroba*	Seeds
Glycyrhiza glabra	Liquorice	Stem
Hibiscus sabdariffa	Roselle	Flowers
Hydrocotyle asiatica	Centella asiatica	Leaves and thalli
Lavandula officinalis	Lavender	Flowers and leaves
Medicago sativa	Alfalfa	Leaves and thalli
Myracrodruon urundeuva	Aroeira	Bark
Myroxylon peruiferum	*Bálsamo do norte*	Bark
Ocotea preciosa	Sassafraz	Stem
Passiflora alata	Passion fruit	Leaves and thalli
Persea gratíssima	Avocado	Leaves
Plantago major	Broadleaf plantain	Leaves and thalli
Polygonum acre	Water Pepper	Leaves and thalli
Ptychopetalum olacoides	Muira Puama	Stem
Punica granathum	Pomegranate	Bark
Quassia amara	Bitter-ash	Stem
Senna alexandrina	Egyptian senna	Leaves
Smilax salsaparrilha	Sarsaparilla	Stem
Stryphnodendron adstringen	*Barbatimão*	Bark
Symphytum asperum	Comfrey	Leaves and thalli
Trichilia catigua	Catuaba	Stem
Urtica dioica	Common nettle	Leaves
Verbena bonariensis	Vervain	Stem
Zea mays	Corn silk	Corn silk
Ziziphus joazeiro	*Juá*	Bark

[a]The identification of scientific names was according to the Brazilian Pharmacopeia (Brasil, 2010), and when not available, the name informed by the suppliers was used.

Intake of methylxanthines and comparison with other foods and beverages

Considering one serving (300 mL) of infusion, prepared with 2 g of the main plants presented in this study, the intake of methylxanthines was estimated. One serving of infused mate tea has 9.60 mg of caffeine and 1.66 mg of theobromine, while the same serving of white tea has 5.50 mg of caffeine and 1.35 mg of theobromine. Black tea has 13.96 mg of caffeine, 0.55 mg of theobromine, while green tea has 7.48 mg of caffeine and 1.22 mg of theobromine. As for theophylline, the same serving of infusion of small-leaved lime, stonebreaker, cow's foot and calendula have 0.10 mg, 0.07 mg, 0.07 mg, and 0.04 mg, respectively.

Considering the dose of 120 mg per day^{-1} as a small intake of caffeine [17], an adult should take around 30 mL of coffee (Rodrigues and Bragagnolo, 2013) or 125 g of chocolate with 42% cocoa (Peralta-Jiménez and Cañizares-Macías, 2013) or 380 mL of an energy drink (Rostagno et al., 2011) or, according to the findings of this study, 2,570 mL of black tea.

For the same amount of theobromine, one would have to take 11 g of chocolate with 42% cocoa or 245 g of milk chocolate, 350 mL of chocolate beverage (Peralta-Jiménez and Cañizares-Macías, 2013) or 21,600 mL of yerba mate infusion, which is equivalent to 72 servings of 300 mL each.

Comparatively, a cup of coffee (50 mL) has around 0.35 mg of theophylline (Rodrigues and Bragagnolo, 2013), which is equivalent to 1,050 mL of small-leaved lime infusion or 1,500 mL of infusion of stonebreaker or cow's foot, or 2,400 mL of calendula infusion. Despite the low concentration of theophylline, this finding is relevant for people who have some degree of sensitivity to caffeine, since these plants have little or no concentration of this compound.

CONCLUSION

This study presented a screening of new sources of methylxanthines (caffeine, theophylline and theobromine) in 100 plants consumed in Brazil, of which, several have not been analyzed for this subject. Among the plants studied, 39% did not present any content of methylxanthines at quantifiable levels. The plants with the highest amount of caffeine were: black tea, green tea, yerba mate and white tea, for theobromine: yerba mate, green tea, white tea, black tea and boldo, and for theophylline: small-leaved lime, stonebreaker, cow's foot and calendula. The content of methylxanthines was lower when compared with other foods and beverages; however, the consumption of infusions is usually in larger amounts, which may contribute to the increased intake of these compounds. From the results obtained it was possible to evaluate the concentration of methylxanthines in herbs commercialized in Brazil and evaluate the exposure and consumption of methylxanthines when these plants are destined to the preparation of infusions. The data obtained allows the elaboration of future studies on bioaccessibility and bioavailability of methylxanthines for use of these plants in food and drugs.

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Authors' contributions

All authors contributed equally to the planning and execution of the research.

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