




Rutin in herbs and infusions: screening of new sources and consumption estimation

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Abstract

Rutin has shown promising results in reducing various human diseases. However, many plants consumed in Brazil do not have their rutin content reported in the literature yet. More than eighty plants were analyzed using aqueous (popularly known as tea) and ethanolic extraction (employed in commercial rutin purification) by liquid chromatography. Rutin was found in 47 samples when ethanol extraction was used. *Echinodorus grandiflorus* had the highest content (14,878.7 mg 1000 g⁻¹), followed by *Sambucus nigra*, *Drimys winteri* and *Taraxacum officinale*. In aqueous infusion, *Echinodorus grandiflorus*, *Sambucus nigra*, *Drimys winteri* and *Ruta graveolens* presented the highest levels of rutin, ranging from 15.5 to 29.7 mg of rutin in 300 mL of infusion from 2 g of the plant. *Echinodorus grandiflorus*, *Sambucus nigra*, *Drimys winteri*, *Taraxacum officinale* and *Ruta graveolens* presented a high amount of flavonoid and might be good alternatives as ingredients in food and pharmaceuticals in order to obtain health benefits.

Keywords: tea; HPLC; phenolic compounds; quantification; bioactive.

Practical Application: This study presented new sources of rutin for enrichment of diets and commercial purification.

1 Introduction

Rutin belongs to the family of flavonoids and could be more specifically classified as a flavonol. It is a molecule of quercetin with the addition of a disaccharide (rhamnose and glucose). Due to rutin's pharmacological activities, associated with different properties, such as anti-inflammatory and antioxidant actions, it became of great importance for the pharmaceutical industry, and many drugs registered worldwide contains rutin in their composition (Abarikwu et al., 2017; Chua, 2013; Ganeshpurkar & Saluja, 2016; Ghorbani, 2017).

Chen et al. (2014) demonstrated that rutin is a potential protective agent against acute lung injury caused by interaction with lipopolysaccharides. The study shows its effect in the inflammatory action in the lungs through inhibition of the activation of MIP-2 (inflammatory protein) and MMP-9 (endopeptidase). The anti-inflammatory effect of rutin was also observed in cases of chronic colitis (Mascaraque et al., 2014). Choi et al. (2015) studied the antithrombotic effect of rutin in vitro and in vivo, suggesting that it could, potentially, be used in the treatment of cardiovascular diseases by reducing thrombin activity, inhibiting platelet aggregation and fibrin coagulation.

Kaur & Muthuraman (2016) found positive effects when they used rutin to treat renovascular hypertension caused by renal ischemia. Some researchers showed promising results for Alzheimer's treatment, in which rutin was correlated with beneficial effects against the neurotoxicity of A β (amyloid beta peptide), besides reducing levels of oxidative stress and brain inflammation (Moghbelinejad et al., 2014; Xu et al., 2014).

Pan et al. (2014) studied the influence of rutin in the treatment of cholestatic liver lesions and Wang et al. (2016) the antifibrotic action on neuropathy in rats. They both concluded that rutin consumption resulted in significant improvement in the studied diseases. Rutin was also considered effective in alleviating the toxic effect of ethanol in neural cells through the activation of ALDH2 (aldehyde dehydrogenase 2) that converts acetaldehyde into acetic acid (Song et al., 2014), besides contributing positively with spinal cord injury treatment (Wu et al., 2016), inhibiting effects of thyroid hormones in rats with hyperthyroidism (Panda & Kar, 2014) and improving neuropathy caused by diabetes (Tian et al., 2016).

Rutin has also been studied for its use in cosmetics for the production of sunscreen, and the result was promising compared to traditional sunscreens (Kamel & Mostafa, 2015).

In Brazil the use of vegetables in infusions is associated with traditional knowledge from native and popular culture and has more recently expanded thanks to miscegenation with emigrants from Africa, Europe and Asia (Bolson et al., 2015). Despite the significant amount of plants used in infusions in Brazil, there are few data on rutin content in these plants and in their aqueous infusions.

Therefore, this work aimed to quantify rutin in more than 80 plants commonly used by the population, taking into consideration the extraction of this compound using aqueous infusion, popularly known as tea, which is the common method of consumption, as well as extraction with ethanol (largely

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employed in commercial purification of rutin), considered a green solvent due to its low cost, toxicity and environmental impact.

2 Materials and methods

2.1 Samples and reagents

The plants were obtained in fairs and public markets of Campinas and Americana, SP, Brazil. Each plant has been acquired from three suppliers in dried form, just like it is commercialized. The plants were identified with both the popular and scientific names, according to the product label description and to the Brazilian Official Pharmacopoeia (Brasil, 2010) and when its name was not found in the publication, we cited their scientific names given by the suppliers. None of the analyzed plants has any consumption restriction according to the Brazilian legislation (Brasil, 2014). This study has access to Brazilian genetic patrimony and was registered under the Ministério do Meio Ambiente (protocol number A3414AD).

The standard of the rutin was obtained from Biopurify (Chengdu, China). Stock solutions were prepared in HPLC grade methanol (J.T. Baker, Brazil), at a concentration of 1 mg mL⁻¹, and stored at -80 °C. Formic acid was purchased from Merck (Brazil), HPLC grade acetonitrile from J.T. Baker (Brazil) and ethanol absolute from Synth (Brazil). Ultrapure water was obtained using Milli-Q® system (Millipore Corporation, France). All solutions were filtered in 0.22 µm PVDF membranes (Millipore Corporation, France).

2.2 Sample preparation

Approximately 40 g of sample from each supplier were weighed, homogenized and ground in a hammermill (Marconi TE 600, Brazil), with a 200 mesh sieve. Samples containing barks, stalks, stems and tubercles were reduced in size with a knife before grinding.

For rutin extraction using solvent, the method was based on the study of Meinhart et al. (2017) where 0.5 g of ground sample were weighed in tubes with lid and 15 mL of solution added containing 74% of water and 26% of ethanol. Tubes were hermetically sealed and heated in water bath at 60 °C, under agitation (240 rpm) for 22 minutes. Then the volume was adjusted to 100 mL, the extracts were filtered and injected.

We weighed 2 g of sample (an equivalent to a tea sachet) to prepare the aqueous infusions (teas), added 300 mL of boiling water and then let them remain still for 16 minutes. After that, the mixture was cooled, its volume adjusted to 500 mL, the extract was filtered and injected. Each extraction was performed three times. The method was based on da Silveira et al. (2017).

2.3 Method for analysis of rutin

All analyses were carried out using ultra performance liquid chromatography employing a Waters Acquity UPLC® (Thermo, USA) equipment, with a Kinetex® C18 column (Phenomenex, USA) - 100 mm length, 2.1 mm i.d. and 1.7 µm particle size. The column temperature was maintained at 30 °C. The mobile phase consisted of acidified water (formic acid 0.1%, pH 2.4) - A and

acetonitrile - B. The linear gradient started with 100% A with linear decrease up to 92% A at 10 minutes, continuing with linear gradient until it reached 70% A at 14 minutes, which was held constant until minute 15. For cleaning the column, a new linear gradient started from 15 to 16 minutes until it reached 100% B at 16 minutes, which was held for 1 minute. From 17 minutes to 23 minutes the column was reconditioned with 100% A. The flow rate was 0.3 mL min⁻¹ and the injection volume was 10 µL. The method was based on studies carried out by Van Hung & Morita (2008) and Meinhart et al. (2017). Identification of the compounds was made comparing standards through retention time, absorption spectrum of the diode arrangement detector (DAD) with a wavelength of 325 nm and through co-chromatography (spiked sample). Quantification was carried out using external calibration curve.

The method was validated for limit of detection and limit of quantification (three times and six times the signal to noise ratio, respectively). The analytical curve was constructed in 7 equidistant concentrations, randomly repeated three times, starting with the limit of quantification and ending with the concentration up until linearity was ensured through the evaluation of the models for lack of fit, and ANOVA regression significance was carried out using Statistica 7.0 (Statsoft, USA). Precision was analyzed between days and on the same day. On the same day it was analyzed through 10 successive determinations, which were repeated for 3 days to determine the precision between days. Precision on the same day and between days was carried out in 3 levels, including the limit of quantification, the intermediate and the maximum point of the analytical curve (n=10 for each). Analytical trueness for the ethanol-based extraction was evaluated through recovery in the same three levels as in the precision assay (n=3 for each level).

3 Results and discussion

3.1 Validation of analytical methodology

The method of analysis has been properly validated showing an adequate linearity, with F values for lack of fit smaller than the critical values of F, with 95% of confidence level. The limits of quantification were 0.05 mg L⁻¹ and the coefficients of variation were a maximum value of 5.4% for precision testings and recovery between 98.3 and 104.7%. The parameters are in accordance with the limits established by IUPAC (International Union of Pure and Applied Chemistry, 2002) and ANVISA (Brasil, 2017), indicating that the method is recommended to perform quantitative analysis with adequate analytical certainty. In Table 1, there are figures of merit for the analytical method.

3.2 Extraction using ethanol for commercial purification

Table 2 shows rutin content in the plants studied using ethanol extraction and aqueous infusion, expressed in mg 1000 g⁻¹. Results were also expressed in mg of rutin in 300 mL of aqueous infusion, considering its consumption when the tea is prepared with a sachet (around 2 g). Figure 1 shows the chromatograms of the winter's bark and *chapéu de couro* samples.

Forty-five of the eighty-three plants analyzed showed quantifiable concentration of rutin between 27.74 mg 1000 g⁻¹ for *Occimum*

Table 1. Figures of merit for the analysis using ultra performance liquid chromatography for determination of rutin in Brazilian plants.

Parameters		Rutin
Linear range (mg L ⁻¹)		0.05 to 35.0
Model		y = 3,511.5x - 155.61
Linear model adjustment (F) ⁽¹⁾		2.17
Precision on the same the day in relative standard deviation (n = 10)	Level 1 ⁽²⁾	5.40%
	Level 2	2.67%
	Level 3	3.09%
Precision between days in relative standard deviation (n = 10)	Level 1	0.59%
	Level 2	1.52%
	Level 3	0.28%
Limit of detection (mg L ⁻¹)		0.025
Limit of quantification (mg L ⁻¹)		0.05
Recovery (n=3 for each level)	Level 1	104.71%
	Level 2	98.28%
	Level 3	99.56%

⁽¹⁾ The model is adequately adjusted when calculated F is smaller than critical F (3.49) with 95% of confidence; ⁽²⁾ Level 1, 2 and 3 concentrations: limit of quantification, intermediate and the maximum point of linear range of analytical curve.

Table 2. Rutin content in plants consumed in Brazil.

Scientific Name	Popular Name	Parts of the plant	Ethanol Extration (mg 1000 g ⁻¹)		Aqueous infusion (mg 1000 g ⁻¹)		Rutin in aqueous infusion (mg 300 mL ⁻¹)	% of infusion extraction in relation to ethanol extraction
			Mean	± RSD	Mean	± RSD		
<i>Rosmarinus officinalis</i>	Rosemary	Leaves	nd		nd		nd	-
<i>Lavandula officinalis</i>	Green Lavender	Flowers	nd		nd		nd	-
<i>Angelica officinalis</i>	Garden angelica	Roots	nd		nd		nd	-
<i>Myracrodruon urundeuva</i>	Aroeira	Bark	nd		nd		nd	-
<i>Adiantum capillus-veneris</i>	Maidenhair	Leaves	nd		nd		nd	-
<i>Cordia verbenácea</i>	Black Sage	Leaves, thalli	nd		nd		nd	-
<i>Myroxylon peruiferum</i>	Bálsamo do norte	Bark	nd		nd		nd	-
<i>Operculina alata</i>	Jalapa	Tubercle	nd		nd		nd	-
<i>Costus spicatus</i>	Spiked spirallflag ginger	Leaves	nd		nd		nd	-
<i>Cymbopogon citratus</i>	Lemongrass herb	Leaves	nd		nd		nd	-
<i>Cirsium vulgare</i>	Common Thistle	Leaves, thalli	nd		nd		nd	-
<i>Rhamnus purshiana</i>	Buckthorn	Bark	nd		nd		nd	-
<i>Erytroxylon catuaba</i>	Catuaba	Bark	nd		nd		nd	-
<i>Equisetum arvensis</i>	Horsetail	Leaves, thalli	nd		nd		nd	-
<i>Hydrocotyle asiatica</i>	Centella Asiatica	Leaves, thalli	nd		nd		nd	-
<i>Polygonum acre</i>	Water Pepper	Leaves, thalli	nd		nd		nd	-
<i>Pimpinella anisum</i>	Anise	Flowers	nd		nd		nd	-
<i>Eucalyptus globulos</i>	Eucalyptus	Leaves, thalli	nd		nd		nd	-
<i>Verbena bonariensis</i>	Vervain	Stem	nd		nd		nd	-
<i>Panax ginseng</i>	Ginseng	Roots	nd		nd		nd	-
<i>Tabebuia impetiginosa</i>	Pau D'arco	Bark	nd		nd		nd	-
<i>Gardenia jasminoides</i>	Gardenia	Flowers	nd		nd		nd	-
<i>Hymenaea courabril</i>	Jatoba	Bark	nd		nd		nd	-
<i>Citrus aurantium</i>	Orange	Leaves	nd		nd		nd	-
<i>Artemisia absinthum</i>	Wormwood	Leaves, thalli	nd		nd		nd	-
<i>Ptychopetalum olacoides</i>	Muira Puama	Stem	nd		nd		nd	-

RSD: relative standard deviation (n = 3), nd: not detected.

Table 2. Continued...

Scientific Name	Popular Name	Parts of the plant	Ethanol Extration (mg 1000 g ⁻¹)		Aqueous infusion (mg 1000 g ⁻¹)		Rutin in aqueous infusion (mg 300 mL ⁻¹)	% of infusion extraction in relation to ethanol extraction
			Mean ±	RSD	Mean ±	RSD		
<i>Melissa officinalis</i>	Lemon balm	Leaves, thalli	nd		nd		nd	-
<i>Mentha sp</i>	Mint	Leaves	nd		nd		nd	-
<i>Trichilia catigua</i>	Catuaba	Stem	nd		nd		nd	-
<i>Galinsoga parviflora</i>	Gallant soldier	Stem	nd		nd		nd	-
<i>Cinchona calisaya</i>	Cinchona	Bark	nd		nd		nd	-
<i>Punica granatum</i>	Pomegranate	Bark	nd		nd		nd	-
<i>Ocotea preciosa</i>	Sassafras	Stem	nd		nd		nd	-
<i>Senna alexandrina</i>	Sene	Leaves	nd		nd		nd	-
<i>Cuphea ingrate</i>	Cuphea	Leaves, thalli	nd		nd		nd	-
<i>Plantago major</i>	Broadleaf plantain	Leaves, thalli	nd		nd		nd	-
<i>Valeriana officinalis</i>	Valerian	Roots	nd		nd		nd	-
<i>Verbena officinalis</i>	Common vervain	Leaves	nd		nd		nd	-
<i>Occimum basilicum</i>	Basil	Leaves, thalli	27.74 ± 1.55		42.50 ± 1.14		0.09	153.21
<i>Maytenus ilicifolia</i>	<i>Espinheira Santa</i>	Leaves, thalli	28.83 ± 1.40		31.92 ± 3.55		0.06	110.73
<i>Mikania glomerata</i>	Guaco	Leaves, thalli	36.39 ± 0.99		32.66 ± 1.40		0.07	89.77
<i>Myrcia sphaerocarpa</i>	<i>Pedra-Ume-Caá</i>	Leaves	36.60 ± 0.97		36.05 ± 1.37		0.07	98.48
<i>Stryphnodendron adstringens</i>	Barbatimão	Bark	40.29 ± 1.64		33.53 ± 1.38		0.07	83.24
<i>Urtica dioica</i>	Common nettle	Leaves	40.91 ± 0.87		Nd ± nd		Nd	0.00
<i>Baccharis trimera</i>	<i>Carqueja</i>	Leaves, thalli	41.81 ± 1.50		37.62 ± 0.30		0.08	89.98
<i>Chenopodium ambrosioides</i>	Wormseed	Leaves, thalli, seeds	43.05 ± 4.49		40.97 ± 1.57		0.08	95.15
<i>Anona muricata</i>	Soursop	Leaves	51.62 ± 3.51		87.44 ± 4.96		0.17	169.39
<i>Cecropia palmate</i>	Pumpwood	Leaves	88.21 ± 2.91		145.25 ± 3.97		0.29	164.67
<i>Persea gratissima</i>	Avocado	Leaves	93.46 ± 5.43		88.42 ± 6.66		0.18	94.61
<i>Tilia cordata</i>	Small-leaved lime	Leaves	94.45 ± 3.23		85.49 ± 1.58		0.17	90.52
<i>Lavandula officinalis</i>	Lavender	Flowers, leaves	96.99 ± 2.22		96.04 ± 3.18		0.19	99.03
<i>Bidens pilosa</i>	Black-jack	Leaves, thalli	101.95 ± 4.13		99.80 ± 4.97		0.20	97.89
<i>Acacia plumose</i>	Cat's claw	Roots	104.07 ± 4.67		32.66 ± 1.62		0.07	31.39
<i>Foeniculum vulgare</i>	Fennel	Seeds	130.14 ± 0.74		95.28 ± 9.33		0.19	73.22
<i>Hibiscus sabdariffa</i>	Roselle	Flowers	150.06 ± 3.25		151.16 ± 10.00		0.30	100.73
<i>Medicago sativa</i>	Alfalfa	Leaves, thalli	161.74 ± 4.31		188.08 ± 1.17		0.38	116.29
<i>Peumus boldus</i>	Boldo	Leaves	172.10 ± 4.41		138.04 ± 7.75		0.28	80.21
<i>Illicium verum</i>	Star anise	Fruit	193.41 ± 10.00		193.15 ± 8.34		0.39	99.87
<i>Salvia officinalis</i>	Sage	Leaves, thalli	214.68 ± 3.19		209.17 ± 5.54		0.42	97.43
<i>Cordia salicifolia</i>	<i>Porangaba</i>	Leaves, thalli	220.08 ± 9.32		170.07 ± 26.98		0.34	77.27
<i>Casearia sylvestris</i>	<i>Guaçatonga</i>	Leaves, thalli	249.28 ± 7.64		240.88 ± 46.71		0.48	96.63
<i>Calendula officinalis</i>	Calendula	Flowers	280.55 ± 12.00		292.62 ± 11.21		0.59	104.30
<i>Artemisia vulgaris</i>	Mugwort	Leaves, thalli	302.23 ± 7.30		46.07 ± 0.76		0.09	15.24
<i>Matricaria chamomilla</i>	Chamomile	Flowers	311.15 ± 13.20		344.49 ± 17.62		0.69	110.72
<i>Juniperus communis</i>	Juniper	Seeds	318.85 ± 14.22		170.59 ± 20.20		0.34	53.50
<i>Cordia sellowiana</i>	Chá de bugre	Leaves	383.84 ± 30.48		257.35 ± 4.19		0.51	67.05
<i>Agerathum conyzoides</i>	Billygoat-weed	Flowers, leaves, thalli	384.65 ± 23.47		261.04 ± 35.25		0.52	67.86
<i>Phyllanthus niruri</i>	Stonebreaker	Leaves, thalli	907.95 ± 19.96		854.84 ± 46.30		1.71	94.15
<i>Camellia sinensis</i>	White tea	Leaves	920.94 ± 33.94		870.90 ± 37.28		1.74	94.57
<i>Camellia sinensis</i>	Black Tea	Leaves	1,115.51 ± 59.19		1,087.46 ± 48.09		2.17	97.49
<i>Jacaranda caroba</i>	Caroba	Leaves, thalli	1,248.49 ± 32.80		1,246.83 ± 25.31		2.49	99.87
<i>Cynara scolymus</i>	Artichoke	Leaves	1,275.81 ± 154.77		1,151.84 ± 144.99		2.30	90.28

RSD: relative standard deviation (n = 3), nd: not detected.

Table 2. Continued...

Scientific Name	Popular Name	Parts of the plant	Ethanol Extraction (mg 1000 g ⁻¹)		Aqueous infusion (mg 1000 g ⁻¹)		Rutin in aqueous infusion (mg 300 mL ⁻¹)	% of infusion extraction in relation to ethanol extraction
			Mean	± RSD	Mean	± RSD		
<i>Camellia sinensis</i>	Green tea	Leaves	1,334.83	± 102.98	1,124.15	± 9.62	2.25	84.22
<i>Bauhinia forficata</i>	Cow's Foot	Leaves	1,540.17	± 27.39	1,318.32	± 69.95	2.64	85.60
<i>Arctium lappa</i>	Greater burdock	Leaves, thalli	1,631.90	± 123.86	2,408.32	± 324.25	4.82	147.58
<i>Sida cordifolia</i>	Flannel weed	Leaves	1,733.38	± 31.82	1,621.73	± 48.47	3.24	93.56
<i>Boehmeria caudate</i>	Assa-Peixe	Leaves, thalli	2,943.22	± 302.56	4,714.01	± 468.19	9.43	160.16
<i>Ilex paraguariensis</i>	Yerba mate	Leaves, thalli	4,645.26	± 313.90	4,120.79	± 37.45	8.24	88.71
<i>Ruta graveolens</i>	Rue	Leaves, thalli	8,291.50	± 210.92	7,763.32	± 398.49	15.53	93.63
<i>Taraxacum officinale</i>	Dandelion	Leaves, thalli	9,148.51	± 908.11		nd	nd	0.00
<i>Drimys winteri</i>	Winter's bark	Bark	9,788.70	± 664.44	7,591.96	± 1,167.61	15.18	77.56
<i>Sambucus nigra</i>	Sabugueiro	Flores	11,576.75	± 519.61	8,316.32	± 145.15	16.63	71.84
<i>Echinodorus grandifloras</i>	Chapéu de couro	Leaves, thalli	14,878.61	± 230.68	14,849.45	± 44.37	29.70	99.80

RSD: relative standard deviation (n = 3), nd: not detected.

basilicum and 14,878.61 mg 1000 g⁻¹ for *Echinodorus grandiflorus*. Besides, *Echinodorus grandiflorus*, *Sambucus nigra*, *Drimys winteri*, *Taraxacum officinale* and *Ruta graveolens* should be highlighted for having rutin content above 8,000.00 mg 1000 g⁻¹, and they can be considered a potential source of rutin. *Ilex paraguariensis* is widely consumed in Brazil as infusion and is highlighted by its high phenolic content, especially those derived from caffeic acid (Silveira et al., 2016; Marques & Farah, 2009). Regarding its rutin content, it was found 4,645.26 mg 1000 g⁻¹ and, although it had the lowest content among the above-mentioned plants, it has a higher content than the popular green tea (1,344.83 mg 1000 g⁻¹), black tea (1,115.51 mg 1000 g⁻¹) and white tea (920.94 mg 1000 g⁻¹) from *Camellia sinensis*.

In the literature, there are works which have reported the quantification of rutin in *Sambucus nigra* and *Ruta graveolens* flowers that were similar to the present work, or even superior (Dawidowicz & Wianowska, 2009; Mohamed & Ibrahim, 2012; Ueng et al., 2015). Differences in this study's results compared to the literature can be explained by the growing conditions of the plants, climate and botanical variety, besides the use of other parts of the plant such as the stem, which has a lower concentration of rutin.

We have not found, so far, studies that determined the concentration of rutin in *Echinodorus grandiflorus* and *Taraxacum officinale*.

The arboreal species *Dimorphandra mollis* is often used for the commercial obtention of rutin in Brazil, and it provides the yield of 8 g per 100 g of the fava pericarp (Becho et al., 2009). Comparing the yielding and the crop characteristics, *Echinodorus grandifloras* presents itself as an excellent source for the commercial obtention due to its high yield (14.9 g per kilo of leaf) and because it is a ground plant, with fast growth, easy to handle and adapted to tropical, subtropical and temperate climates.

3.3 Extraction using aqueous infusion for human consumption

The plants which presented higher content of rutin transferred from the plant to the aqueous infusion were *Echinodorus grandifloras* (14,849.45 mg 1000 g⁻¹), *Sambucus nigra* (8,316.32 mg 1000 g⁻¹), *Ruta graveolens* (7,763.32 mg 1000 g⁻¹) and *Drimys winteri* (7,591.96 mg 1000 g⁻¹).

The percentage of extraction of seventeen plants ranged from 90 to 99.99% when hot water was used compared to ethanol extraction. The percentage of extraction of seven plants ranged from 80 to 89.99%, four from 70 to 79.99%, and two from 60 to 69.99%. *Acacia plumosa*, *Artemisia vulgaris* and *Juniperus communis* percentages were lower than 60%, whereas only *Taraxacum officinale* and *Urtica dioica* had non-quantifiable results of rutin using infusion.

Although *Taraxacum officinale* contains a high amount of rutin when extracted using solvent, it did not have a quantifiable content when using aqueous infusion. The transfer rate of compounds from the leaves to the infusion may be affected by the amount of leaves or stems used, particle size, water volume, emperature, presence or absence of stirring and the duration of infusion (Astill et al., 2001; Nishiyama et al., 2010), showing the necessity of optimizing the way aqueous infusion is prepared to increase the migration of this compound.

On the other hand, nine of the forty-five samples that had quantifiable content of rutin in ethanol extraction had a higher concentration when extracted using only aqueous infusion. *Calendula officinalis* concentration was 4% higher in aqueous infusion, *Matricaria chamomilla* and *Maytenus ilicifolia* concentration was 10% higher, *Medicago sativa* 16%, *Arctium lappa* 47%, *Occimum basilicum* 53%, *Boehmeria caudata* 60%, *Cecropia palmata* 64% and *Anona muricata* 69%.

The results show that when we compare ethanol extraction and aqueous infusion, more elaborate exploratory studies are necessary for each plant in order to obtain the maximum extraction of

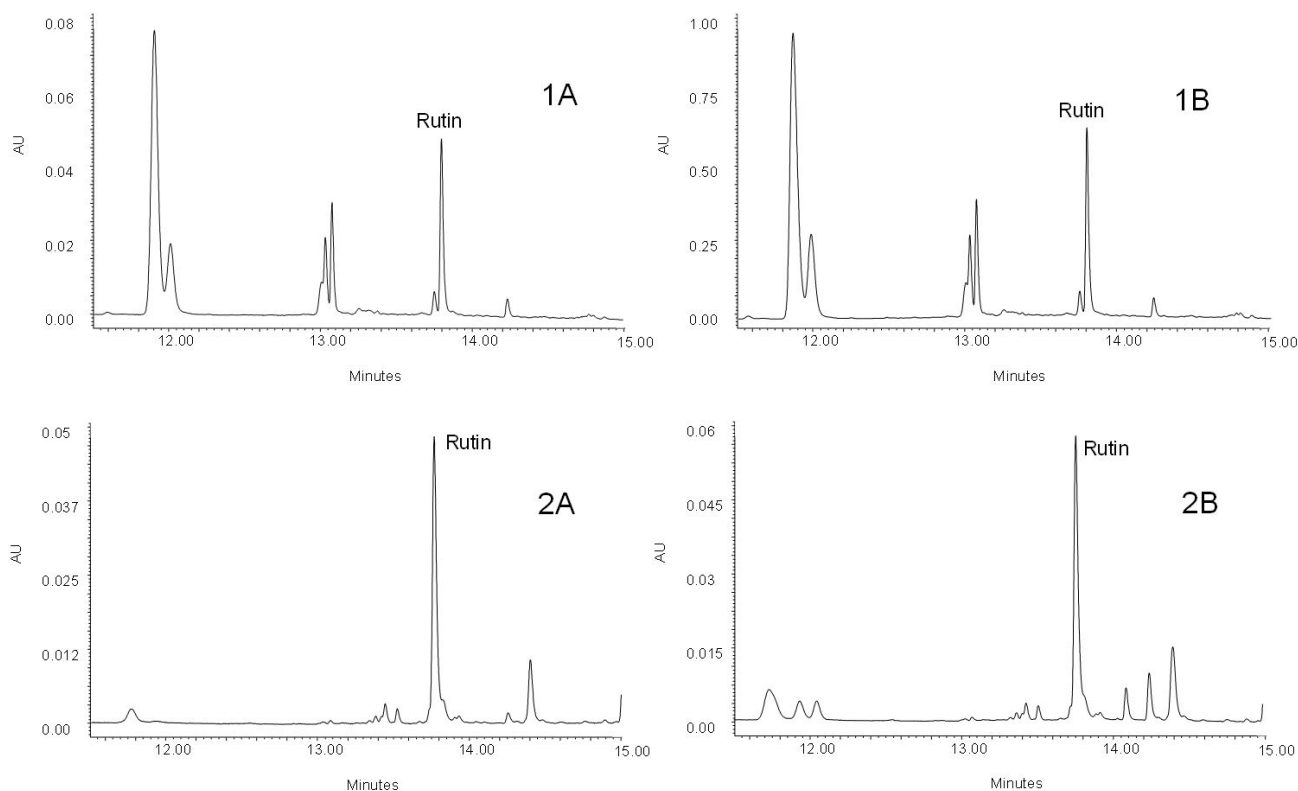


Figure 1. Chromatogram of rutin in Winter's bark (*Drimys winteri*) – ethanol:água (1A) and aqueous infusion (1B) and Chapéu de couro (*Echinodorus grandifloras*) – ethanol:água (2A) and aqueous infusion (2B). UPLC analysis conditions: C18 column, column oven at 30°C, mobile phase (0.3 mL min⁻¹): water acidified with formic acid (0.1%, pH 2.4) – A and acetonitrile – B with a linear gradient beginning at 100% A, with a linear decrease to 92% A at 10 minutes, following the linear gradient until 70% A at 14 minutes, which was kept constant until 15 minutes.

rutin. Several parameters may influence the extraction difference observed when the samples were extracted only with water or water: ethanol mixture. One of the most important factors is the capacity of the extracting solution to permeate the plant structure and solubilize the compound. Such effect may be altered by the individual or synergistic action of the composition of the extraction solution (whose polarity change influences the intensity of the attractive forces between the rutin molecules and the extraction solution), agitation, particle size, volume of extraction and, in particular, the composition of the plant matrix (which may cause physical barrier effects or competitive effects among the other matrix compounds - as seen in Figures 1A and 1B) (Astill et al., 2001; Silveira et al., 2014; Nishiyama et al., 2010; Damoraran & Parkin, 2019; Martins et al., 2013). To further elucidate the chemical effects that occur in each plant, future species-specific optimization studies may be relevant.

Table 2 gives the concentration of rutin per 300 mL of beverage prepared by infusion from 2 g of plant to determine rutin consumption per portion of beverage. Five infusions with higher levels of rutin were *Ilex paraguariensis* (8.24 mg), *Boehmeria caudata* (9.43 mg), *Drimys winteri* (15.18 mg), *Ruta graveolens* (15.53 mg), *Sambucus nigra* (16.63 mg) and *Echinodorus grandiflorus* (29.70 mg). In order to consume the same amount of rutin present in 300 mL of *Echinodorus grandiflorus* beverage, a person must consume 600 mL of beverage made of *Sambucus nigra*, *Drimys winteri* or *Ruta graveolens*, approximately 1000 mL

of *Ilex paraguariensis* infusion, approximately 5200 mL of white tea (*Camellia sinensis*) infusion or 46000 mL of *Lavandula officinalis* infusion.

Compared to other foods and beverages, the consumption of 300 mL of *Echinodorus grandiflorus* aqueous infusion has a concentration of rutin equal to 400 g of cherry tomatoe (Sánchez-Rodríguez et al., 2012), 70 g of olives or 70 mL of olive oil (Yorulmaz et al., 2012), 550 mL of tangerine juice (Milella et al., 2011), 20 g of acerola (Nunes et al., 2011) or 6000 mL of red wine (Garaguso & Nardini, 2015). These data show the potential of *Echinodorus grandiflorus* infusion as a source of rutin when compared to other foods and beverages.

Studies have reported that rutin offers beneficial effects on health, such as antihyperglycemic, in doses from 5.0 mg per kg of body mass per day (Ghorbani, 2017). Thus, for a 70 kg adult, it is required a minimum intake of approximately 350.0 mg of rutin, although this value can vary for each individual according to their metabolism and bioavailability of rutin (Lesser et al., 2015). The content is found in only 23.6 g of the plant, indicating that its consumption through dishes such as salads, seasoning or hot dishes is an easy way of obtaining the supply of this flavonol from alimentation. In addition, future studies may be useful to optimize the preparation form of the infusion, so that a greater amount of plant is used in relation to the volume of water, thus, allowing the ingestion of this quantity of rutin in a lower volume of infusion.

4 Conclusion

This study highlights the importance of plants used in Brazil regarding rutin content, since it was found in 45 of 83 plants studied. The method of extraction using solvent (most used in commercial purification processes) or aqueous infusion (common method of consumption) has proved itself to be quite relevant regarding the content of rutin, since we observed a difference ranging from 0 to 169% among extractions, which shows the necessity of optimizing extraction for each source. The plants that showed the highest content of rutin in the aqueous infusions were *Echinodorus grandiflorus*, *Sambucus nigra*, *Ruta graveolens* and *Drimys winteri* (all above 7,000.00 mg of rutin per 1000 g of plants), emphasizing the potential of plants that had not been studied as new sources of rutin when compared to other foods and beverages, especially *Echinodorus grandifloras* (14,849.45 mg 1000 g⁻¹).

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