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Determination of phenolic compounds and evaluation of cytotoxicity in *Plectranthus barbatus* using the *Allium cepa* test

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Abstract. *Plectranthus barbatus*, popularly known as *Brazilian boldo* or *false boldo* is mainly used for digestive problems. The aim of our study was to evaluate the proliferative and genotoxic potential of aqueous extracts obtained from fresh and dry leaves and stems of *P. barbatus* using the *in vivo Allium cepa* test. For the treatments, 6 g of fresh material was first weighed. This was then dried in the microwave, resulting in 0.9 g stems and 0.64 g leaves. The same amount of material was dried naturally at room temperature. We prepared the aqueous extracts by infusion of the leaves and decoction of the stems. Water was used as a negative control and glyphosate 1.5% as a positive control. The extracts were analyzed by high performance liquid chromatography (HPLC). Statistical analysis was performed using the Chi-square and Scott-Knott tests ($p < 0.05$). The results showed that the extracts increased the cellular division of roots. No treatment found genotoxicity. The HPLC showed the predominance of isoquercitrin and kaempferol in the leaves, and isoquercitrin, kaempferol, and quercitrin in the stems. We concluded that the aqueous extracts from both leaves and stems of *P. barbatus* have proliferative and non-genotoxic activity on the cell division of *A. cepa*, which can be extrapolated to other eukaryotic cell types.

Keywords: Brazilian boldo, proliferation, liquid chromatography, mitotic index, medicinal plants.

INTRODUCTION

People have used medicinal plants for the treatment of ailments for thousands of years. Though current medicine is well developed, the World Health Organization (WHO) has found that a large part of the population in developing countries (85%) still uses traditional folk medicine for their basic health care (Brasil, 2006). However, the use of some plants to treat diseases is still based on empirical knowledge, thus without science-based data. The indiscriminate consumption of these plants as herbal teas or other products can

result in negative side effects, mainly due to the lack of knowledge on their chemical constituents. Only plants whose effects are known should be consumed, thus understanding putative toxic effects (Martins *et al.*, 2003).

Plectranthus barbatus Andrews is a species used in traditional medicine, known in Brazil as *boldo, fake-boldo*, and *malva-santa*. This species has a characteristic bitter taste when using its leaves in herbal teas, which is absent in its stems, but the substance causing this taste has not yet been identified (Lorenzi and Matos, 2008). Aqueous extracts of the leaves are used to treat heartburn, gastric discomfort, dyspepsia, hangovers, as a laxative (Lorenzi and Matos, 2008), and to prevent or control oral diseases (Figueiredo *et al.*, 2010). It has also been shown to have contraceptive activity in rats (Almeida and Lemonica, 2000), and potential use as an antimalarial drug (Kiraithe *et al.*, 2016).

Studies that validate putative genotoxic and proliferative effects of medicinal plants are necessary, and the use of cytogenetic bioassays - which use plants for detecting chromosomal damage that may be caused by other plant extracts, complex mixtures, chemicals, etc. - are highly recommended. The *Allium cepa* L. test is one of the few direct methods to measure damage to cells exposed to mutagenic or potentially carcinogenic agents and to assess the effects of such damage by observing chromosomal changes (Tedesco and Laughinghouse IV, 2012). Several studies have used the *A. cepa* test to evaluate plant extracts for cytotoxicity, such as Tedesco *et al.* (2015), Hister *et al.* (2017), and Sousa *et al.* (2018).

Rank and Nielsen (1994) found an 82% correlation between the *A. cepa* test and the mouse carcinogenicity test, concluding that *A. cepa* was even more sensitive than the Ames test. Teixeira *et al.* (2003) found the same results in tests comparing meristematic cells of onion roots, bone marrow cells of rats, and human lymphocytes as bioindicators, validating the safety of *A. cepa* for cytogenetic studies.

Extracts from medicinal plants are a complex mixture of various bioactive compounds. To better understand these mixtures, High Performance Liquid Chromatography (HPLC) can be used for phytochemical analyses (Trapp *et al.*, 2016). This technique is able to analyze the amount of chemical compounds in a sample in one single analysis, revealing the chromatographic or fingerprint profile of the extract (Alaerts *et al.*, 2007).

Our work aimed to evaluate the possible genotoxic and proliferative activities of fresh and dried leaves and stems of *P. barbatus*. This was undertaken using two different methods: 1) analyzing the cell cycle of root tips of *A. cepa* and 2) determining the phenolic compounds in the extracts.

MATERIAL AND METHODS

Sampling of the material

Material was obtained from an adult plant grown in a natural garden and acclimatized for 4 years in a 10kg large pot. The plant was collected in Santa Maria, Rio Grande do Sul, Brazil (29°42'54.8"S and 53°43'12.0"W) and taxonomically identified by Dr. TS Canto-Dorow.

Treatments and criteria for analysis

The meristematic cells of roots of *A. cepa* (onion, 2n = 16) were used as a test system to evaluate morphological and structural changes in chromosomes and to determine mitotic indices. Groups of four bulbs, each corresponding to a treatment, were placed in tap water for rooting.

After rooting, one group remained in water (negative control), another group was placed in a 1.5% glyphosate solution (positive control), since this herbicide induces chromosomal alterations and inhibits cell division in meristematic cells (Dimitrov *et al.*, 2006; Souza *et al.*, 2010; Rodrigues *et al.*, 2017). The other bulbs were transferred to the treatment solutions for 24 hours with the different extracts of *P. barbatus*. We used 24 hours because this is the duration of the mitotic cycle in *A. cepa* (Matagne, 1968).

The experiment was divided into two steps, using both dried and fresh material:

Step 1: 6 g (Brasil, 2011) of fresh material (leaves and stems) was weighed. A microwave was used to dry 6 g for comparison (5 minutes for leaves and 7 minutes for stems), which resulted in 0.64 g of leaves and 0.9 g of stems. The following treatments were then prepared: T1- water (negative control); T2- aqueous extract of 6 g L^{-1} of fresh stems; T3- aqueous extract of 0.9 g L^{-1} of microwave dried stems; T4- aqueous extract of 6 g L^{-1} of fresh leaves; T5- aqueous extract of 0.64 g L^{-1} of microwave dried leaves; T6- glyphosate 1.5% (positive control).

Step 2: Stems and leaves were dried naturally at room temperature, resulting in the same dry weight as those dried in the microwave. The following treatments were used: T1- water (negative control); T2- aqueous extract of 0.64 g L^{-1} of room temperature dried leaves; T3- aqueous extract of 0.9 g L^{-1} of room temperature dried stems; T4- glyphosate 1.5% (positive control).

After treatment, *A. cepa* rootlets were collected, fixed in ethanol:acetic acid (3:1) and stored in 70% ethanol at 4°C for further analysis. For slide preparation, a modified squashing technique was used, and acetic acid was used to stain the genetic material (Guerra and Souza 2002).

Slide analyses were performed using a Leica ICC50 HD light microscope, at 400x magnification and for each bulb two replicates were used (two slides). Five hundred cells per slide were counted, totaling 1000 cells per bulb and 4000 cells per treatment. Mitotic index (MI) values were calculated based on the percentage of dividing cells, according to the formula: MI = (number of cells in mitosis / total number of cells) x 100. In addition, we observed the phases of cell division to verify possible irregularities, such as chromosomal breakage, chromosome bridges, laggard chromosomes, and micronuclei.

Analysis of extracts by high performance liquid chromatography (HPLC-DAD)

A sample of each extract of *P. barbatus* was run on an HPLC-DAD at the Phytochemical Laboratory of the Department of Industrial Pharmacy at UFSM.

General Chemistry, Apparatus and Procedures

All chemical reagents were of analytical grade. Methanol, formic acid, gallic acid, caffeic acid, ellagic acid, and boldine were purchased from Merck (Darmstadt, Germany). Quercetin, quercitrin, isoquercitrin, rutin, and kaempferol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). HPLC was performed with a Shimadzu (Kyoto, Japan) and Shimadzu self-injector system (SIL-20A) equipped with alternative pumps (Shimadzu LC-20AT) attached to a degasser (20A5 DGU) with an integrator (CBM 20A), diode arrangement detector (SPD-M20A) and software (LC solution SP1 1.22).

Quantification by HPLC

Chromatographic analyses were performed in reverse phase under gradient conditions using a C_{18} column (4.6 mm x 150 mm) loaded with particles of 5 µm diameter; the mobile phase used water containing 1% formic acid (A) and methanol (B), and the gradient was: 13% B for 10 minutes, following 20%, 30%, 50%, 60 %, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 minutes, respectively, following the method described by Abbas *et al*. (2014) with minor modifications. The aqueous extracts of the leaves of *P. barbatus* (fresh and dry), stems of *P. barbatus* (fresh and dry), and the mobile phase were filtered with a 0.45 µm membrane filter (Millipore) and then degassed with an ultrasonic bath before use. The extracts were analyzed at a concentration of fresh leaves at 6 g L^{-1}, microwave dried leaves at 0.64 g L^{-1}, fresh stems at 6 g L^{-1}, microwave dried stems at 0.9 g L^{-1}. The flow used was 0.7 mL min^{-1}, injection volume of 40 µl, and the wavelength was 254 nm for gallic acid, 302 nm for boldine, 327 nm for caffeic and ellagic acids, and 366 nm for quercetin, quercitrin, isoquercitrin, kaempferol, and rutin.

The reference solutions were prepared in the mobile phase for HPLC at 0.025 - 0.300 mg mL^{-1} for quercetin, quercitrin, isoquercitrin, rutin, and kaempferol; 0.05 - 0.45 mg mL^{-1} for ellagic, gallic, and caffeic acids; and 0.006 - 0.250 mg mL^{-1} for boldine. Chromatographic peaks were confirmed by comparison of their retention time to standards and by DAD spectra (200 to 500 nm). Calibration curve for gallic acid: Y = 13174x + 1273.6 (r = 0.9999), boldine: Y = 12583x + 1274.9 (r = 0.9999), caffeic acid: Y = 11992x + 1367.1 (r = 0.9999), ellagic acid: Y = 13286x + 1264.1 (r = 0.9999), quercetin: Y = 12837x + 1364.5 (r = 0.9999), isoquercetin: Y = 12769x + 1326.5 (r = 0.9999), rutin: Y = 13158x + 1173.9 (r = 0.9998), quercetin: Y = 13627x + 1292.5 (r = 0.9996), and kaempferol: Y = 13271x + 1324.6 (r = 0.9999).

The extracts of the naturally dried leaves and stems were also analyzed at a concentration of room temperature dried leaves at 0.64 g L^{-1} and room temperature dried stems at 0.9 g L^{-1}. In this case, the flow used was 0.7 mL min^{-1}, injection volume of 40 µl, and the wavelength was 254 nm for gallic acid, 302 nm for boldine, 327 nm for caffeic and ellagic acids, and 366 for quercetin, quercitrin, isoquercitrin, kaempferol, luteolin, and rutin.

The reference solutions were prepared in the mobile phase for HPLC at the concentrations of 0.045-0.300 mg mL^{-1} for quercetin, quercitrin, isoquercitrin, rutin, luteolin, and kaempferol; 0.02 - 0.35 mg mL^{-1} for ellagic, gallic, and caffeic acids; and 0.006 - 0.250 mg mL^{-1} for boldine. Chromatographic peaks were confirmed by comparing their retention time to standards and by DAD spectra (200 to 600 nm). Calibration curve for gallic acid: Y = 12683x + 1197.5 (r = 0.9998), boldine: Y = 12481x + 1238.9 (r = 0.9999), caffeic acid: Y = 11983x + 1267.1 (r = 0.9999), ellagic acid: Y = 12670x + 1325.8 (r = 0.9992), quercetin: Y = 13056x + 1264.5 (r = 0.9999), isoquercitrin: Y = 11979x + 1286.5 (r = 0.9996), rutin: Y = 12758x + 1345.3 (r = 0.9999), Y = 12629x + 1198.6 (r = 0.9999), luteolin: Y = 13540x + 1317.1 (r = 0.9993), and kaempferol: Y = 11978x + 1257.6 (r = 0.9997).

All chromatographic operations were performed at room temperature and in triplicate. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope, using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 $\sigma.S^{-1}$, respectively, where σ is the standard deviation of

the response, and S is the slope of the calibration curve (Boligon *et al*., 2013).

Statistical analysis

The experimental design was completely randomized. Mitotic index values were compared by the Chi-square test ($\chi 2$) ($p < 0.05$), using BIOESTAT 5.O (Ayres *et al*., 2007). The means of phenolic compounds from HPLC were compared using the Scott-Knott's Test ($p < 0.05$) using Assistat®, beta version 7.7 (Silva and Azevedo, 2016).

RESULTS AND DISCUSSION

The *A. cepa* test is an ideal bioindicator to identify potential cytotoxic or mutagenic effects from medicinal plants, which can be harmful to human health (Bagatini *et al*. 2007).

Table 1 shows that the treatments with the aqueous extracts increased cell division, compared to the negative control. This means that both the leaves and stems from fresh and microwave dried material induced cell proliferation in meristematic cells of *A. cepa* rootlets. Thus, the substances tested can be considered cytotoxic, since they modify normal MI, increasing or decreasing it (Leme and Marin-Morales, 2009; Vieira and Silveira, 2018). In the case of *P. barbatus*, the aqueous extracts increased the cell division of *A. cepa* roots. This is similar to data by Iganci *et al.* (2006) on aqueous extracts of fresh leaves of *P. barbatus* (30 g L^{-1}) on seeds of *A. cepa*, where the authors found a significant increase in cell division after 6 days of treatment.

The highest mitotic index was induced by the extracts of stems of *P. barbatus*, with T2 = 5.5% (extract from the decoction of fresh stems). For leaf extracts, the microwave-dried leaf extract (T5) differed the most from the other extracts, but did not differ ($p < 0.05$) from the treatment with microwave-dried stems (T3). In addition, all the mitotic indices of the treatments differed ($p < 0.05$) from the positive control, which had a high cytotoxic effect.

In Table 2, we see that extracts from stems of *P. barbatus* dried at room temperature decreased cell proliferation in relation to water (T1), except T2 (aqueous extract by infusion of 0.64 g L^{-1} dry leaves), which was not significantly different from the negative control.

The mitotic indices of naturally dried stems and microwave-dried stems were different, suggesting that forced drying is able to intensify the cell division of *A. cepa* (Table 1 and Table 2). Still, the extract from fresh stems had the highest overall mitotic index.

Although the extracts induced cell division in *A. cepa*, we found no cells with chromosomal alterations in any of the treatments. Probably the secondary compounds in the extracts, which are complex mixtures with numerous bioactive secondary metabolites, induced the cell division. Iganci *et al.* (2006) also observed an increase in MI with no genotoxic effects when testing aqueous extracts of *P. barbatus* on seeds of *A. cepa*. In contrast, a study by Costa (2002) found that *P. barbatus* leaves were toxic to the liver and kidneys of mice treated over seven days. Similarly, Souza and Maia (2000) reported chronic toxicity of hydroalcoholic extracts of *P. barbatus* in rats treated with doses 20x greater than those used in folk medicine (680 mg kg^{-1}).

Therapeutic actions of medicinal plants are determined by their secondary metabolites (Taiz and Zeiguer, 2013), producing phenolic compounds with several functions, such as defense against pests and diseases, protection from ultraviolet radiation, and they can also attract pollinators (Ignat *et al*., 2011). As a secondary function, the phenolic compounds can have anti-inflammatory (Smolarek *et al*., 2009) and antimicrobial effects (Medina

Table 1. Number of analyzed *Allium cepa* cells and mitotic indices (MI) of treatments with fresh and dried leaves and stems of *Plectranthus barbatus*.

Treatments – step 1	TCO	Cells in interphase	P	M	A	T	Mitotic index (%)
T1 – negative control	4000	3893	66	19	8	14	2.68[d*]
T2 – decoction of 6 g $L^{-1}$of fresh stems	4000	3780	102	46	36	36	5.5[a]
T3 – decoction of 0.9 g $L^{-1}$of microwave-dried stems	4000	3840	77	22	30	31	4.0[b]
T4 – infusion of 6 g $L^{-1}$of fresh leaves	4000	3877	91	15	7	10	3.1[c]
T5 – infusion of 0.64 g $L^{-1}$of microwave-dried leaves	4000	3846	70	36	24	24	3.9[b]
T6 – positive control	4000	3975	10	8	2	5	0.63[e]

TCO = Total cells observed; P= prophase; M= metaphase; A= anaphase; T= telophase. *Means followed by the same letter are not significantly different among themselves, using the Chi-Square test at 5% probability.

Table 2. Number of analyzed *Allium cepa* cells and mitotic indices (MI) of treatments of dried leaves and stems of *Plectranthus barbatus* at room temperature.

Treatments – step 1	TCO	Cells in interphase	P	M	A	T	Mitotic index (%)
T1 – negative control	4000	3847	84	28	15	26	3.83 [a*]
T2 – infusion 0.64 g L^{-1} naturally dried leaves (room temperature)	4000	3860	75	19	18	28	3.5 [a]
T3 – decoction de 0.9 g L^{-1} of naturally dried stems (room temperature)	4000	3918	50	12	6	14	2.05 [b]
T4 – positive control	4000	3946	36	6	7	5	1.3 [c]

TCO = Total cells observed; P= prophase; M= metaphase; A= anaphase; T= telophase. *Means followed by the same letter are not significantly different among themselves, using the Chi-Square test at 5% probability.

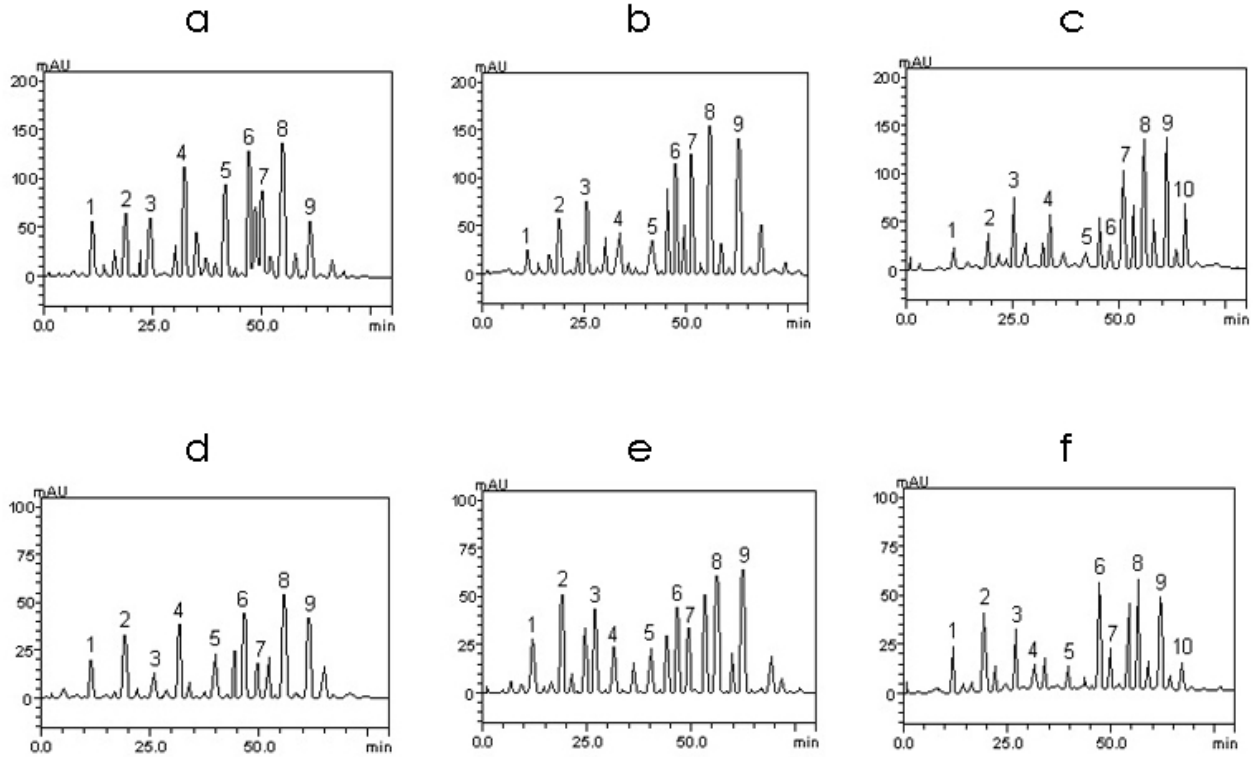

Figure 1. Representative HPLC profile of aqueous extracts of leaves and stems of *Plectranthus barbatus*: a) Infusion of fresh leaves; b) Infusion of dry leaves (microwave); c) Infusion of dry leaves (room temperature); (d) Decoction of fresh stems; e) Decoction of dry stems (microwave); (f) Decoction of dry stems (room temperature). UV detection at 327 nm. gallic acid (peak 1), boldine (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), rutin (peak 5), quercitrin (peak 6), quercetin (peak 7), isoquercitrin (peak 8), kaempferol (9) and luteolin (peak 10).

et al., 2011). In our study, HPLC analyses revealed different phenolic compounds in the aqueous extracts of leaves and stems: gallic acid (peak 1), boldine (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), rutin (peak 5), quercitrin (peak 6), quercetin (peak 7), isoquercitrin (peak 8), kaempferol (9), and luteolin (peak 10) (Figure 1a, 1b, 1c, 1d, 1e and 1f).

Table 3 shows the mean amount of each of the phenolic compounds present in the aqueous extracts of *P. barbatus* leaves analyzed by HPLC.

We found that in aqueous extracts of fresh leaves, isoquercitrin was the most abundant compound, followed by quercitrin and ellagic acid. In extracts from the microwave-dried leaves, isoquercitrin was also the pre-

Table 3. Phenolic compounds in aqueous extracts by infusion of *Plectranthus barbatus* leaves using high performance liquid chromatography (HPLC).

Compound	Extract of fresh leaves mg g^{-1}	Extract of microwave-dried leaves µg mL^{-1}	LOD mg g^{-1}	LOQ µg mL^{-1}	Extract of naturally dried leaves	LOD	LOQ
Gallic acid	5.28 ^{h*}	2.02 ^{h}	0.023	0.075	1.98 ^{f}	0.020	0.067
Boldine	7.21 ^{f}	5.64 ^{f}	0.008	0.026	3.76 ^{e}	0.012	0.039
Caffeic acid	5.11 ^{h}	7.05 ^{e}	0.015	0.049	7.12 ^{c}	0.019	0.061
Ellagic acid	15.29 ^{c}	3.89 ^{g}	0.027	0.093	5.70 ^{d}	0.027	0.093
Rutin	12.82 ^{d}	3.86 ^{g}	0.019	0.062	1.87 ^{g}	0.015	0.049
Quercitrin	16.44 ^{b}	14.92 ^{d}	0.013	0.042	2.02 ^{f}	0.034	0.113
Quercetin	10.14 ^{e}	16.09 ^{c}	0.024	0.079	9.92 ^{b}	0.008	0.026
Isoquercitrin	17.82 ^{a}	19.65 ^{a}	0.017	0.056	13.57 ^{a}	0.017	0.056
Kaempferol	5.63 ^{g}	17.23 ^{b}	0.035	0.115	13.62 ^{a}	0.025	0.081
Luteolin	-	-			7.03 ^{c}	0.013	0.042
TOTAL	95.74	90.35			66.59		

Results are expressed as mean of three determinations. LOD is the limit of detection and LOQ is the limit of quantification. * Means followed by different letters differ by the Scott-Knott test ($p < 0.01$).

Table 4. Phenolic compounds in aqueous extracts by decoction of *Plectranthus barbatus* stems using high performance liquid chromatography (HPLC).

Compound	Extract of fresh leaves mg g^{-1}	Extract of microwave-dried leaves µg mL^{-1}	LOD mg g^{-1}	LOQ µg mL^{-1}	Extract of naturally dried leaves	LOD	LOQ
Gallic acid	1.95 ^{g*}	3.08 ^{f}	0.023	0.075	2.81 ^{e}	0.020	0.067
Boldine	3.44 ^{e}	7.12 ^{c}	0.008	0.026	4.99 ^{c}	0.012	0.039
Caffeic acid	1.10 ^{i}	6.50 ^{d}	0.015	0.049	4.08 ^{d}	0.019	0.061
Ellagic acid	4.75 ^{d}	2.37 ^{g}	0.027	0.093	1.34 ^{g}	0.027	0.093
Rutin	2.15 ^{f}	2.34 ^{g}	0.019	0.062	1.41 ^{g}	0.015	0.049
Quercitrin	6.07 ^{b}	6.52 ^{d}	0.013	0.042	7.87 ^{a}	0.034	0.113
Quercetin	1.35 ^{h}	3.97 ^{e}	0.024	0.079	2.22 ^{f}	0.008	0.026
Isoquercitrin	8.25 ^{a}	8.32 ^{b}	0.017	0.056	7.88 ^{a}	0.017	0.056
Kaempferol	5.98 ^{c}	9.06 ^{a}	0.035	0.115	7.34 ^{b}	0.025	0.081
Luteolin	-	-			1.49 ^{g}	0.013	0.042
TOTAL	35.09	49.28			41.43		

Results are expressed as mean of three determinations. LOD is the limit of detection and LOQ is the limit of quantification. * Means followed by different letters differ by the Scott-Knott test ($p < 0.01$).

dominant compound (including more than in the fresh leaf extract), followed by kaempferol and quercetin. However, in the aqueous extracts of naturally dried leaves, both isoquercitrin and kaempferol were most abundant, followed by quercetin, caffeic acid, and luteolin. Luteolin is a phenolic compound found only in the extract of naturally dried leaves. These results are similar to those by Grayer *et al.* (2010), who analyzed the chemical composition of *P. barbatus* and observed kaempferol and quercetin, in addition to Pires *et al.* (2016) who detected quercitrin, quercetin, and kaempferol.

The extract of fresh leaves had the highest amount of total quantified phenolic compounds, followed by the extract of microwave-dried leaves and then the extract of naturally dried leaves. Thus, slowly drying the leaves caused a greater loss of compounds compared to fresh leaves. Studies by Pereira *et al.* (2000) support this finding, where they identified that higher levels of bioactive compounds are linked with rapid drying.

In Table 4, we see that in fresh stems there were low amounts of phenolic compounds, which is a different pattern than seen in the fresh leaves. Isoquercitrin was the

most abundant compound in fresh stems, followed by quercitrin and kaempferol. The extracts of microwave-dried stems contained the highest amount of compounds among the stems, with kaempferol being the major compound, followed by isoquercitrin and boldine. In the aqueous extract of the naturally dried stems, isoquercitrin and quercitrin were both the most abundant, followed by kaempferol and boldine. As in the leaf extracts, luteolin was also found in the extract of naturally dried stems.

Stem extracts had nearly 50% less phenolic compounds than the leaves, while inducing higher mitotic indices. Phenolic compounds such as flavonoids (quercitrin and isoquercitrin) are able to sequester free radicals (Decker, 1997) and their abundance has been positively correlated with antimicrobial action in *Salmonella enteritidis* (Medina *et al*., 2011). Interestingly, a study by Boligon *et al.* (2012) found that isoquercitrin isolated from *Scutia buxifolia* Reissek had a protective effect against human lymphocyte damage caused by hydrogen peroxide. The authors hypothesize that this was due to the reduction of oxidative stress due to its antioxidant nature. Carvalho *et al.* (2007) found that phenols, such as ellagic acid and gallic acid, can inhibit seed germination, plant growth, and fungi. According to Tomás-Barberán and Espín (2001), this group of secondary metabolites is related to the prevention of cardiovascular diseases and cancer.

In summary, aqueous extracts prepared with naturally dried material at room temperature did not affect the mitotic index of *A. cepa*. On the other hand, fresh and microwave-dried extracts of leaves and stems increased cell division (mitotic index). No cells with chromosomal alterations were found in any of the treatments.

Phytochemical analyses found a high amount of isoquercitrin in the aqueous extracts of leaves, with the major compound in naturally dried leaves being kaempferol. The total amount of phenolic compounds was much lower in stems. Isoquercitrin was the major compound in the extracts of fresh and dried stems at room temperature, and in the latter quercitrin was also predominant. The extracts of the microwave-dried stems were dominated by kaempferol.

Our results are preliminary, but we emphasize the importance to identify putative harmful compounds found in plants, to increase the safety for their use in folk medicine.

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