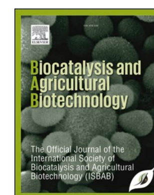
Biocatalysis and Agricultural Biotechnology 24 (2020) 101536

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: http://www.elsevier.com/locate/bab



Antioxidant capacity and phytochemical characterization of *Spathodea campanulata* growing in different climatic zones in Brazil

Valter H.M. Santos [a,*], Igor O. Minatel [b], Giuseppina P.P. Lima [b], Regildo M.G. Silva [c], Chung-Yen O. Chen [d]

[a] *Faculdade Pouso Alegre - Unis Group, Pouso Alegre, Minas Gerais, Brazil*
[b] *São Paulo State University (Unesp), Institute of Biosciences, Botucatu, Department of Biochemistry, Botucatu, São Paulo, Brazil*
[c] *São Paulo State University (UNESP), School of Sciences, Humanities and Languages, Assis, Department of Biotechnology, Laboratory of Herbal Medicine and Natural Products, Assis, São Paulo, Brazil*
[d] *Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA, United States*

A R T I C L E I N F O

Keywords:
Spathodea campanulata
Total phenolic
Antioxidant activity
Phytochemical tests
Climatic zones

A B S T R A C T

The aim of present study was evaluating the antioxidant activity and real content of phytochemicals of *Spathodea campanulata* from different climatic regions (two subtropical and one tropical area/cities) at Brazil. Samples of leaves, flowers and nectars from *S. campanulata* were tested for total phenolic content and *in vitro* antioxidant activity by ORAC (Oxygen Radical Absorbance Capacity), FRAP (Ferric Reducing Antioxidant Power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The phytochemical tests were carried out by two different chromatographic methods of detection (PDA and ECD) in HPLC for quantify the phenolic compounds content and one methods (PDA) in HPLC for quantify the carotenoids content. The results of the study indicate that mainly the samples of leaves and flowers of *S. campanulata* have significant total phenolic content and the antioxidant activity independent of analyzed city. In relation to phytochemical tests, the leaves samples from Assis city presented the higher total content of phenolic compounds among analyzed cities independent of detector (PDA and ECD). Total content of carotenoids was higher the leaves samples from Londrina city. This study reveals that the flowers and leaves of *S. campanulata* present substances with antioxidant potential, as noted in the antioxidant tests and phytochemical screening. Thus, *Spathodea campanulata* can be considered a good source of antioxidants independent of the occurrence of the individual in different climatic regions of Brazil.

1. Introduction

The *Spathodea campanulata* P. Beauv. (popularly named African tulip tree) belongs to the Bignoneaceae family, is native from African continent and was widely introduced in Brazil for ornamental purposes. Plants from Bignoneaceae family are known by producing compounds with aromatic, medicinal, and nutritional properties (Godzien et al., 2011; Manach et al., 2005). When these compounds are isolated, purified and incorporated in food supplements or medicaments, they can avoid metabolic deficiencies and prevent many kind of diseases. However, its leaves, flowers, and stem bark are used in folk medicine to treat edema, diarrhea, stomach complications, as well as, diuretic and anti-inflammatory (Choudhury et al., 2011; Heim et al., 2012; Ilodigwe et al., 2010). There has been increasing interest in bioactive compounds produced by plants, especially when they have beneficial effects to human health, such as antioxidant activity. The antioxidant compounds, when isolated and identified, may be used for development and formulation of several products related to food, cosmetology and pharmaceuticals (Boligon et al., 2009; Zadra et al., 2012). Antioxidants are essentials for preventing the oxidative damage in living cells and oxidative deterioration in foods. These compounds can delay oxidative degradation reactions by complexing to pro-oxidant metals or reduce cellular structures oxidation by inhibiting the production or scavenging free radical (Anissi et al., 2014; Pietta, 2000).

Previous studies have demonstrated the presence of phenolic acids and flavonoids as the main phytochemicals in *S campanulata* aerial parts (Elusiyan et al., 2011; Nazif, 2007). However, as far as we know, there are no reports concerning antioxidant capacity, phenolic acids,

* Corresponding author. Faculdade Pouso Alegre – Unis Group, Pouso Alegre, Minas Gerais, Brazil. Rua Pedro Bechara, 140 - Centro, 37550-000, Pouso Alegre, Minas Gerais, Brazil.
E-mail addresses: valter.vhmds@gmail.com, valter.santos@professor.unis.edu.br (V.H.M. Santos).

https://doi.org/10.1016/j.bcab.2020.101536
Received 30 November 2019; Received in revised form 4 January 2020; Accepted 7 February 2020
Available online 8 February 2020
1878-8181/© 2020 Elsevier Ltd. All rights reserved.

flavonoids, and carotenoids, from leaves, flowers, and nectar of *S. campanulata.* Furthermore, plants from different climatic regions (two subtropical and one tropical area/cities) and two different chromatographic methods of detection were evaluated the possible climatic and analytical influences on the real content of phytochemicals.

2. Methods

2.1. Chemicals and reagents

Analytical grade standards and organic solvents were obtained from Sigma Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade and purchased from J.T. Baker (JT Baker, Mexico).

2.2. Plant material and climate zones

The leaves, flowers, and nectars were collected from specimens *of S. campanulata*, randomly selected, during 2016 year, and located in the urban perimeter of three different cities: Limeira (22°34′34.0″S, 47°21′54.1″W, climate - tropical), Assis (22°38′53.2″S, 50°25′12.8″W, climate - transition zone tropical/subtropical), and Londrina (23°00′57.3″S, 51°11′28.4″W, climate - subtropical) (Fig. 1). Leaves and flowers were collected and then dried in a forced air incubator at a temperature of 40 °C until reach constant weight. Dried samples were ground in a knife mill and the resulting powder was stored in amber flasks at −80 °C. The nectar was collected from completely opened flowers, and later freeze-dried and stored at −80 °C.

2.3. Extract preparation

Two-hundred milligrams of dried leaves, flowers, and nectar were homogenized in vortex for 30 s with 2 mL of HCl:H_2O: methanol (2:18:80, v/v/v) and incubated for 30 min in an ultrasonic bath (Branson, Georgia, USA). After centrifugation for 10 min at 4 °C, the supernatant was collected and stored in amber flask. The pellet was re-extracted thrice and the supernatants combined to obtain the methanolic extract (MEx). These extracts were filtered through 0,22 μm syringe filter and used for antioxidant tests, total phenols quantification, and phenolic and flavonoids HPLC (UV/VIS -PDA detection and electrochemical detection - ECD) analysis. For carotenoids analysis in HPLC, 200 mg of powdered and dried samples were extracted with methanol and tetrahydrofuran (MeOH:THF extract) (Minatel et al., 2014). Antioxidant assays were performed in leaves and flowers MEx diluted 1:20 (v/v) in methanol. MEx of nectars were diluted 1:10 (v/v) in methanol only for ORAC assay.

2.4. Antioxidant assays

2.4.1. ORAC (Oxygen Radical Absorbance Capacity)

The ORAC values were determined using AAPH - 2,2′-Azobis (2-methylpropionamidine) dihydrochloride to generate the peroxyl radical, as previously described (Bolling et al., 2012). Samples were incubated with 0.32 M AAPH and 10 μM of fluorescein for 70 min. Fluorescence was monitored at 485 nm excitation wavelength and 520 nm emission using FLUOstar OPTIMA spectrofluorimeter (BMG LABTECH Inc., Cary, N.C., USA). A standard curve constructed in the range of 5 to 50 μmolTrolox/L was used to determine the ORAC values, based on the area under the response curve of each sample. The standard curve was linear between 0 and 50 μM Trolox and the results are expressed as μM/TEAC.mg^{-1} by dry weight of leaves, flowers and nectar.

2.4.2. FRAP (Ferric Reducing Antioxidant Power)

The FRAP assay was done according to Benzie and Strain (1996) with some modifications. Flowers and leaves methanolic extracts were diluted (1:20, v/v) in methanol. No dilution were made in nectars extracts. Samples were incubated in the dark at room temperature with the FRAP reagent for 1 h, and the absorbance at 593 nm was then recorded. FRAP values were calculated from standard curves using Trolox at 31.25–1000 μM/L. Results were expressed as uM Trolox/mg by dry weight of leaves, flowers and nectar.

2.4.3. DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The antioxidant activity of the leaves, flowers, and nectars were evaluated by monitoring their ability in quenching the stable free radical DPPH (Brand-Williams et al., 1995). Briefly, 900 μL of 100 μmol/L DPPH in ethanol was mixed with 100 μL of sample and the decrease in absorbance was monitored at 517 nm until a constant reading was obtained. The % inhibition was calculated by:

$$\%inhibition = \frac{AB - AS}{AB} x\ 100$$

where, AB = blank absorbance and AS = sample absorbance.

2.5. Total phenols

Total phenol of extracts were determined according to Singleton

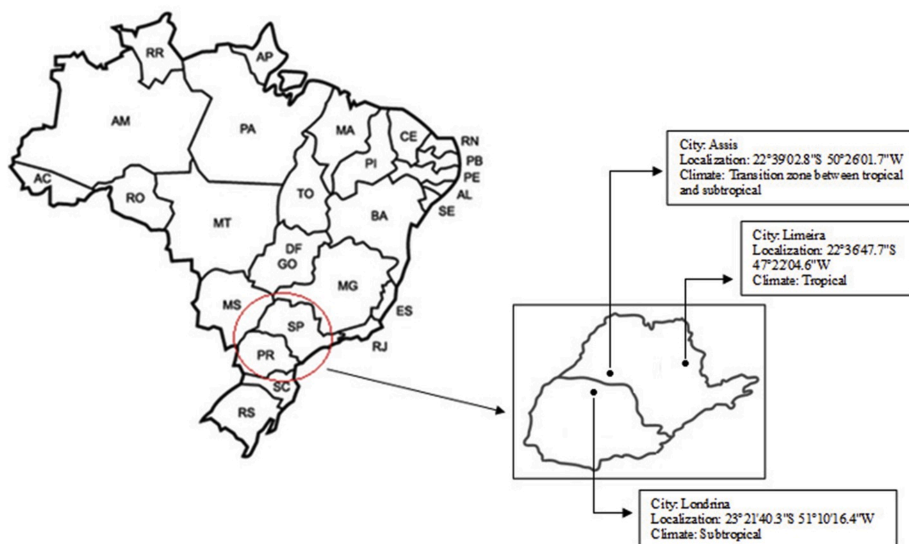


Fig. 1. Brazilian map showing the location of the study and the cities location.

et al. (1999). Two-hundred microliters of 10% Folin-Ciocalteu solution and 800 μL of Na_2CO_3 700 mM were mixed to 100 μL of each sample and incubated in the dark for 1 h at room temperature. Results were measured at 765 nm and expressed as micromol per liter gallic acid equivalents (GAE). The gallic acid standard curve ranged from 0.0125 to 0.2 mg GAE/mL.

2.6. HPLC/PDA analysis of phenols and flavonoids

The HPLC analysis of leaves, flowers, and nectar MEx were performed on a Thermo-Dionex UltiMate 3000 HPLC system (Thermo, Germany) equipped with quaternary pump, autosampler, column compartment, photodiode array detector and software Chromeleon version 7.1. A reversed phase ACE5 C18 (4.6 mm × 250 mm) column was used in the experiment. The injection volume was 10 μL, the column temperature maintained at 30 °C and the detection was set to 225, 280, 300, and 340 nm. The mobile phases used were: (A) phosphoric acid/ultrapure water (1:99, v/v) and (B) methanol 70%, and the gradient was set up for 1.0 mL/min to begin at 100% solvent A followed by 70% solvent A over a 10-min linear gradient. This is followed by an 40-min linear gradient to 5% solvent A and 3-min hold at 5% solvent A, and finally, a 2-min linear gradient back to 100% solvent A. The system is held at 100% solvent A for 3 min for equilibration back to initial conditions. The compounds were identified by chromatographic comparisons with authentic standards.

2.7. HPLC/ECD analysis of phenolic acids and flavonoids

Phenolic acids and flavonoids were determined by HPLC with electrochemical detection (ECD), using equipment and conditions previously described (Chen et al., 2005). MEx of leaves and flowers were dissolved in the aqueous mobile phase at 1:10 (v/v) and 1:4 (v/v), respectively. The nectar MEx was not diluted.

2.8. HPLC/PDA analysis of carotenoids

Ten milliliters of MeOH:THF extracts, for each sample, were dried under nitrogen, resuspended in 100 μL of ethanol and injected onto HPLC (equipment Thermo described above) equipped with a C30 column (3 μm, 150 × 3.0 mm; YMC, EUA), to determine the carotenoids content. The mobile phases A and B, running conditions, injection volume and flow rate were identical to previously described (Minatel et al., 2014).

2.9. Statistical analysis

All extractions and analysis were performed in triplicate. The results are expressed as means ± standard deviations, and were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey's test to multiple comparisons, using the SPSS software, version 19.0 (IBM Inc).

3. Results

3.1. Antioxidant capacity and total phenols

The antioxidant capacity and total phenols of leaves, flowers and nectar from *S. campanulata* growing in Limeira, Assis, and Londrina are showed in Table 1. The highest antioxidant capacity of each plant material were present in the climatic transition zone. In fact, for all climatic zones evaluated the antioxidant capacity and total phenols were higher in leaves, flowers and nectars, respectively.

Probably the temperature and rain incidence in transition zone is more favorable to antioxidant compounds synthesis by *S. campanulata* as showed by antioxidant assays and total phenols analysis. The ORAC assay was the most efficient procedure to assess the antioxidant capacity of the samples. This assay is more efficient to quantify the power of radical scavenging in samples, once it has the ability to react with hydrophilic and hydrophobic compounds in a high extend than DPPH and FRAP assays.

Table 1
Antioxidant capacity and total phenols of *Spathodea campanulata.*

Samples	Assis (transition)			
	DPPH (% inhibition)	FRAP[a] (uM TEAC/mg)	ORAC[a] (uM TEAC/mg)	Total phenols[b] (mg/g GAE)
Leaves	3.98 ± 0.002a	16.40 ± 0.001a	44.43 ± 0.005a	6.28 ± 0.002a
Flower	2.40 ± 0.002b	10.17 ± 0.001b	30.07 ± 0.004b	5.77 ± 0.001b
Nectar	0.08 ± 0.001c	0.281 ± 0.004c	1.086 ± 0.007c	0.25 ± 0.003c
Londrina (subtropical)				
Leaves	3.74 ± 0.002a	14.71 ± 0.003a	40.37 ± 0.008[a]	5.82 ± 0.002a
Flower	2.06 ± 0.003b	8.477 ± 0.002b	27.54 ± 0.005b	5.21 ± 0.002b
Nectar	0.07 ± 0.001c	0.243 ± 0.002c	0.956 ± 0.002c	0.24 ± 0.003c
Limeira (tropical)				
Leaves	3.54 ± 0.003a	13.71 ± 0.002a	32.26 ± 0.003a	5.35 ± 0.003a
Flower	1.80 ± 0.001b	6.708 ± 0.002b	23.29 ± 0.005b	4.62 ± 0.002b
Nectar	0.05 ± 0.001c	0.178 ± 0.001c	0.727 ± 0.001c	0.22 ± 0.002c

Values are expressed as mean ± standard deviation; means with the same letter in the column do not differ by Tukey test ($\alpha \leq 0.05$).
[a] The antioxidant capacity is expressed in uM Trolox (TEAC)/mg of samples.
[b] The total phenols are expressed in mg of samples per g of gallic acid equivalent.

3.2. Phenolic acids and flavonoids analysis by HPLC with PDA or ECD detector

In order to have a more efficient approach regarding the differences in phenolic acids and flavonoids profile, samples were evaluated by HPLC using two different detection systems. Employing both detection methods, PDA or ECD, the highest amounts of phenolic acids and flavonoids were found in leaves, flowers, and nectar, respectively. Despite the detection system employed and climatic zone, the leaves were the plant parts with the higher amounts of phenolic compounds, followed by flowers and nectar (Tables 2 and 3).

The PDA detection methods allowed the quantification of 5 compounds in leaves, 8 in flowers and 3 in nectar, independently of city analyzed. In relation to leaves, the most abundant compounds were caffeic acid and rutin, in flowers were caffeic acid and chlorogenic acid and in the nectar were identified only caffeic acid, p-cumaric and vitexin (Table 2).

Quantification and identification of phenolic compounds by HPLC/ECD allowed to observed the presence of four compounds for leaves and flowers and three for nectar, independent of city. In leaves was observed the higher concentration of rutin and ferulic acid, for flowers' samples the most abundant compounds were chlorogenic and ferulic acid. The nectar's samples presented lower number of identified compounds, being the p-cumaric acid the major compound (Table 3).

Taking into account carotenoids analysis, it was observed the presence of six compounds for leaves and flowers samples and two compounds for nectar samples, for the cities Assis and Londrina. In the case of Limeira samples, the leaf presented three compounds, the flower showed two compounds and in the nectar sample does not possible identify none compound. Independently of analyzed city, the compounds the most abundant for leaves samples was the lutein (Assis - 178,9 μg g^{-1}; Londrina - 378,2 μg g^{-1}; Limeira - 186 μg g^{-1}), flowers

Table 2
Phenolic acids and flavonoids identified by HPLC/PDA in leaves, flowers, and nectars of *Spathodea campanulata*.

Phenolic compounds (μg/g dw)											Total
City (climate)	Samples	Caffeic acid	Chlorogenic acid	Ferulic acid	p-Cumaric acid	Gallic acid	Rutin	Isoorientin	Vitexin	Orientin	
Assis (transition)	Leaf	1127.8 ± 0.18a	ND	46.1 ± 0.03b	ND	ND	1127.0 ± 0.01a	ND	31.8 ± 0.03c	0.3 ± 0.09d	2333
	Flower	571.4 ± 0.01a	857.1 ± 0.04b	55.1 ± 0.08c	535.6 ± 0.05d	39.3 ± 0.02e	ND	0.86 ± 0.12f	0.85 ± 0.05f	1.3 ± 0.03g	2061
	Nectar	0.097 ± 0.11a	ND	ND	0.5 ± 0.01b	ND	ND	ND	0.01 ± 0.01c	ND	0.61
Londrina (subtropical)	Leaf	961.9 ± 0.05a	ND	40.1 ± 0.07b	ND	ND	1084.9 ± 0.04c	ND	27.1 ± 0.02d	0.2 ± 0.04e	2114
	Flower	532.3 ± 0.01a	696.4 ± 0.09b	33.0 ± 0.05c	488.0 ± 0.08d	21.7 ± 0.03e	ND	4.55 ± 0.07f	0.73 ± 0.02g	1.3 ± 0.02h	1973
	Nectar	0.608 ± 0.06a	ND	ND	2.3 ± 0.10b	ND	ND	ND	0.02 ± 0.03c	ND	2.93
Limeira (tropical)	Leaf	750.1 ± 0.02a	ND	40.7 ± 0.09b	ND	ND	1045.1 ± 0.03c	ND	27.5 ± 0.05d	0.2 ± 0.11e	1864
	Flower	336.0 ± 0.11a	406.2 ± 0.11b	33.0 ± 0.04c	305.0 ± 0.02d	14.7 ± 0.09e	ND	0.17 ± 0.01f	0.13 ± 0.03f	0.4 ± 0.07f	1096
	Nectar	0.327 ± 0.04a	ND	ND	2.3 ± 0.08b	ND	ND	ND	0.01 ± 0.01c	ND	2.64

ND - Not detected.
Values are expressed as mean ± standard deviation; means with the same letter in the line do not differ by Tukey test ($\alpha \leq 0.05$).

Table 3
Phenolic acids and flavonoids identified by HPLC/ECD in leaves, flowers and nectars of *Spathodea campanulata*.

Phenolic compounds (μg/g dw)									
City (climate)	Samples	Gallic acid	Chlorogenic acid	Caffeic acid	p-Coumaric acid	Ferulic acid	Rutin	Quercetin 3-glucoside	Total
Assis (transition)	Leaf	ND	ND	68.4 ± 0.03a	ND	1415 ± 0.01b	238.4 ± 0.03c	38.46 ± 0.05d	1760
	Flower	ND	466.9 ± 0.02a	13.1 ± 0.03b	350.7 ± 0.01c	410.4 ± 0.01d	ND	ND	1241
	Nectar	0.026 ± 0.01a	ND	0.11 ± 0.01b	1.4 ± 0.02c	ND	ND	ND	1.5
Londrina (subtropical)	Leaf	ND	ND	61.03 ± 0.03a	ND	1351 ± 0.01b	176.8 ± 0.01c	35.92 ± 0.03d	1624
	Flower	ND	438.4 ± 0.04a	13.85 ± 0.04b	353.8 ± 0.02c	404.6 ± 0.02d	ND	ND	1210
	Nectar	0.020 ± 0.03a	ND	0.093 ± 0.02b	0.3 ± 0.02c	ND	ND	ND	0.4
Limeira (tropical)	Leaf	ND	ND	54.37 ± 0.07a	ND	1299 ± 0.03b	143.5 ± 0.01c	40.99 ± 0.02d	1497
	Flower	ND	233.9 ± 0.02a	14.65 ± 0.02b	128.4 ± 0.02c	327.9 ± 0.02d	ND	ND	705
	Nectar	0.006 ± 0.01a	ND	0.051 ± 0.03b	1.4 ± 0.06c	ND	ND	ND	1.5

ND - Not detected.
Values are expressed as mean ± standard deviation; means with the same letter in the line do not differ by Tukey test ($\alpha \leq 0.05$).

samples was the *trans*-beta-carotene (Assis - 32,32 μg g^{-1}; Londrina - 30,82 μg g^{-1}; Limeira - 4,29 μg g^{-1}). In relation to nectars samples, the compound the most abundant was the *trans*-luteín (Table 4).

4. Discussion

The present study is the first to assess the bioactive compounds in leaves, flowers, and nectar of *S. campanulata* comparing different methods of analysis and climate zones. The maximum distance between cities was 484 km for Limeira and Londrina. In addition, the maximum and minimum temperature registered in 2016 for Assis, Londrina and Limeira cities were 29.3–9.7 °C; 29.6–10.7 °C, and 28.4–9.9 °C, respectively. Although the three cities showed temperatures relatively similar in the 2016 year, the phenolic compounds analyzed and the antioxidant activity from obtained extracts were always higher in plants growing in Assis city. Based in these results, we hypothesized that phenolic compounds in *S. campanulata* are mainly synthesized when the trees are located in transition areas between warm (Limeira) and cold (Londrina) climates.

To measure, *in vitro*, the antioxidant capacity of a plant extract several laboratory methods are employed, such as DPPH, ORAC, FRAP, ABTS, and TBARS (Arnao et al., 1999; Pisoschi et al., 2016; Thaipong et al., 2006; Drouet et al., 2018). The evaluation of antioxidant activity is a complex process, because normally it can be occasioned for many mechanisms, therefore, it is advisable the utilization of more than one test. In this study, the DPPH, FRAP and ORAC tests, with samples of *S. campanulata*, showed significant antioxidant activity in all tests, mainly to flowers and leaves, giving emphasis to Assis, that showed the best results.

The test of total phenols provide great evidences to support the idea

Table 4
Carotenoids identified by HPLC/PDA in leaves, flowers and nectars of *Spathodea campanulata.*

Carotenoids (μg/g dw)										
City (climate)	Samples	Lutein	*Trans*-lutein	Zeaxanthin	Cryptoxanthin	13cis β-carotene	Alpha-carotene	*trans*-beta-carotene	9-cis-beta-carotene	Total
Assis (transition)	Leaf	179.0 ± 0.03a	ND	5.3 ± 0.04b	ND	4.5 ± 0.05b	13.3 ± 0.05c	62.7 ± 0.04d	8.6 ± 0.01e	273.4
	Flower	1.4 ± 0.02a	ND	ND	8.5 ± 0.01b	11.3 ± 0.03c	4.4 ± 0.01d	32.3 ± 0.02e	4.7 ± 0.05d	62.6
	Nectar	ND	0.06 ± 0.02a	ND	ND	ND	ND	0.05 ± 0.01a	ND	0.1
Londrina (subtropical)	Leaf	378.2 ± 0.01a	ND	15.9 ± 0.05b	ND	8.8 ± 0.01c	27.4 ± 0.08d	114.3 ± 0.03e	16.1 ± 0.01b	560.7
	Flower	2.3 ± 0.01a	ND	2.7 ± 0.02a	8.3 ± 0.01b	5.4 ± 0.07c	4.6 ± 0.02c	30.8 ± 0.02d	ND	54.1
	Nectar	ND	0.05 ± 0.08a	ND	ND	ND	ND	0.03 ± 0.02a	ND	0.1
Limeira (tropical)	Leaf	186.0 ± 0.05a	ND	2.8 ± 0.03b	ND	ND	ND	7.1 ± 0.09c	ND	155.9
	Flower	3.2 ± 0.03a	ND	ND	ND	ND	ND	4.3 ± 0.01b	ND	7.5
	Nectar	ND	ND	ND	ND	ND	ND	ND	ND	–

ND - Not detected.
Values are expressed as mean ± standard deviation; means with the same letter in the line do not differ by Tukey test (α ≤ 0.05).

that antioxidant activity present in the *S.campanulata* is linked to the presence of polyphenols, since that the aim of test is measure the content of phenolic compounds in the evaluated sample (Pisoschi et al., 2016). In this study, the total phenols presented significant results for flowers and leaves samples, mainly to city of Assis, like noted previously in the antioxidant tests.

Spathodea campanulata becomes one interesting specie of study, once previous researches has demonstrated potential antioxidant activity from some parts of this plant (Heim et al., 2012; Sangeetha et al., 2016). Nevertheless, few information regarding the phytochemical associated to this antioxidant activity are available. In the present study, we identified and quantified phenolic compounds by applying two different liquid chromatography detection systems (PDA and ECD). Each detector can present more or less capacity to detect certain substances, depending on the chemical origin of compound (Williamson et al., 2003; Elwekeel et al., 2013; Chambers et al., 2017). Both detectors were efficient, but compounds as trans ferulic acid, isoorientin, vitexin and orientin were identified only by PDA detector. On the other hand, ferulic acid and quercetin 3-glucoside were identified only by ECD. The other metabolites present in Tables 2 and 3 differed with regard their concentrations.

Despite of electrochemical (EC) detector is considered one of the most sensitive and selective HPLC detectors available and the PDA detector is considered more generalist (Kissinger and Heineman, 1984; Ackworth, 1997), in this study the photodiode (PDA) detector was more sensitive, in the number of compounds as in the concentration of identified compounds. EC detectors require the use of electrically conductive mobile phases capable of oxidize and reduce the compounds present into the analyzed samples, by any chance the reduction or oxidation do not happen, others detector, such as PDA, it is become more efficient and sensitive than ECD. In the present study, maybe the mobile phase used in the analysis with EC detector was not capable of oxidize and reduce the phenolic compounds present in samples, once these compounds are present in many oxidation and reduction reactions. Due the limitations and particularities of both detectors, some studies of chemical investigation of complex samples such as plant samples, it advises the use EC and PDA detectors, in order to maximize the identification of the compounds in the sample (Gamborg et al., 1961; Pereira et al., 2009).

The synthesis of secondary compounds in general suffers the direct influence of intrinsic factors of ambient, for example, solar radiation, photoperiod, temperature, soil types, rainfall and even biotic factors as action of herbivorous, in this way, plants of same species can present greater or lesser capacity of synthesis of bioactives depending on where it is inserted (Verpoorte et al., 1999; Inderjit and Duke, 2003; Carrier et al., 2003; Khan et al., 2009). A study carried out in leaves of 93 plants, located in two African rain forests, showed a remarkable difference in phenolic content between forests (Gartlan et al., 1980). The authors attributed this difference to soil nutrients and the region climate.

Plants with low levels of Ni can present decreased production of anthocyanins (Hawrylak et al., 2007). Trace metals obviously limit anthocyanin biosynthesis by inhibiting activity of L-phenylalanine ammonia-lyase (PAL) (Krupa et al., 1996). In studies performed by Angelova (et al., 2006) the nutrients $AgNO_3$ and $CdCl_2$ elicited overproduction of two tropane alkaloids, scopolamine and hyoscyamine, by in hairy root cultures of *Brugmansia candida*. According to Chambers et al. (2017), soils with higher quantities of macro elements, such as calcium and magnesium, can impact on the content of crude fiber and in the concentration of flavonolignans in *Silybum marianum*. Other abiotic factor, temperature variations, it has multiple effects on the metabolic regulation, permeability, rate of intracellular reactions in plant cell cultures, likewise, it may influence in the secondary metabolite production of plants, as reported in studies performed with *Melastoma malabathricum* cell cultures incubated at a lower temperature range (20 ± 2 °C) grew better and had higher anthocyanin production than those grown at 26 ± 2 °C and 29 ± 2 °C (Chan et al., 2010) and studies carry out by Szakiel et al. (2011), authors noted that lower soil temperatures caused an increase in levels of steroidal furostanol and spirostanol saponins.

Despite of some antioxidant tests as ORAC and Folin-Ciocalteu method have a higher sensibility in the detection of hydrophilic compounds, these methods also can interact with others compounds of lipophilic origin, such as carotenoids, terpenes and some classes of vitamins, being necessary more sensitive tests as high-performance liquid chromatography (HPLC) to identification of bioactives present in the test samples (Antolovich et al., 2002; De Oliveira et al., 2009).

Phytochemical investigations performed with species of Bignoneaceae family showed the presence of many classes of secondary metabolites, which goes from tannins, flavonoids, phenolic acids until lipophilic compounds as carotenoids (De Oliveira et al., 2009; Antonisamy et al., 2012). In relation to investigation carried out in this study, the phenolic compounds were identified and quantified by two detectors, the photodiode (PDA) and electrochemical (ECD), because each detector can present more or less capacity of detect of certain substances, depending on the chemical origin of compound (Williamson et al., 2003). Both detectors were efficient, but compounds as trans ferulic acid, isoorientin, vitexin and orientin were identified only by PDA detector, ferulic acid and quercetin 3-glucoside were identified only by ECD. The other metabolites present in Tables 2 and 3 differed with regard their concentrations.

Despite of electrochemical (EC) detector is considered one of the most sensitive and selective HPLC detectors available (Kissinger and Heineman, 1984; Ackworth, 1997), in this study the photodiode (PDA) detector presented higher effectiveness, in either in the number of identified compounds and concentration of identified compounds. EC detectors require the use of electrically conductive mobile phases capable of oxidize and reduce the compounds present into the analyzed samples, by any chance the reduction or oxidation do not happen, others detector, such as PDA, it is become more efficient and sensitive than ECD (Michael, 2010). In the present study, maybe the mobile phase used in the analyze with EC detector was not capable of oxidize and reduce the phenolic compounds present in samples, once these compounds are present in many oxidation and reduction reactions (Gamborg et al., 1961; Pereira et al., 2009).

Among the identified polyphenols by both detectors, we can highlight the rutin, caffeic acid, ferulic acid, compounds noted in previous studies carried out with the *S. campanulata* and known in scientific literature as antioxidant substances (Kowti et al., 2011; Heim et al., 2012; Coolborn et al., 2015). Ilodigwe et al. (2010) showed that leaves of species present polyphenols in its chemical composition, such as phenolic acids (caffeic acid, ferulic acid, gallic acid) and some classes of flavonoids (kaempferol, quercetin, quercetin 3-glucoside and rutin). In the case of screening performed with flowers, it was possible identify others classes of polyphenols as tannins and some flavonoids, as quercetin and rutin (Banerjee and DE, 2001).

In relation to therapeutic potential of polyphenols, studies performed with rutin showed that the compound has capacity of acts by inhibiting the formation of free radicals in many levels, because the flavonoid can react with anion superoxide and lipid peroxyl radicals neutralizing them, and it can also form a complex with iron that catalyze the formation of radical of non-toxic oxygen (Kim et al., 2011; Radjabian and Huseini, 2010; Poppe and Petersen, 2016). The caffeic acid, other class of phenolic compounds, also present many therapeutic properties with highlight for its antioxidant activity (Heim et al., 2012). Nardini et al. (1995) showed that the caffeic acid inhibited, in a dose/dependent manner, the lipid peroxidation induced by cupric ions. In the same study, the authors showed that the phenolic acid is capable of reduction the lipoperoxyl radicals (ROO•) (by means of donation of a hydrogen) and imped the propagation of lipid peroxidation. Finally, the ferulic acid, it has an antioxidant action due its chemical structure that facilitate the neutralization of free radicals, mainly the hydrogen peroxide, superoxide anions and hydroxyl radicals, reducing the degradation of proteins and lipids, caused mainly by hydroxyl radicals. Moreover, the ferulic acid also showed effective in the regulation and renovation of enzymes involved in the combat to the oxidative stress, such as superoxide dismutase (SOD) and catalase (Coolborn et al., 2015)).

Others compounds with therapeutic potential that were identified in this study were the carotenoids (Table 4). The results of analyzed samples of cities (Assis, Londrina e Limeira) showed the presence mainly of lutein and *trans*-beta-carotene, substances known for their medicinal potential. There are no studies reporting the presence of carotenoids in *S. campanulata*, Tinoi et al. (2006), showed the presence this class in *Pyrostegia venusta* and *Tabebuia chrysantha*, species of Bignoneaceae family, and the majority compounds were the lutein, zeaxanthin, cryptoxanthin and β-carotene. Foods rich in carotenoids as lutein, zeaxanthin and β-carotene may help ophthalmic damage prevention, mainly due their antioxidant activity, by scavenging free radical and working as filters for blue light and high ultraviolet energy (Rodriguez-Amaya et al., 2008).

Together, the antioxidant tests and phytochemical screening, can complement other potentiality assigned to *S. campanulata*, the insecticidal activity from nectar (Amusan et al., 1996; Santos et al., 2017). Researchers believe that the nectar of specie present compounds in its chemical composition with insecticidal activity and oxidant and does not compounds with antioxidant activity (Portugal-Araújo, 1963; Santos et al., 2017). This idea is supported, because in studies of count of dead insects inside the flowers of *S. campanulata* showed the presence of many species, as *Plebeia droryana*, *Tetragonisca angustula*, *Scaptotrigona postiça* and *Trigona spinipes* (Nogueira-Neto, 2002). Portugal-Araújo (1963) considered also the toxic effects of nectar from *S. campanulata*, where the researcher noted the presence of approximately 200 dead insect, among ants, bees and dipterous in just a single inflorescence this specie. Finally, Santos et al. (2017) showed the toxic effect of gross and dialyzed nectar in the insecticide tests with *E. heros*, *H. zea* and *A. gemmatalis*. The authors believe that insecticidal activity from nectar be caused indirectly by classes of identified proteins in study, as glycosyl transferase family and serine-threonine-protein phosphatase, since the proteins are involved in the biosynthesis of secondary compounds with insecticidal activity (Ferreyra et al., 2013).

In summary, this study reveals that the flowers and leaves of *S. campanulata* present substances with antioxidant potential, as noted in the antioxidant tests and phytochemical screening. Thus, *Spathodea campanulata* can be considered a good source of antioxidants independent of the occurrence of the individual in different climatic regions of Brazil.

Declaration of competing interest

The authors this article have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Acknowledgments

We are thankful for financial support by The São Paulo Research Foundation - FAPESP. Grant numbers - 2012/24440-7 and 2015/13855-0.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2020.101536.

References

Ackworth, I.N., 1997. Coulametric Electrode Aray Detectors for HPLC (Progress in HPLC-HPCE, vol. 6. Brill Academic, Boston.

Amusan, O.O.G., Adesogan, E.K., Makinde, J.M., 1996. Antimalarial active principles of *Spathodea campanulata* stem bark. Phytother Res. 10 (8), 692–693. https://doi.org/10.1002/(SICI)1099-1573(199612)10:8<692::AID-PTR928>3.0.CO;2-O.

Angelova, Z., Georgiev, S., Roos, W., 2006. Elicitation of plants. Biotechnol. Biotechnol. Equip. 20 (2), 72–83. https://doi.org/10.1080/13102818.2006.10817345.

Anissi, J., El Hassouni, M., Ouardaoui, A., Sendide, K., 2014. A comparative study of the antioxidant scavenging activity of green tea, black tea and coffee extracts: a kinetic approach. Food Chem. 150, 438–447. https://doi.org/10.1016/j.foodchem.2013.11.009.

Antolovich, M., Prenzler, P.D., Patsalides, E., McDonald, S., Robards, K., 2002. Methods for testing antioxidant activity. Analyst 127 (1), 183–198. https://doi.org/10.1039/B009171P.

Antonisamy, J.M., Aparna, J.S., Jeeva, S., Sukumaran, S., Ananthan, B., 2012. Preliminary phytochemical studies on the methanolic flower extracts of some selected medicinal plants from India. Asian Pacific Journal of Tropical Biomedicine 2 (1), 79–82. https://doi.org/10.1016/S2221-1691(12)60134-8.

Arnao, M.B., Cano, A., Acosta, M., 1999. Methods to measure the antioxidant activity in plant material. A comparative discussion. Free Radic. Res. 31 (Suppl. l), 89–96. https://doi.org/10.1080/10715769900301371.

Banerjee, A., DE, B., 2001. Anthocyanins in some flowers of West Bengal. JMAPS (J. Med. Aromat. Plant Sci.) 23, 600–604.

Benzie, I.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239 (1), 70–76. https://doi.org/10.1006/abio.1996.0292.

Boligon, A.A., Pereira, R.P., Feltrin, A.C., Machado, M.M., Janovik, V., Rocha, J.B.T., Athayde, M.L., 2009. Antioxidant activities of flavonol derivatives from the leaves

and stem bark of *Scutia buxifolia* Reiss. Bioresour. Technol. 100 (24), 6592–6598. https://doi.org/10.1016/j.biortech.2009.03.091.

Bolling, B.W., Chen, Y.Y., Kamil, A.G., Chen, C.Y.O., 2012. Assay dilution factors confound measures of total antioxidant capacity in polyphenol-rich juices. J. Food Sci. 77 (2), H69–H75. https://doi.org/10.1111/j.1750-3841.2011.02538.x.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 28 (1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Carrier, D.J., Crowe, T., Sokhansanj, S., Wahab, J., Branka, B., 2003. Milk Thistle, *Silybum marianum* (L.) Gaertn., flower head development and associated marker compound profile. J. Herbs, Spices, Med. Plants 10 (1), 65–74. https://doi.org/10.1300/J044v10n01_08.

Chambers, C.S., Hole, V., Petrásková, L., Biedermann, D., Buchta, M., Vladimír, K., 2017. The silymarin composition … and why does it matter??? Food Res. Int. 100 (3), 339–353. https://doi.org/10.1016/j.foodres.2017.07.017.

Chan, L.K., See, K.S., Boey, P.L., Bhatt, A., 2010. Effect of biotic stress on biomass and anthocyanin production in cell culture of *Melastoma malabathricum*. Biol. Res. 43 (1), 127–135. https://doi.org/10.4067/S0716-97602010000100014.

Chen, C.Y., Milbury, P.E., Lapsley, K., Blumberg, J.B., 2005. Flavonoids from almond skins are bioavailable and act synergistically with vitamins C and E to enhance hamster and human LDL resistance to oxidation. J. Nutr. 135 (6), 1366–1373. https://doi.org/10.1093/jn/135.6.1366.

Choudhury, S., Datta, S., Das Talukdar, A., Choudhury, M.D., 2011. Phytochemistry of the family bignoniaceae-A review. Assam university. Journal of Science & Technology Biological and Environmental Sciences 7, 975–2773.

Coolborn, A.F., Bolatito, B., Omolara, A.V., Adetuyi, F.C., 2015. Phytochemical and antioxidant effect of *Spathodea campanulata* leaf extracts. Int. J. Biochem. Res. Rev. 7 (3), 148–159. https://doi.org/10.9734/IJBCRR/2015/16371.

Drouet, S., Abbasi, B.H., Falguières, A., Ahmad, W.S., Ferroud, C., Doussot, J., Vanier, J. R., Lainé, E., Hano, C., 2018. Single laboratory validation of a quantitative core shell-based LC separation for the evaluation of silymarin variability and associated antioxidant activity of Pakistani ecotypes of milk thistle (Silybum marianum L.). Molecules 23 (4), 904. https://doi.org/10.3390/molecules23040904.

Elusiyan, C.A., Ani, N.C., Adewunmi, C.O., Olugbade, T.A., 2011. Distribution of iridiod glucosides and anti-oxidant compounds in Spathodea campanulata parts. Afr. J. Tradit., Complementary Altern. Med. 8 (1), 27–33. https://doi.org/10.4314/ajtcam.v8i1.60491.

Elwekeel, A., Elfishawy, A., Abouzid, S., 2013. Silymarin content in Silybum marianum fruits at different maturity stages. J. Med. Plants Res. 7 (23), 1665–1669.

Ferreyra, M.L.F., Rodriguez, E., Casas, M.I., Labadie, G., Grotewold, E., Casati, P., 2013. Identification of a bifunctional maize C- and O-glucosyltransferase. J. Biol. Chem. 288 (44), 31678–31688. https://doi.org/10.1074/jbc.M113.510040.

Gamborg, O.L., Wetter, L.R., Neish, A.C., 1961. The role of plant phenolic compounds in the oxidation of reduced diphosphopyridine nucleotide by peroxidase. Can. J. Biochem. Physiol. 39 (7), 1113–1124. https://doi.org/10.1139/o61-115.

Gartlan, J.S., McKey, D.B., Waterman, P.G., Struhsaker, T.T., 1980. A comparative study of the phytochemistry of two African Rain Forests. Biochem. Systemat. Ecol. 8 (4), 401–422. https://doi.org/10.1016/0305-1978(80)90044-7.

Godzien, J., Ciborowski, M., Angulo, S., Ruperez, F.J., Martínez, M.P., Señorans, F.J., Cifuentes, A., Ibañez, E., Barbas, C., 2011. Metabolomic approach with LC-QTOF to study the effect of a nutraceutical treatment on urine of diabetic rats. J. Proteome Res. 10 (2), 837–844. https://doi.org/10.1021/pr100993x.

Hawrylak, B., Matraszek, R., Szymanska, M., 2007. Response of lettuce (*Lactuca sativa* L.) to selenium in nutrient solution contaminated with nickel. Veg. Crops Res. Bull. 67 (1), 63–70. https://doi.org/10.2478/v10032-007-0031-7.

Heim, S.C., Guarnier, F.A., Ferreira, D.T., Braz-Filho, R., Cecchini, R., Cecchini, A.L., 2012. Antioxidant activity of *Spathodea campanulata* (Bignoneaceae) extracts. Brazilian Journal of Medicinal Plants 14 (2), 287–292. https://doi.org/10.1590/S1516-05722012000200006.

Ilodigwe, E.E., Akah, P.A., Okoye, T.C., Omeje, E.O., 2010. Anticonvulsant effects of a glycoside isolated from the leaf of *Spathodea campanulata* P. Beauv. Journal of Medicinal Plants 4 (18), 1895–1900. https://doi.org/10.5897/JMPR10.360.

Inderjit, Duke, S.O., 2003. Ecophysiological aspects of allelopathy. Planta 217 (4), 529–539. https://doi.org/10.1007/s00425-003-1054-z.

Khan, M.A., Blackshaw, R.E., Marwat, K.B., 2009. Biology of milk thistle (Silybum marianum) and the management options for growers in north-western Pakistan. Weed Biol. Manag. 9 (2), 99–105. https://doi.org/10.1111/j.1445-6664.2009.00326.x.

Kim, W., Gilet, T., Bush, J.W.M., 2011. Optimal concentrations in nectar feeding. Proc. Natl. Acad. Sci. U.S.A. 108 (40), 16618–16621. https://doi.org/10.1073/pnas.1108642108.

Kissinger, P.T., Heineman, W.R., 1984. Laboratory Techniques in Electroanalytical Chemistry. Marcel Dekker, New York.

Kowti, R., Joshi, V., Dabadi, P., Thammanna, G.S.S., Satish, B.P., Dinesha, R., 2011. Antioxidant activity of *Spathodea campanulata* in prevention of T-BOOH and H_2O_2 induced DNA damage. Int. J. Curr. Pharmaceut. Res. 3 (1), 87–89.

Krupa, D.J., Weng, J., Thompson, R.F., 1996. Inactivation of brainstem motor nuclei blocks expression but not acquisition of the rabbit's classically conditioned eyeblink response. Behav. Neurosci. 110 (2), 219–227. https://doi.org/10.1037/0735-7044.110.2.219.

Manach, C., Williamson, G., Morand, C., Scalbert, A., Rémésy, C., 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am. J. Clin. Nutr. 81 (1 Suppl. l), 230S–242S. https://doi.org/10.1093/ajcn/81.1.230S.

Minatel, I.O., Han, S.I., Aldini, G., Colzani, M., Matthan, N.R., Correa, C.R., Fecchio, D., Yeum, K.J., 2014. Fat-soluble bioactive components in colored rice varieties. J. Med. Food 17 (10), 1134–1141. https://doi.org/10.1089/jmf.2014.3146.

Nardini, M., D'Aquino, M., Tomassi, G., Gentili, V., Di Felice, M., Scaccini, C., 1995. Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. Free Radical Biol. Med. 19 (5), 541–552. https://doi.org/10.1016/0891-5849(95)00052-Y.

Nazif, N.M., 2007. Phytochemical and antioxidant activity of *Spathodea campanulata* P. beauvois. Growing in Egypt. Natural Product Sciences 13 (1), 11–16.

Nogueira-Neto, P., 2002. Management of plants to maintain and study pollinating bee species, and also to protect vertebrate frugivorous fauna. In: Kevan, P., Imperatriz Fonseca, V.L. (Eds.), Pollinating Bees - the Conservation Link between Agriculture and Nature - Ministry of Environment/Brasília, pp. 21–28.

Oliveira, A.C., Valentim, I.B., Goulart, M.O.F., Silva, C.A., Bechara, E.J.H., Trevisan, M.T. S., 2009. Fontes vegetais naturais de antioxidante. Quím. Nova 32 (3), 689–702. https://doi.org/10.1590/S0100-40422009000300013.

Pereira, D.M., Valentão, P., Pereira, J.A., Andrade, P.B., 2009. Phenolics: from chemistry to biology. Molecules 14 (6), 2202–2211. https://doi.org/10.3390/molecules14062202.

Pietta, P.G., 2000. Flavonoids as antioxidants. J. Nat. Prod. 63 (7), 1035–1042. https://doi.org/10.1021/np9904509.

Pisoschi, A.M., Pop, A., Cimpeanu, C., Predoi, G., 2016. Antioxidant capacity determination in plants and plant-derived products: a review. Oxidative Medicine and Cellular Longevity 2016, 1–36. https://doi.org/10.1155/2016/9130976.

Poppe, L., Petersen, M., 2016. Variation in the flavonolignan composition of fruits from different Silybum marianum chemotypes and suspension cultures derived therefrom. Phytochemistry 131, 68–75. https://doi.org/10.1016/j.phytochem.2016.09.003.

Portugal-Araujo, V., 1963. O perigo de dispersão da tulipeira do gabão (*Spathodea campanulata* Beauv.). Chacaras e Quintaes 107, 562–563.

Radjabian, T., Huseini, H.F., 2010. Anti-hyperlipidemic and anti-atherosclerotic activities of silymarins from cultivated and wild plants of Silybum marianum L. With different content of flavonolignans. Iran. J. Pharmacol. Ther. 9 (2), 63–67.

Rodriguez-Amaya, D.B., Kimura, M., Godoy, H.T., Amaya-Farfan, J., 2008. Updated Brazilian database on food carotenoids: factors affecting carotenoid composition. J. Food Compos. Anal. 21 (6), 445–463. https://doi.org/10.1016/j.jfca.2008.04.001.

Sangeetha, S., Meenakshi, S., Akshaya, S., Vadivel, V., Brindha, P., 2016. Evaluation of total phenolic content and antioxidant activity of different solvent extracts of leaf material of *Spathodea campanulata* P. Beauv. and investigation of their proliferation inhibition potential against EAC cell line. J. Appl. Pharmaceut. Sci. 121–127. https://doi.org/10.7324/JAPS.2016.60918.

Santos, V.H.M., Minatel, I.O., Reco, P.C., Garcia, A., Lima, G.P.P., Silva, R.M.G., 2017. Peptide composition, oxidative and insecticidal activities of nectar from flowers of *Spathodea campanulata* P. Beauv. Industrial Crops and Products 97, 211–217. https://doi.org/10.1016/j.indcrop.2016.12.025.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 299, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.

Szakiel, A., Paczkowski, C., Henry, M., 2011. Influence of environmental biotic factors on the content of saponins in plants. Phytochemistry Rev. 10 (4), 471–491. https://doi.org/10.1007/s11101-010-9177-x.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D.H., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compos. Anal. 19 (6–7), 669–675. https://doi.org/10.1016/j.jfca.2006.01.003.

Tinoi, J., Rakariyatham, N., Deming, R.L., 2006. Determination of major carotenoid constituents in petal extracts of eight selected flowering plants in the north of Thailand. Chiang Mai J. Sci. 33 (3), 327–334.

Verpoorte, R., Van Der Hejden, R., Ten Hoopen, H.J.G., Memelink, J., 1999. Metabolic engineering of plant secondary metabolite pathways for the production of fine chemicals. Biotechnol. Lett. 21 (6), 467–479. https://doi.org/10.1023/A:1005502632053.

Williamson, K.S., Hensley, K., Floyd, R.A., 2003. HPLC with electrochemical and photodiode array detection analysis of tocopherol oxidation and nitration products in human plasma. Methods in Biological Oxidative Stress 67, 76. https://doi.org/10.1385/1-59259-424-7:67.

Zadra, M., Piana, M., Brum, T.F., Boligon, A.A., Freitas, R.B., Machado, M.M., Stefanello, S.T., Soares, F.A., Athayde, M.L., 2012. Antioxidant activity and phytochemical composition of the leaves of *Solanum guaraniticum* A. St.-Hil. Molecules 17 (11), 12560–12574. https://doi.org/10.3390/molecules171112560.