

## Determination of phenolic compounds and evaluation of antioxidant capacity of *Campomanesia adamantium* leaves

I. D. Coutinho<sup>1</sup>, R. G. Coelho<sup>1</sup>, V. M. F. Kataoka<sup>2</sup>, N. K. Honda<sup>1</sup>, J. R. M. Silva<sup>1</sup>,  
W. Vilegas<sup>3</sup> and C. A. L. Cardoso<sup>1,2,\*</sup>

<sup>1</sup>Universidade Federal de Mato Grosso do Sul, Departamento de Química,  
Caixa Postal 549, 79070-900, Campo Grande/MS, Brazil

<sup>2</sup>Universidade Estadual de Mato Grosso do Sul, Curso de Química,  
Caixa Postal 351, 79804-970, Dourados/MS, Brazil

<sup>3</sup>Universidade Estadual Paulista, Departamento de Química Orgânica,  
Caixa Postal 355, 14801-970, Araraquara/SP, Brazil

\*claudia@uems.br

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**Abstract:** Five flavanones and three chalcones were isolated from *Campomanesia adamantium* Berg. (Myrtaceae) leaves. The contents of these compounds were determined by HPLC. The phenolic contents were also determined. The monitoring of the antioxidant activity was carried out by inhibition of peroxidation using the linoleic acid system and radical-scavenging (DPPH). The plants were collected from 4 distinct cities of the Mato Grosso do Sul State, Brazil. The different samples exhibited a range of 4.67-232.35 mg/g chalcones and 15.62-50.71 mg/g flavanones and phenolic contents of the 7.24-21.19 mg/g gallic acid. All extracts showed high antioxidant activity with a wide range of the radical-scavenging (DPPH) from 52.0 to 92.2 % and inhibition oxidation of linoleic acid from 14.6 to 94.2%.

**Keywords:** *Campomanesia adamantium*; Chalcones; Flavanones; Antioxidant activity..

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### Introduction

Many plants used in folk medicine contain a wide variety of phenolic compounds. The phenolic compounds act in the scavenging of free radical and in the inhibition of lipid peroxidation, especially the flavonoids [1]. Epidemiological studies support the belief that flavonoids in the human diet can reduce the risk of various cancers, especially hormone-dependent breast and

prostate cancer [2]. Studies “in vitro” activity of flavanones and chalcones demonstrated that these compounds have antitumoral effects [3]. The chalcones also exhibit numerous beneficial activities such as antibacterial, antifungal, anti-inflammatory, antileishmanial, antimalarial [4] and anti-HIV protease [5].

Given the importance of dietary habits and food components to health, studies of the identification of compounds in plants associated

with antioxidant activity are important to support the protective status of fruits and vegetables for people. The chemical composition and antioxidant activity of the *Campomanesia* species, a Brazilian "Cerrado" plant, has been investigated by our group.

*Campomanesia adamantium* (Myrtaceae) is a small tree with edible fruit and commonly known as guavira or guabiroba [6]. The fruits and leaves are widely used to make liqueurs, juices and sweets. In popular medicine the leaves are used as depurative, anti-diarrhoeic, cleanser, anti-rheumatic and reducer of blood cholesterol [7] and the roots are also used by people in the treatment of diabetes.

The HPLC technique has been shown to be a very efficient system for separating complex mixtures of plants. HPLC methods have been reported for the determination of substances and for monitoring variation in the composition of samples [8,9]. A procedure suitable for HPLC-UV analysis of flavanones and chalcones contents in *C. adamantium*, has been developed by this research team.

This paper describes the isolation of flavanones and chalcones and the evaluation of antioxidant activity from *C. adamantium* leaves during flowering stage. And also the quantification of these compounds, the determination of the phenolic contents and antioxidant activity in samples collected from four cities of the Mato Grosso do Sul State, Brazil during fruit bearing stage.

## Materials and methods

### *Chemical and spectral measurements*

Materials used and the suppliers were: quercetin, gallic acid, 2,6-Di-*tert*-butyl-4-methylphenol (BHT), naringenin, 2,2-diphenyl-1-picryl-hydrazil (DPPH) and linoleic acid were obtained from Sigma-Aldrich; folin-Cicauteau from Merck;  $\beta$ -carotene from Calbiochem; tanic acid from CETUS; chloroform-*d* from Cambridge Isotope Laboratories, Inc; spectroscopy-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany); water was purified using a Milli-Q system (Millipore). The solvents employed in other

analyses were grade analytic. NMR 1D and 2D spectra were recorded in chloroform-*d* used as an internal reference, employing a Bruker DPX-300 ( $^1\text{H}$  300 MHz;  $^{13}\text{C}$  75 MHz). The antioxidant activity assays were recorded in MeOH, employing 700 S Femto UV Spectrophotometer of 517 nm wavelength. Column chromatography was performed with Kieselgel 60 (70-230 mesh, Merck). Preparative TLC was carried out on silica gel PF<sub>254</sub>. Spots were visualized under ultraviolet light of 254 nm wavelength, sprayed with *p*-anysaldeyde and heated. Sephadex LH-20 (Pharmacia) was used for GPC.

### *The collection of the vegetable material*

For the phytochemical work, the leaves of the *C. adamantium* were collected in Mato Grosso do Sul State, Brazil, in the city of Jardim (**CAIF** latitude 21° 25' 02.0" S and longitude 056° 13' 77.0" W) during the flowering stage. For quantification and chemistry analyses the leaves were collected in the cities of Dourados (**CAE**; latitude 22° 11' 813" S and longitude 054° 55' 801" W); Bela Vista (**CAL**; latitude 22° 06' 35.8" S and longitude 056° 33' 00.8" W); Bonito (**CAB**; latitude 21° 07' 50.0" S and longitude 056° 24' 68.0" W) and Jardim (**CAI**; latitude 21° 25' 02.0" S e longitude 056° 13' 77.0" W) during the fruit bearing stage. The species were identified by Marcos Sobral (UFMG) and voucher specimens 5196 (Dourados), 5198 (Bela Vista), 5197(Bonito) and 5195 (Jardim) have been deposited in the Herbarium of Mato Grosso do Sul - HMS, Campo Grande, MS, Brazil.

### *Isolation of chemical constituents and purification*

The air-dried and powdered *C. adamantium* leaves (804.19 g) collected in the city of Jardim (**CAIF**), were extracted exhaustively with hexane, chloroform and methanol at room temperature (seven days for each solvent). The solvent was removed from the extract under vacuum yielding the hexane (5.32 g), chloroform (8.86 g) and methanol (58.88 g) extracts. For this paper only the methanol extract is discussed. The methanol extract (**EM**) was partitioned using water/hexane (1:1 v/v) and water/ethyl acetate (1:1 v/v). From that liquid-liquid partition were obtained the hexane phase and a mixture of the

three phases denominated ethyl acetate phase, water phase and inter-phase **IP**, the latter phase being insoluble in ethyl acetate and water.

The sample **IP** (675.60 mg) was dissolved in 15 mL of methanol/ethyl acetate and fractionated by Sephadex LH-20 CC (3.5 cm x 40 cm) and eluted with methanol/ethyl acetate isocratic systems. Two-hundred and five fractions of 10 mL were collected and were combined according to their behavior using TLC  $F_{254}$  in hexane/ethyl acetate/chloroform (4:4:2 v/v/v). The fractions 46-51 and 60-62 were purified on CC using silica gel 70-230 mesh (1.5 cm x 15 cm) and eluted with toluene/ethyl acetate/ethanol gradient systems. Compounds **1** and **2** were isolated from fractions 46-51 and compounds **3**, **4**, **5**, **6** and **7** were isolated from fractions 60-62. The fractions 90-98 were purified in CC using silica RP18 (1.5 cm x 15 cm) and eluted with water/acetonitrile/methanol gradient systems, resulting in the isolation of compound **8**. All compound structures were determined by NMR 1D and 2D and compared with data in the literature [5, 10-15].

#### *Preparation of the extracts from the plant collected in different regions for quantitative chemical analyses*

For the antioxidant activity assay in **ME** and **IP** the samples were prepared from 20 mg in 10 ml of methanol. Samples **CAE**, **CAL**, **CAI** and **CAE** collected during the fruit bearing stage were prepared with 50 mg of leaves in 25 mL methanol (maceration in sonication for two hours), filtered through a Millex filter (0.22  $\mu\text{m}$  diameter pore) and reconstituted in a 25 mL volumetric flask with methanol (n=3) for the determination of phenolic contents, antioxidant activity and the quantification of flavonones and chalcones.

#### *Determination of the flavanones and chalcones contents by HPLC*

##### *Analysis by HPLC*

The methanolic extracts obtained from *C. adamantium* leaves collected during the fruit bearing stage were analysed by an analytical HPLC (Varian 210) system with a ternary solvent delivery system, equipped with an autosampler and a photodiode array detector. The Star WS

(Workstation) software was used for chromatograms and measuring peak areas. The HPLC column was a RP18 (25 cm x 4.6 mm x 5  $\mu\text{m}$ ) reversed-phase column, with a small precolumn (2.5 cm x 3 mm) containing the same packing, used to protect the analytical column. Elution was carried out with a gradient solvent program of methanol/water/acetonitrile (40:50:10) taking 40 minutes to reach 80% methanol, 10% water and 10% acetonitrile, returning after that in 20 minutes to the initial conditions. The flow rate of 1.0 mL/min and the volume injected was of 50  $\mu\text{L}$ . All chromatographic analyses were performed at 22° C.

#### *The quantification of flavanones and chalcones*

The estimation of the contents of compounds **2**, **3**, **4**, **5**, **6**, **7** and **9**, the last compound was isolated from *Campomanesia pubescens* fruits [16], in the samples **CAE**, **CAB**, **CAI** and **CAL**, was performed by external calibration. The standards (**2-7** and **9**) were purified by HPLC (purity was  $\geq 90\%$ ) as is standard for HPLC analysis.

In this study the compounds were dissolved separately in spectroscopy-grade methanol in order to obtain stock solutions, which were appropriately diluted in 5 concentrations for each substance. Aliquots (50  $\mu\text{L}$ ) of 5 dilutions for each standard were analyzed by HPLC (n=3). For each standard was obtained a corresponding chromatogram and a graphic plot was constructed of the means of areas made against the weight of each substance. Linear least squares regression of the peak areas as a function of the weight was performed to determine correlation coefficients. The equation parameters (slope and intercept) of each standard curve were used to obtain concentration values for samples.

#### *Determination of the phenolic contents*

Aliquots of 0.1 mL samples of **CAE**, **CAB**, **CAI** and **CAL** were mixed with 0.5 mL (1/10 dilution) of the Folin-Cicauteau reagent and 1 mL of ultrapurified water. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, 1.5 mL of 20% sodium carbonate solution was added and incubated at room temperature for 40 min. The reaction mixture absorbance was measured at 760 nm on a spectrophotometer. The

blanks were prepared with all the reagents except the sample. Gallic acid was chosen as a standard using 6 point standard curve (10, 50, 100, 150, 300 and 400  $\mu\text{g/mL}$ ). All the tests were conducted in triplicate. The data were expressed as milligram gallic acid /g extract.

#### Determination of the antioxidant activity

##### Free radical scavenging activity (DPPH)

The free radical scavenging assay of samples **ME** and **IP** in the concentrations of 10.0, 20.0, 40.0, 80.0 and 160.0  $\mu\text{g/mL}$  and samples **CAL**, **CAI**, **CAB** and **CAE** in the concentrations of 160.0, 480.0, 800.0 and 1120.0  $\mu\text{g/mL}$  were determined based on their ability to react with the stable DPPH free radical. The assay was carried out with aliquots of 1mL of each sample added to 2 mL of a methanol solution of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) 0.004% and the mixture was incubated at a temperature of 25°C for 30 min and the absorbance of each solution was determined at 517 nm. The scavenging effect of the radical of each sample was calculated and compared with the scavenging effect of quercetin, naringenin, BHT and tanic acid in the concentrations of 10.0, 20.0, 40.0, 80.0 and 160.0  $\mu\text{g/mL}$ . All the tests were conducted in triplicate. The scavenging effect of the DPPH radical was calculated using the following equation [17]:

$$\text{DPPH scavenging effects (\%)} = [(A_o - (A - A_A)) / A_o] \times 100$$

$A_o$  is  $A_{517}$  of DPPH without a sample (control).  $A$  is  $A_{517}$  of a sample and DPPH.  $A_A$  is the  $A_{517}$  of a sample without DPPH (blank).

##### $\beta$ -carotenelinoleic acid assay

The  $\beta$ -carotene-linoleic acid assay was performed as reported by TEPE *et al.* [18] with some modifications. A stock solution of  $\beta$ -carotene-linoleic acid mixture was prepared as follows: 0.5 mg  $\beta$ -carotene was dissolved in 1 mL of chloroform and 25  $\mu\text{L}$  linoleic acid and 200 mg of Tween 20 were added. The chloroform was removed under nitrogen. The volume of the resulting mixture was completed up to 100 mL with aerated water. Aliquots (2.5 mL) of this reaction mixture were dispensed into different test

tubes containing 300  $\mu\text{L}$  of test samples in the concentrations of 10.0, 20.0, 40.0, 80.0 and 160.0  $\mu\text{g/mL}$  for the **ME** and **IP** samples and concentrations of 48.0, 144.0, 240.0 and 336.0  $\mu\text{g/mL}$  for the **CAL**, **CAI**, **CAB** and **CAE** samples. The absorbance in 470 nm was measured in the initial time of the reaction and after two hours. The BHT, tanic acid, naringenin and quercetin were used as standards in concentrations of 10.0, 20.0, 40.0, 80.0 and 160.0  $\mu\text{g/mL}$ . A control containing 300  $\mu\text{L}$  of methanol and 2.5 mL of the above mixture was prepared. The tubes were placed at 45 °C in a water bath for two hours. All the tests were conducted in triplicate. The inhibition of peroxidation of the solutions tested was calculated using the following equation:

$$\text{Inhibition of peroxidation (\%)} = (\beta\text{-carotene content after 2 h assay} / \text{initial } \beta\text{-carotene content}) \times 100.$$

##### Stability Study

The stability of the working standard solutions was tested at 22 °C (working temperature) and -20 °C (storage temperature). The stability of substances in the samples was evaluated during all the storage steps (*i.e.* at room temperature and at -20 °C). Stability was defined as being less than 2% loss of the initial drug concentration in the stated time.

## Results and Discussion

### General

The substances isolated from *C. adamantium* leaves collected in the flowering stage are here described for first time. Compounds **1-4** and **6** were characterized as flavanones and **5** and **7-9** as chalcones: 7-hydroxy-5-methoxy-flavanone (**1**), 7-hydroxy-5-methoxy-6-methylflavanone (**2**), 5,7-dihydroxy-6-methylflavanone (**3**), 5,7-dihydroxy-8-methylflavanone (**4**), 2',4'-dihydroxy-6'-methoxychalcone (**5**), 5,7-dihydroxy-6,8-dimethylflavanone (**6**), 2',4'-dihydroxy-6'-methoxy-5'-methylchalcone (**7**), 2',4'-dihydroxy-6'-methoxy-3'-methylchalcone (**8**) and 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (**9**).

### Quantification of flavanones and chalcone

A number of preliminary HPLC experiments employing samples of *C. adamantium* leaves were performed to establish optimal conditions for HPLC analysis of this species.

HPLC analysis showed baseline separation for the compounds under consideration. The observed retention time to isolate compounds is shown in Table 1. The quantification of compounds **2**, **3**, **4**, **5**, **6**, **7** and **9** was made by comparison of their retention time with those of authentic standards and by standards addition in the same samples analyzed using HPLC. The photodiode array detector was employed in the samples in the determination of compounds and in the evaluation of interference in all peaks of the chromatograms. The analysis of the precision of the assay intra- day CVs were less than  $\pm 2\%$ .

The compounds were stable in the working solutions after 24 h at 22 °C, two months at -20 °C. The calibration curves were determined by linear regression (Table 1). Average standard

errors for the peak areas of replicate injections (n=3) were smaller than 2% showing good repeatability of the calibration curve. The peak areas for the calibration curve of compound **2** were determined at 284 nm, compounds **3** and **4** at 296nm, compound **5** at 350 nm, compound **6** at 299 nm, and compounds **7** and **9** at 340 nm.

Table 2 shows the contents of the compounds identified in samples in distinct cities during the fruit bearing stage. The data displayed showed that **CAL** contains larger amounts of the flavanones and chalcones followed by **CAB**, **CAI** and **CAE**. The samples show higher amounts of the chalcones than that of flavanones.

Though the flavanones and chalcones contents showed a difference quantitatively in different samples, those consumed by the Bela Vista, Bonito and Jardim populations exhibited higher contents, mainly chalcones **7** and **9**. Studies of the latter exhibited individually activity against *Staphylococcus aureus* and *Bacillus cereus* and increased activity in synergy with the antibiotics berberin and

**Table 1.** Retention time and regression data of calibration curve for quantitative determination by HPLC.

Compounds	Retention time (min. $\pm$ S.D. <sup>a</sup> )	LR <sup>b</sup>	B <sup>c</sup>	A <sup>d</sup>	R <sup>e</sup>	N <sup>f</sup>
2	15.33 $\pm$ 0.16	0.04-10.80	4.95919.106	-161369.02552	0.99962	6
3	22.02 $\pm$ 0.24	0.16-4.10	6.58015.106	-1.3073.106	0.99889	5
4	23.51 $\pm$ 0.27	0.12-3.67	1.80351.107	-2.37114.106	0.99978	5
5	24.70 $\pm$ 0.21	0.20-5.00	5.60768.106	-1.29557.106	0.99825	5
6	26.22 $\pm$ 0.26	0.21-5.00	5.15469.106	-564964.75892	0.99921	5
7	30.18 $\pm$ 0.25	5.65-22.66	500747.0648	-35037.81908	0.9995	5
9	33.67 $\pm$ 0.24	3.00-28.00	507205.62017	-22757.06765	0.99860	5

<sup>a</sup>S.D.: standard deviations. <sup>b</sup>LR: linear range. Linear regression. formula:  $y = A + Bx$ . where  $y$  = peak areas ratio. <sup>c</sup>B: slope. <sup>d</sup>A: intercept.  $x$ :  $\mu\text{g}$ . <sup>e</sup>R: correlation coefficient. <sup>f</sup>N: number of samples. Compounds: 7-hydroxy-5-methoxy-6-methylflavanone (**2**), 5,7-dihydroxy-6-methylflavanone (**3**), 5,7-dihydroxy-8-methylflavanone (**4**), 2',4'-dihydroxy-6'-methoxychalcone (**5**), 5,7-dihydroxy-6,8-dimethylflavanone (**6**), 2',4'-dihydroxy-6'-methoxy-5'-methylchalcone (**7**) and 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (**9**).

**Table 2.** Contents of compounds identified in the methanolic extracts from the *C. adamantium* leaves.

Compounds	Flavanones (mg/g $\pm$ S.D. <sup>a</sup> )					Chalcones(mg/g $\pm$ S.D. <sup>a</sup> )	
	2	3	4	5	6	7	9
CAI	6.72 $\pm$ 0.44	22.82 $\pm$ 0.95	6.38 $\pm$ 0.30	5.74 $\pm$ 0.41	3.71 $\pm$ 0.28	82.42 $\pm$ 5.01	11.24 $\pm$ 0.78
CAB	8.27 $\pm$ 0.42	12.74 $\pm$ 0.33	6.26 $\pm$ 0.24	6.39 $\pm$ 0.12	3.49 $\pm$ 0.09	94.70 $\pm$ 2.81	15.01 $\pm$ 0.35
CAL	10.12 $\pm$ 0.11	30.17 $\pm$ 0.62	10.42 $\pm$ 0.21	10.62 $\pm$ 0.25	6.37 $\pm$ 0.24	194.66 $\pm$ 2.32	31.32 $\pm$ 0.76
CAE		12.67 $\pm$ 0.28	2.95 $\pm$ 0.03		4.67 $\pm$ 0.23		

<sup>a</sup>S.D.: standard deviations.

tetracycline [4]. The samples from Bela Vista, Bonito and Jardim show chalcone **5**, which have anti-HIV-1 protease activity. Although, the sample consumed by the Dourados population does not showed the presence of **2**, **5**, **7** and **9**.

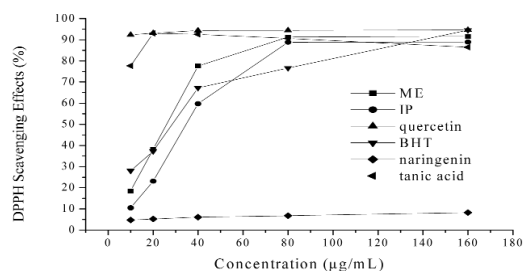
Hence supposing that the production and concentration of the secondary metabolites in the guavira leaves can be influenced by environmental strain of each region, these changes might be associated with the variation of the medicinal properties of the vegetables in each municipality.

#### Determination of the free radical scavenging activity

The use of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) as a reagent for screening the antioxidant activity of molecules has been reported [19]. In this assay the scavenging of the DPPH radical is followed by monitoring the decrease in absorbance at 517 nm, which occurs due to the reduction by antioxidants and has been used to assess the ability of phenolic compounds to transfer labile H atoms to radicals [1].

The antioxidant activity of **ME** and **IP** using the DPPH assay displayed in figure 1 shows that the *C. adamantium* leaves exhibit very good radical scavenging activities. But only the concentration from the 80.0  $\mu\text{g/mL}$  samples showed similar activity with quercetin and tannic acid standards and that more active than BHT and naringenin, all in the same concentration. The **ME** and **IP** show the highest activity with 91.6% and 88.9% at 160.0  $\mu\text{g/mL}$  and quercetin standard of 94.6% in some concentrations.

The **CAI** and **CAB** samples showed an scavenging effect larger than 80.0 % as of 480.0  $\mu\text{g/mL}$ , while **CAL** and **CAE** showed that the scavenging effect was larger than 80.0 % as of 160.0



**Figure 1.** Free radical scavenging activity of **ME**, **IP**, quercetin, BHT, naringenin and tannic acid.

$\mu\text{g/mL}$ . The antioxidant activity in all extracts in 160.0  $\mu\text{g/mL}$  were lower than the quercetin standard in 160.0  $\mu\text{g/mL}$  (94.6 %), except the **CAL** sample that showed the same scavenging effect as quercetin in the lower concentration. The analysis of the precision of the assay intra- day CVs were less than  $\pm 2\%$ , except for **CAI** (160.0  $\mu\text{g/mL}$ ) and **CAB** (160.0  $\mu\text{g/mL}$ ), 5% and 6%, respectively.

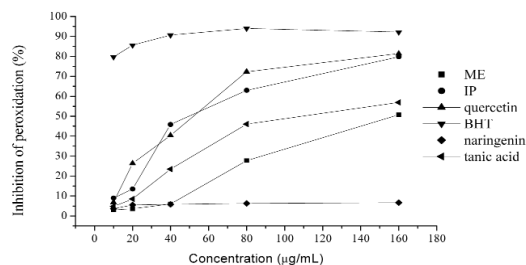
The compounds analyzed by extracts did not show direct correlation with antioxidant activity, except the **CAL** sample.

The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors and singlet oxygen quenchers. The chemical nature of the flavonoids depends on structural class, degree of hydroxylation, other substituents and conjugations [20].

Studies of structure-radical scavenging activity of phenolic compounds in Chinese plants showed that the lowest antiradical activity of the flavonoids was attributed to the flavanones and isoflavanones, therefore the antioxidant activity depends on the number and position of hydroxyl groups in rings A and B as well as 2,3-double bond (insaturation) and the 4-oxo group in ring C [21].

#### Inhibitory effect on lipid peroxidation

In the  $\beta$ -carotene/linoleic acid assay the antioxidant capacity is determined by measuring the inhibition of the organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [18]. This assay has been used to simulate the oxidation of the membrane lipid components in the presence of antioxidants inside the cell [22]. The results obtained from extracts of *C. adamantium* leaves are exhibited in figure 2.



**Figure 2.** Antioxidant activity of **ME**, **IP**, quercetin, BHT, naringenin and tannic acid by  $\beta$ -carotene/linoleic acid system.

Sample **ME** shows low inhibition of peroxidation 50.8% in a concentration of 160.0  $\mu\text{g/mL}$ , while the **IP** shows appreciable inhibition of 79.7% with values similar to the quercetin (81.3%), higher than tanic acid (56.9%) and naringenin (6.7%) and lower than BHT (92.1%) all the same concentrations.

The samples collected in distinct cities exhibited antioxidant activity based on inhibition of peroxidation assay ranging from 14.6-94.2 % in a concentration of 48-336  $\mu\text{g/mL}$  and a lower variation between samples.

Samples **CAL**, **CAI**, **CAE** and **CAB** in the 144.0  $\mu\text{g/mL}$  concentrations show inhibition of peroxidation of 89.8, 84.8, 66.3 and 46.5 %, respectively. These results indicate that of the *C. adamantium* leaves the samples **CAL** and **CAI** exhibited appreciable inhibition of peroxidation compared to BHT of 160.0  $\mu\text{g/mL}$  (92.1%). The analysis of the precision of the assay intra- day CVs were less than  $\pm 5\%$ .

Analysis of the structure-antioxidant activity relationship of the standards shows that the lipophilic compounds with the BHT are more potent than the hydrophilic compounds such as quercetin and tanic acid, as well as compounds of low insaturation and absence of the lipophilicity substituent with naringenin. Compounds **3**, **4**, **6**, **7** and **9** show correlation which enhances the inhibition of peroxidation, mainly chalcones **7** and **9** in the **CAL**, **CAI** and **CAB** samples and flavanones **3**, **4**, **6** with methyl substituents in all samples.

#### *Determination of total phenolic content*

The results showed **CAL** (21.2 mg/g) and **CAE** (15.9 mg/g) with high phenolic content in comparison to **CAI** (9.0 mg/g) and **CAB** (7.2 mg/g). The precision analysis of the assay intra-day CVs was less than  $\pm 5\%$  showing reproducibility of the extraction procedure. The compounds identified in the samples, in general, did not show direct correlation to phenolic contents. The **CAL** sample showed direct correlation to the compounds under consideration in relation to phenolic content. The **CAE**, **CAB** and **CAI** samples did not show this profile.

The antioxidant activity by inhibition of peroxidation shows correlation to phenolic contents, except the **CAE** sample. And also with

antioxidant activity by DPPH radical scavenging in a concentration of 160.0  $\mu\text{g/mL}$ , but in higher concentration the **CAI** sample showed no correlation.

These results showed that in high concentrations, the extract exhibited high antioxidant activity and that as it increases the concentration of the extract, the activity is practically constant, even with a variation of phenolic content. The higher antioxidant activity showed in the extracts might be associated with other compound phenolics that were not isolated, mainly the **CAE** samples, which show higher phenolic content and lower phenolic content among the compounds isolated.

## Conclusions

On the basis of the results it is suggested that the *Campomanesia adamantium* leaves evaluated here can be used as a source of natural antioxidant. However, the results showed a distinct composition quantitative in this vegetable of some species collected in distinct cities, but a similar chemical composition qualitative (except the Dourados sample). The different samples exhibited a range of total phenolics contents varying from 7.2 to 21.2 mg/g. Phenolic compounds seem to have important role in stabilizing lipid oxidation and to be associated with antioxidant activity. The phenolic compounds may contribute directly to antioxidative action. Therefore, further work could be done on the isolation and purification of the active components from the crude extracts of *Campomanesia* species for showing the mode of action of them.

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I. D. Coutinho, R. G. Coelho, V. M. F. Kataoka, N. K. Honda, J. R. M. Silva, W. Vilegas and C. A. L. Cardoso. Determinação de compostos fenólicos e avaliação da capacidade antioxidante das folhas de *Campomanesia adamantium*.

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**Resumo:** Cinco flavanonas e três chalconas foram isoladas das folhas de *Campomanesia adamantium* Berg. (Myrtaceae). O teor desses compostos foram determinados por CLAE. O teor de compostos fenólicos também foi determinado. O monitoramento da atividade antioxidante foi feito pela inibição da peroxidação usando o sistema do ácido linoleico e método do radical livre 2,2-difenil-1-picril-hidrazila - DPPH•. As espécies vegetais foram coletadas em 4 cidades diferentes do estado de Mato Grosso do Sul, Brasil. As diferentes amostras exibiram uma variação de 4,67-232,35 mg/g chalconas e 15,62-50,71 mg/g flavanonas e teor de compostos fenólicos de 7,24-21,19 mg/g ácido gálico. Todos os extratos exibiram alta atividade antioxidante com uma variação do efeito radical livre (DPPH) de 52,0-92,2 % e da inibição da oxidação do ácido linoleico de 14,6-94,2%.

*Palavras-chave:* *Campomanesia adamantium*; chalconas; flavononas; atividade antioxidante.

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