International Journal of Secondary Metabolite
2023, Vol. 10, No. 1, 59–70
https://doi.org/10.21448/ijsm.953427
Published at https://dergipark.org.tr/en/pub/ijsm
Research Article

Promising antioxidant activity of crude extract from *Calliandra tweedii* Benth

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Abstract: The interest in medicinal plants encourages new research studies on plant extracts, as they are sources for treatments in medicine, agriculture, and veterinary. *Calliandra tweedii* is a native Brazilian species with broad use in folk medicine. This study aimed to examine the leaf crude extract (CE) of C. tweedii and its methanolic (MP) and hexane (HP) phases concerning total phenolic compounds (TPC), proanthocyanidins (PRO), and flavonoid profile, and correlate it to their antioxidant activity (DPPH and FRAP). CE and MP revealed high contents of TPC, PRO and high antioxidant activity (DPPH and FRAP) in relation to HP. Contrarily, HP showed reduced antioxidant activity, according to the scarce phenolic constituents obtained from the partition. Five flavonols were detected by HPLC-UV-DAD, being quercitrin the major constituent. Also, one kaempferol derivate and a series of three quercetin derivates were detected. Strong positive correlations were observed between DPPH, FRAP, and phenolic compounds. These discoveries are important to highlight the promising antioxidant activity of CE of C. tweedii, which could contribute to the expansion of popular herbal medicines and new drug discovery.

ARTICLE HISTORY
Received: June 18, 2022
Accepted: Mar. 02, 2023

KEYWORDS
Medicinal plants,
Flavonoid,
HPLC-UV-DAD,
Proanthocyanidin,
Antioxidant activity.

1. INTRODUCTION

Brazil is one of the countries with the largest number of plant species (~50.000), of which half are considered as medicinal. Nevertheless, less than 1% of the plants have been studied concerning their biological activities, so research studies are necessary to confirm their efficacy and reduce the risks of poisoning (Ferreira de Souza et al., 1999). According to the World Health Organization (WHO), 88% of the regions of the Americas recognize the use of traditional medicines due to low cost, affordability, and fewer side effects (WHO, 2019).

Calliandra tweedii Benth, commonly called sponge, belongs to Fabaceae (Mimosoideae) family, genus *Calliandra* (in Greek, beautiful stamens) is one of the 132 species of *Calliandra*,

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ISSN-e: 2148-6905 / **© IJSM 2023**

ocurrences of shrubs and trees distributed across several countries in South America and Africa (Barbeny & Grimes, 1998). Brazil registers 75 species of *Calliandra*, of which 59 are endemic distributed mainly in the Northeast and Southeast regions (Souza, 2015). *C. tweedii*, earlier called *Inga pulcherrima*, is a Brazilian native species with occurrences in three of the seven phytogeographic domains (Cerrado, Mata Atlântica and Pampa) located in the Southeast (Espírito Santo, Minas Gerais, Rio de Janeiro and São Paulo) and South (Paraná, Rio Grande do Sul and Santa Catarina) regions (Flora do Brasil, 2020). *C. tweedii* is highly sought after by bees and, due to its fast growth, it is used as livestock feed, ornamental plant, garden fences and firewood (Riswan et al., 1996).

C. tweedii has been poorly studied when compared to *C. portoricensis*, *C. calothryrsus,* and *C. haematocephala* (Lorenzi & Souza, 2001; Kumar et al., 2002; Paiva, 2003). In general, people prepare infusions from the bark to use them in problems of rheumatism, arthritis, colds, uterine disorders and edema (fluid retention). In other cases, bark tinctures are made with schnapps for the same purposes (Araujo, 2010).

Previous studies have shown that the active compounds present in plant extracts can mediate biological activities, interacting and modulating the activities of proteins, nucleic acids, and biomembranes. Ethanolic and aqueous extracts of *C. portoricensis* leaves showed constituents identified as tannins, flavonoids, glycosides, and triterpenoid saponins, which inhibited the ulcerogenic effects and gastric lesions in rats at a dose of 50 mg/kg. In addition, these extracts have limited the growth of *Escherichia coli*, *Staphylococcus aureus*, and *S. faecalis* at a concentration of 0.3 to 0.5 mg/mL (Aguwa & Lawal, 1988). Orishadipe et al. (2010) corroborated the phytochemical screening of hexane extract from leaves, stem, and root of *C. portoricensis* reporting the presence of steroids, fatty acids, saponins, and digitalis glycosides with strong antimicrobial activity against *S. aureus*, *E. coli*, and *S. gallinallum*, but not against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. Also, the analgesic activity of the methanolic extracts of the roots and leaves of *C. portoricensis* has been correlated to a dose-dependent of 200, 400, and 600 mg/kg. Da Silva & Parente (2013) reported hemolytic activity from a new triterpenoid saponin of leaves of *C. pulcherrima* and Sikder et al. (2012) described the antimicrobial, cytotoxic, and thrombolytic effect of the methanol extract of *C. surinamensis.*

Additionally, the evaluation of condensed tannins (CT) in *Calliandra* is most important for livestock production, since native species with high CT concentrations (>5% dry matter (DM)) reduce feed intake, digestive efficiency and animal productivity. In contrast, species with moderate concentrations of CT (2-4% DM) improve protein utilization in ruminants (Addisu, 2016). The content and composition of CT can vary according to local weather conditions. CT of *C. haematocephala* and *C. calothyrsus* has been extensively reviewed and correlated to strong antioxidant activity (Rakhmani et al., 2005; Wei et al., 2015).

Considering the lack of studies about the chemical composition of phenolic substances of *C. tweedii* and problems associated with their consumption, this work aimed to investigate the total phenolic compounds, condensed tannins, the profile of flavonoids and their antioxidant activity in crude extract (CE) and methanolic (MP) and hexane (HP) phases of leaves of *C. tweedii.*

2. MATERIAL and METHODS

2.1. Chemical and Reagents

Folin-Ciocalteu reagent, gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH$^{\cdot}$(2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine), quercetin, kaempferol, ferric ammonium sulfate, potassium persulfate, ferric chloride, ferrozine (3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p′-disulfonic acid monosodium salt hydrate) were purchased from Sigma-Aldrich; sodium carbonate was purchased from

Merck; methanol, hydrochloric acid were obtained from Synth, n-butanol, quebracho tannin. All solvents and chemicals were of analytical or HPLC grade.

2.2. Plant Materials

Leaves of three specimens of C. tweedii were collected around the Institute of Biosciences at the University of São Paulo, São Paulo, Brazil (23°34'1" S, 46°43'49" W; 783 m above mean sea level). A voucher specimen (SPF 185861) was deposited at the Herbarium SPF, Institute of Biosciences, University of São Paulo, SP.

2.3. Preparation of Extracts

Leaves were dried at 40°C for 24 hours and powdered to the extraction process. Dried leaves (30 g) were extracted three times under reflux with 300 mL of 80% methanol for one hour. After pooling the solvent and filtration, extracts were concentrated under reduced pressure at 40°C and then freeze-dried. One hundred milligrams of the freeze-dried extract were taken for analysis as crude extract and the remaining mass was partitioned with 100 mL of methanol and 100 mL of hexane to separate polar and nonpolar substances, respectively. The methanolic and hexane phases were collected and concentrated under reduced pressure at 40°C and immediately freeze-dried. Finally, the crude extract (CE), methanolic phase (MP), and hexane phase (HP) were resuspended in MeOH and submitted to chemical analysis and antioxidant assays.

2.4. Determination of Total Phenolic Compounds

Total phenolic compounds (TPC) were determined using the Folin-Ciocalteu colorimetric method in a microplate reader (SynergyTM H1), according to Furlan et al. (2015). Fifty microliters of CE, MP, and HP solubilized in methanol (0.25 mg/mL) were mixed with 190 µL of ultrapure water, 10 µL of Folin-Ciocalteu reagent, 50 µL of 10% sodium carbonate and incubated in dark for 30 min at 40°C under stirring. The estimation of total phenolic compounds was performed at 760 nm from an analytical curve using gallic acid as a standard (0-80 µg/mL). Results were expressed as mg of gallic acid equivalents (GAE) per g of dry weight (DW).

2.5. Determination of Condensed Tannins

Condensed tannins (CT) content was determined using the HCl/n-butanol method adapted from Waterman & Mole (1994).

In a screw-capped glass tube, 3 mL of 5% HCl/n-butanol was mixed with 100 µL of each sample in the following concentration: CE (2 mg/mL), MP (2 mg/mL) and HP (6 mg/mL) solubilized in methanol. The mixtures were heated at 95ºC for one hour, cooled and the absorbance was read in a spectrophotometer (UV - 1650 PC - Shimadzu) at 550 nm. CT content was estimated using quebracho tannin (0-320 µg/mL) as a standard. Results were expressed in mg of quebracho tannin equivalents (QTE)/ g of dry weight (DW).

2.6. Characterization and Quantification Flavonoids by HPLC

Flavonoids in CE, MP, and HP were investigated using high-performance liquid chromatography (HPLC). Samples (0.5 mg/mL) were solubilized in methanol. CE, MP, and HP were analyzed on an Agilent 1260-HPLC equipped with a diode array detector (G4212B 1260 DAD). Polar substances were separated using a Zorbax C18 column (4.6 x 150 mm, 3.5 µm) (Agilent, Brazil), operated at 40°C. The injection volume for all samples was 3 µL. The mobile phase consisted of 0.1% (v/v) acetic acid in water (eluent A) and acetonitrile (eluent B). The gradient program was as follows: 0-20 min 15% B in A; 20-25 min 15-100% B in A; 25-30 min 100% B in A; 30-32 min 100-15% B in A; 32-35 min 15% B in A. The flow rates were from 0-25 min 1.5 mL/min, 25.1-25.2 min 1.5-1 mL/min, 25.2-26.9 min 1 mL/min, 26.9-27 min 1-1.5 mL/min, 27-35 min 1.5 mL/min. Constituents were monitored at 352 nm and the diode array

detector (DAD) wavelength range was 200-600 nm. Flavonoids were identified by comparison of their retention time with kaempferol, quercetin and quercitrin standards, and UV-Vis spectra.

The amount of each flavonoid was determined in relation to the quercetin calibration curve (3-120 µg/mL). Results were expressed as mg of quercetin equivalents (QE)/ g of dry weight (DW).

2.7. Antioxidant Assays

2.7.1. *DPPH radical scavenging capacity*

Free radical scavenging activity of CE, MP, and HP was determined according to the DPPH radical method by Furlan et al. (2015). DPPH solution in methanol (0.2 mM) was freshly prepared and 200 µL were mixed with 20 µL of each sample at different concentrations (50-250 µg/mL). The reaction mixture was incubated for 20 min at room temperature and in the dark. Absorbance was measured at 515 nm in a microplate reader (SynergyTM H1). As positive controls, Trolox (6-200 µg/mL) and quercetin (7.5-120 µg/mL) were used. Methanol was used as a negative control.

2.7.2. *Ferric reducing antioxidant power* (FRAP)

The ferric reducing power of samples was determined according to Furlan et al. (2015). FRAP solution was prepared daily by mixing 25 mL of acetate buffer (0.3 M, pH 3.6), 2.5 mL of 10 mM TPTZ, and 2.5 mL of 20 mM ferric chloride. Aliquots of 265 µL of FRAP solution were mixed with 10 µL of each sample at different concentrations (50-250 µg/mL) and 25 µL of ultrapure water. After incubation of 30 min at 37°C, the absorbance was detected by a microplate reader (SynergyTM H1) at 595 nm. Trolox (6-200 mg/mL) and quercetin (7.2-180 mg/mL) were used as positive control. Methanol was used as a negative control.

The antioxidant potential of samples was expressed as a percentage of antioxidant activity (%) and the effective concentration of each sample to achieve 50% of the antioxidant activity (EC_{50}).

2.8. Statistical Analysis

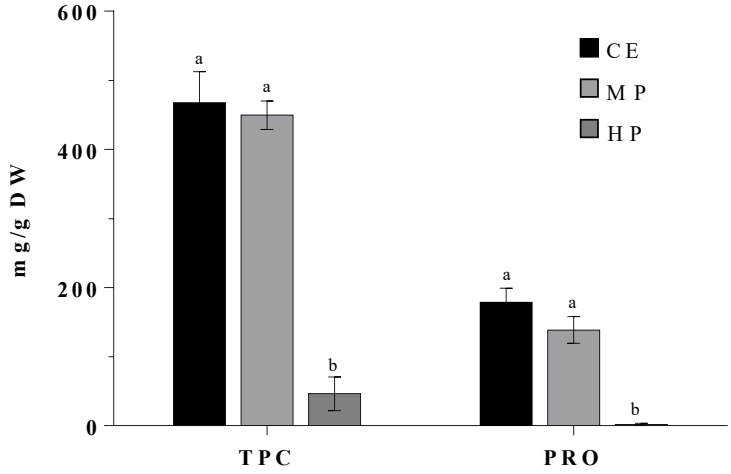
All assays were performed in triplicate and expressed as mean values ± SD (standard deviation). Differences between mean values were assessed by the Tukey's test with a significance level of $p < 0.05$. Data were analyzed using the R Statistics Software (version 3.5.0). Pearson's ($p < 0.05$) was performed for correlation analysis.

3. RESULTS and DISCUSSION

3.1. Total Phenolic Compounds and Proanthocyanidins

Contents of total phenolic compounds (TPC) and condensed tannins (CT) of crude extract (CE), methanolic phase (MP), and hexane phase (HP) of *C. tweedii* are shown in Figure 1. As expected, CE and MP showed high contents of TPC (CE, 467.2 ± 36.5 mg/g DW; MP, 449.2 ± 16.7 mg/g DW; and HP, 46.3 ± 20.0 mg/g DW). Comparing to Firmansyah et al. (2019) and Ahn et al. (1997), who quantified phenolic compounds in *C. calothyrsus* (159.8 mg/g and 188.2 mg/g, respectively), the present results suggest ***C.*** *tweedii* as possessing higher content of phenolic substances than other species from the same genus. However, Wei et al. (2015), evaluating water, 30% acetone, and 70% acetone extracts of *C. haematocephala,* found 199 mg/g, 433 mg/g, and 455 mg/g of TCP, respectively. These last data are very close to those found in this study. Therefore, the content of phenolic compounds could be more related to the extraction methods instead of a defined characteristic for each species.

Figure 1. Contents of total phenolic compounds (TPC, mg GAE/g DW) and total proanthocyanidins (PRO, mg QT/g DW) of crude extract (CE), methanol phase (MP) and hexane phase (HP) of *Calliandra tweedii*. The values correspond to the mean ± standard deviation (n = 3). Different letters represent significant differences among extracts for test.



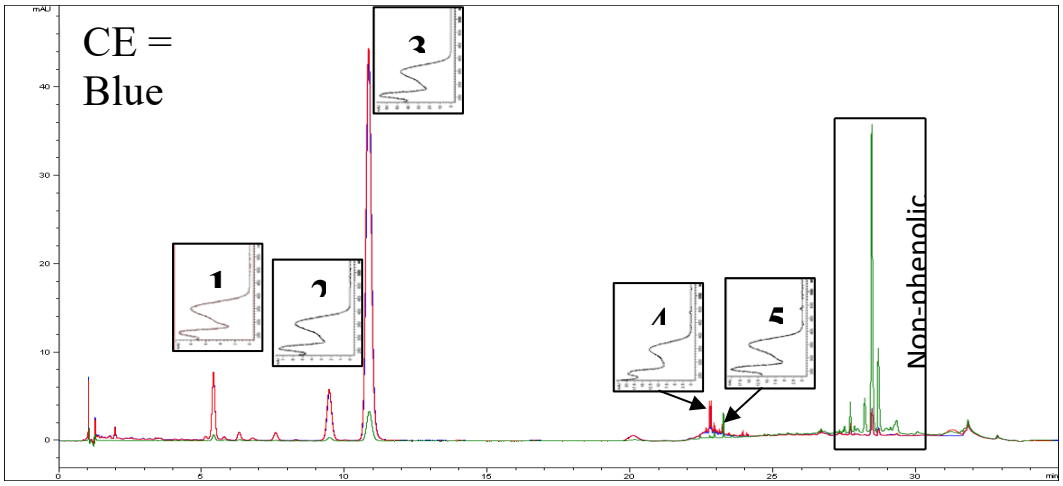
Contents of PRO in CE (178.6 ± 16.7 mg/g DW) and MP (138.5 ± 15.8 mg/g DW) were close to the values reported for *C. calothyrsus* (85.2-117.1 mg/g) (Ahn et al., 1997, Firmansyah et al. 2019), but lower than the ones found in *C. haematocephala* (450-504 mg/g) (Wei et al., 2015). These values suggest that the leaves of ***C.*** *tweedii* could perform a biological activity similar to *C. calothyrsus*, which reduces ruminal methanogenesis due to its high content of tannins (Hess et al., 2004). In addition, these results could be used for discoveries of biological controllers against pathogenic microorganisms that cause great economic losses in horticulture (Hess et al., 2004).

HP phase showed lower content of TPC and PRO, due to the reduced solubility of these compounds in non-polar solvents as hexane. Complementary studies by gas chromatography-mass spectrometry (GC-MS) could be performed with the hexane extract, to test some antimicrobial properties, as Orishadipe et al. (2010) reported for *C. portoricensis*. In any case, partition was effective to eliminate phenolic substances from HP.

3.2. HPLC Analysis of *C. tweedii* Extracts

The profile of flavonoids analyzed by HPLC-UV-DAD from the CE, MP, and HP extracts is shown in Figure 2. The retention times (t_R), the wavelength of maximum absorption (UV λ_{max}), commercial standards, and relevant information about the techniques of flavonoid identification described by Mabry et al. (1970) and Markham (1982) were used to differentiate the flavonoids from leaf extracts of *C. tweedii*. Five flavonols were detected (Table 1).

Figure 2. HPLC-DAD-UV chromatograms at 352 nm of crude extract (CE), methanol (MP) and hexane phases (HP) of *Calliandra tweedii*.



The UV spectrum of flavonol 1 exhibited maximum absorption at 256 and 350 nm and a shoulder at 295 nm, suggesting that it is a kaempferol derivate. Flavonol 2 had UV λ_{max} at 256 and 352 nm and two shoulders at 264 and 308 nm, distinguishing it as a quercetin derivate. Flavonol 3 showed UV λ_{max} at 256 and 348 nm and two shoulders at 264 and 308 nm, identified using co-chromatography with a commercial standard of quercitrin (quercetin 3-*O*-rhamnoside). The UV absorption bands at 256 and 346 nm and two shoulders at 262 and 306 nm of flavonol 4, suggest the presence of a quercetin derivate. Flavonol 5 showed UV λ_{max} at 256 and 350 nm and two shoulders at 264 and 306 nm, reveling another quercetin derivate. This peak 5 was detected in MP and only in one sample of CE and HP (Table 1).

Table 1. Flavonols (mg QE/g DW) detected by HPLC-DAD in *Calliandra tweedii* crude extract (CE), methanol phase (MP), and hexane phase (HP). Values are expressed as means ± standard deviation. t_R: retention time. UV λ_{max} (nm): wavelength of maximum absorption.

Peak	t_R (min)	UV λ_{max} (nm)	Constituent	CE	MP	HP
1	5.35	256, 264sh, 350	Kaempferol derivative	2.5 ± 0.5[a]	2.9 ± 0.6[a]	-
2	9.40	256, 264sh, 308sh, 352	Quercetin derivative	2.0 ± 1.1[a]	2.3 ± 1.1[a]	-
3	10.78	256, 262sh, 308sh, 348	Quercitrin	20.3 ± 6.0[a]	23.6 ± 5.7[a]	0.9 ± 0.9[b]
4	22.44	256, 262sh, 306sh, 346	Quercetin derivative	0.5 ± 0.4[a]	0.6 ± 0.5[a]	-
5	23.25	256, 264sh, 306sh, 350	Quercetin derivative	0.7*	0.3 ± 0.4	0.1*

*Detected in one sample.
For each compound, different letters represent significant differences among extracts.

These data corroborate with the work performed by Moharram et al. (2006), that reported quercetin derivatives as major flavonoids in leaves and stem of *Calliandra* spp., including quercitrin (*C. haematocephala*). These flavonoids have been associated with strong antioxidant potential by scavenging DPPH radical, showing EC_{50} of 18 µM (Yamazaki et al., 2007). Besides that, the antiproliferative and apoptotic activities of quercitrin were demonstrated in colorectal adenocarcinoma cell lines, as well as in lung cancer cells (Cincin et al., 2014). Silva et al. (2012) tested the interaction of the flavonols quercetin, quercitrin, and isoquercitrin with the enzyme arginase from *Leishmania amazonenses* demonstrating that these substances inhibit this enzyme's activity, which is essential for the parasite development. These substances were considered potential molecules for the study of antileishmanial drugs. Furthermore, it has been found the quercitrin efficacy in treating periodontal disease (Gómez-Florit et al., 2014).

Encarnación & Ochoa (1994) isolated and reported antimicrobial activity of two methoxylated flavones (7,2',4',5'-tetramethoxyflavone and 5-Hydroxy-7,2',4',5'-tetramethoxyflavone) from *C. californica.* In a previous study, Encarnación & Keer (1991) observed the antimicrobial activity of ethanol extract of *C. californica* against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and pathogenic yeasts (*Candida albica*ns).

The estimation of each flavonol amount was summarized in Table 1. Quercitrin (flavonol 3) was the major constituent of CE (20.3 ± 6.0 mg QE/g DW) and MP (23.6 ± 5.7 mg QE/g DW), followed by kaempferol derivative (flavonol 1), quercetin derivate (flavonol 2), and another quercetin derivate (flavonol 4).

3.3. Antioxidant Assays

Antioxidant activity assays of plant extracts have been performed as a form of bioprospecting potential of medicinal plant species, since the prevention of free radical formation, as well as the conversion and stabilization of these molecules in non-oxidizing forms, is essential to prevent oxidative stress. Oxidative stress is associated with many diseases such as cancer, diabetes, atherosclerosis, and autoimmune diseases, among others (Valko et al., 2007).

In this study, the antioxidant activity of *C. tweedii* was evaluated by two different assays: DPPH and FRAP. DPPH is a stable radical, which quench radicals by transferring either a hydrogen atom or a single electron, depending on the antioxidant. The reducing ability of antioxidants toward DPPH radical is measured at 515 nm since the original purple color of DPPH is lost (Prior et al., 2005; Schaich & Xie, 2015). On the other hand, FRAP is a method totally based on the single-electron transference, in which the ferric 2,4,6-tripyridyl-s-triazine (TPTZ) is reduced to an intense blue-colored product (Prior et al., 2005).

The results obtained in the determination of EC_{50} DPPH and EC_{50} FRAP, expressed in µg/mL, are listed in Table 2. In the DPPH assay, EC_{50} of CE and MP were 83.8 µg/mL and 100.5 µg/mL, respectively, while EC_{50} for the standards Trolox and quercetin were 152.2 and 68.4 µg/mL, respectively. In the FRAP assay, EC_{50} of CE and MP were 218.5 µg/mL and 201.6 µg/mL, respectively, while EC_{50} for the standards Trolox and quercetin were 74.8 and 40.6 µg/mL, respectively. EC_{50} of CE and MP were statistically similar; however, these extracts were different from HP in DPPH and FRAP assays.

Table 2. EC_{50} of crude extract (CE), methanol phase (MP), and hexane phase (HP) of *Calliandra tweedii* determined using DPPH and FRAP assay. Values are expressed as means ± standard deviation.

µg/mL	Trolox	Quercetin	CE	MP	HP
EC_{50} DPPH*	152.2	68.4	83.8 ± 9.7^{a}	100.5 ± 1.7^{a}	17259.0 ± 26431.4^{b}
EC_{50} FRAP**	74.8	40.6	218.5 ± 42.5^{a}	201.6 ± 23.6^{a}	3381.2 ± 1103.1^{b}

*EC_{50} was calculated in relation to the negative control; **EC_{50} was calculated in relation to Trolox standard.
For each assay, different letters represent significant differences among extracts.

Considering that CE and MP showed lower EC_{50} compared to Trolox in DPPH assay, and that CE showed an EC_{50} close to the best-known reference antioxidant quercetin (Chen et al., 2013), *C. tweedii* can be considered as a promising source of natural antioxidant compounds. This result highlights the CE extract as promising to carry out complementary biological activities assays.

The results of this study corroborate to those obtained by Adaramoye et al. (2015), who found high levels of inhibition of free radicals by DPPH assay using methanol fraction of leaf extract of *C. portoricensis*. Moharram et al. (2006) associated the observed activity of radical captors in the DPPH test to quercitrin compounds identified in *C. haematocephala*. Therefore,

it is believed that the presence of high levels of phenolic compounds and proanthocyanidins contributed to the high antioxidant activity of CE and MP in this study.

Proanthocyanidins or condensed tannins are polyphenols that play a role in the defense of plants, especially against herbivory (Taiz & Zieger, 2009). Studies evaluating the effects of proanthocyanidins of grape seed extracts have shown that these metabolites have strong antioxidant activity, acting as chemo protectors in rats treated with cisplatin, a potent drug used in the treatment of various types of tumors, but recognized for its toxicity for the patient (Yousef et al., 2009). The effects of proanthocyanidins have also been reported in tests with mice treated with a collagen-induced arthritis compound (Cho et al., 2009). In the same study, animals receiving the plant extract showed decreased inflammation, decreased bone loss and cartilage, and decreased production of H_2O_2, which are parameters of the disease progression.

In many cases, the antioxidant activity is attributed to the synergy among the phenolic compounds in plant extracts. In flavonoids, which have the phenyl rings A and B connected through a pyran C-ring (Dewick, 2009), the antioxidant activity appears to increase with the presence of hydroxyl groups attached to the B-ring. Thus, a single hydroxyl group (OH) substituent can generate little or no antioxidant capacity.

3.4. Correlation Analysis

The analysis of Pearson's correlation coefficient demonstrated how the evaluated phenolic substances of *C. tweedii* might contribute to the antioxidant activity assays DPPH and FRAP (Table 3).

Table 3. Pearson's correlation coefficients for phenolic compounds (TPC), proanthocyanidins (PRO), DPPH, and FRAP with their respective *p-values*. Higher correlation values are highlighted in bold. *P*-values lower than 0.05 were considered significant and appear in italics.

	TPC	PRO	EC_{50}DPPH	EC_{50}FRAP	Flavonol1	Flavonol2	Flavonol3	Flavonol4
PRO	**0.970**							
P-value	***0.000***							
EC_{50}DPPH	-0.562	-0.529						
P-value	*0.115*	*0.143*						
EC_{50}FRAP	**-0.951**	**-0.907**	0.474					
P-value	***0.000***	***0.001***	*0.198*					
Flavonol1	**0.808**	**0.851**	-0.468	**-0.804**				
P-value	***0.008***	***0.004***	*0.204*	***0.009***				
Flavonol2	**0.745**	**0.790**	-0.406	**-0.704**	**0.824**			
P-value	***0.021***	***0.011***	*0.278*	***0.034***	***0.006***			
Flavonol3	**0.890**	**0.895**	-0.507	**-0.831**	**0.880**	**0.959**		
P-value	***0.001***	***0.001***	*0.164*	***0.005***	***0.002***	***0.000***		
Flavonol4	0.486	0.364	-0.298	-0.510	0.418	-0.051	0.202	
P-value	*0.184*	*0.336*	*0.437*	*0.161*	*0.263*	*0.896*	*0.602*	
Flavonol5	0.218	0.142	-0.142	-0.246	0.402	0.509	0.470	0.114
P-value	*0.574*	*0.715*	*0.715*	*0.523*	*0.284*	*0.162*	*0.202*	*0.771*

EC_{50} of FRAP assay showed higher negative correlations for TPC and PRO (-0.951 and -0.970, respectively, *p-value* $\leqslant$0.001), compared to flavonols 1, 2 and 3 (-0.704$\leqslant$r$\leqslant$-0.831, *p-value* $\leqslant$0.034). This result could indicate that phenolic compounds other than flavonoids influence more the antioxidant activity. Among these phenolic compounds, we can point out the PRO content, which presented higher negative correlation with EC50 of FRAP assay than any other flavonoid. Since we have focused on the analysis of individual flavonoids instead of

proanthocyanidins, a deeper study concerning the later substances could confirm this promising antioxidant activity from leaves of *C. tweedii*. Flavonols 4 and 5 showed no significant correlation with TPC, EC_{50} DPPH, EC_{50} FRAP and flavonols 1, 2 and 3 ($-0.051 \leqslant r \leqslant 0.509$, *p-value* >0.16), probably due to its reduced content in CE, MP, and HP. Phenolic compounds are recognized as potent antioxidants and have already demonstrated moderate to strong scavenging properties on DPPH assay by leaves of another *Calliandra* species. More specifically, the antioxidant activity has been correlated with quercetin rhamnosides detected in these species (Moharram et al., 2006). Although we have identified quercetin derivatives in the methanol extract and phase, the correlation analysis pointed out that the proanthocyanidins could be responsible for this role.

Also, no significant correlation between FRAP and DPPH was observed (r=0.474, *p-value* = 0.198). This is an intriguing result since the basic principle of both antioxidant assays is to measure the capacity of the extract constituents in transferring an electron and in reducing an oxidant compound.

4. CONCLUSION

To our knowledge, *C. tweedii* flavonoids have not been reported in other studies, being this work precursor in providing important data regarding preliminary identification to further elucidate the molecular structure of these flavonoids. It is important to highlight that the antioxidant activity of the crude extract (CE) and the methanolic phase (MP) are promisors and they were not statistically different from each other. In addition, their EC_{50} values were similar to quercetin's. Therefore, a partition of the crude extract would not be mandatory in order to obtain a potent antioxidant extract. This is an interesting aspect because higher amounts of material can be wasted in the partition process. These results reveal a potential use of leaves of *C. tweedii* as herbal medicine. Although flavonoids, mainly quercetin rhamnosides, have already been related to the promising antioxidant activity, the correlation of the antioxidant assays from this study was higher with TPC and PRO. Therefore, more studies must be performed to investigate the role of other phenolic compounds in the antioxidant activity of *C. tweedii*.

Acknowledgments

The authors thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES; Finance Code 001) for funding this research. Also, authors thank Sacramento, G.N., Vasconcelos, M.C.V., Silva, C.A.P., Souza, B.C.E., students of the III Winter Botany course offered by the Botany Department of the University of São Paulo.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

This study was developed as a part of the III Winter Botany course offered by the Botany Department of the University of São Paulo. **Kátia Dos Santos**: Conceptualization, Investigation, Formal analysis, Writing – original draft; **Alice Nagai**: Investigation, Formal analysis, Writing – original draft, Writing – review & editing; **Carmen Palacios**: Investigation, Formal analysis, Writing – original draft, Writing – review & editing; **Bruno Evangelista**: Formal analysis, Writing – original draft; **Carlos Priante**: Formal analysis, Writing – original draft; **Débora Zamban**: Formal analysis, Writing – original draft; **Claudia Furlan**: Writing – review & editing, Supervision.

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