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Artigo Original

Antioxidant activity and quantification of phenols, flavonoids and total tannins of *Cinnamomum triplinerve* (Lauraceae)

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Abstract

The present study evaluated the antioxidant activity and determined the total phenolic, flavonoid and tannin contents of the extracts from leaves, barks and fruits of *Cinnamomum triplinerve*. The antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) free radical scavenging method and the quantification of total phenols, flavonoids and tannins by spectrophotometry in the visible region. The extract from the barks was the most active in relation to the antioxidant activity (IC_{50} = 11.42 ± 0.41 μg/mL) and also the one with the highest total phenol content (36.38 ± 1.11 mg GAE/g extract) and total tannins, 14.58 ± 1.48 mg TAE/g extract. The results suggest that the antioxidant activity of *C. triplinerve* is mainly related to the presence of phenolic compounds, such as flavonoids and tannins, which are included in the category of free radical scavengers and efficient in the prevention of oxidative processes.

Keywords: *Cinnamomum triplinerve*; *Bioactivity; Phenolic compounds*

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1 Introduction

The relationship between the etiology of various diseases and the action of excessive reactive oxygen species in the body has aroused interest in the discovery of new antioxidants of natural origin. Detection of antioxidant activity in plants can provide a large number of secondary metabolites capable of capturing free radicals (DUARTE, 2014). Radicals originate from chemical reactions or biochemical processes and may cause oxidative damage to various biomolecules such as proteins and deoxyribonucleic acid (SOUSA et al. 2007; ATOUI et al. 2005). This process favors tissue aging, as well as the appearance of degenerative diseases such as cancer, cardiovascular diseases and brain dysfunction (BARBOSA et al. 2010).

Phenolic compounds from plants stand out among the classes of antioxidant substances of natural occurrence and fall into several categories, such as simple phenols, flavonoids, tannins, coumarins, lignans and lignins. The antioxidant property of these compounds depends on their chemical structure, and can be determined by the action of the molecule as reducing agent (rate of inactivation of the free radical, reactivity with other antioxidants and potential chelation of metals) (SOUSA et al. 2007). Effective treatments against cardiovascular diseases and certain types of cancer were performed using sources of phytochemical, in particular phenolic compounds (TULIO et al. 2014).

Cinnamomum, a genus belonging to the family Lauraceae comprises 12 species in Brazil (QUINET et al. 2015). Many species of *Cinnamomum* are considered medicinal and of varied use, performing different functions against diverse diseases. Chemically, flavonoids, anthraquinones, saponins, terpenoids and coumarins are described for species of the genus (RAMSHINI et al. 2015; NGOC et al. 2012; AHMAD et al. 2013) and broad biological activity is reported for their essential oils, extracts and/or secondary metabolites, especially antioxidant activity, among others (ANDRADE et al. 2012; AKTER et al. 2015; THAMIZHSELVAM et al. 2012; MUSTAFFA et al. 2014; PRASAD et al. 2009).

Cinnamomum triplinerve (Ruiz & Pav) Kosterm is a tree widely distributed throughout the Americas. In Brazil, it can be found in the Atlantic Forest, Amazon and Cerrado. There are few reports on its chemical composition and biological activity (CUCA-SUÁREZ et al. 2012; ARGOTI et al. 2011). Thus, this study aimed to evaluate the antioxidant activity, as well as to determine the total phenolic, flavonoid and tannin contents of the crude ethanol extracts from the leaves, bark and fruits of *C. triplinerve* occurring in the southern region of Mato Grosso do Sul.

2 Material and methods

2.1 Collection and identification of plant material

The plant material (leaves, barks and fruits) was collected during the fruiting period, in August 2017 in a Atlantic Forest fragment located at Três Fronteiras Farm, Japorã, Mato Grosso do Sul (23°53'30.18"S and 54°24'16.30"W). The identification was carried out by Dr. Flávio Macedo Alves of the Federal University of Mato Grosso do Sul (UFMS) and the voucher specimen was deposited in the CGMS herbarium (UFMS) under registration number 46208.

2.2 Preparation of extracts

Leaves, barks and fruits were air-dried and milled in Wiley mill. Subsequently, they were cold extracted exhaustively with ethanol. Each resulting extract was filtered and concentrated under reduced pressure. In this way, samples of 25.85 g leaves, 7.03 g barks and 19.99 g fruits were obtained.

2.3 Total phenols

The content of total phenolic compounds was determined by the Folin-Ciocalteau spectrophotometric method (BONOLI et al. 2004). To a 1.0 mL aliquot of sample methanolic solution (1mg/mL) was added 5 mL distilled water and Folin- Ciocalteau reagent (0.2 mL). The solution was stirred for 1 minute and 0.6 mL 15% Na_2CO_3 was added. The volume was made up to 10 mL with distilled water. After 1h and 30 minutes, the absorbance of the samples was red at 750 nm in a UV/vis Tecnal spectrophotometer. Total phenol content was determined by interpolating the absorbance of the samples against a calibration curve constructed with gallic acid standards (2.5 to 7.5 μg/mL). The equation of the obtained curve was y = 0.068x + 0.138; R2 = 0.996, where y is the absorbance and x is the concentration of gallic acid. The analyses were performed in triplicate, the results expressed as milligram of gallic acid equivalent (GAE) per gram of sample.

2.4 Total flavonoids

The flavonoid determination was performed by the colorimetric method with aluminum chloride (LIN AND TANG, 2007). 1 mL of the sample methanolic solution (1 mg/mL) was mixed with 0.2 mL 2.5% aluminum chloride ($AlCl_3$) in MeOH and the volume completed to 10 mL. After standing for 30 minutes, a UV/vis Tecnal spectrophotometer was read at 425 nm. The total flavonoid content was determined using a quercetin calibration curve (2 to 10 μg/mL). The curve equation was y = 0.099x + 0.029, R2 = 0.998, where y is the absorbance, x is the quercetin concentration. The analyses were performed in triplicate, and the results expressed in milligram of QE (quercetin equivalents) per gram of sample.

2.5 Total tannins

The total tannin dosage was determined by the Folin-Denis spectrophotometric method (PANSERA et al. 2003). To 1.0 mL of sample solution (1 mg/mL) Folin-Denis reagent (1 mL) was added, then the solution was stirred for 1 minute and 0.5 mL of 25% Na_2CO_3 was added. The

volume was made up to 10 mL with distilled water. After 30 minutes, the absorbance of the samples was measured at 725 nm in a UV/vis Tecnal spectrophotometer. The total tannin content was determined using a calibration curve constructed with tannic acid standards (2.0 to 10.0 µg/mL). The equation of the curve was y = 0.0275x + 0.0047; R2 = 0.990, where y is the absorbance and x is the concentration of the tannic acid. The analyses were performed in triplicate, the results expressed as milligrams of tannic acid equivalent (TAE) per gram of sample.

2.6 Antioxidant activity

One of the methods for assessing oxidant activity in extracts is free radical scavenging method 2,2-diphenyl-1-picrylhydrazyl (DPPH). The assay is based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine. DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the moleculeas a whole. The delocalisation also gives rise to the deep violet colour, with an absorption at around 520 nm. The reduced DPPH shows a yellow color and a consequent disappearance of the absorption (KEDARE AND SINGH, 2011; ALVES et al. 2010).

The quantitative evaluation of the antioxidant activity was done following a methodology described in the literature (SOUSA et al., 2007) with modifications, evaluating the consumption of the DPPH radical by the samples, through the reduction in absorbance of solutions of different concentrations (200 to 25 µg/mL). The absorbance measurements of the reaction mixtures (0.5 mL of the sample solution and 2.5 mL of 40 µg/mL DPPH methanolic solution) were recorded at the end of 60 minutes in a UV/vis Tecnal spectrophotometer at 515 nm, having gallic acid as a positive control. From the absorbance values, the percentages of oxidation inhibition of the radical were calculated according to Equation:

$$\% \text{ Reduction (DPPH consumed)} = [A_{control} - A_{sample}/A_{control}] \times 100$$

Where, $A_{control}$ is the initial absorbance of DPPH at the concentration of 40 µg/mL and A_{sample} corresponds to the absorbance of DPPH in the medium, after the reaction with the sample.

The inhibitory concentration (IC_{50}) was determined from a first-order exponential curve obtained by plotting on the abscissa the sample concentrations (µg/mL) and, on the ordinate, the percentages of DPPH reduction.

2.7 Statistical analysis

The results were expressed as mean ± standard deviation (n = 3) for each extract. The statistical treatment of the data was done by analysis of variance (ANOVA) and Tukey's test, using the program Sisvar 5.6. The p value < 0.05 was considered statistically significant.

3 Results and discussion

The results concerning the antioxidant activity of leaf, bark and fruit ethanolic extracts were expressed as the capacity to reduce the DPPH radical in percentage (Figure 1) and the IC_{50} value (Table 1), which is a parameter indicative of the inhibitory concentration required to decrease the DPPH free radical by 50%. The lower the IC_{50} value, the higher the antioxidant activity (EL AND KARAKAYA, 2004; ARBOS et al. 2010).

In relation to the ability to scavenge the DPPH radical (% DPPH reduction) from the analyzed samples, it was verified that, when evaluated separately, leaf, bark and fruit extracts presented differences in the concentrations tested ($p < 0.05$). Only leaf extract showed no significant difference in the reduction of DPPH at concentrations of 50 and 25 µg/mL.

Among the extracts, the bark extract was considerably more active at the concentrations tested. It was possible to verify that, in the concentrations of 200 and 100 µg/mL, this extract was significantly more effective in the reduction of DPPH, reducing in more than 90% the radical. This behavior was also observed at concentrations of 50 and 25 µg/mL, but with a reduction of less than 90%. At the concentrations tested, leaf extract was the ones that reduced DPPH the least, about 30% at the highest concentration (200 µg/mL), therefore with the lowest potential as sources of free radical scavengers.

Based on the IC_{50} values produced by extracts of *C. triplinerve*, the extract from the bark was effective in the antioxidant capacity (11.42 ± 0.41 µg/mL). Fruit extract produced IC_{50} of 130.9 ± 4.09 µg/mL and IC_{50} > 400 µg/mL. All samples showed less antioxidant activity than the gallic acid standard (IC_{50} = 3.91 ± 0.71 µg/mL).

The results obtained in the determination of the total phenols indicated that the highest content was presented by the bark extract (36.38 ± 1.11 mg GAE/g). This sample had low flavonoid content (1.23 ± 0.01 mg QE/g), but the highest tannin concentration (14.58 ± 1.48 mg TAE/g). The lowest concentration of total phenols was observed for leaf extract (8.08 ± 1.83 mg GAE/g), which recorded 2.55 ± 0.17 mg QE/g flavonoids and 4.60 ± 0.89 mg TAE/g total tannins (Table 1). Phenolic substances have higher affinity with polar solvents such as ethanol (TIWARI et al. 2011). In this sense, in the extraction process ethanol was used in order to obtain higher content of these compounds.

Some authors describe that the antioxidant activity of plants is related to the presence of phenolic compounds such as flavonoids and tannins. These compounds are included in the category of free radical scavengers, being very efficient in the prevention of the oxidative process (PERES et al. 2009; SOUSA et al. 2007).

In the case of *C. triplinerve*, this approach can be applied because the extract from the barks, the most active in relation to the antioxidant activity (IC_{50} = 11.42 ± 0.41 µg/mL) was also the one with the highest phenolic content (36.38 ± 1.11 mg GAE/g). Leaf extract yielded an IC_{50} value above 400 µg/mL, the highest concentration obtained in this evaluation and the lowest phenolic content (8.08 ± 1.83 mg GAE/g). Considering the total phenol contents, it is

Figure 1 – Percentage of DPPH reduction of crude ethanolic extracts from leaves (ExtBF), barks (ExtBC) and fruits (ExtBFr) of *C. triplinerve*

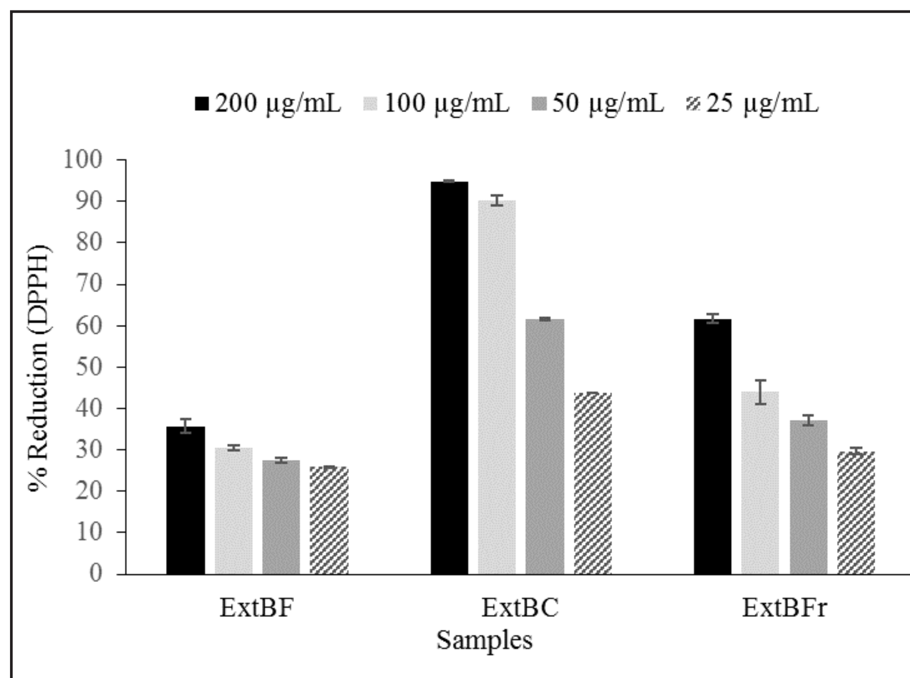

Table 1 – Total phenolic content, total flavonoids, total tannins and IC_{50} of crude extracts of *C. triplinerve* (ExtBFr) of *C. triplinerve*

Samples	Total phenolic (mg GAE/g)	Total flavonoids (mg QE/g)	Total tannins (mg TAE/g)	IC_{50} (µg/mL)
ExtBF	8.08 ± 1.83^{c}	2.55 ± 0.17^{b}	4.60 ± 0.89^{c}	447.56 ± 50.22^{a}
ExtBC	36.38 ± 1.11^{a}	1.23 ± 0.01^{b}	14.58 ± 1.48^{a}	11.42 ± 0.41^{c}
ExtBFr	12.80 ± 0.32^{b}	4.72 ± 0.92^{a}	7.21 ± 0.43^{b}	130.9 ± 4.09^{b}
Gallic acid	-	-	-	3.91 ± 0.71

Values expressed as mean ± standard deviation (n = 3). Means followed by same letters in the same column are not significantly different after ANOVA followed by Tukey's test ($p > 0.05$). Crude ethanolic extracts from leaves (ExtBF), barks (ExtBC) and fruits (ExtBFr).

possible that the main phenolic constituents are flavonoids and mainly tannins, compounds known as antioxidants (SANTOS AND RODRIGUES, 2017; DEGÁSPARI AND WASZCZYNSKYJ, 2004) and quantified in this study.

Previous studies report that phytochemical screening of *C. triplinerve* revealed the presence of flavonoids in extracts from different parts of the plant, but the presence of tannin compounds has not been reported (SILVA et al. 2017). In another study, the preliminary chemical analysis detected flavonoids in the leaves, bark and wood ethanolic extracts and tannins only in the leaves of the species (CUCA-SUÁREZ et al. 2012). The synthesis of secondary metabolites in plants is frequently influenced by several factors. Thus, these variations of secondary metabolites for the same species can be justified by environmental factors such as seasonality, circadian rhythm, stage of development, temperature, water availability, UV radiation, soil nutrients, altitude, atmospheric composition, among others (GOBBO-NETO AND LOPES, 2007; NUNES et al. 2016).

In the study carried out in Colombia the ethanolic extract from the wood of *C. triplinerve* tested with superoxide and DPPH presented a relevant antioxidant activity (CE_{50} = 12.51 ± 0.60 µg/mL) attributed to the content of phenolic compounds, suggesting that the species is promising for future investigations (ARGOTI et al. 2011).

Significant antioxidant activity similar to that found for the extract of the *C. triplinerve* barks in this study was also reported for other species of the genus. Akter et al. (2015) evaluated the antioxidant potential of *Cinnamomum tamala* and obtained for the leaf ethanolic extract an IC_{50} of 13.55 µg/mL. The methanolic extract from *Cinnamomum iners* leaves showed an IC_{50} of 15 µg/mL. According to the authors, the study proves that the plant can potentially be used as an antioxidant in the pharmaceutical and nutraceutical industries (UDAYAPRAKASH et al. 2015).

4 Conclusions

Our findings indicate that the antioxidant activity of *C. triplinerve* is related to the presence of phenolic compounds such as flavonoids and tannins quantified in leaf, bark and fruit extracts. Among the analyzed extracts, the bark extract showed a considerable DPPH radical scavenging power, which stimulates the continuity of

the studies to isolate and characterize the substances responsible for this antioxidant capacity.

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