



Quantification of phenolic derivatives and antioxidant activity of the leaves of *Chamaecrista diphylla* (L.) Greene (Fabaceae)

Quantificação de derivados fenólicos e atividade antioxidante das folhas de *Chamaecrista diphylla* (L.) Greene (Fabaceae)

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Based on chemotaxonomy, the specie *Chamaecrista diphylla* (L.) Greene became the object of study of this work, since it is part of the same genus of *C. nictitans*, where there are proanthocyanidins, which act against the Herpes Simplex Virus. Thus, this work aimed to determine the compounds phenol content and antioxidant potential of extracts and fractions of leaves *C. diphylla*. Quantitative analysis showed ethyl acetate fraction (F-AcOEt) and ethanolic extract (EE) showed the highest levels of phenolic compounds, flavonoids and condensed tannins. Referent at antioxidant activity, the EC₅₀ value for F-AcOEt (0.11 mg.mL⁻¹) was better than the ascorbic acid standard (0.13 mg.mL⁻¹), indicating a strong activity. It can also be noted that the content of flavonoids and condensed tannins are strongly correlated with antioxidant activity. Therefore, it is suggested that *C. diphylla* specie have secondary metabolites, flavonoids and tannins, with promising biological actions for application in the pharmaceutical and cosmetic industry.

Keywords: medicinal plants, phenolic compounds, antioxidant activity.

Baseando-se na quimiotaxonomia, a espécie *Chamaecrista diphylla* (L.) Greene virou objeto de estudo deste trabalho, visto que faz parte do mesmo gênero da *C. nictitans*, onde encontram-se proantocianidinas, que agem contra o Vírus Herpes Simplex. Desta forma, este trabalho objetivou determinar o teor de fenóis e o potencial antioxidante dos extratos e frações das folhas da espécie *C. diphylla*. As análises quantitativas demonstraram que o fracionado em acetato de etila (F-AcOEt) e o extrato etanólico (EE) apresentaram os maiores teores de compostos fenólicos, flavonoides e taninos condensados. Referente a atividade antioxidante, o valor de CE₅₀ para F-AcOEt (0,11 mg.mL⁻¹) se apresentou melhor que o padrão ácido ascórbico (0,13 mg.mL⁻¹), indicando uma forte atividade. Pode-se notar ainda que o teor de flavonoides e taninos condensados estão fortemente correlacionados com atividade antioxidante. Portanto, sugere-se que a espécie *C. diphylla* apresenta metabólitos secundários, flavonoides e taninos, com promissoras ações biológicas para aplicação na indústria farmacêutica e cosmética.

Palavras-chave: plantas medicinais, compostos fenólicos, atividade antioxidante.

1. INTRODUCTION

The diversity of active molecules in plants represents a challenge for the “chemical” who seeks the isolation and structural determination of organic compounds, since an extract of a given plant may contain many secondary metabolites such as flavonoids, alkaloids, coumarins, aglycones, anthraquinones, triterpenes, saponins, tannins among others. Thus, Natural Products Chemistry plays an important role in obtaining and discovering compounds that have some biological activity [1].

But, how to select a medicinal plant? Several approaches to the selection of plant species have been presented in the literature, such as ethnopharmacological and ethnobotany, which seek information from the knowledge of different peoples and ethnicities. However, it is worth noting that not all medicinal plants have been investigated, as many are not part of traditional medicine. There are, in this sense, other avenues for the study of medicinal plants, such as the chemotaxonomic or phylogenetic approach, which is based on the selection of a species correlated with the occurrence of a given chemical class of substances in a genus or family [2].

Based on chemotaxonomy, the specie *Chamaecrista diphylla* (L.) Greene (Figure 1) belonging at Fabaceae Lindl family. and the Caesalpinioideae DC subfamily became the object of this study.

Although little studied, it is part of the same genus as *Chamaecrista nictitans* (L.) Moench, which according to Mateos-Martín et al. (2014) [3] are proanthocyanidins class of polyphenols responsible for their antiviral activity, specifically against Herpes Simplex Virus (HSV).

Based on research with *C. nictitans* and other studies with species of the same genus [4, 5, 6, 7], this study aimed to determine the phenolics compounds content and the antioxidant potential of the leaves extracts and fractions specie *Chamaecrista diphylla* (L.) Greene.

2. 2. MATERIALS AND METHODS

2.1 Plant material

The specie *C. diphylla* (Figure 1) was collected from January to March 2018 in the coastal strip of the Bacurizal Ecological Reserve (048°31'07.0"W; 00°46'41.0"S), in the municipality of Salvaterra, located in Marajó Island, Pará, Brazil. The specie was identified by Dr. Ana Claudia Caldeira Tavares Martins and deposited in the Herbarium Prof. Dr. Marlene Freitas da Silva (n° 008383), linked to the State University of Pará (SUP).

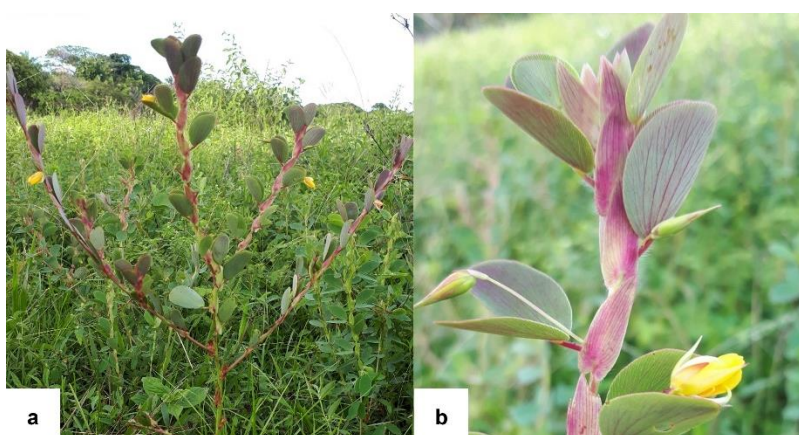


Figure 1: *Chamaecrista diphylla* (L.) Greene: (a) shrub; (b) flowering branch.

2.2 Preparation of extracts and fractions

The leaves of the specie were dried in an air circulation oven at 40 °C (\pm 0.5) for a period of 72 hours and ground in a Becker ® Cutter. The pulverized material (200 g) was macerated with 99.8% (v / v) ethanol in 72 hour (3 cycles), totaling a period of 9 days. After, concentration of the ethanolic extract (EE), a portion (5.0 g) was fractionated by liquid-liquid partition into a separatory funnel using solvents in increasing order of polarity: hexane and ethyl acetate. After total solvent removal, three fractions were originated: hexane (F-Hex), ethyl acetate (F-AcOEt) and hydroalcoholic (F-WOH).

The aqueous extract (AE) was obtained by adding 500 mL of distilled water (100 ° C) to 100 g of the dried leaves. The solution was infused for 10 min and then filtered. Then, the material was dried in the fume hood [8].

2.3 Determination of total phenols, flavonoids and condensed tannins

Total phenol content was quantified by the spectrophotometric method described by Wilczynska (2010) [9] using the Folin-Ciocalteu reagent. Flavonoids quantification was performed according to Woisy methodology according Salatino (1998) [10], with the use of aluminum chloride. The determination of condensed tannins was performed according to the methodology proposed by Lugo (2015) [11], using the sulfuric vanillin method. To determine the antioxidant activity of *C. diphylla* extracts and fractions, the method described by Bastos (2016) [12] was adapted.

2.4 Statistical Analysis

The results obtained in this study correspond to the average of three repetitions \pm standard deviation of the mean. Means were statistically analyzed by Tukey test at 5% probability ($P < 0.05$) by applying ANOVA. All analyzes were performed using the STATISTICA 8.0 program (StatSoft, Inc.).

3. 3. RESULTS AND DISCUSSION

3.1 Phenolic compounds, flavonoids and condensed tannins

Quantifications of the contents of total phenols, flavonoids and condensed tannins in each of the extracts and fractions prepared from leaves *C. diphylla* are presented in Table 1.

Table 1: Contents of total phenols, flavonoids and tannins in extracts and fractions of leaves of *Chamaecrista diphylla*.

Sample	Total phenols (mg GAE/g extract)	Flavonoids (mg RE/g extract)	Condensed tannins (mg CE/g extract)
EE	184.90 \pm 0.00 ^b	410.56 \pm 1.96 ^b	57.17 \pm 0.35 ^a
AE	177.81 \pm 0.91 ^b	151.53 \pm 1.96 ^d	27.98 \pm 0.02 ^c
F-Hex	83.61 \pm 0.91 ^c	ne	ne
F-AcOEt	776.52 \pm 2.74 ^a	885.76 \pm 0.00 ^a	50.42 \pm 0.18 ^b
F-WOH	175.87 \pm 5.47 ^b	252.22 \pm 0.00 ^c	24.24 \pm 0.00 ^d

Caption: Values expressed as mean \pm standard deviation. The results of each test were analyzed separately. Averages with different letters in the same column are statistically different by Tukey's test, being $p < 0.05$. EE – ethanolic extract; AE – Aquoso extract; F-Hex – hexane fraction; F-AcOEt – ethyl acetate fraction; F-WOH – hydroalcoholic fraction; GAE – gallic acid equivalents; RE – rutin equivalents; CE – catechin equivalents; ne – not evaluated due to chlorophyll interference.

According to the results obtained in Table 1, it was observed that the F-AcOEt sample presented the highest content of phenolic compounds (776.52 \pm 2.74 mg GAE/g), followed by EE, AE and F-WOH (184.90 \pm 0.00, 177.81 \pm 0.91 and 175.87 \pm 5.47 mg GAE/g, respectively), which exhibited very close concentrations with each other. The hexane fraction, F-Hex, had the lowest total phenol content, equivalent to 83.61 \pm 0.91 mg GAE/g.

Regarding the flavonoid content, the F-AcOEt and EE samples showed the highest concentrations, with values equivalent to 885.76 \pm 0.00 and 410.56 \pm 1.96 mg RE/g, respectively, highlighting the double found for F-AcOEt compared at EE, followed by F-WOH (252.22 \pm 0.00 mg RE/g) and EE (151.53 \pm 1.96 mg RE/g), which had the lowest concentration of flavonoids.

In the determination of the content of condensed tannins (proanthocyanidins), the EE extract showed the highest concentration (57.17 \pm 0.35 mg CE/g), followed by F-AcOEt (50.42 \pm 0.18 mg CE/g), AE (27.98 \pm 0.02 mg CE/g) and finally F-WOH (24.24 \pm 0.00 mg CE/g).

Phenolic compounds are widely distributed in plants and are responsible for several proven biological activities in the prevention and treatment of many diseases, such as cardiovascular and cerebrovascular diseases, liver disease, inflammation, cancer and AIDS [13].

It is a chemically heterogeneous group that can be extracted from its matrices through several different methods and organic solvents. This methodological diversity makes it difficult to compare the results obtained with reports in the literature. Most phenolic constituents are soluble in polar solvents such as water and ethanol, however many of them solubilize only in medium polarity solvents such as ethyl acetate [12].

Flavonoids, in turn, have wide distribution in plants, and are responsible for several proven biological activities, highlighting antimicrobial, antiviral, antiulcerogenic, cytotoxic, antineoplastic, antioxidant, antihepatotoxic, antihypertensive, hypolipidemic, anti-inflammatory action, antiplatelet, etc [14, 15].

Silva (2017) [7] determined the content of total phenols and flavonoids in the ethanolic extract and fractions in hexane, dichloromethane and ethyl acetate of the aerial parts of *Chamaecrista* sp. In this study it was found that the ethyl acetate phase had a polyphenol content equal to 267 mg GAE/g, followed by the ethanolic extract (104.6 mg GAE/g), dichloromethane phase (101 mg GAE/g) and hexane phase (3.33 mg GAE/g). For the flavonoids, the ethyl acetate phase exhibited a concentration of 8.73 mg RE/g, followed by the ethanolic extract (4.37 mg RE/g), dichloromethane phase (1.46 mg RE/g) and hexane (0.40 mg RE/g).

The results found for total phenols in the ethyl acetate phase were higher than those found for EE, AE and F-WOH in the present study, but the latter were slightly higher than those obtained in the ethanolic extract and dichloromethane phase. In the case of F-AcOEt, the result was much higher compared to the researcher's work. Regarding the total flavonoid content, it was observed that the results found in extracts and fractions of leaves *C. diphylla* were higher when compared to the study by Silva (2017) [7].

Nancy and Ashlesha (2016) [6] performed the determination of the content of total phenols and flavonoids in the hexane, ethyl acetate, ethanol and hydroalcoholic extracts of Chaksu (*Chamaecrista absus*) seeds, a species found throughout India and Sri Lanka, and continents like Oceania and Africa. The researchers found that the hydroalcoholic extract had a polyphenol content of 31 mg GAE/g, followed by ethyl acetate (8.05 mg GAE/g), ethanolic (7.26 mg GAE/g) and hexane (5.25 mg GAE/g). For flavonoids, ethyl acetate extract showed a concentration of 160.54 mg RE/g, followed by ethanolic (103.4 mg RE/g), hexane (87 mg RE/g) and hydroalcoholic (25.87 mg RE/g).

The results found for total phenols of *C. diphylla* leaf extracts and fractions were superior when compared to work Nancy's and Ashlesha (2016) [6]. Regarding the flavonoid content, it was observed that the quantification obtained for the ethyl acetate extract was very close to that found in the AE (151.53 mg RE/g). In the case of the F-AcOEt, EE and F-WOH samples, the results were much higher (885.76, 410.56 and 252.22 mg RE/g, respectively), when comparing this literature.

The determination of total phenols and flavonoids of *C. absus* seeds has been previously performed by Sebei et al. (2014) [5], who observed a polyphenol content of 7.56 mg GAE/g and for flavonoids of 1.113 mg CE/g.

Adewusi et al. (2011) [4] reported that *Chamaecrista mimosoides* has a polyphenol content of 141.53 mg GAE/g for DCM: MeOH (1: 1) extract and 64.16 mg GAE/g for aqueous root extract. For total flavonoids, a concentration of 16.86 mg RE/g in DCM: MeOH extract (1: 1) and 5.32 mg RE/g in aqueous extract was found. It can be observed that the values of polyphenols and flavonoids found for AE are much higher (177.81 mg GAE/g and 151.53 mg RE/g, respectively) compared to this work.

In addition to simpler molecules such as phenolic acids and flavonoids, tannins have been the subject of several studies, most of which address the different ecological interactions between vegetable and herbivorous tannins. The complexation between tannins and proteins forms the basis for both their pharmacological properties as anti-inflammatory, healing and reverse transcriptase inhibitor activity in HIV virus [16].

Sebei et al. (2014) [5] determined the content of condensed tannins in seeds of specie *C. absus*. The authors found a concentration of 0.527 mg CE/g in the ethanolic extract of the seeds. The value presented for tannin content was much lower than that obtained for EE (57.17 mg CE/g) in the present study. Adewusi et al. (2011) [4] reported the total proanthocyanidin content of *C. mimosoides* root extracts, and found concentrations of 98.83 mg CE/g for DCM: MeOH (1: 1) and 16.19 mg CE/g for aqueous extract of the roots.

Correlating the results of chemical constituents, the type of solvent used in extraction / fractionation and the chemical characterizations performed in this study, we suggest the presence of very polar phenolic compounds in EE, AE and F-WOH, with emphasis on those which are in heterosidic form, and which therefore have a much higher solubility in more polar solvents such as ethanol and water. However, in F-AcOEt, it is suggested the presence of flavones, flavonoids and low molecular weight flavanones in higher concentrations. Already the content of condensed tannins, can be justified by the fact that they are phenolic compounds soluble in water and polar organic solvents [17].

The differences found in the contents of total phenols, flavonoids and condensed tannins of the present work, in relation to the mentioned literature, are due to the modifications of several parameters employed in obtaining the extracts. As parameters, we can highlight the processes of collection of the plant material (date, time, place), the extraction method used, the extraction solvent, the age and the stage of development of the plant. In addition, environmental conditions such as luminosity, temperature, rainfall, radiation, altitude, seasonality, water availability and atmospheric composition directly affect the production of secondary metabolites of biological and pharmacological interest [18].

3.2 Evaluation of antioxidant activity by DPPH method

The best interpretation of the results of the DPPH method is by CE_{50} (effective concentration required to sequester 50% of the DPPH radicals from the solution). This method was introduced because it is easy to interpret and accurate, being widely used in the analysis of antiradical compounds obtained from fruits and plant extracts [12]. In this sense, the concentration values necessary to exert 50% of the antiradical activity in *C. diphylla* extracts and fractions were determined. Results are expressed in Table 2.

Table 2: Concentrations, percentage inhibition index of DPPH radical and efficient concentration in extracts and fractions of leaves of *Chamaecrista diphylla*.

Sample	Concentration (mg.mL ⁻¹)	Inhibition of DPPH (%)	CE ₅₀ (mg.mL ⁻¹)
EE	0.08	13.54	0.35
	0.1	18.73	
	0.12	19.27	
	0.2	32.06	
	0.4	54.50	
AE	0.9	11.41	5.42
	1.5	17.03	
	1.8	18.22	
	3.0	27.74	
	6.0	55.48	
F-Hex	0.8	15.12	2.62
	1.2	22.69	
	1.6	30.28	
	2.0	39.27	
	3.0	56.77	
F-AcOEt	0.02	13.53	0.11
	0.04	23.57	
	0.08	36.36	
	0.1	49.09	
	0.2	81.44	
F-WOH	0.6	10.25	3.86
	0.9	15.84	
	1.8	27.41	
	3.0	38.82	
	4.0	51.76	

Caption: EE - ethanol extract; AE - aqueous extract; F-Hex - hexane fraction; F-AcOEt - ethyl acetate fraction; F-WOH - hydroalcoholic fraction.

Analyzing the data in Table 2, attention is drawn to the F-AcOEt and EE samples, which showed the best inhibitory efficiency indexes of DPPH free radical. Treatment with concentrations ranging from 0.02 to 0.2 mg.mL⁻¹ (AcOEt) and 0.08 to 0.4 mg.mL⁻¹ (EE) promoted an inhibition of 13.53 to 81.44% for the first one, 13.54 to 54.50% for the second, with respective CE₅₀ values of 0.11 and 0.35 mg.mL⁻¹. The tests with AE, F-WOH and F-Hex showed, respectively, the highest values of CE₅₀, 5.42, 3.86 and 2.62 mg.mL⁻¹, meaning that their antioxidant properties are inferior compared to the others.

Noting the results obtained in the determination of chemical constituents (Table 1), it is suggested that the excellent antioxidant activity may be related to the high levels of flavonoids, such as those observed in F-AcOEt and EE (885.76 ± 0.00 and 410.56 ± 1.96 mg RE/g, respectively). Quercetin, for example, is a flavonoid found in high amounts in onion, tea and apple, meets all these characteristics and is one of the strongest natural antioxidants known in recent years [19].

Silva (2017) [7] performed the DPPH antiradical activity test on ethanolic extract and phases in hexane, dichloromethane and ethyl acetate of the aerial parts of *Chamaecrista* sp. The CE₅₀ values for the extract and phases were, respectively, 0.097, 0.265, 0.104 and 0.009 mg.mL⁻¹, being considered more significant results than those obtained for extracts and fractions of leaves *C. diphylla*. Bastos (2016) [12] points out that the fractionation of extracts in different organic solvents could concentrate the antioxidant compounds and, thus, obtain more expressive results. Thus, it can be inferred that if the compound responsible for the excellent antioxidant activity in F-AcOEt is isolated, this activity may be higher than that found in the present study.

Nancy and Ashlesha (2016) [6] evaluated the antiradical activity by the DPPH method in the hexane, ethyl acetate, ethanol and hydroalcoholic extracts of Chaksu (*Chamaecrista absus* Linn.) Seeds, and found that all extracts did not exhibit high inhibitory powers. However, among all extracts, hydroalcoholic was the one that showed the highest antioxidant activity, with an inhibitory index of 15.41%, and the lowest inhibition was observed in hexane, with an index of 6.8%. The ethyl acetate and ethanol extracts exhibited intermediate values of 7.77 and 8.43%, respectively. Sebei et al. (2014) [5] determined the CE₅₀ value for the ethanolic extract of *C. absus* Linn seeds, and found an CE₅₀ value of 0.016 mg.mL⁻¹, being more significant when compared to *C. diphylla* extracts and fractions.

Analyzing the results obtained in the antiradical assay of *C. diphylla* extracts and fractions, it can be verified that the EE and F-AcOEt presented the highest contents of phenolic compounds, flavonoids, condensed tannins and, consequently, greater ability to sequester free radicals. It is also noteworthy that the CE₅₀ values of the two samples matched the ascorbic acid standard, which had an CE₅₀ value of 0.13 mg.mL⁻¹, especially F-AcOEt (0.11 mg.mL⁻¹), which showed a better inhibitory efficiency index than ascorbic acid, a substance widely used in medicine [20].

The correlation coefficients between antioxidant activity versus total phenols, antioxidant activity versus flavonoids and antioxidant activity versus condensed tannins of *C. diphylla* extracts and fractions are shown in Figures 2, 3 and 4. A negative correlation was found for three intersections, being $r_1 = -0.5655$, $r_2 = -0.8212$ and $r_3 = -0.9221$ with a 95% reliability index in the results. According to Shimakura (2006) [21], a negative or inverse correlation indicates that the two variables move in opposite directions, and the correlation becomes stronger the closer to -1.

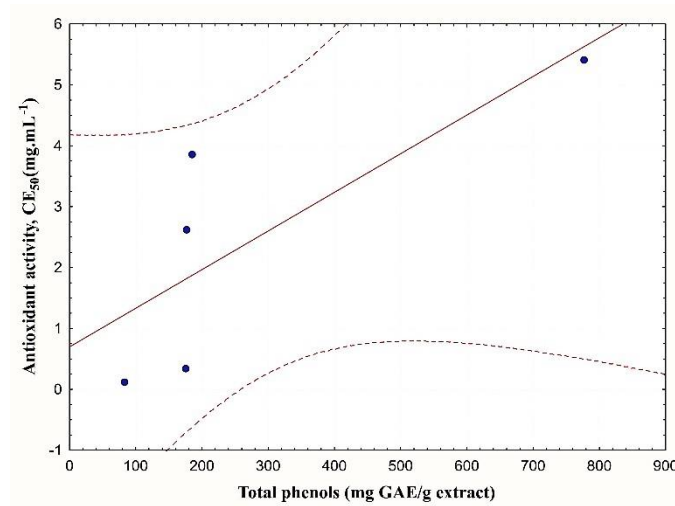


Figure 2: Correlation between antioxidant activity and total phenols with $r1 = -0.5655$.

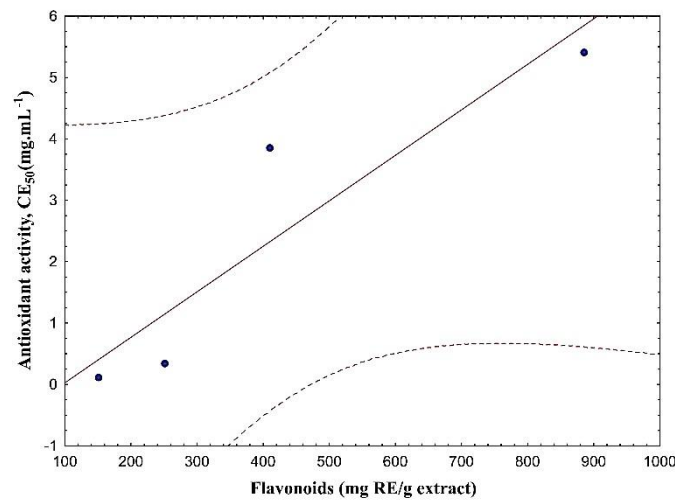


Figure 3: Correlation between antioxidant activity and flavonoids with $r2 = -0.8212$.

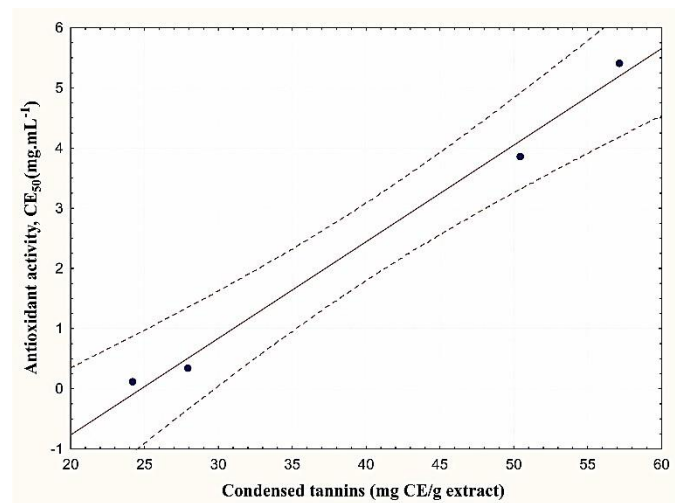


Figure 4: Correlation between antioxidant activity and condensed tannins with $r3 = -0.9221$.

Moderate correlation was observed between total phenols and antioxidant activity (CE₅₀) of the samples. According to the literature, there are great difficulties in establishing rules for these

correlations. as phenolic compounds can interact with each other or with other secondary metabolites in the composition of plant material. These interactions can lead to synergism of these metabolites. increasing or decreasing their ability to sequester free radicals [22].

The correlation between flavonoids and antioxidant activity (CE_{50}) of the samples was considered strong. The anti-radical activity of flavonoids depends on the arrangement of functional groups in the molecular structure of the compound. Ring configuration. degree of substitution and number of hydroxyl groups can influence in mechanisms of antioxidant activity. such as the elimination of free radicals and the ability to complex some metals [12, 16].

The correlation between the condensed tannins and the antioxidant activity (CE_{50}) of the samples was considered very strong. This analysis suggests that the presence of proanthocyanidins contributes in a particular and more effective way to the action of free radical elimination in *C. diphylla* extracts and fractions. Therefore. these results may be used in the future for identification and isolation studies of bioactive compounds that can be applied in drug development.

4. CONCLUSIONS

The chemical study of extracts and fractions of leaves *C. diphylla* showed that F-AcOEt and EE samples had the highest levels of phenolic compounds. flavonoids and condensed tannins. The importance of these determinations was to correlate the levels found with the type of extract and the various proven biological activities of these compounds.

In the determination of antiradical activity. the CE_{50} values for EE ($0.35 \text{ mg}\cdot\text{mL}^{-1}$) and F-AcOEt ($0.11 \text{ mg}\cdot\text{mL}^{-1}$) were similar to those obtained with the ascorbic acid standard ($0.13 \text{ mg}\cdot\text{mL}^{-1}$). especially the fractionated in ethyl acetate. which showed a higher inhibitory efficiency index than the standard. Regarding the samples AE. F-Hex and F-WOH. a lower activity was observed. and in none of them the percentage of DPPH radical sequestration was unsatisfactory. Thus. the results indicate that. for the selective extraction of natural antioxidants. a study on the most appropriate solvent for the extractive process is of great importance.

Regarding the correlation between antioxidant activity and compounds derived from secondary metabolism of plants. it can be noted that the content of flavonoids and condensed tannins are strongly correlated with the antioxidant activity. extracts and fractions of *C. diphylla*. Regarding the total polyphenol content. a moderate correlation with antioxidant activity was observed.

From the results obtained in this research. characterized by the viable and environmentally correct application of the resources of the Amazon biome. it can be concluded that *C. diphylla* presents secondary metabolites with potential biological actions. such as flavonoids and tannins. mainly for application in the pharmaceutical industry and cosmetic.

It is noteworthy that this study presents a novelty regarding the chemical constituents and antioxidant activity of extracts and fractions of specie *C. diphylla*. Therefore. further studies will be necessary for the isolation and elucidation steps of the chemical constituents responsible for antioxidant activity or to prove the hypothesis of a possible synergism between the compounds found.

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6. REFERENCES

1. Marques JA. Borges CPF. Práticas de Química Orgânica. Campinas: Editora Átomo; 2012.
2. Albuquerque UP. Hanazaki N. As pesquisas etnodirigidas na descoberta de novos fármacos de interesse médico e farmacêutico: fragilidades e perspectivas. Rev Bras Farmacogn. 2006 Dez;16:678-689. doi: 10.1590/S0102-695X2006000500015.
3. Mateos-Martín ML. Fuguet E. Jiménez-Ardón A. Herrero-Urbe L. Tamayo-Castillo G. Torres JL. Identification of polyphenols from antiviral *Chamaecrista nictitans* extract using high-resolution LC-ESI-MS/MS. Anal Bioanal Chem. 2014 Sep;406(22):5501-5506. doi: 10.1007/s00216-014-7982-6.

4. Adewusi EA, Moodley N, Steenkamp V. Antioxidant and acetylcholinesterase inhibitory activity of selected Southern African medicinal plants. *South Afr J Bot.* 2011 Aug;77(3):638-644. doi: 10.1016/j.sajb.2010.12.009.
5. Sebei K, Sbissi I, Zouhir A, Herchi W, Sakouhi F, Boukhchina S. Phylogenetic identification, phytochemical analysis and antioxidant activity of *Chamaecrista absus* var. *absus* seeds. *J Plant Biol Res.* 2014 Jan;3(1):1-11.
6. Nancy P, Ashlesha V. Pharmacognostic and phytochemical studies of *Cassia absus* seed extracts. *Int J Pharm Pharm Sci.* 2016 Aug;8(1):325-332.
7. Silva TS. Estudo fitoquímico, atividade antioxidante e fotoprotetora de *Chamaecrista* sp. e *Senna splendida*. Tese (Doutorado em Produtos Naturais e Sintéticos Bioativos) – Universidade Federal da Paraíba, João Pessoa. 2017.
8. Martins MCB, Santos CDG. Ação de extratos de plantas medicinais sobre juvenis de *Meloidogyne incognita* raça 2. *Rev Ci Agron.* 2016 Jan./Mar;47(1):135-142. doi: 10.5935/1806-6690.20160016.
9. Wilczynska A. Phenolic content and antioxidant activity of different types of polish honey – a short report. *Polish J Food Nutrit Sci.* 2010 Jan;60(4):309-313.
10. Woisky RG, Salatino A. Analysis of própolis: some parameters and procedures for chemical quality control. *J Apic Res.* 1998;37:99-105. doi: 10.1080/00218839.1998.11100961.
11. Lugo YO. Estudo da atividade antioxidante, teor de fenóis totais e proantocianidinas do extrato etanólico e composição química do óleo essencial de *Diospyros hispida* A. DC. Dissertação de Mestrado em Química, Urbelândia (MG): Universidade Federal de Urbelândia, Urbelândia. 2015.
12. Bastos RG. Caracterização fitoquímica e avaliação das atividades biológicas dos extratos obtidos das folhas de *Eugenia florida* DC. (Myrtaceae). Dissertação de Mestrado em Ciências Farmacêuticas, Alfenas (MG): Universidade Federal de Alfenas. 2016.
13. Fang X, Wang J, Hao J, Li X, Guo N. Simultaneous extraction, identification and quantification of phenolic compounds in *Eclipta prostrata* using microwave-assisted extraction combined with HPLC–DAD–ESI–MS/MS. *Food Chem.* 2015 Dez;188:527-536. doi: 10.1016/j.foodchem.2015.05.037.
14. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. *Sci World J.* 2013;1-16. doi: 10.1155/2013/162750.
15. Simões CMO, Schenkel EP, Mello JCP, Mentz LA, Petrovick PR. Farmacognosia: da planta ao medicamento. Porto Alegre: Artmed. 2017.
16. Monteiro JM, Albuquerque UP, Araujo EL, Amorim ELC. Taninos: uma abordagem da química à ecologia. *Quím Nova.* 2005 Set/Out; 28(5):892-896. doi: 10.1590/S0100-40422005000500029.
17. Azevedo LFP, Faria TSA, Pessanha FF, Araujo MF, Lemos GCS, Azevedo LFP. Triagem fitoquímica e atividade antioxidante de *Costus spicatus* (Jacq.) S.w. *Rev Bras Pl Med.* 2014;16:209-215. doi: 10.1590/S1516-05722014000200007.
18. Gobbo-Neto L, Lopes NP. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Quím Nova.* 2007 Mar/Apr;30(2):374-381. doi: 10.1590/S0100-40422007000200026.
19. Giada MLR. Uma abordagem sobre a capacidade antioxidante *in vitro* de alimentos vegetais e bebidas. *Demetra.* 2014;9(1):137-146. doi: 10.12957/demetra.2014.8256.
20. Vannucchi H, Rocha MM. Funções plenamente reconhecidas de nutrientes: ácido ascórbico (Vitamina C). *ILSI Brasil.* 2012.21:3-11.
21. Shimakura SE. Interpretação do coeficiente de correlação. 2006. Disponível em: <http://leg.ufpr.br/~silvia/CE003/node74.html>. Acesso em: 21 nov. 2018.
22. Cabello-Hurtado F, Gicquel M, Esnault M. Evaluation of the antioxidante potential of cauliflower (*Brassica oleracea*) from a glucosinolate content perspective. *Food Chem.* 2012 May;132(2):1003-1009. doi: 10.1016/j.foodchem.2011.11.086.