

Pharmacognosy

Phytochemical study and antioxidant activity of *Dalbergia ecastaphyllum*

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Abstract

The chemical profile of *Dalbergia ecastaphyllum* has been indicated as the botanical origin of Brazilian red propolis, an apicultural product with proven therapeutic properties. However, few studies have investigated this plant species. This study evaluated and compared microbiological quality, chemical composition, and antioxidant activity of stem and leaf samples of *D. ecastaphyllum*. The samples were collected in February 2015, in the southern region of the state of Bahia, Brazil. We performed the microbiological analyses, determined the contents of fatty acid, total phenol and flavonoid, and identified the chemical profile and antioxidant activity. *Escherichia coli*, *Salmonella* spp. and sulfite reducing clostridial spores were not detected in the samples. Acids of the family $\omega 3$ were recorded in the stems and $\omega 6$ in the leaves. The leaves presented better nutritional quality of the fraction, better antioxidant capacity in the tests by the DPPH method and β -carotene bleaching. There were 49 chemical compounds, of which 38 belonged to the class of flavonoids. The results indicate that stems and leaves of *D. ecastaphyllum* have biological properties. Leaves particularly are better for functional food formulation and as natural antioxidant.

Key words: DPPH, fatty acids, flavonoids, LC-MS, omega-6.

Resumo

O perfil químico de *Dalbergia ecastaphyllum* tem sido indicado como a provável origem botânica da própolis vermelha brasileira, um produto apícola com inúmeras propriedades terapêuticas comprovadas. No entanto, existem poucas pesquisas dedicadas a esta espécie de planta. O objetivo deste estudo foi avaliar e comparar a qualidade microbiológica, composição química e atividade antioxidante de amostras de caule e folhas de *D. ecastaphyllum*. As amostras foram coletadas em fevereiro de 2015, na região sul do estado da Bahia, Brasil. Análises microbiológicas, determinação de ácidos graxos, conteúdo total de fenóis e flavonoides, identificação do perfil químico e atividade antioxidante foram realizados. *Escherichia coli*, *Salmonella* spp. e esporos de clostrídios redutores de sulfito estavam ausentes em todas as amostras. Ácidos da família $\omega 3$ foram registrados nos caules e $\omega 6$ nas folhas. A folha apresentou melhor qualidade nutricional da fração, melhor capacidade antioxidante nos testes pelo método DPPH e branqueamento β -caroteno. Foram encontrados 49 compostos químicos, dos quais 38 pertenciam à classe dos flavonoides. Os resultados indicam que o caule e a folha de *D. ecastaphyllum* possuem propriedades biológicas, particularmente as folhas são melhores para uso na formulação de alimentos funcionais e como antioxidante natural.

Palavras-chave: DPPH, ácidos graxos, flavonoides, LC-MS, ômega-6.

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Introduction

Dalbergia ecastaphyllum (L.) Taub. belongs to the Fabaceae family and is widely distributed along the coast of the Americas (from southern Florida to southern Brazil) as well as on the eastern coast of the African continent. The occurrence of this species is usually associated with areas of riverbeds, mangroves and restinga vegetation (Carvalho 1997). *Dalbergia ecastaphyllum* has a growth of liana shrub, incandescent or semi-prostrate and presents a tangle of branches and a stem that contributes to better fixation (Lima 2015).

This species is widely used in the recovery of areas degraded by floods (Bohrer *et al.* 2009). It is also used in traditional medicine a diuretic, emetic remedy as well as in inhalations, control of uterine inflammation and anemia (Guedes *et al.* 2014). In the phytochemical screening of extracts from various parts in plants of this genus, metabolites with broad biological performance were identified, such as antioxidant (Lianhe *et al.* 2012), anti-inflammatory (Ganga *et al.* 2012), antimicrobial (Mutai *et al.* 2013; Guedes *et al.* 2014), cytotoxicity (Saha *et al.* 2013), photoprotective (Martins *et al.* 2016), among others. Souza *et al.* (2015) argue that plant parts may present different chemical compositions and consequently different biological activities.

According to the chromatographic methods, isoflavonoids are the main constituent metabolites of *D. ecastaphyllum*, namely biochanin A, daidzein, dalbergin, formononetin, isoliquiritigenin, medicarpin, 3-Hydroxy-8,9-dimethoxypterocarpan and liquiritigenin (Donnelly *et al.* 1973; Matos *et al.* 1975; Dausch *et al.* 2008; Piccinelli *et al.* 2011). Some of these compounds have also been identified in samples of red propolis whose chemical profile *D. ecastaphyllum* is indicated as the botanical origin of this bee product (Mendonça-Melo *et al.* 2017).

Many studies have identified and elucidated numerous biological actions of red propolis (Rufatto *et al.* 2017); however, few studies have investigated its primary source. Although some studies have investigated *D. ecastaphyllum*, its bioactive compounds, microbiological quality and antioxidant activity are not well known. This study evaluated and compared microbiological quality, chemical composition and antioxidant activity of stem and leaf samples of *D. ecastaphyllum*.

Materials and Methods

Sampling

Samples of stems (n = 5) and leaves (n = 5) of *D. ecastaphyllum* (L.) Taub. were collected in February 2015 in different sites in the municipalities of Canavieiras (15°40'30"S, 38°56'50"W) and Ilhéus (14°47'20"S, 39°02'58"W) in the state of Bahia, Brazil, located in the region with phytophysiognomy of the Atlantic Forest and mangrove vegetation. The sampling sites were linked to the environment of red propolis apiaries. For each site, a plant was selected randomly to collect samples of stems with 10 cm in length X 3 cm in diameter and adult leaves. These samples were dried in air circulating greenhouses (40 °C) for 72 h and then ground in knife mill.

The specimens were herborized and sent to the Herbarium of the Federal University of Recôncavo da Bahia (HURB), where they were identified and deposited in vouchers 11,507 and 11,509, respectively, collected in the municipality of Canavieiras and Ilhéus.

Microbiological analyses

Ten grams of the material *in natura* were weighed 90 ml of sterile peptone saline solution and homogenized for 2 min in Stomacher Lab-Blender (Seward type 400, London, United Kingdom). For the quantification of the colony forming units of aerobic mesophilic microorganisms, using the methodology recommended by Silva *et al.* (2010) and for molds and yeasts in ISO 21527-2 (2008). The quantification of Coagulase-positive *Staphylococcus* and Clostridium Sulphite Reduction Spores was performed according to Bennett and Lancette (2001) and ISO 15213 (2003). The counts of coliforms and *Escherichia coli* were obtained by the SimPlate method (BioControl) and the detection of *Salmonella* spp. by the Immunodiffusion 1-2 Test (AOAC 2005).

Chemical composition

Hydro-alcoholic extract

In the extraction, 1 g of the dried and ground samples were used in 12.5 mL of 70% ethanol at constant shaking at room temperature for 14 d. Afterwards, the samples were placed in ultrasonic bath (1h) and centrifuged at 3,000 rpm/10 min, filtered and evaporated under low pressure. After solvent evaporation and stability of samples weight, the dried extract was placed in a freezer

(-20 °C). The extracts used in the analyses were solubilized in methanol at a concentration of 4 mg/mL (Park *et al.* 1998).

Fatty acids

The profile of fatty acids in the samples was determined by gas-liquid chromatography with flame ionization detection (GC-FID at 260 °C) according to Barros *et al.* (2013). A capillary column was used in DANI GC 1,000 (Izasa, Barcelona, Spain) instrument, equipped with a split/splitless injector, with a flame ionization detector (FID) and a Macherey-Nagel column (25 × 0.32 Mm ID × 0.25 µm df). The method involves the transesterification process, which consists of solubilization of samples in methanol, followed by the addition of sulfuric acid and toluene (at a ratio 2: 1: 1 (v / v / v)) for at least 12 h in bath at 50 °C and 160 rpm. It was added 3 mL of deionized water and 3 mL of diethyl ether to this mixture for phase separation. After separation, the lipid phase was extracted, filtered on a column containing sodium sulphate and packed in an amber vessel.

The oven temperature ranged from 30 °C to 220 °C, totaling 35.16 min per analysis. The gas (hydrogen) was kept at a flow rate of 4.0 mL/min (0.61 bar), measured at 50 °C. A volume of 1 µL of transesterified sample was injected into the GC. Fatty acids were identified by comparing the retention times relative to the peaks of standard FAME samples. The results were recorded and processed using CSW 1.7 software (DataApex 1.7, Prague, Czech Republic) and expressed as a relative percentage of each fatty acid calculated by internal normalization of the peak area of the chromatographic peak.

Nutritional quality index of lipid fraction

To assess the nutritional quality of lipids, we determined the Atherogenicity Index (IA), Thrombogenicity Index (IT) (Ulbricht & Southgate 1991) and the relationship between hypocholesterolemic/hypercholesterolemic (H/H) (Santos-Silva *et al.* 2002).

Total phenols and total flavonoids

The total phenol content and total flavonoids of the methanolic extract of stems and leaves were determined by the colorimetric method, Folin-Ciocalteu (Singleton *et al.* 1999) and Aluminum Chloride (Alencar *et al.* 2007), respectively.

The extracts (0.5 mL) were mixed with 2.5 mL of Folin-Ciocalteu reagent (1:10) and 2.0 mL of Na₂CO₃ (4% sodium carbonate). After incubation for 2 h at room temperature, absorbance at 760 nm was determined. The results were expressed as milligram equivalents of Gallic acid per gram of extract (mg GAE / g) from the standard curve ($y = 0.0093x + 0.0287$; $R^2 = 0.9999$). To obtain the total flavonoid content, 2 mL of the extract was mixed with 2 mL of AlCl₃ (2% aluminum chloride). Absorbance was measured at 420 nm after 1 h of incubation at room temperature and the result expressed as milligram equivalent of quercetin per gram of extract (mg QE / g) from the standard curve ($y = 0.0353x + 0.0073$; $R^2 = 0.9995$).

Identification of chemical compounds by LC / MS

The identification of the chemical compounds in stem and leaf samples of *D. ecastaphyllum* were performed using Liquid Chromatography coupled to the LTQ Orbitrap XL™ mass spectrometer (Thermo Fischer Scientific, Bremen, Germany), controlled by LTQ Tune Plus 2.5.5, for the control and acquisition of the data the system Xcalibur 2.1.0. The mobile phase consisted of a system gradient: 0.1% formic acid in Milli-Q (A) water and HPLC-grade methanol (B). The separation was performed using the Gemini C18 reverse phase column (250 × 4.6 mm, 5 µm) of Phenomenex in negative mode, kept at 25 °C, the chromatographic data acquired by three channels of 280 nm, 320 nm and 360 nm readings and fractions to the UV profile display (Falcão *et al.* 2013).

Antioxidant activity

The antioxidant capacity was determined by the physical method, known as radical sequestering activity (DPPH), and the chemical method, based on the transfer of hydrogen atoms (β-carotene/linoleic acid system) (Alves *et al.* 2010). Butylhydroxyanisole (BHA) and ascorbic acid, synthetic antioxidant and natural antioxidant respectively, were used as reference substances for both methods.

Free radical scavenging activity - DPPH (1,1-diphenyl-2-picrylhydrazyl)

The measurement of the DPPH radical sequestering activity of methanolic extracts at different concentrations were performed according to methodology established by Barros *et al.* (2013).

In this physical method, the reaction mixture consisted of 0.3 mL of the sample at different concentrations (0.004 to 0.4 mg/mL) and 2.7 mL of the 0.5 mM solution of DPPH in methanol. After incubation for 1 h at room temperature, the absorbance reading was performed at 517 nm. The antioxidant activity was expressed by EC50, that is, the concentration that induces half the maximum effect.

β -carotene bleaching assay

The coupled oxidation of β -carotene/linoleic acid was performed according to Barros *et al.* (2013). In this chemical method, 5 mg of β -carotene were weighed and diluted in 25 mL of chloroform. A 4-mL aliquot was added, and 80 μ L of linoleic acid and 800 μ L of Tween 20 were added. The reaction mixture was subjected to complete chloroform evaporation using a rotary evaporator, the residue obtained was redissolved in 200 mL of water Deionized, previously saturated with oxygen for 30 min. Aliquots of 4.5 mL of the β -carotene/linoleic acid emulsion were mixed with 0.2 mL of 0.1 mg/mL methanolic extracts. Emulsion oxidation was monitored spectrometrically (UV-visible - Unicam Hekios Alpha), absorbance was measured at 470 nm at the initial time ($t = 0$) and after 2 h of incubation at 50°C. The antioxidant activity (AA) was expressed by inhibition percentage in relation to the control (emulsion + methanol) after a 2-h incubation period.

Statistical analyses

The experimental results were presented as means \pm standard deviation. For the inferential analysis, normality was verified by the Shapiro-Wilks test and the variance homogeneity by the Levene's and Bartlett's tests. The unpaired *Student t*-test was used to compare the differences between the means of treatment groups (stem and leaf), when values were within the normal distribution (fatty acids, phenol content, DPPH and β -carotene/Linoleic acid) or the non-parametric Mann-Whitney test, when values did not fit into the normal distribution (flavonoid content and microorganism counts). The multivariate analysis of canonical correlation was performed to establish correlation between two groups of variables, that is, the group formed by the content of phenols and flavonoids with the second group formed by the different antioxidant methods in stems and leaves (β -carotene/linoleic acid and DPPH). The first group represents the dependent variables (Y), while the second group

represents the independent variables (X). The Hotelling multivariate test (approximation of the F distribution) was used to verify the significance of pairs of canonical variables (Johnson & Wichern 1992). The variance percentage explained by the canonical variable was determined by the square of the canonical correlation. All analyses were performed in the statistical program R (version 3.3.0) at 5% ($p < 0.05$) significance level.

Results and Discussion

Microbiological analyses

As seen in Figure 1, the total colony-forming unit count for the mesophilic aerobic microorganisms (ranging to 9×10^3 to 2×10^5 cfu.g⁻¹), molds and yeasts (ranging to 2×10^2 to 3×10^4 cfu.g⁻¹) (Mann-Whitney U test, $p < 0.00001$), were significantly higher in the stems than in the leaves of *D. ecastaphyllum*. Plants normally have a microbial load from the soil, water and air; however, as the stem is located in a more shaded and moist area of the plant, it has more favorable conditions for the proliferation of microorganisms (Huang *et al.* 2012).

The determination of fungi in vegetables and processed foods is of great importance due to risks to human and animal health, since some fungi produce toxic secondary metabolites. On the other hand, fungi such as endophytes, associated to medicinal plant tissues, may contain bioactive compounds economically useful to human health (Toghueo *et al.* 2017).

The count of aerobic mesophilic microorganisms, as well as of molds and yeasts, is commonly used as a general indicator of product quality. Total coliforms are also indicators of contamination during production, harvesting, and processing stages, and the best indicators are the presence of pathogenic microorganisms of enteric origin, such as *Escherichia coli* (Brasil 2010). No *Escherichia coli*, *Staphylococcus* spp., clostridium sulphite reducing spores and *Salmonella* spp. were found in the samples. The absence of these microorganisms indicates that plant tissues of *D. ecastaphyllum* are safe, from a microbiological viewpoint. This is important because these microorganisms are potentially pathogenic and of interest to public health (De-Melo *et al.* 2015).

Fatty acids

In the lipid composition of stems and leaves of *D. ecastaphyllum*, 21 types of methyl ester acids were identified, with higher percentages for

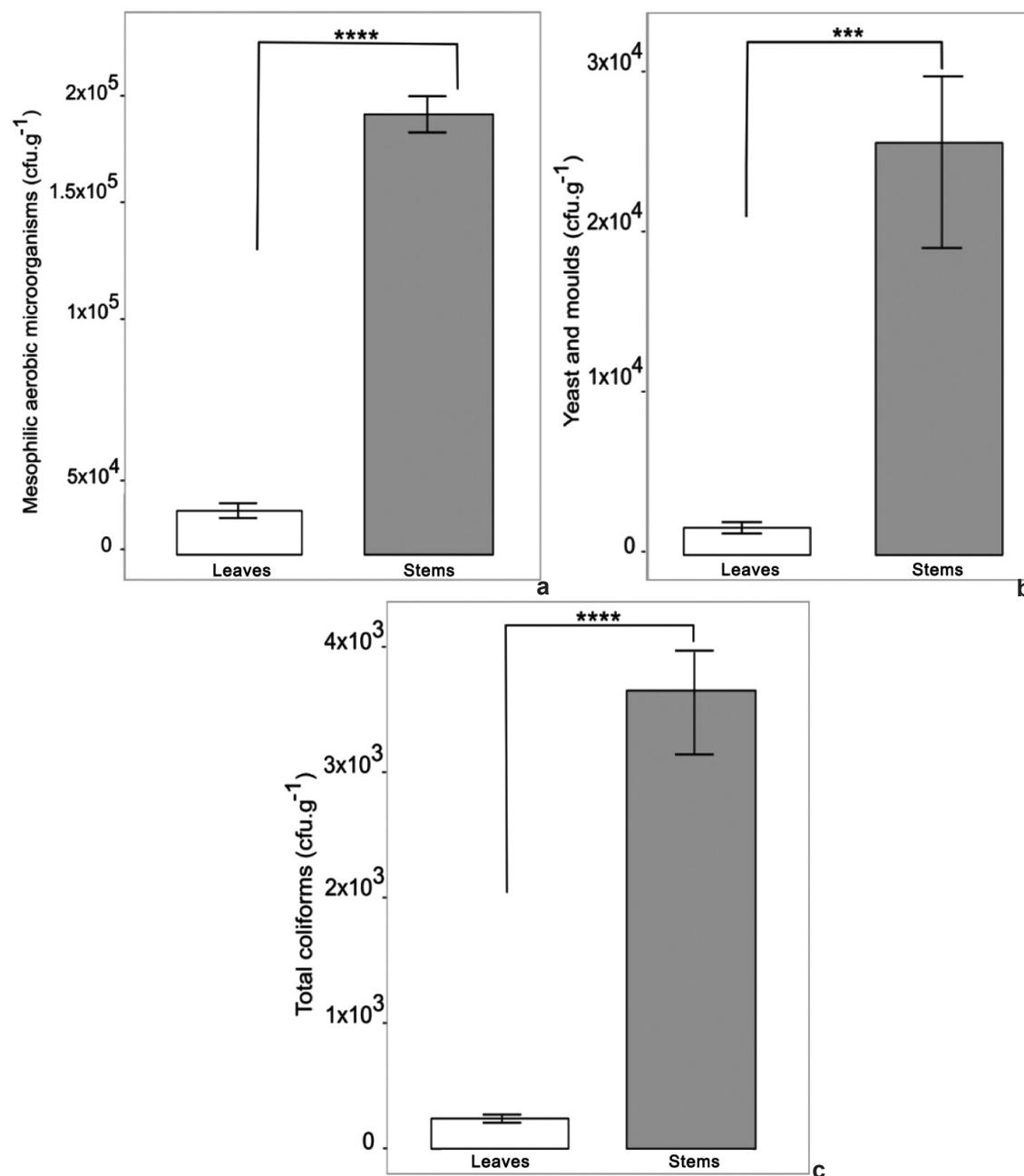


Figure 1 – a-c. Contamination level in leaves and stems samples of *Dalbergia ecastaphyllum* – a. of aerobic mesophilic microorganisms; b. of molds and yeasts; c. of total coliforms. Statistical significance was determined using the Mann-Whitney U test (***: $p < 0.001$; ****: $p < 0.0001$).

saturated acids (SFA), followed by polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA). The amount of SFA in stems ($58.81 \pm 7.65\%$) was higher ($p < 0.05$) than in leaves, PUFA were higher ($p < 0.05$) in leaves ($46.06 \pm 4.43\%$) than in stems, and for MUFA, there was no

statistical difference between the samples. Among the SFA, C16: 0 (palmitic acid) presented higher levels and did not differ statistically between the samples. For PUFA, C18: 2n6c (linoleic acid) was more present ($p < 0.05$) in the stem ($24.50 \pm 5.16\%$), while in the leaves the most abundant

was C18: 3n3 (linolenic acid ($41.54 \pm 4.06\%$)). These PUFA contributed the highest percentage of omega-6 ($\omega 6$) in the stems and of omega-3 ($\omega 3$) in the leaves (Tab. 1).

In a comparative study of leaves, stems and the entire plant of *Ilex paraguariensis*, C18: 3n3 was pronounced in leaves ($60.3 \pm 0.3\%$) and C16: 0 was higher in stems ($25.2 \pm 1.8\%$) (Souza *et al.* 2015), with difference in the chemical composition in the different plant parts. In this study, we also observed difference for C18: 3n3 between the stems and leaves, with the highest concentration observed in leaves. However, no difference was found in the C16: 0 content between the stems and leaves.

In *D. odorifera* T. Chen seed oils, C18: 2n6c was predominant when compared to C18: 3n3 (Lianhe *et al.* 2012). This result was similar to our study for stem samples of *D. ecastaphyllum*, while an inverse relationship for acids was observed in the leaves. These differences may be related to the characteristics of each species, as well as the soil and climatic conditions where the samples were collected.

Fatty acids are energetic sources and play important metabolic functions in the constitution of the cell membrane phospholipid bilayer, in regulating intracellular signaling pathways, transcription factors, and gene expression, as well as in the production of bioactive lipid mediators (Calder 2015). Among fatty acids, the palmitic acid is the most abundant in the human body and has the function of guaranteeing the physical properties of the cell membrane (Carta *et al.* 2017). It has also been reported as a precursor of the sapienic acid (C16: 1n10), which is present in sebaceous glands in the skin (Ge *et al.* 2003). Factors, such as excessive dietary carbohydrate and alcoholic beverages associated to a sedentary lifestyle, can trigger a dietary imbalance of C16: 0 with polyunsaturated acids, generating negative effects on human health (Carta *et al.* 2017).

The stems and leaves of *D. ecastaphyllum* evaluated in this study presented values that could improve the diet characteristics, although the leaves were nutritionally better ($p < 0.05$) with higher PUFA: SAT ratio (0.92 ± 0.15) and lower ratio n -6: n -3 (0.10 ± 0.03). The stems and leaves of *D. ecastaphyllum* evaluated in this study presented values that could improve the characteristics of the diet, although the leaves were nutritionally better ($p < 0.05$) with higher PUFA: SAT ratio (0.92 ± 0.15) and lower ratio n -6: n -3 (0.10 ± 0.03). According to the Department of Health and Social Security of

London (United Kingdom) (1994) and Santos-Silva *et al.* (2002), foods with values lower than 0.45 PUFA: SAT favor the increase cholesterol levels in the blood and values higher than 4.0 n-6: n-3 ratio contribute to cardiovascular diseases. The presence of the omega-6 and omega-3 in polyunsaturated acids, respectively in the stem and leaf, contributed to the nutritional quality of the samples, since diets rich in these acids have a preventive action on diseases, such as cardiovascular diseases (Zhuang *et al.* 2018).

The AI (Atherogenicity Index) and TI (Thrombogenicity Index) indexes in the leaves of *D. ecastaphyllum* were lower ($p < 0.05$) than in the stems and an inverse relationship was found for the HH (Hypocholesterolemic/Hypercholesterolemic) index (Tab. 1). These indices indicate that diets with low AI and TI values may decrease the risk of coronary heart disease associated in isolation or synergy, respectively, with atherosclerosis and thrombosis (Ulbricht & Southgate 1991; Souza *et al.* 2015). HH is directly related to cholesterol metabolism and studies on leafy vegetables have shown that HH are effective in reducing the hypercholesterolemic state in experimental models (Cheurfa & Allem 2015). For these indices, no reference values have yet been established.

Total phenols and total flavonoids

The leaves had higher phenolic contents ($698.52 \pm 9.64\%$, *Student t*-test, $p = 0.00001$, Fig. 2a) and flavonoids ($47.51 \pm 2.21\%$, Mann-Whitney test, $p = 0.00001$; Fig. 2b) than the stems did. Chaitra *et al.* (2015), analyzing the levels of phenolic compounds by spectrophotometric method with standards similar to that of this study, found higher values of phenols in stems (9.32 mg GAE / g) than in leaves (8.54 mg GAE / g) of *D. paniculata* Roux. Similar result was reported for stem flavonoids (13.22 mg QE / g) of *Grewia carpinifolia* (Adebiyia *et al.* 2017). Liu *et al.* (2017) observed that the environment temperature could be a relevant factor to quantify phenol and flavonoid contents in the different plant parts, not obtaining, exclusively, a preponderant value for stems or leaves of the species *Lycium chinense* Miller.

Comparing the content of phenolic compounds in methanolic extracts of stems and leaves of *D. ecastaphyllum* with the ethanolic extract of red propolis from Sergipe state (300.36 ± 0.01 mg GAE / g and 57.6 ± 0.01 mg QE / g) and from Alagoas state (197.77 ± 0.01 mg GAE / g and 58.19 ± 0.01 mg QE / g) (Machado *et al.* 2016), there was a high

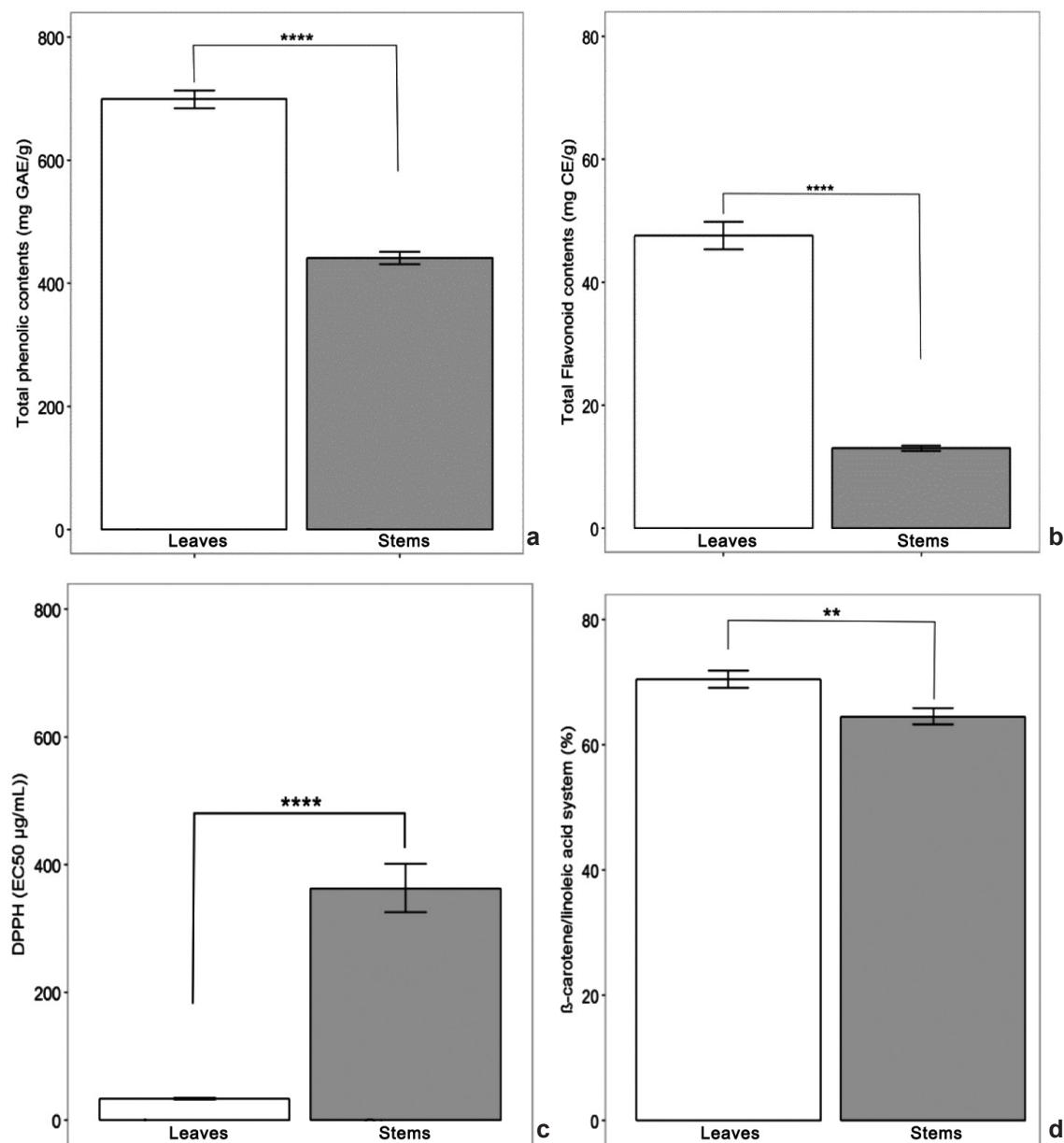


Figure 2 – a. Total phenol content; b. total flavonoid content; c. antioxidant activity of the stem and leaf extracts of *Dalbergia ecastaphyllum* by DPPH; d. β -carotene/linoleic acid. Statistical significance was determined using the *Student t*-test for the total phenol variables (***: $p < 0.0001$) and β -carotene/linoleic acid (**: $p = 0.01$) and the Mann-Whitney U test for Variable total flavonoids (****: $p < 0.0001$) and DPPH (****: $p < 0.0001$). GAE = Gallic acid Equivalent; QE = Quercetin Equivalent; DPPH = 1,1-diphenyl-2-picrylhydrazyl; EC 50 = concentration that induces half the maximum effect.

concentration of methanolic extracts in the plant parts, mainly in the leaves, in relation to the propolis extract. These data corroborate the results in this study, indicating that the leaves are an important source of phenolic compounds.

The levels of total phenols and total flavonoids in our study were higher than those reported by Lianhe *et al.* (2012) and Chaitra *et al.* (2015) in other species of the genus *Dalbergia* and in red propolis. This fact can be associated with several

Table 1 – Chemical composition of fatty acids in stem and leaf samples of *Dalbergia ecastaphyllum*. The results of the fatty acids were expressed in relative percentage. Averages followed by an asterisk (*) differ statistically by the *Student t*-test ($p < 0.05$); ^a = Standard deviation; ^b = Not found; ^c = Total saturated fatty acids; ^d = Total monounsaturated fatty acids; ^e = Total polyunsaturated fatty acids; ^f = Total omega-6 fatty acids; ^g = Total omega-3 fatty acids; ^h = Proportion of polyunsaturated and saturated fatty acids; ⁱ = Proportion of omega-6 and omega-3 fatty acids; ^j = Atherogenicity index; ^k = Thrombogenicity index; ^l = Relation of hypocholesterolemic / hypercholesterolemic fatty acid.

| Fatty acids | Stems | Leaves |
|---|----------------------------|----------------------------|
| | Mean (%) ± SD ^a | Mean (%) ± SD ^a |
| Caprylic Acid (C8:0) | 4.21 ± 1.20* | 2.57 ± 0.70 |
| Lauric Acid (C12:0) | 0.69 ± 0.40* | 0.25 ± 0.07 |
| Myristic Acid (C14:0) | 1.10 ± 0.23 | 0.96 ± 0.11 |
| Pentadecanoic Acid (C15:0) | 0.65 ± 0.10* | 0.15 ± 0.04 |
| Cis-10-Pentadecenoic Acid (C15:1 CIS-10) | 0.34 ± 0.11 | NF ^b |
| Palmitic Acid (C16:0) | 38.19 ± 4.47 | 37.68 ± 2.72 |
| Heptadecanoic Acid (C17:0) | 2.75 ± 0.57* | 1.04 ± 0.24 |
| Cis-10-Heptadecenoic Acid (C17:1 CIS-10) | NF | 0.05 ± 0.03 |
| Stearic Acid (C18:0) | 6.92 ± 1.35* | 5.26 ± 0.96 |
| Oleic Acid (C18:1n9ct) | 2.50 ± 1.51 | 3.07 ± 0.65 |
| Linolelaidic Acid (C18:2n6t) | 0.90 ± 0.35 | NF |
| Linoleic Acid (C18:2n6c) | 24.50 ± 5.16* | 4.22 ± 1.41 |
| Linolenic Acid (C18:3n3) | 11.02 ± 2.29 | 41.54 ± 4.06* |
| Arachinic Acid (C20:0) | 1.59 ± 0.65* | 0.27 ± 0.10 |
| Cis-11,14-Eicosadienoic Acid (C20:2 CIS) | 0.51 ± 0.14 | NF |
| Cis-8,11,14-Eicosatrienoic Acid (C20:3n6) | 0.38 ± 0.23 | NF |
| Eicosatrienoic Acid (C20:3n3) | NF | 0.30 ± 0.20 |
| Behenic Acid (C22:0) | 0.97 ± 0.53* | 0.12 ± 0.06 |
| Tricosanoic Acid (C23:0) | 0.60 ± 0.32* | 0.14 ± 0.07 |
| Lignoceric Acid (C24:0) | 1.14 ± 0.82* | 0.39 ± 0.13 |
| Nervonic Acid (C24:1) | 1.03 ± 0.51 | NF |
| Sums and proportions of fatty acids | | |
| SAT ^c | 58.81 ± 7.65* | 50.82 ± 4.28 |
| MUFA ^d | 3.87 ± 1.75 | 3.11 ± 0.64 |
| PUFA ^e | 37.32 ± 6.49 | 46.06 ± 4.43* |
| Total | 100 | 100 |
| n-6 ^f | 25.78 ± 5.16* | 4.22 ± 1.41 |
| n-3 ^g | 11.02 ± 2.29 | 41.84 ± 4.06* |
| PUFA:SAT ^h | 0.65 ± 0.21 | 0.92 ± 0.15* |
| n-6:n-3 ⁱ | 2.23 ± 0.40* | 0.10 ± 0.03 |
| Nutritional quality indexes of the lipid fraction | | |
| AI ^j | 1.15 ± 0.30* | 0.86 ± 0.08 |
| TI ^k | 1.02 ± 0.30* | 0.32 ± 0.03 |
| HH ^l | 1.00 ± 0.32 | 1.27 ± 0.14* |

Table 2 – Identification of chemical compounds in samples of *Dalbergia ecastaphyllum* stems and leaves using LC / MS. L = Leaf; S = Stem.

| RT | [M-H] ⁻ (m/z) | Formulae | Tentative Identification | Class of compounds | Samples |
|-------|--------------------------|---|--------------------------------------|--------------------------|---------|
| 2.41 | 289 | C ₁₅ H ₁₄ O ₆ | Catechin | Flavanol | S |
| 2.43 | 289 | C ₁₅ H ₁₄ O ₆ | Epicatechin | Flavanol | S |
| 2.87 | 387 | C ₂₀ H ₂₀ O ₈ | Pentamethoxy hydroxy flavonol | Flavanol | S |
| 2.88 | 461 | C ₂₁ H ₁₈ O ₁₂ | Luteolin-O-glucuronide | Flavone | L |
| 2.89 | 179 | C ₉ H ₈ O ₄ | Caffeic acid | Hydroxycinnamic acid | S |
| 3.33 | 165 | C ₉ H ₁₀ O ₃ | Ethoxy benzoic acid | Hydroxybenzoic acid | L |
| 3.33 | 133 | C ₄ H ₆ O ₅ | Malic acid | Organic acid | L |
| 4.24 | 147 | C ₉ H ₈ O ₂ | Cinnamic acid | Hydroxycinnamic acid | L |
| 4.42 | 207 | C ₁₀ H ₈ O ₅ | 7,8-Dihydroxy-6-methoxycoumarin | Hydroxycinnamic acid | S; L |
| 6.55 | 169 | C ₇ H ₆ O ₅ | Galic acid | Hydroxybenzoic acid | S; L |
| 8.4 | 343 | C ₁₅ H ₂₀ O ₉ | Homovanilic acid-O-hexoside | Hydroxyphenylacetic acid | S |
| 9.82 | 137 | C ₇ H ₆ O ₃ | Salicylic acid | Hydroxybenzoic acid | L |
| 11.63 | 153 | C ₇ H ₆ O ₄ | Protocatechuic acid | Hydroxybenzoic acid | S; L |
| 14.96 | 609 | C ₂₇ H ₃₀ O ₁₆ | Kaempferol-diglucoside | Flavanol | L |
| 15.12 | 609 | C ₂₇ H ₃₀ O ₁₆ | Rutin | Flavanol | S; L |
| 16.43 | 255 | C ₁₅ H ₁₂ O ₄ | Pinocembrin | Flavanone | S |
| 17.89 | 593 | C ₃₀ H ₂₆ O ₁₃ | Tiliroside | Flavanol | L |
| 19 | 447 | C ₂₁ H ₂₀ O ₁₁ | Kaempferol-3-O-glucoside | Flavanol | S |
| 19.46 | 121 | C ₇ H ₆ O ₂ | 4-hydroxybenzaldehyde | Hydroxybenzaldehyde | S |
| 20.13 | 593 | C ₂₇ H ₃₀ O ₁₅ | Kaempferol-3-O-rutinoside | Flavanol | S; L |
| 24.82 | 435 | C ₂₁ H ₂₄ O ₁₀ | Phloridzin | Dihydrochalcone | L |
| 26.12 | 579 | C ₂₇ H ₃₂ O ₁₄ | Naringin | Flavanone | S |
| 26.65 | 447 | C ₂₁ H ₁₉ O ₁₁ | Kaempferol 7- O-glucoside | Flavanol | L |
| 26.96 | 447 | C ₂₁ H ₂₀ O ₁₁ | Kaempferol-3-O-glucoside | Flavanol | L |
| 27.03 | 593 | C ₃₀ H ₂₆ O ₁₃ | Tiliroside | Flavanol | S |
| 29.9 | 289 | C ₁₅ H ₁₄ O ₆ | Catechin | Flavanol | L |
| 29.9 | 289 | C ₁₅ H ₁₄ O ₆ | Epicatechin | Flavanol | L |
| 32.39 | 271 | C ₁₅ H ₁₂ O ₅ | Naringenin | Flavanone | S |
| 34 | 625 | C ₂₇ H ₃₀ O ₁₇ | Quercetin 3-sophoroside | Flavanol | L |
| 35.8 | 223 | C ₁₁ H ₁₂ O ₅ | Sinapic acid | Hydroxycinnamic acid | S; L |
| 39.14 | 595 | C ₂₇ H ₃₂ O ₁₅ | Naringenin-O-dihexoside | Flavanone | L |
| 43.79 | 463 | C ₂₁ H ₂₀ O ₁₂ | Quercetin-3-O-glucoside | Flavanol | L |
| 45.35 | 817 | C ₂₇ H ₃₀ O ₁₆ | Kaempferol 3-diglucoside-7-glucoside | Flavanol | S; L |
| 48.7 | 255 | C ₁₅ H ₁₂ O ₄ | Isoliquiritigenin | Chalcone | S |
| 50.05 | 253 | C ₁₅ H ₁₀ O ₄ | Daidzein | Isoflavonoid | S |
| 51.2 | 479 | C ₂₁ H ₂₀ O ₁₃ | Myricetin-3-O-glucoside | Flavanol | S |
| 51.48 | 283 | C ₁₆ H ₁₂ O ₅ | Bichanin A | Isoflavonoid | S |
| 53 | 285 | C ₁₅ H ₁₀ O ₆ | Kaempferol | Flavanol | S |
| 53.25 | 285 | C ₁₆ H ₁₄ O ₅ | Vestitone | Isoflavonoid | S |

| RT | [M-H] ⁻ (m/z) | Formulae | Tentative Identification | Class of compounds | Samples |
|-------|--------------------------|---|----------------------------|----------------------|---------|
| 53.38 | 329 | C ₁₇ H ₁₄ O ₇ | Quercetin-dimethyl-ether | Flavonol | L |
| 55.64 | 269 | C ₁₆ H ₁₄ O ₄ | Medicarpin | Isoflavonoid | S; L |
| 56.38 | 285 | C ₁₅ H ₁₀ O ₆ | Kaempferol | Flavonol | L |
| 56.64 | 301 | C ₁₅ H ₁₀ O ₇ | Quercetin | Flavonol | S; L |
| 58.8 | 271 | C ₁₆ H ₁₆ O ₄ | Neovestitol | Isoflavonoid | S |
| 58.91 | 271 | C ₁₅ H ₁₂ O ₅ | Naringenin | Flavanone | L |
| 60 | 255 | C ₁₅ H ₁₂ O ₄ | Pinocembrin | Flavanone | S; L |
| 60.2 | 267 | C ₁₆ H ₁₂ O ₄ | Formononetin | Isoflavonoid | S; L |
| 61.07 | 271 | C ₁₆ H ₁₆ O ₄ | Vestitol | Isoflavonoid | S |
| 63.09 | 315 | C ₃₃ H ₃₈ O ₂₀ | Quercetin-7-methyl-ether | Flavonol | S |
| 63.11 | 313 | C ₁₆ H ₁₂ O ₆ | Luteolin 3'-methyl ether | Flavone | L |
| 66.37 | 283 | C ₁₆ H ₁₂ O ₅ | Galangin-5-methyl ether | Flavonol | L |
| 69.87 | 285 | C ₁₇ H ₁₈ O ₄ | (3S)-7-O-methylvestitol | Isoflavonoid | S |
| 71.76 | 369 | C ₂₁ H ₂₁ O ₆ | Pinobanksin-3-O-hexanonate | Flavonol | S |
| 73.94 | 353 | C ₁₆ H ₁₈ O ₉ | Chlorogenic acid | Hydroxycinnamic acid | S |
| 76.57 | 435 | C ₂₁ H ₂₄ O ₁₀ | Phloridzin | Dihydrochalcone | S |

factors, namely sample extraction and preparation procedures, seasonal variations, storage and quality of raw material (Rasheed *et al.* 2012).

Identification of chemical compounds by LC/MS

The chromatographic profile of extracts of stems and leaves of *D. ecastaphyllum* recorded at 280 nm and identification based on the deprotonated molecule, its fragmentations (MS) and comparison with molecular masses described in the literature are summarized in Table 2.

The LC/MS method allowed identification of 49 chemical compounds, of which 38% was compounds found exclusive in the stem, 30% in the leaf and 32% common to the samples. These compounds are distributed in 38 flavonoids and have phenolic acids. An organic acid (malic acid) was also detected in the leaf and aldehyde (4-hydroxybenzaldehyde) was observed in the stem. Among the flavonoids, the highest number of compounds are in the flavonols class (n = 18), followed by isoflavonoids (n = 8), flavanones (n = 5), flavanol (n = 3), flavones and with less representation to chalcone (n = 1) and dihydrochalcone (n = 1). Only the flavones (derived from luteolin) were assimilated into the leaves. Isoflavonoids, formononetin, and medicarpin were present both in the stem and in the leaves.

Donnelly *et al.* (1973) and Matos *et al.*

(1975), analyzing the content of flavonoids in woody extract of the species *D. ecastaphyllum*, recorded the compounds formononetin (C₁₆H₁₂O₄), isoliquiritigenin (C₁₅H₁₂O₄), daidzein (C₁₅H₁₀O₄), vestitol (C₁₆H₁₆O₄), which were also found in this study. Few investigations have been carried out exclusively on *D. ecastaphyllum* and most are related in a comparative way to its resin with the chemical composition of red propolis. Several studies on chemical compositions show that resin of *D. ecastaphyllum* is the main raw material for Brazilian red propolis, mainly due to the presence of compounds like formononetin (m/z 267), biochanin A (m/z 283), pinocembrin (m/z 255), daidzein (m/z 253), medicarpin (m/z 269), isoliquiritigenin (m/z 255), (Daugsch *et al.* 2008; Piccinelli *et al.* 2011; López *et al.* 2014; Mendonça-Melo *et al.* 2017).

In the other species of the genus *Dalbergia*, studies reported isoflavonoid formononetin in the shell of *D. melanoxylon* (Mutai *et al.* 2013), pinocembrin, liquiritigenin, galangin, naringenin and isoiquiritigenin in ethanolic extracts of *D. conchichinensis* (Liu *et al.* 2016). biochanin A and genistein were the main compounds present in the leaves of *D. odorifera* (Zhang *et al.* 2011); however, in our study, biochanin A was only recorded in the stem. Catechin, epicatechin, luteolin, naringenin, quercetin, daidzein and the caffeic acid are considered antioxidant compounds (Cömert & Gökmen 2018).

Table 3 – Canonical correlation and canonical pairs between the variables (phenols, flavonoids and β -carotene / linoleic acid and DPPH) of the groups, stem and leaf of *Dalbergia ecastaphyllum*.

^a = 1,1-Difenyl-2-picrylhydrazyl; ** = $p < 0.001$; ^{ns} = not significant ($p < 0.05$); ^b = Canonical correlation; ^c = Approximate value of the statistic F; ^d = Level of significance.

| Variables | Pair canonical | |
|--------------------------------|--------------------------|--------------------------|
| | 1 ^a Dimension | 2 ^a Dimension |
| Phenols | 0.98 | 0.21 |
| Flavonoids | 0.96 | -0.27 |
| β carotene/linoleic acid | 0.53 | 0.12 |
| DPPH ^a | -0.88 | -0.02 |
| r ^b | 0.89 | 0.15 |
| F ^c | 15.74** | 0.577 ^{ns} |
| α ^d | 0.00 | 0.45 |

In the stem and leaf samples of *D. ecastaphyllum*, promising compounds for human health were identified, such as formononetin, which has a potential effect on dermatitis attenuation (Li *et al.* 2018a), inhibition of cancer cell proliferation and metastasis (Zhang *et al.* 2018), neuroprotective (Li *et al.* 2018b) and antibactericidal properties (Mutai *et al.* 2015). Pinocembrin suppressed autophagy in melanoma cells (Zheng *et al.* 2018), demonstrating a protective effect on ischemia-induced brain injury (Tao *et al.* 2018) and neurovascular area with deficits related to Alzheimer's (Liu *et al.* 2014). Medicarpin could be used as a chemopreventive or cytotoxic agent for chemotherapeutic agents in resistant cancer cells (Gatouillat *et al.* 2015). Rutin reduced plasma and glucose levels associated to diabetes, it prevented degenerative changes in the heart, anti-inflammatory effect against intestinal diseases, anti-allergic properties against rhinitis and dermatitis, anti-tumor and antimicrobial features. These activities are linked mainly to the high antioxidant property of this compound (Gullón *et al.* 2017).

Antioxidant Activities

Antioxidants are substances capable of preventing and fighting oxidative damage caused by free radicals (Alves *et al.* 2010). Excess of these radicals could cause deleterious damage to mitochondria (Mailloux *et al.* 2014), plasma membrane (Neto & Cordeiro 2016), and DNA deoxyribonucleic acid (Citron *et al.* 2016),

triggering loss of cellular homeostasis and several pathogenic diseases (Alves *et al.* 2010).

Secondary metabolites produced by plants are sources of active principles with potential to block free radicals. They are of interest in the production of drugs, nutraceuticals, functional foods and food additives (Atanasov *et al.* 2015).

The methanolic extract of the leaves analyzed revealed a greater power reduction of the DPPH radical ($33.53 \pm 2.14 \mu\text{g} / \text{mL}$, Mann-Whitney test, $p = 0.00001$) (Fig. 2c) and better results for the β -carotene bleaching ($70.37 \pm 1.36\%$, Student *t*-test, $p = 0.0034$) (Fig. 2d), compared to the stems. Reference substances, butyl hydroxyanisole (BHA) ($11 \pm 0.001 \mu\text{g} / \text{mL}$) and ascorbic acid ($22 \pm 0.002 \mu\text{g} / \text{mL}$), have shown to be more efficient in the reduction ($p < 0.05$) of DPPH. The β -carotene bleaching test showed that the antioxidant activity of BHA ($95.62 \pm 1.15\%$) was higher than in our extracts, while ascorbic acid was less expressive ($43.63 \pm 0.95\%$).

The antioxidant activity of the hydroalcoholic extract of *D. monetaria* bark at a concentration of $5.46 \pm 0.17 \mu\text{g}/\text{mL}$ presented better DPPH sequestering efficacy than in this study (Martins *et al.* 2016). Ganga *et al.* (2012) observed that the free radical sequestering activity of the methanolic extract of *D. paniculata* leaf is related to the anti-inflammatory action tested in rat edema. For the antiradical effect (DPPH), the EC₅₀ value ($70.6 \mu\text{g}/\text{mL}$) found by these authors needed a higher concentration of methanolic extract of leaves

of *D. paniculata* than the methanolic extract of leaves of *D. ecastaphyllum* (33 µg/ml).

The carotenoid β-Carotene helps in the inhibition of cellular lipid auto-oxidation; however, at high amounts of reactive oxygen species, it can undergo oxidation. The β-carotene/linoleic acid system allows evaluating the ability of an antioxidant to protect β-carotene during the peroxidation of linoleic acid (Alves *et al.* 2010). Souza *et al.* (2015) also observed that extracts from leaves of *I. paraguariensis* prevented β-carotene degradation better than the stem extracts did.

The results of the multivariate analysis of canonical correlation show that the first canonical pair was significant ($p < 0.01$) and with a high correlation value, which was used to interpret the data (Tab. 3). The results show that 89% of the variance between the groups of characteristics are explained by the canonical function 1. Therefore, the contents of phenols and flavonoids presented a positive correlation with the β-carotene/linoleic acid method and a high negative correlation with DPPH. These results indicate, respectively, that the higher the phenolic compounds content, the greater the inhibition of lipid peroxidation and the lower the concentration of antioxidant in free radical sequestration DPPH.

The A canonical correlation analysis corroborates observations that the leaves showed better antioxidant activity, regardless of the method used, in comparison with the stems, which can be explained by the high content of phenolic compounds (Fig. 2a,b), the main secondary compounds of plants to control or delay the damage caused by free radicals to genetic material or lipid peroxidation (Farzaneh & Oliveira 2015).

Conclusion

The stems and leaves of *D. ecastaphyllum* presented high levels of phenolic compounds and lipid contents with valuable acids, such as the linoleic and linolenic acids (omega family), respectively. The presence of the flavonoids formononetin, pinocembrin, kaempferol, rutin, naringenin and medicarpin among others characterizes chemical compositions that deserve attention, due to the versatility of pharmacological properties reported by the literature and antioxidant activity pointed out in this study. However, leaf samples showed significant higher results for total phenolic compounds and antioxidant activity when compared to stem samples. The absence

of pathogenic bacteria in the samples is also a positive result.

The results indicate that the stems and leaves of *D. ecastaphyllum* have biological properties. Leaves are particularly better for functional food formulations and as natural antioxidant.

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