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# ***Dalbergia ecastaphyllum* leaf extracts: *in vitro* inhibitory potential against enzymes related to metabolic syndrome, inflammation and neurodegenerative diseases**

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**ABSTRACT.** For the first time, the anti-hemolytic activity and the enzyme inhibitory activities of *Dalbergia ecastaphyllum* leaves extracts were tested against  $\alpha$ -amylase,  $\alpha$ -glucosidase, lipase, acetylcholinesterase, butyrylcholinesterase, tyrosinase and hyaluronidase. The phenolic profile of the obtained extracts was also investigated by high-performance liquid chromatography with photodiode array detection (HPLC-PAD). The extracts showed inhibitory activity against all enzymes evaluated, with the highest inhibitory activity reported for the enzyme hyaluronidase ( $28.28 \pm 2.43$  to  $72.19 \pm 1.40 \mu\text{g mL}^{-1}$ ). The obtained extracts also demonstrate anti-hemolytic activity ( $52.22 \pm 1.62$  to  $71.17 \pm 1.82\%$ ). Among the phenolic compounds identified, protocatechuic, vanillic and  $\beta$ -resorcylic acids were the most abundant ( $1.13 \pm 0.06$  to  $2.53 \pm 0.06$ ,  $0.90 \pm 0.06$  to  $2.19 \pm 0.06$  and  $1.03 \pm 1.62$  to  $22.11 \pm 1.62 \text{ mg L}^{-1}$ , respectively). In the statistical analysis, a significant correlation was found between the flavonoids content and all enzymes inhibitory activities. The present study showed that *D. ecastaphyllum* leaves extracts may have the potential to be used in the therapeutic treatment of several diseases such as Alzheimer, Parkinson, type 2 diabetes mellitus, hyperglycemia, and pigmentation, as well as those associated with oxidative stress.

**Keywords:** acetylcholinesterase;  $\alpha$ -glucosidase; hyaluronidase; preclinical research.

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## **Introduction**

Researches conducted along the years have confirmed that several diseases, such as Parkinson, Alzheimer, and cancer are intimately related to the dysfunction of the metabolic synthesis process involving specific substances known as enzymes. The effectiveness of enzyme activities depends on some features considered optimal conditions for acting in bodies, such as substrate concentration, pH, ionic strength and temperature, as they are sensitive substances. Thus, changes occurred in these factors significantly affect their performance and consequently the severity of the disease (Bisswanger, 2014). In this sense, the bioprospecting studies have advanced over the past few decades and recognized the enzyme inhibitors isolated from plants in the preventative medicine without or with a minimum side effect (Dalar, Uzun, Mukemre, Turker, & Konczak, 2015).

Diverse substances found in the plants, such as phenolic acids, flavonols and flavonones, are reported to have the capacity to modulate the physiological effects of enzymes related to several diseases namely diabetes, obesity, neurodegenerative diseases and inflammation (Lunagariya, Patel, Jagtap, & Bhutani, 2014; Zengin et al., 2015; Im, Nguyen, Choi, & Lee, 2016).

Nowadays, the pharmaceutical industry has invested in research on natural products, especially for being considered the most successful source of drug, it leads for the treatment of many diseases and illnesses. The use of natural products has recently increased through traditional medicine due to the side effects caused by synthetic drugs and for their inexpensive purchase (David, Wolfender, & Dias, 2014). Additionally, it has contributed to value the biodiversity of the ecosystem in different countries where these products can be found and supported strategies to preserve them.

*Dalbergia ecastaphyllum* is a native species found along the east coast of the American continent, from South Florida (USA) to the South of Brazil and in West Africa (Carvalho, 1997). In South America, Brazil stands out for presenting records in a wide range of its territory, from the Amazon in the Northern region to

the Atlantic Forest in the Southern. In the Northeast region, particularly in the state of Bahia, it is located the longest area with this species across its coastline. *D. ecastaphyllum* is known as the main resin source Brazilian red propolis (Bueno-Silva, Marsola, Ikegaki, Alencar, & Rosalen, 2017), an important natural product with nutraceutical and pharmacological properties studied over the years, such as anti-inflammatory, antioxidant, antiseptic and antineoplastic properties (Freires, Alencar, & Rosalen, 2016).

Although *D. ecastaphyllum* shows ecological and economic importance for beekeeping, to date no studies have been reported concerning its enzyme inhibitory activities and phenolic composition, mainly of the species located in South America. The aim of the present study was to evaluate the enzyme inhibitory activity, as well as to determine the phenolic composition of hydroalcoholic extracts of *D. ecastaphyllum* leaves collected in seven municipalities located in the state of Bahia, Brazil.

## Material and methods

### Chemicals

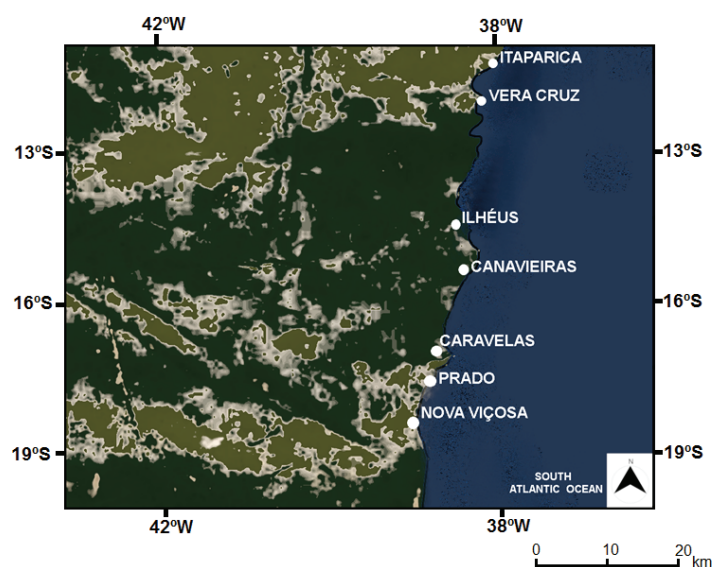
HPLC standards were purchased from Sigma-Aldrich (Sternheim, Germany), and all the solvents employed were HPLC purity grade, filtrated through a nylon filter of 0.20 µm pore size (Supelco, Bellefonte, PA, USA) under vacuum and degassed prior to their use. All enzymes were purchased from Sigma-Aldrich (Sternheim, Germany).

### Plant material

Fresh leaves samples of *D. ecastaphyllum* were collected in the following places: Itaparica (12° 53' 18" S; 38° 40' 43" W), Vera Cruz (13° 5' 60" S; 38° 45' 00" W), Nova Viçosa (17° 57' 40.8" S; 39° 27' 53.8" W) Prado (17° 06' 11.8" S; 39° 15' 54.9" W), Caravelas (17° 43' 57.4" S; 39° 15' 54.9" W); Canavieiras (15° 40' 38" S; 38° 56' 42" W) and Ilhéus (14° 47' 36"; 39° 2' 46" W) located in the state of Bahia, Brazil (Figure 1). Only healthy mature leaves were harvested (not insect damaged, diseased and mechanically injured); the leaf had approximately the same size. Upon receipt into the laboratory, none of the samples had signs of any visible contamination namely fermentation, spoilage or field residues. The species confirmation was carried out in the Herbarium of the Center for Agrarian, Environmental and Biological Sciences of the *Universidade Federal do Recôncavo da Bahia*, Brazil. The samples were oven-dried with air circulation (Fortinox) at 40°C for 72 hours, ground in a grinder (Tecnal, TE-625), stored in closed polycarbonate flasks, and kept under room temperature protected from light.

### Extracts preparation

For the preparation of hydro-ethanol extracts, 15 g of each dried sample was macerated with 135 mL of ethanol (70%) at room temperature for 14 days. Subsequently, the extracts were filtered in filter paper and evaporated (IKA® RV-USA) at 40°C until dryness of the solvent. The extracts were stored at room temperature in darkness.



**Figure 1.** Geographic data of *Dalbergia ecastaphyllum* collected from seven municipalities located in the state of Bahia, Brazil.

### Total phenolic and flavonoids content

The total phenolics (TP) were estimated using a modified colorimetric Folin-Ciocalteu method based on the procedure described by Wolfe, Wu, and Liu (2003). The results were expressed in mg of gallic acid equivalents (GAE) per g of dry weight. The measurements were performed in triplicate.

The quantification of total flavonoids (TFL) was performed according to the method described by Zhishen, Mengcheng, and Jianming (1999). The results were expressed in mg quercetin equivalents (QE) per gram of dry weight. The measurements were performed in triplicate.

### Phenolic compounds determination by HPLC-PDA analysis

The identification and quantification of phenolic compounds were performed according to the method previously described by Nastić et al. (2018). In brief, *D. ecastaphyllum* extracts (20 µL) were injected into the HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with an LC-20AD prominence pump, a DGU-20AS prominence degasser, a CTO-10AS VP column oven, a SIL-20A HT prominence autosampler, and an SPD-M20A photodiode array detector (PDA). Separations were performed at 25°C on a Phenomenex Gemini C<sub>18</sub> column (250 x 4.6 mm, 5 µm) and the chromatograms were recorded at 280, 320 and 360 nm depending on the phenolic compound maximum wavelength. Phenolic compounds were analyzed using a gradient elution at 1.0 mL min.<sup>-1</sup> with the following program: 85% B in 0 min., from 85 to 70% B in 20 min., from 70 to 55% B in 20 min., from 55 to 50% B in 5 min., from 50 to 45% B in 5 min., from 45 to 30% B in 15 min., from 30 to 0% B in 10 min., followed by 100 A for 5 min. and back to 85% B in 10 and 10 min. of reconditioning before the next injection. The mobile phase was composed of methanol (solvent A) and water (solvent B) both with 0.1% formic acid. Phenolic compound identification was carried out by comparing the UV absorption spectra and retention time of each compound with those of pure standards injected in the same HPLC conditions. For the quantification of phenolics, different concentrations of each standard were prepared from the respective stock solution (2000 mg L<sup>-1</sup> in methanol), and the results were expressed as milligrams of compound per liter (mg L<sup>-1</sup>).

### Enzyme inhibitory activities

The extracts were submitted to different enzymes inhibition assays. For α-amylase and α-glucosidase inhibition, assays were evaluated according to the method described by Loizzo, Tundis, Menichini, Statti, and Menichini (2008) and acarbose was used as a positive control. The pancreatic lipase inhibition assay was performed as previously reported by Conforti et al. (2012). Orlistat was used as a positive control.

The acetylcholinesterase (ACh) and butyrylcholinesterase (BCh) inhibitory activities were measured using the spectrophotometric method developed by Ellman, Courtney, Andres, and Featherstone (1961) and esterine was used as a positive control. The tyrosinase inhibitory activity was performed as previously described by Orhan and Khan (2014) using L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate. Kojic acid was used as a positive control.

For all enzymes assays cited above, the percentage of enzyme inhibition (%) was calculated according to the expression  $(\%) = 100 \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$ , where A control was the absorbance of the control reaction and A sample was the absorbance of the examined samples. All reactions were carried out in triplicate and the results were expressed in IC<sub>50</sub> values (concentration needed to inhibit 50% enzyme activity, µg mL<sup>-1</sup>) as calculated from the log-dose inhibition curve.

The anti-inflammatory activity was evaluated spectrophotometrically by measuring the inhibitory effect of leaf extracts on the reactions catalyzed by hyaluronidase using the method described by Silva, Rodrigues, Feás, and Estevinho (2012). Epigallocatechin-3-gallate was used as a positive control.

The anti-hemolytic assay was performed as described by Valente, Baltazar, Henrique, Estevinho and Carvalho (2011). The anti-hemolytic activity (%) was calculated from  $\% = 100 \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$ , where A control was the absorbance of the control reaction and A sample was the absorbance of the examined samples. All measurements were carried out in triplicate.

### Statistical analysis

Independent triplicates were used in all analyses and the data obtained were subjected to statistical analysis expressed as mean value ± standard deviation (SD). One way analysis of variance

(ANOVA) was performed to assess differences between the samples at the level of  $p < 0.05$ . The relationships between pairs of variables were analyzed by the Pearson correlation coefficient and considered statistically significant when  $p < 0.05$ . In addition, principal components analysis (PCA) was performed to identify groups of interrelated variables and better understand their relationship. All analyses were performed using the program R version 3.3.0 (R Core Team, 2010).

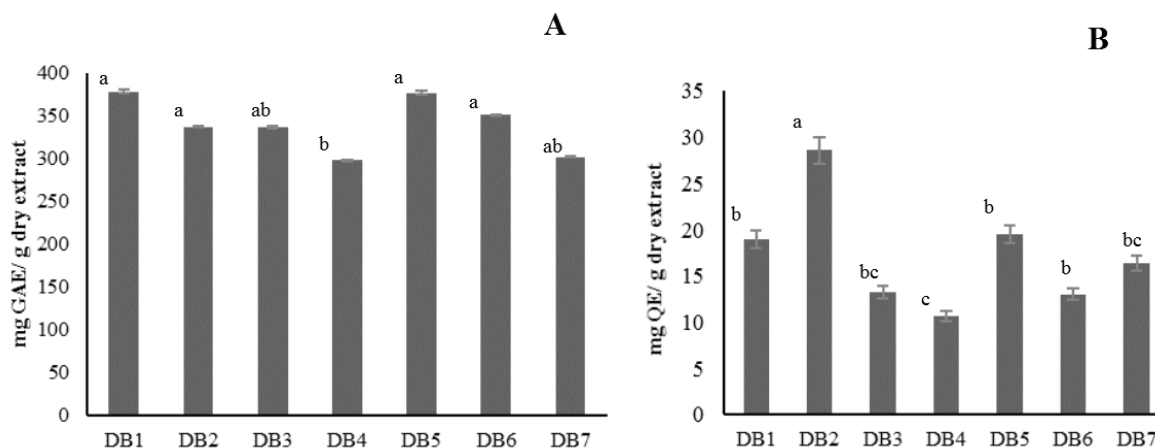
## Results and discussion

### Total phenolic, flavonoid content and phenolic profile

The obtained results of the TP and TFL content for *D. ecastaphyllum* samples are shown in Figure 2. The TP content ranged from  $297.89 \pm 4.54$  to  $377.17 \pm 10.50$  mg GAE  $g^{-1}$  dry extract. The obtained results are at least 38 times higher than the observed in methanol extracts of *Dalbergia odorifera* ( $7.76 \pm 0.30$  mg GAE  $g^{-1}$  dry extract) (Gan, Xu, Song, Kuang, & Li, 2010). Moreover, the present results are also higher than ethanolic extracts leaves of *D. odorifera* prepared under different extraction methods, such as heat-refluxing extraction, ultrasound-assisted extraction and negative pressure cavitation ( $39.84 \pm 0.80$ ,  $31.45 \pm 0.76$ , and  $35.34 \pm 0.71$  mg GAE  $g^{-1}$  dry extract, respectively) (Zhang et al., 2011).

It is important to highlight that the great content of TP obtained in our study may be related to the temperature and solvent concentration employed. As previously reported in the literature, the yield of polyphenols is correlated with these two factors (Jovanović et al., 2017). The high temperatures associated with a long time of exposure also lead to a decrease in these compounds due to the degradation of some phenolic glycosides and flavonols (Segovia, Lupo, Peiró, Gordon, & Almajano, 2014; Tay, Tan, Abas, Yim, & Ho, 2014). Therefore, the applied extraction method in the present study may be responsible for the efficient recovery of phenolic compounds of *D. ecastaphyllum* leaves. Regarding the TFL content, the highest value was observed to sample DB2 ( $28.59 \pm 2.10$  mg QE  $g^{-1}$  dry extract), while the lowest for sample DB4 ( $10.62 \pm 3.05$  mg QE  $g^{-1}$  dry extract), even though this sample was not significantly different from samples DB3, DB6, and DB7. In *D. sissoo* Roxb. ex DC. leaves extract higher amounts of these compounds was observed,  $41.23 \pm 0.21$  mg QE  $g^{-1}$  dry extract, (Chandra, Sachan, & Pal, 2015) similar to the results found in methanolic extract of *Dalbergia sissoo* leaves (Yasmeen & Gupta, 2016).

The identification and quantification of phenolic compounds in *D. ecastaphyllum* hydroalcoholic extracts were performed by HPLC-PDA analysis (Table 1). According to the reported results, it was possible to identify different hydroxybenzoic acid derivatives as protocatechuic, vanillic and  $\beta$ -resorcylic acids, whose last compounds were the main contributors for analysis. Sinapic acid, a hydroxycinnamic acid derivative, was also identified and quantified in all samples, the content was ranging from  $0.28 \pm 0.02$  to  $1.28 \pm 0.20$  mg  $L^{-1}$ . Concerning the flavonoid family, it was possible to quantify four compounds, namely catechin, rutin, and the flavanones naringin and naringenin. From the previously mentioned compound, catechin and rutin were the main contributors to the phenolic composition. It is observed a trend with samples DB5, DB6 and DB7 presenting the highest content of the phenolic compounds quantified.



**Figure 2.** Total phenolic (A) and flavonoid (B) contents of hydroalcoholic extracts of *Dalbergia ecastaphyllum*. DB1 = Caravelas; DB2 = Ilhéus; DB3 = Vera Cruz; DB4 = Itaparica; DB5 = Nova Viçosa; DB6 = Canavieiras; DB7 = Prado. Means  $\pm$  standard deviation followed by the same letters do not differ significantly ( $p > 0.05$ ).

The important differences observed in flavonoid content, even though samples belonged to the same species, might be attributed to specific environmental conditions of the harvest place. *D. ecastaphyllum* species grow along beaches, dunes, and mangroves, therefore, the levels of salinity, temperature, light, nutrients, and water availability diverge. It has been demonstrated in several studies that these abiotic factors effectively influence the chemical composition of plants, particularly the production of secondary metabolites among which flavonoids (Prinsloo & Nogemane, 2018).

### Enzyme inhibitory activities

The ability of *D. ecastaphyllum* hydroalcoholic extracts to inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes were evaluated (Table 2). The decrease of the glucose absorption with reduction of postprandial hyperglycemia by inhibition of these enzymes consist in the therapeutic alternative for the treatment of early-stage diabetes (Tundis et al., 2011).

For the  $\alpha$ -amylase inhibitory activity, DB1 and DB7 samples were the most efficient with  $IC_{50}$  values of  $28.35 \pm 2.40$  and  $25.09 \pm 1.33 \mu\text{g mL}^{-1}$ , respectively. For the  $\alpha$ -glucosidase inhibitory activity, the  $IC_{50}$  values ranged from  $21.56 \pm 0.78$  to  $68.37 \pm 2.75 \mu\text{g mL}^{-1}$ . Fei et al. (2014) investigated the inhibitory effects and possible mechanisms of Oolong tea polyphenols, specifically (–)-epigallocatechin gallate (EGCG) and (–)-epigallocatechin 3-O-(3-O-methyl) gallate (EGCG3"Me) on pancreatic  $\alpha$ -amylase concluding that the inhibitory effects against  $\alpha$ -amylase could be related to the cooperative effects of hydrophobic association and hydrogen bond formation between polyphenols and  $\alpha$ -amylase.

The pancreatic lipase is a lipophilic enzyme secreted by the pancreas responsible for 50 to 70% hydrolysis of the total fat intake on the diet. This enzyme is capable of removing fatty acids from the C-1 and C-3 position of the triglycerides. These acids are directed to the small intestine through the micelles, where they are absorbed and conducted to the circulation by molecules known as chylomicrons. The inhibition of lipase activity leads to an intervention on these processes that results in a reduction of fat absorption, and consequently low consumption of calories and weight loss (Seyedan, Alshawsh, Alshagga, Koosha, & Mohamed, 2015). The values found for the inhibition of lipase in the studied extracts of *D. ecastaphyllum* are reported in Table 3. All the extracts demonstrated the ability to inhibit the pancreatic lipase. Within the samples analyzed, DB1 was the most efficient ( $IC_{50}$ :  $1.15 \pm 0.17 \mu\text{g mL}^{-1}$ ) and DB6 shows the lower inhibition ( $IC_{50}$ :  $4.37 \pm 0.17 \mu\text{g mL}^{-1}$ ). The ability of lipase inhibition in some plants is related to its constituent composition, such as glycosides, terpenes, polyphenols, carotenoids, alkaloids and, saponins (Lunagariya et al., 2014). In the present study, the correlation between the phenolic composition and the lipase inhibitory activity will be further evaluated and discussed (Section Multivariate analysis).

Alzheimer disease (AD) is known as a degenerative and progressive disorder in the brain, considered one of the most common types of dementia that affect old people. It is associated with the decrease of acetylcholine neurotransmitter content in the central and peripheral nervous system due to its hydrolysis by Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE). So, the interruptions on these enzymes activities leading accumulate acetylcholine, with hyperstimulation of nicotinic and muscarinic receptors, and continuous neurotransmissive processes (Colovic, Krstic, Lazarevic-Pasti, Bondzic, & Vasic, 2013). The results of the capacity of *D. ecastaphyllum* extracts to inhibit AChE and BChE evaluated in this study are presented in Table 2. The  $IC_{50}$  values reported for inhibition of AChE ranged from  $81.85 \pm 1.34$  to  $252.9 \pm 3.08 \mu\text{g mL}^{-1}$ , while for inhibition of BChE a higher range was observed, with  $IC_{50}$  values between  $169.30 \pm 3.79$  to  $706.26 \pm 5.86 \mu\text{g mL}^{-1}$ . There are no reports of the cholinesterase inhibition properties of any *Dalbergia* genus. However, species belonging to the Fabaceae family have demonstrated cholinesterase (ChEs) inhibitory activity. *Mimosa pudica*, *Crotalaria asiatica*, and *C. pumila* exhibited AChE inhibitory activity, with  $IC_{50}$  values ranging from  $11.4 \pm 12$  to  $45.1 \pm 3 \mu\text{g mL}^{-1}$  for leaves and  $16.8 \pm 1$  to  $42.0 \pm 2 \mu\text{g mL}^{-1}$  for roots (Nour et al., 2014). In the methanolic and aqueous extract of *Cynometra cauliflora* L. leaves was observed  $IC_{50}$  values of  $65.0 \pm 8$  and  $52 \pm 15 \mu\text{g mL}^{-1}$ , respectively (Ado, Abas, Ismail, Ghazali, & Shaari, 2015).

Parkinson's disease is a neurodegenerative disorder resulting from the neurotransmitter dopamine deficiency in the brain. Abundant accumulation of tyrosinase results in an increase of this neurotransmitter content and reactive oxygen species located in the cytosol and mitochondria, which consequently leads to melanin production and apoptosis of cells (Hasegawa et al., 2008; Hasegawa, 2010). Considering this assumption, the finding of inhibitors of tyrosinase is extremely important for the treatment of melanogenesis and Parkinson's disease. In Table 2 the results obtained for the inhibition of



tyrosinase (IT) for *D. ecastaphyllum* extracts ( $IC_{50}$  values ranged from  $124.62 \pm 3.73$  to  $473.81 \pm 3.51 \mu\text{g mL}^{-1}$ ) are reported. Comparing the obtained  $IC_{50}$  values to the one of the control standard kojic acid ( $5.92 \pm 0.29 \mu\text{g mL}^{-1}$ ), all the prepared extracts were less efficient, but still capable of inhibiting the enzyme.

The hydroalcoholic extracts of *D. ecastaphyllum* demonstrated to be efficient for the anti-inflammatory activity (Table 2). It was revealed that all the samples were more efficient in inhibiting the hyaluronidase activity than the positive standard epigallocatechin-3-gallate, ( $IC_{50}$ :  $98.85 \pm 0.53 \mu\text{g mL}^{-1}$ ). The sample DB6 was the most efficient followed by DB3 presenting  $IC_{50}$  values of  $28.28 \pm 2.43$  and  $31.03 \pm 1.42 \mu\text{g mL}^{-1}$ , respectively. On the other hand, the DB7 sample presented the lowest values ( $IC_{50}$ :  $72.19 \pm 1.40 \mu\text{g mL}^{-1}$ ), but still more effective than the standard control. To the best of our knowledge, our study is the first to report anti-hyaluronidase activity of extracts from *D. ecastaphyllum*.

In the present study, the anti-hemolytic activity (AA) efficiency reported for all the *D. ecastaphyllum* extracts was above 50% (Table 2). The samples DB1 and DB7 showed the highest activities,  $77.10 \pm 1.64$  and  $71.17 \pm 1.82\%$ , respectively. On the opposite side, sample DB3 presented the lowest activity ( $52.22 \pm 1.62\%$ ). These results may support future studies on the antioxidant capacity of this species.

### Multivariate analysis

Pearson's correlation coefficient between chemical components contents and enzyme inhibitory activities are displayed in Table 3. A significant correlation was found between total flavonoids content and all enzymes inhibitory activities, with a stronger correlation between lipase inhibitory activity ( $r = 0.81$ ) and moderate correlation between  $\alpha$ -amylase inhibitory activity ( $r = 0.59$ ), according to Taylor (1990). These results corroborate with the obtained in other studies, confirming the inhibition of some enzymes by flavonoids compounds (Demirkiran, Sabudak, Ozturk, & Topcu, 2013; Yao, Zhu, Chen, Tian, & Wang, 2013; Torab, Takahashi, & Yamaki, 2015; Zeng, Ma, Yang, Jing, & Qu, 2015). It is important to highlight that flavonoids are substances that have a phenolic nucleus, a structural unit, which favors molecular (non-covalent) interactions with enzymes. Therefore, some effects resulting from these interactions, added to their antioxidant properties, are important characteristics and denote their beneficial potential in health (Batra & Sharma, 2013).

**Table 1.** Quantification of phenolic compounds in *Dalbergia ecastaphyllum* hydroalcoholic extracts by HPLC-PDA analysis. Results correspond to means of triplicates  $\pm$  standard deviation and were expressed as mg of compound  $L^{-1}$ .

Phenolic compounds	Sample Code*						
	DB1	DB2	DB3	DB4	DB5	DB6	DB7
PRT	$2.34 \pm 0.06^{ab}$	$1.60 \pm 0.06^{ab}$	$1.13 \pm 0.06^b$	$1.65 \pm 0.06^{ab}$	$2.53 \pm 0.06^a$	$1.66 \pm 0.06^{ab}$	$1.37 \pm 0.06^{ab}$
CAC	$1.56 \pm 0.15^d$	$3.67 \pm 0.15^d$	$6.62 \pm 0.15^{cd}$	$12.49 \pm 1.52^{cd}$	$28.03 \pm 1.53^a$	$26.31 \pm 1.52^{ab}$	$19.94 \pm 1.52^{abc}$
VAC	$0.98 \pm 0.05^b$	$1.42 \pm 0.05^{ab}$	$0.90 \pm 0.06^b$	$1.35 \pm 0.05^{ab}$	$2.19 \pm 0.06^a$	$2.06 \pm 0.05^a$	$1.44 \pm 0.05^{ab}$
BRS	$8.66 \pm 1.62^{cd}$	$8.03 \pm 0.16^{cd}$	$6.72 \pm 0.16^d$	$1.03 \pm 1.62^{cd}$	$14.53 \pm 1.62^{bc}$	$20.72 \pm 1.63^{ab}$	$22.11 \pm 1.62^a$
SIC	$0.65 \pm 0.02^{bc}$	$0.50 \pm 0.02^{bc}$	$0.28 \pm 0.02^c$	$0.63 \pm 0.02^{bc}$	$0.95 \pm 0.02^{ab}$	$1.28 \pm 0.20^a$	$0.94 \pm 0.02^{ab}$
NGI	$1.28 \pm 0.06^a$	$3.72 \pm 0.60^a$	$1.67 \pm 0.06^b$	$1.99 \pm 0.06^b$	$1.70 \pm 0.06^b$	$3.72 \pm 0.60^a$	$1.28 \pm 0.06^b$
RUT	$11.04 \pm 0.63^{ab}$	$7.99 \pm 0.63^b$	$4.83 \pm 0.63^b$	$10.49 \pm 0.63^{ab}$	$18.52 \pm 0.63^a$	$20.22 \pm 0.63^a$	$17.97 \pm 0.63^a$
NGE	$0.26 \pm 0.03^c$	$0.77 \pm 0.03^{abc}$	$0.77 \pm 0.03^c$	$0.64 \pm 0.03^{bc}$	$1.38 \pm 0.03^a$	$1.39 \pm 0.03^a$	$1.23 \pm 0.03^{ab}$

\*Sample code: DB1 = Caravelas; DB2 = Ilhéus; DB3 = Vera Cruz; DB4 = Itaparica; DB5 = Nova Viçosa; DB6 = Canavieiras; DB7 = Prado. Chemical compounds: PRT = protocatechuic acid; CAC = Catechin; VAC = vanillic acid; BRS =  $\beta$ -resorcylic acid; SIC = sinapic acid; NGI = naringin; RUT = rutin; NGE = narigenin. Means  $\pm$  standard deviation followed by the same letters on the lines do not differ significantly ( $p > 0.05$ ).

**Table 2.** Enzyme inhibitory activities of hydroalcoholic extracts of *Dalbergia ecastaphyllum*.

Samples	$IC_{50} (\mu\text{g mL}^{-1})$							
	IA	IG	IL	IAC	IBC	IT	IH	AA (%)
DB1	$28.35 \pm 2.40^{ef}$	$21.98 \pm 1.34^f$	$1.15 \pm 0.17^f$	$109.00 \pm 8.13^f$	$181.91 \pm 1.92^f$	$124.62 \pm 3.73^f$	$66.85 \pm 3.07^a$	$77.10 \pm 1.64^a$
DB2	$52.62 \pm 1.79^c$	$42.31 \pm 1.41^c$	$2.91 \pm 0.14^c$	$154.60 \pm 3.72^c$	$201.79 \pm 5.51^e$	$201.79 \pm 2.41^b$	$52.14 \pm 1.60^c$	$66.55 \pm 1.12^c$
DB3	$77.86 \pm 1.05^a$	$52.95 \pm 2.11^b$	$3.67 \pm 0.08^b$	$252.90 \pm 3.08^a$	$706.26 \pm 5.86^a$	$473.81 \pm 3.51^a$	$31.03 \pm 1.42^e$	$52.22 \pm 1.62^e$
DB4	$31.71 \pm 1.44^e$	$27.84 \pm 0.94^e$	$2.45 \pm 0.18^{cd}$	$128.20 \pm 3.01^e$	$230.05 \pm 7.14^d$	$184.41 \pm 3.35^c$	$44.54 \pm 0.87^d$	$63.92 \pm 1.65^{cd}$
DB5	$40.68 \pm 1.68^d$	$33.40 \pm 1.27^d$	$2.59 \pm 0.12^d$	$142.00 \pm 1.89^d$	$276.43 \pm 3.44^c$	$171.72 \pm 3.64^d$	$60.51 \pm 2.83^b$	$67.69 \pm 0.89^{bc}$
DB6	$63.22 \pm 2.74^b$	$68.37 \pm 2.75^a$	$4.37 \pm 0.17^a$	$212.10 \pm 2.58^b$	$534.07 \pm 11.29^b$	$463.22 \pm 5.62^a$	$28.28 \pm 2.43^e$	$59.75 \pm 1.93^d$
DB7	$25.09 \pm 1.33^f$	$21.56 \pm 0.78^f$	$1.63 \pm 0.20^e$	$81.85 \pm 1.34^g$	$169.30 \pm 3.79^f$	$147.88 \pm 2.20^e$	$72.19 \pm 1.40^a$	$71.17 \pm 1.82^b$
Acarbose	$0.02 \pm 0.005^g$	$0.02 \pm 0.005^g$						
Orlistat			$0.018 \pm 0.00^g$					
Esterine				$0.005 \pm 0.001^h$	$0.005 \pm 0.001^g$			
EGCG <sup>31</sup>							$98.85 \pm 0.53^f$	
Kojic acid						$5.92 \pm 0.29^g$		

\*Sample code: DB1 = Caravelas; DB2 = Ilhéus; DB3 = Vera Cruz; DB4 = Itaparica; DB5 = Nova Viçosa; DB6 = Canavieiras; DB7 = Prado. Inhibitory activity: IA = inhibition of  $\alpha$ -amylase; IG = inhibition of  $\alpha$ -glucosidase; IL = inhibition of lipase; IAC = inhibition of acetylcholinesterase; IBC = inhibition of butyrylcholinesterase; IT = inhibition of tyrosinase; IH = inhibition of hyaluronidase; AA = anti-hemolytic activity. Means  $\pm$  standard deviation followed by the same letters on the lines do not differ significantly ( $p > 0.05$ ).

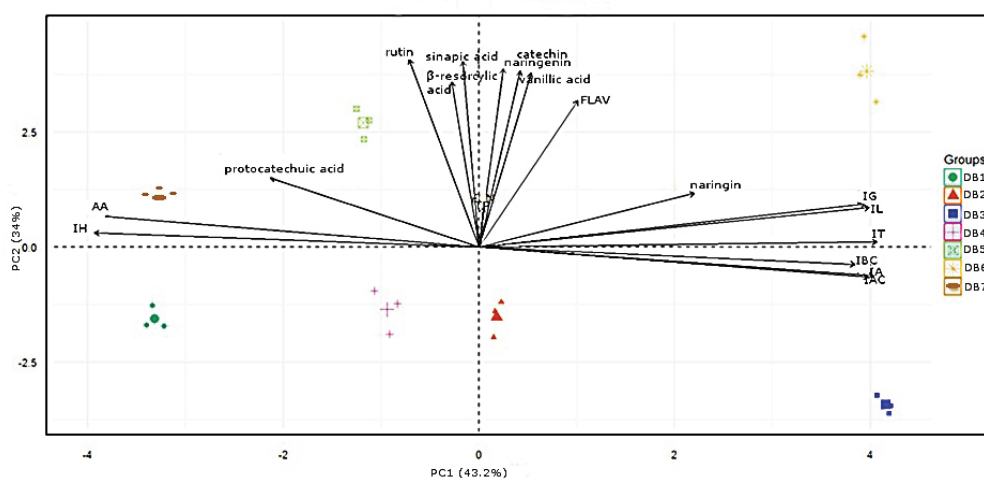
Concerning the correlation between total phenols content and some enzymes inhibitory activities, namely  $\alpha$ -amylase ( $r = 0.50$ ) and butyrylcholinesterase ( $r = 0.49$ ), a low significant correlation was reported. Regarding the correlation between the individual phenolic compounds and the enzyme inhibitory activities, a moderate correlation between the flavonoid naringin and  $\alpha$ -glucosidase inhibitory activity ( $r = 0.68$ ) or anti-inflammatory activity ( $r = -0.50$ ) or inhibition of lipase ( $r = 0.64$ ) was obtained, demonstrating the biological activity of *D. ecastaphyllum* extracts. For the phenolic acids family, protocatechuic acid showed significant correlations between anti-hemolytic activity ( $r = 0.62$ ) or butyrylcholinesterase ( $r = -0.45$ ) or hyaluronidase ( $r = 0.46$ ) or tyrosinase activity ( $r = -0.52$ ).

A principal components analysis (PCA) was also performed in the present study (Figure 3). The data of hydroalcoholic extracts was used to visualize the variation in component contents and enzyme inhibitory activities of the samples. This multivariate analysis method enables the reduction of the information from a large number of variables to a small number of principal components, and thus determining the similarity between them and contributing to a better interpretation of the data (Jolliffe & Cadima, 2016). The first component (PC1) was strongly represented by chemical component contents, while the second component (PC2) was associated with enzyme inhibitory activities. The seven groups formed representing the samples characterized by chemical component contents and biological properties. The samples DB5, DB6, and DB7 were distinctly characterized by positive values of PC2, while the other by negative values. The negative side of PC1 contains the samples DB1, DB4, DB5, and DB7 while the positive side characterizes the rest of the samples.

**Table 3.** Matrix of correlation between chemical components contents and enzyme inhibitory activities.

	AA	TFL	TP	IA	IAC	IBC	IG	IH	IL	IT	NGE	NGI	PRT	RUT	SIC	BRS	VAC	CAC
AA	1																	
TFL	0.04	1																
TP	0.47*	0.30	1															
IA	-0.86*	0.59*	0.50*	1														
IAC	-0.87*	0.65*	0.44	0.97*	1													
IBC	-0.87*	0.67*	0.49*	0.90*	0.94*	1												
IG	-0.75*	0.46*	0.34	0.88*	0.87*	0.80*	1											
IH	0.87*	-0.72*	-0.14	-0.83*	-0.89*	-0.83*	-0.86*	1										
IL	-0.84*	0.81*	0.40	0.86*	0.86*	0.79*	0.95*	-0.90*	1									
IT	-0.87*	0.75*	0.39	0.91*	0.93*	0.95*	0.91*	-0.89*	0.89*	1								
NGE	0.00	0.53*	-0.00	-0.07	-0.11	-0.07	0.23	0.05	0.28	0.04	1							
NGI	-0.28	0.33	0.02	0.45	0.39	0.18	0.68*	-0.50*	0.64*	0.39	0.30	1						
PRT	0.62*	0.31	0.72*	-0.45	-0.40	-0.45*	-0.34	0.46*	-0.40	-0.52*	0.15	-0.15	1					
RUT	0.29	0.70*	0.15*	-0.30	-0.31	-0.20	0.04	0.22	0.01	-0.10	0.87*	0.08	0.37	1				
SIC	0.22	0.77*	0.14	-0.23	-0.19	-0.11	0.19	0.07	0.14	0.02	0.82*	0.27	0.31	0.95*	1			
BRS	0.14	0.59*	-0.15	-0.24	-0.25	-0.09	0.08	0.16	0.06	0.02	0.83*	0.09	-0.01	0.90*	0.87*	1		
VAC	0.01	0.58*	0.24	-0.02	-0.02	-0.05	0.29	-0.05	0.34	0.03	0.91*	0.40	0.41*	0.82*	0.80*	0.63*	1	
CAC	0.10	0.56*	0.04	-0.04	-0.00	0.10	0.24	-0.05	0.31	0.14	0.92*	0.09	0.20	0.87*	0.82*	0.80*	0.88*	1

AA = anti-hemolytic activity; TFL = flavonoids content; TP = total phenolics; IA = inhibition of  $\alpha$ -amylase; IAC = inhibition of acetylcholinesterase; IBC = inhibition of butyrylcholinesterase; IG = inhibition of  $\alpha$ -glucosidase; IH = inhibition of hyaluronidase; IL = inhibition of lipase; IT = inhibition of tyrosinase; NGE = naringenin; NGI = naringin; PRT = protocatechuic acid; RUT = rutin; SIC = sinapic acid; BRS =  $\beta$ -resorcylic acid; VAC = vanillic acid; CAC = Catechin. \* Significantly different ( $p < 0.05$ ).



**Figure 3.** Principal component analysis for phenolic compounds identified and enzyme inhibitory activities of *Dalbergia ecastaphyllum* hydroalcoholic extracts (coded as DB1 to DB7). IA = inhibition of  $\alpha$ -amylase; IG = inhibition of  $\alpha$ -glucosidase; IL = inhibition of lipase; IAC = inhibition of acetylcholinesterase; IBC = inhibition of butyrylcholinesterase; IT = inhibition of tyrosinase; IH = inhibition of hyaluronidase; AA = anti-hemolytic activity.



## Conclusion

The results shown in this work demonstrate the capacity of *D. ecastaphyllum* hydroalcoholic extracts in inhibiting different enzyme activities for the first time. These data suggest the potential use of these samples in preclinical research to prevent neurodegenerative diseases, such as Parkinson, and Alzheimer, in addition to hyperglycemia, anti-inflammatory, hyperpigmentation, and obesity disorders. Although the total chemical composition of the *D. ecastaphyllum* extracts have not been analyzed in the present study, remarkable phenolic substances, mainly flavonoids, were found. In this sense, other studies are necessary in order to evaluate the bioaccessibility and bioavailability of these compounds and understand their effective health benefits.

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