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# Reserach Article
# Antioxidant Activity and Chemical Characterization of Extracts from the Genus *Hymenaea*

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## Abstract

**Background and Objective:** The genus *Hymenaea* August 6, 2016(Fabaceae, Caesalpinioideae) is comprised of 14 species, 12 of which occur in several Brazilian regions commonly known as "Jatobá". Several scientific studies of these species are being conducted to demonstrate their potential for pharmaceutical and food industries. **Methodology:** This study assessed antioxidant activity and sun protection by several parts of two endemic medicinal plants from Brazil, *Hymenaea courbaril* and *Hymenaea stigonocarpa*, through DPPH, FRAP and ORAC-FL assays. Fractionation of 70% ethanolic seed extract from *H. courbaril* was performed. The flavonoids profile by HPLC-PAD was performed with 70% ethanol fractions that showed better results for antioxidants tests and total phenols content. **Results:** All extracts showed antioxidant activity and high phenolic compound content, except the pulp extract. The fraction ethyl acetate methanol (EAM) 70:30 showed better results for DPPH, FRAP and total phenolic content (72.21±1.08%, 3029.97±09.78 μM TE $g^{-1}$ extract and 491.56±02.62 mg GAE $g^{-1}$ of dry extract, respectively). The HPLC-PAD analysis revealed a spectrum with typical absorption bands of flavonoids for the fraction ethyl acetate:methanol 70:30. The results of this study indicate that the 70% ethanol extract from seeds of the specie *H. courbaril* and its fraction EAM 70:30 showed high antioxidant activity, as well as the presence of phenolic compounds. **Conclusion:** These results suggest that *H. courbaril* should be further explored with a focus on use in cosmetic, pharmaceutical and food formulations.

Key words: *Hymenaea courbaril*, *Hymenaea stigonocarpa*, ORAC, FRAP

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The genus *Hymenaea* (Fabaceae, Caesalpiniodeae) is comprised of 14 species, 12 of which occur in several Brazilian regions. Most species of this genus have economic value, mainly due to the high quality of their woods, resins, bark and edible fruits[1,2]. The fruits have amealy pulp that is greatly appreciated by rural populations and also sold in popular markets and fairs, being consumed fresh or as jam, liquor, cake flour, breads and porridges[3,4]. Several species of this genus are used in folk medicine for the treatment of anemia, diarrhea, ulcer, gastric disorder, kidney and lung problems, leukemia, prostate problems and weakness[5,6].

The species from the genus *Hymenaea* are commonly known as "Jatobá". Currently several scientific studies are being conducted onthese species, toassess their potential for the cosmetic, pharmaceutical and food industries[4,7,8].

Studies have reported that the species *Hymenae acourbaril* contains such compound classes as flavonoids and diterpenoids in their resins, leaves, bark, fruit pulps and seeds[9-12]. Previous studies demonstrated therapeutic potentialities of *H. courbaril* such as anti-inflammatory and lipid peroxidation inhibitory activity[13], myorelaxant[14] and antioxidant activity[12].

Phytochemical studies upto the present focusing on constituent compounds of *H. stigonocarpa* have demonstrated the presence of sesquiterpenes, flavonoids, eperuic acid and copalic acid[15-17]. The demonstrated biological activities attributed to this species include gastroprotective, antidiarrheal, cicatrising, anti-inflammatory, antioxidants and antitermitic activity[17-19].

Currently there is growing interest in natural antioxidants present in medicinal and food plants[19-22], because these compounds protect the cells from damage caused by oxidative stress, which implies an excessive production of reactive oxygen and nitrogen species compared to the production of enzymatic and non-enzymatic antioxidants, thus preserving their functional integrity[23,24]. In addition to antioxidant action, several studies have demonstrated photoprotective activity by plant extracts that protect against damage caused by ultraviolet radiation, animportant exogenous source of reactive oxygen species[25]. Thus, antioxidants help reduce the risk of developing various diseases associated with oxidative stress, such as cardiovascular, inflammatory, neurodegenerative diseases, premature aging and cancer[26-28]. Therefore, this study aimed to evaluate the antioxidant and photoprotective potential, as well as to determine preliminary quantities of phenolic compounds from 70% ethanol extract and methanolic extracts of *H. courbaril* and *H. stigonocarpa*. In addition, this study sought to determine the profile of flavonoids derived from 70% ethanol extract fractions of *H. courbaril* seeds.

## MATERIALS AND METHODS

**Reagents:** The 2,2-diphenyl-1-picrylhydrazyl(DPPH), 2, 2′-azobis(2-amidinopropane)dihydrochloride(AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4, 6-tris(2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade.

**Plant material:** There was no necessity to obtain specific permission to collect plant parts, because the studied species were grown at the University for this purpose and the field studies did not involve endangered or protected species. The leaves and fruits of *Hymenaea courbaril* and *Hymenaea stigonocarpa* were collected in the city of Assis, located in Southeast Brazil, between November, 2012 and February, 2013 (*H. courbaril*-22°39'00,66"S and 50°26'15,86"W at an altitude of about 845 m, *H. stigonocarpa*-22°37'18,01"S and 50°27'28,89"W at an approximate altitude of 941 m). The plants (*H. courbaril* and *H. stigonocarpa*) were taxonomically identified by Dr. Renata Giassi Udulutsch from Universidade Estadual Paulista (UNESP-Assis, SP, Brazil) and a voucher specimen has been deposited in the Herbarium Assisense (HASSI) under the respective numbers 186 and 187. The plant parts (leaves, fruit rinds, pulp and seeds) were separated, cleaned and dried at 40°C in an oven with air circulation (Cienlab, Brazil) and subsequently ground in a Wiley type mill (Cienlab, CE-430, Brazil).

**Preparation of extracts:** The ground material (50 g) was macerated with metanol (500 mL) and ethanol solution (70% ethanol-30% water) (500 mL) as solvents for 24 h, at room temperature in the dark with constant stirring. The process was repeated twice with the same powder. The resulting extracts were combined, filtered and concentrated in avacuum (Marconi, MA-120, Brazil). After this process, the hydroethanolic extracts were frozen and subsequently lyophilized (Liotop, L101, Brazil). The dried crude extracts were kept in small opaque bottles at room temperature until used.

### Antioxidant activity assays

**DPPH radical scavenging activity:** The DPPH radical scavenging activity of the extracts was determined according to the technique described by Blois[29]. One milliliter of the 100 mM acetate buffer solution (pH 5.5) was mixed with

1.25 mL of absolute ethanol; then, 250 µL of 500 µM DPPH solution (in ethanol) and 50 µL of tested samples (25-3000 µg $mL^{-1}$ in ethanol) or standard (gallic acid (10-60 µg $mL^{-1}$ in ethanol) was added. After incubation at room temperature for 30 min, the absorbance of each sample or standard was determined at 517 nm in as pectrophotometer UV-vis (Femto, 800XI, Brazil). All determinations were performed in triplicate. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

where, Acontrol is the absorbance of the control and Asample is the absorbance of the sample. The $EC_{50}$ was calculated as the concentration of extracts causing a 50% inhibition of DPPH, where in a lower $EC_{50}$ value corresponds to a higher antioxidant activity of samples.

**Ferric Reducing Antioxidant Power (FRAP):** The FRAP assay was performed as previously described by Benzie and Strain[30]. The FRAP reagent was prepared as required by mixing 25 mL acetate buffer (300 mM, pH 3.6), 2.5 mL TPTZ solution (10 mM in HCl solution (40 mM)) and 2.5 mL $FeCl_3$ solution (20 mM). Sample (500 µg $mL^{-1}$) or Troloxstandard (25-500 µM $L^{-1}$) 90 µL was mixed with 270 µL of distilled water and 2.7 mL of freshly prepared FRAP reagent and incubated at 37°C for 30 min. Maximum absorbance values were read at 595 nm. The results were expressed as micromoles of Trolox Equivalents (TE) per gram of dry extract or fraction.

**Oxygen radical absorbance capacity (ORAC-FL):** The oxygen radical absorbance capacity was determined as described by Davalos *et al.*[31] with slight modi cations. Peroxyl radicals were generated by decomposition of AAPH at 37°C. Fluorescein was used as a fluorescent probe, in which fluorescence indicates a damage reduction to fluorescein by reaction with peroxyl radicals. All reagents were prepared in 75 mM sodium phosphate buffer. Twenty microlittler of sample (1.56-25 µg $mL^{-1}$, final concentration) was mixed with 50 mL of 0.001 mM fluorescein solution in a black 96 well microplate; then, 130 µL, 18.5 mM AAPH solution was added. The microplate was immediately placed in the reader and the uorescence recorded every 5 min for 120 min using a microplate fluorescence reader (Bio-TekInstruments, Synergy HT, Inc.) at excitation wavelength 485 nm and emission wavelength 520 nm. The microplate was automatically shaken prior to each reading. A control sample with fluorescein and AAPH using sodium phosphate buffer instead of the antioxidant solution and five calibration solutions using trolox (0.25-2.5 µg $mL^{-1}$, final concentration) as standard antioxidant were also used in each assay. The ORAC value was expressed as Trolox Equivalents, and is quantified by integrating the area under the curve (AUC).

**Determination of total phenolic content:** The phenolic content of the extracts was determined by the colorimetric method of Folin-Ciocalteu[32]. First, 0.1 mL of the plant extracts (500 µg $mL^{-1}$ in ethanol) was added to 5 mL of deionized water and 0.5 mL of Folin-Ciocalteu reagent. In the blank sample, 5.1 mL of deionized water and 0.5 mL of Folin-Ciocalteu reagent were added. After mixing, the samples were left for 3 min at room temperature; then 1.4 mL of a 25% w/v solution of sodium carbonate ($Na_2CO_3$) and 3 mL of deionized water were added. The mixture was left for 1 h at room temperature in the dark and the absorbance was measured at 765 nm. The total phenolic content was determined by a standard curve of absorbance values derived from standard concentration solutions of gallic acid. The total phenol content was expressed as milligram of gallic acid equivalent per gram of dried extract (mg GAE $g^{-1}$ dry extract). Each sample was tested in triplicate.

**Fractionation of the crude extract:** The dried extract (2 g) that showed the best antioxidant result (70% ethanol extract from *Hymenaea courbaril* seeds) was subjected to adsorption chromatographic process in the filter column using 48 g of silica gel (70-230 mesh). About 300 mL of different solvents and mixtures in order of increasing polarity were used: Hexane, dichloromethane, ethyl acetate, ethyl acetate/methanol (70:30 v/v), ethyl acetate/methanol (50:50 v/v), ethyl acetate/methanol (30:70 v/v) and methanol. The tests used for the antioxidant evaluation (DPPH and FRAP) and determination of total phenolic content of fractions were carried out according to a methodology previously described in this study.

**Analysis of flavonoids by HPLC:** Aiming to identify classes of compounds that may be linked to the activities evaluated in this study, the fraction (ethyl acetate:methanol (70:30 v/v) fraction-EAM 70:30) was chose that showed the best results for antioxidant activity and totalphenolcontent assays, which was analyzed by analytical HPLC.

A sample (10 mg) of EAM 70:30 fraction was dissolved in 1 mL of methanol/water (7:3 v/v) solution and filtered through a syringe filter with PTFE membrane (0.45 µm). The

filtered solution was applied tosolid-phase extraction cartridges (Strata-X, Phenomenex®) containing 500 mg of C18 silica for removing apolar compounds and tannins.

Chromatographic separation was performed in HPLC, quaternary gradient pump PU-2089S plus Jasco®, coupled photodiode array detector (PAD) with scan range 200-900 nm, MD-2015 Plus (Jasco®), AS-2055 autosampler (Jasco®) and CO-2060 plus column oven. The HPLC analysis was performed using a reverse phase column (Phenomenex® Luna $C_{18}$, 250×4.6 mm internal diameter; 5 µm) and Phenomenex® (4×3 mm internal diameter). The mobile phase was composed of solvent A (0.1% formic acid in methanol) and solvent B (0.1% formic acid in water), ranging from 20-50% A in 30 min and 30-35% A in 5 min at a flow rate of 1.0 mL $min^{-1}$. Injection volume: 40 µL, column oven: 40°C, λ = 350 nm. The data acquisition and treatment were conducted using the jasco chrom pass chromatography data system (Version 1.8.1.6). The sample was monitored in a PDA detector in the visible region of the spectrum (UV-vis) in the range of 200-600 nm.

**Statistical analysis:** The data are expressed as the Mean±SD by measuring three independent replicates. Analysis of variance using one-way ANOVA followed by Tukey's test was performed to test the significance of differences between means obtained among the treatments at the significance level of $\alpha \leq 0.05$ using the software Bioestat, version 5.0[33].

## RESULTS AND DISCUSSION

### Antioxidant activity

**DPPH radical scavenging assay:** The $EC_{50}$ values are displayed in Table 1. The 70% ethanol extract from *Hymenaea courbaril* seeds produced the lowest $EC_{50}$ value (149.45 µg $mL^{-1}$). Lower $EC_{50}$ values indicate higher antioxidant activity[34,35].

The methanolic extract from the seeds showed lower $EC_{50}$ (179.43 µg $mL^{-1}$) than the other methanolic extracts of this species. However, this value was higher than the control (Gallic acid = 43.82 µg $mL^{-1}$). Bezerra *et al.*[14] found antioxidant activity attributable to the extracts and fractions of this species, whereas the ethanolic extract of *H. courbaril* showed $EC_{50}$ = 3.07 µg $mL^{-1}$, while the ethyl acetate and methanol fractions presented, respectively $EC_{50}$ = 5.05 and $EC_{50}$ = 5.12 µg $mL^{-1}$. Veggi *et al.*[36] also evaluated the antioxidant activity of the species *H. courbaril* and found $EC_{50}$ = 200 µg $mL^{-1}$ (supercritical fluid extraction).

All extracts of the species *Hymenaea stigonocarpa* presented $EC_{50}$ values higher than the gallic acid control. The

Table 1: Antioxidant activity as $EC_{50}$ (µg $mL^{-1}$) of the hydroethanolic and methanolic extracts from *H. courbaril* and *H. stigonocarpa* measured in DPPH assay

| | Antioxidant activity $EC_{50}$ (µg $mL^{-1}$) | | | |
|---|---|---|---|---|
| | *H. courbaril* | | *H. stigonocarpa* | |
| Plant parts | 70% EtOH | MeOH | 70% EtOH | MeOH |
| Leaves | 415.80[a] | 392.05[a] | 209.37[a] | 193.61[a] |
| Fruit rind | 428.10[a] | 395.44[a] | 235.90[a] | 236.24[a] |
| Seeds | 149.45[b] | 179.43[b] | 470.55[b] | 2150.25[b] |
| Pulp | - | - | - | - |
| Gallic acid | 43.82[c] | | | |

-: Value not detected by the test, values are expressed as mean, same letters within the same column indicate non significant diferences among samples by Tukey test ($\alpha \leq 0.05$)

70 % ethanol extracts from leaves and fruit rind of species *H. stigonocarpa* did not differ statistically and presented the lowest $EC_{50}$ values (209.37 and 235.90 µg $mL^{-1}$, respectively) (Table 1). Rocha *et al.*[37] also found antioxidant activity for this specie, $EC_{50}$= 1050 mg $L^{-1}$ (ethanolic extract) and $EC_{50}$ = 1554 mg $L^{-1}$ (aqueous extract).

Recent studies have demonstrated antioxidant properties of the genus *Hymenaea*, Dias *et al.*[38] evaluated the antioxidant potential of the lipid fraction from seeds and pulp of *H. courbaril* and found a correlation between bioactive substances and the DPPH-scavenging action (0.94 and 0.78, respectively). In the present study, it was observed that other vegetative parts (leaves and fruit rinds) also exhibit considerable antioxidant activity and are amenable to studies of their use in different industrial sectors.

Despite little scientific information on the species *H. stigonocarpa*, Orsi *et al.*[18] and Maranhao *et al.*[17] showed different biological activities including antioxidant activity of extracts from different plant parts (sap, pulp of the fruit and bark of the trunk) from these species.

The 70% ethanol extracts of seeds from *H. courbaril* and *H. stigonocarpa* showed higher antioxidant potential than methanolic extracts from the seeds of these species, thus demonstrating the influence of the extraction process on antioxidant capacity.

**Ferric reducing antioxidant power (FRAP):** According to Boulekbache-Makhlouf *et al.*[39] and Benzie and Choi[40], the antioxidant compounds present in plant-derived extracts can act on different mechanisms *in vivo*. Furthermore, the methods for evaluation are diverse and may present interference, which has led to the recommendation to use more than one methodology. Therefore, in this study for the evaluation of antioxidant extracts, the FRAP test was used in

Table 2: Antioxidant activity of hydroethanolic and methanolic extracts from *H. courbaril* and *H. stigonocarpa* by FRAP assay

| | FRAP (μM TE $g^{-1}$ of extract) | | | |
|---|---|---|---|---|
| | *H. courbaril* | | *H. stigonocarpa* | |
| Plant parts | 70% EtOH | MeOH | 70% EtOH | MeOH |
| Leaves | 632.64±08.20[a] | 1112.63±53.24[a] | 1404.57±42.79[a] | 1267.98±68.60[a] |
| Fruit rinds | 1274.42±59.42[b] | 614.31±21.72[b] | 1716.55±27.00[b] | 1271.48±23.31[a] |
| Seeds | 3073.51±66.73[c] | 2797.90±28.83[c] | 1372.08±88.27[a] | 474.69±51.41[b] |
| Pulp | - | - | - | - |

-: Value not detected by the test. Values are expressed as Mean±SD. Same letters within the same column indicate non significant diferences among samples by Tukey test ($\alpha \leq 0.05$)

addition to the DPPH test, because according to Benzie and Choi[40] the FRAP test allows greater representativeness of the results, which is of great importance in nutritional science.

Table 2 shows the results of the FRAP test on the extracts of species assessed. The 70% ethanolic extract from *H. courbaril* seeds demonstrated the highest reduction potential of $Fe^{3+}$ (3073.51 μM TE $g^{-1}$ of extract) thus corroborating the results obtainedbythe DPPH assay. A similar result was obtained from the methanol extract of this species; it was also the seed extract that showed the greatest potential for reducing $Fe^{3+}$ (2797.90 μM TE $g^{-1}$ of extract). Contreras-Calderon *et al.*[41] found in the pulp from this species 7.60 μM TE $g^{-1}$ wet weight.

With respect to the species *H. stigonocarpa* the hydroethanolic extract from fruit rinds showed the highest reduction potential of iron (1716.55 μM TE $g^{-1}$ of extract), thus differing significantly from the other hydroethanolic extracts from this species. The hydroethanolic extract from the leaves and seeds of this species did not differ significantly (Table 2). Among the methanolic extracts, the extracts from leaves and fruit rinds displayed the highest potential for iron reduction, and did not differ significantly from each other (1267.98 and 1271.48 μM TE $g^{-1}$ of dry extract respectively). Rufino *et al.*[42] reported high levels of antioxidant activity by the FRAP test to evaluate other fruits native to Brazil, namely camu-camu (2502 μM $Fe_2SO_4$ $g^{-1}$ of dry fruit) and acerola (1996 μM $Fe_2SO_4$ $g^{-1}$ of dry fruit).

According to De Oliveira *et al.*[43] there is great interest in substituting natural antioxidants in place of synthetic ones due to their implications in health and functionality. This has attracted the attention of the pharmaceutical, food and cosmetic industries in search of plant material for identification and isolation of new bioactive compounds from natural sources of antioxidants.

**Oxygen radical absorbance capacity (ORAC-FL):** Figure 1 shows the decay curve of the AAPH induced fluorescence for both the control (fluorescein+AAPH+sodium phosphate buffer) and samples (fluorescein+AAPH+sample). The highest concentration of trolox (2.5 μg $mL^{-1}$) promoted maximum protection for about 45 min before the fluorescence intensity began to decrease (Fig. 1a). The increase in AUC values in the ORAC-FL test reflects a higher antioxidant potential in preventing the damage induced by AAPH peroxyl radicals[44]. The fluorescence decay curves of all *H. courbaril* extracts showed antioxidant activity compared to the control (Fig. 1b), and the highest AUC was obtained for the 70% ethanol extract from leaves of this species. All extracts from the species *H. stigonocarpa* presented higher AUC than control whereas the 70% ethanol extract from the leaves showed highest AUC (Fig. 1c). Among all samples, the 70% ethanol extract from *H. stigonocarpa* leaves demonstrated the highest antioxidant potential (0.56 TE), whereas the methanolic extract of the seeds of *H. stigonocarpa* presented the lowest antioxidant potential (0.07 TE). The hydroethanolic and methanolic extracts from *H. courbaril* leaves showed the same value (0.34 TE). These results are consistent with a work by Xi *et al.*[45] that demonstrated the applicability of the ORAC test for analysis of fruit pulp extracts (Table 3).

**Determination of the total phenolic content:** The total phenolic content showed variation among the different extracts and species. The 70% ethanol extract from *H. courbaril* seeds showed higher total phenolic content (464.34 mg GAE $g^{-1}$ dry extract) compared to the other *H. courbaril* extracts. About 70% ethanol extracts from the fruit, rind and leaves of this species did not differ statistically (211.26 and 208.17 mg GAE $g^{-1}$ extract, respectively), however, these values were higher than those produced by the pulp extract (3.37 mg GAE $g^{-1}$ dry extract). The total phenolic contents of the methanol extracts of *H. courbaril* varied significantly according to the plant part used, with the seed extract showing the highest total phenol content (396.68 mg GAE $g^{-1}$ dry extract) (Table 4).

The various plant parts of *H. stigonocarpa* showed different total phenolic content (Table 4) while the leaf extract showed the highest contentof phenols (278.33 mg GAE $g^{-1}$ dry extract). Studies demonstrated that other legumes also present phenolic compounds; Ramli *et al.*[46] found in the extract from leaves of *Acacia farnesiana* (209.78 mg GAE $g^{-1}$ of ethanolic extract). Matuda and Netto[47] found in seeds of *H. stigonocarpa*, 48 mg equivalent catechin per gram of seed flour. In the present study, 70% ethanol seed extract presented 117.73 mg GAE $g^{-1}$

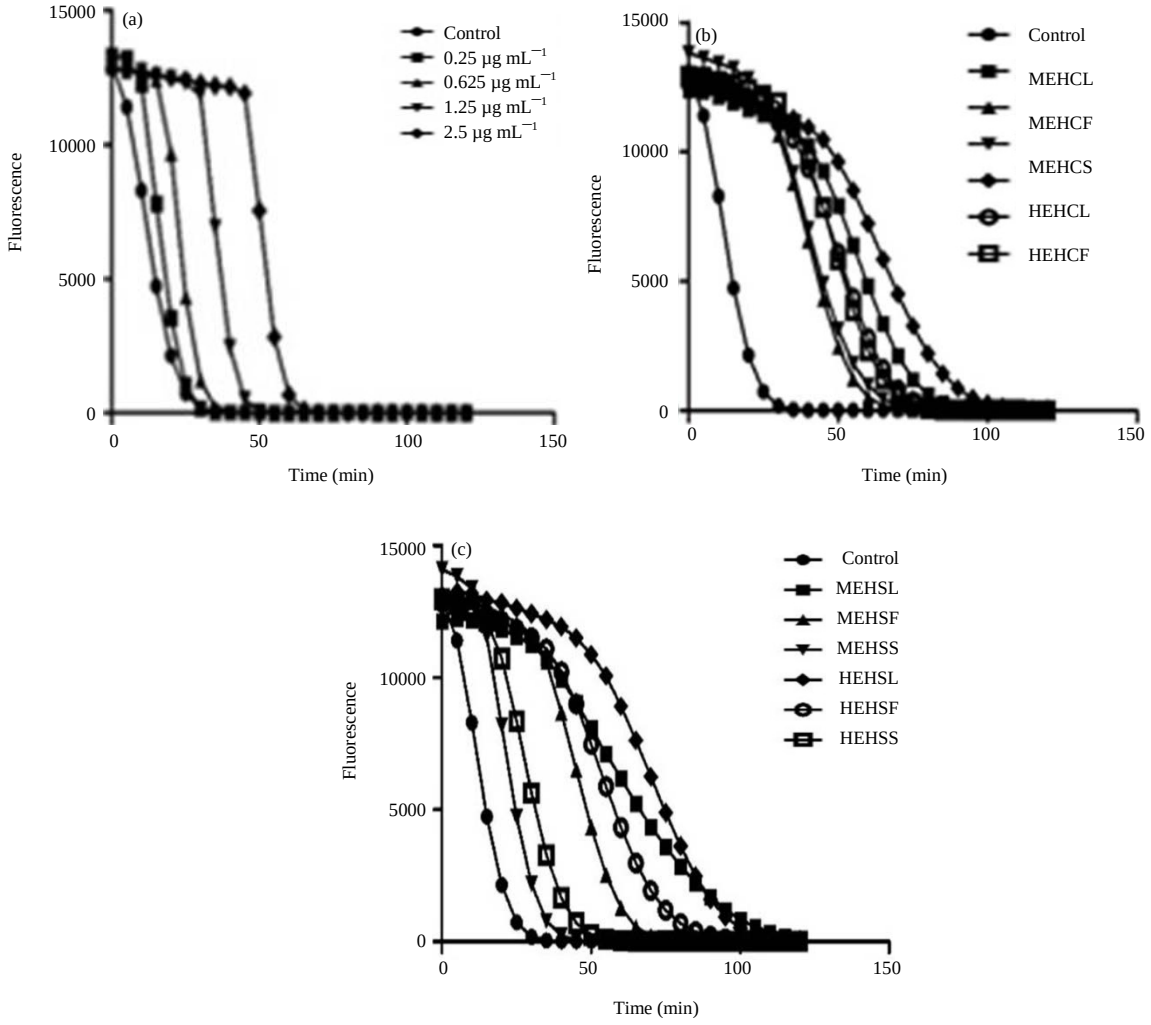


Fig. 1(a-c): Kinetic curves of ORAC-FL assay, (a) Kinetic curves of the trolox standard, (b) Kinetic curves of *H. courbaril* extracts and (c) Kinetic curves of *H. stigonocarpa* extracts, MEHSL: Methanolic extract from *H. stigonocarpa* leaves, MEHSF: Methanolic extract from *H. stigonocarpa* fruit rinds, MEHSS: Methanolic extract from *H. stigonocarpa* seeds, HEHSL: Hydroethanolic extract from *H. stigonocarpa* leaves, HEHSF: Hydroethanolic extract from *H. stigonocarpa* fruit rinds, HEHSS: Hydroethanolic extract from *H. stigonocarpa* seeds, MEHCL: Methanolic extract of *H. courbaril* leaves, MEHCF: Methanolic extract of *H. courbaril* fruit rinds, MEHCS: Methanolic extract from *H. courbaril* seeds, HEHCL: Hydroethanolic leaf extract from *H. courbaril*, HEHCF: Hydroethanolic extract from *H. courbaril* fruit rinds, HEHCS: Hydroethanolic extract from *H. courbaril* seeds

Table 3: Oxygen radical absorbance capacity (ORAC-FL)

| | ORAC-FL (Trolox equivalent) | | | |
|---|---|---|---|---|
| | *H. courbaril* | | *H. stigonocarpa* | |
| Plant parts | 70% EtOH | MeOH | 70% EtOH | MeOH |
| Leaves | 0.34 | 0.34 | 0.56 | 0.25 |
| Fruit rinds | 0.28 | 0.16 | 0.36 | 0.27 |
| Seeds | 0.25 | 0.12 | 0.14 | 0.07 |
| Pulp | - | - | - | - |

-: Value not detected by the test. Values are expressed as mean

of extract. As for methanol extracts of *H. stigonocarpa*, the highest extracts from *H. stigonocarpa* pulp did not present phenolic content.

Contreras-Calderon *et al.*[41] studied total phenols in *H. courbaril* seeds and found high content of phenolic compounds (2013 mg GAE/100 g of fresh weight). In the present study, high levels of total phenols in extracts from this plant part as well as extracts from the leaves and fruit rinds were also found (Table 4).

**Fractionation of the crude extract:** The 70% ethanol extract from the *H. courbaril* seeds showed the best results in the antioxidant tests and measurements of the levels of total phenols; therefore, this extract was fractionated. The highest yield was obtained from the fraction ethyl acetate: methanol 50:50 (v/v) (21.25%) (Table 5).

**Determination of antioxidant activity and total phenols from fractions of *H. courbaril*:** The antioxidant activities measured by DPPH scavenging and FRAP methods as well as the spectrophotometric estimation of the total phenols are shown in Table 6. For DPPH radical scavenging assay, the fractions were used at the concentration of 250 μg $mL^{-1}$. All fractions evaluated showed a lower value of DPPH activity than the gallic acid standard (Table 6). The highest scavenging activity was at 72.21% for fraction EAM 70:30. Dias *et al.*[38] found antioxidant capacity of 83.49% in the seed lipid fraction of *H. courbaril*. There was no significant difference between the EAM 50:50, EAM 30:70 and FM fractions in relation to DPPH scavenging activity. The highest FRAP value was found in the EAM 70:30 (3029.97 μM TE $g^{-1}$ dry extract) and the lowest in the EAM 50:50 and EAM 30:70, which did not differ significantly.

The antioxidant activity of plants is mainly attributable to the active compounds they contain[48] the extraction of these compounds significantly depends on the type of extraction solvent, solvent composition and extraction temperature[49]. The fraction EAM 70:30 showed the highest total phenol content, while the other fractions did not differ significantly (Table 6). The results of total phenols presented by the fraction EAM 70:30 corroborate the results obtained in the antioxidant tests.

Table 4: Phenolic total content of the hydroethanolic and methanolic extracts from *H. courbaril* and *H. stigonocarpa*

| | Phenolic total (mg GAE $g^{-1}$ of dry extract) | | | |
|---|---|---|---|---|
| | *H. courbaril* | | *H. stigonocarpa* | |
| Plant parts | 70% EtOH | MeOH | 70% EtOH | MeOH |
| Leaves | 208.17±04.04[a] | 236.25±16.04[a] | 278.33±04.53[a] | 227.76±01.86[a] |
| Fruit rinds | 211.26±01.87[a] | 187.31±03.77[b] | 237.72±08.87[b] | 198.29±02.99[b] |
| Seeds | 464.34±04.62[b] | 396.68±13.16[c] | 117.73±14.54[c] | 35.04±03.55[c] |
| Pulp | 3.37±04.10[c] | 13.54±01.15[d] | - | - |

-: Value not detected by the test. Values are Mean±SD same subscript letters within the same column indicate no significant diferences among samples by Tukey test ($\alpha \leq 0.05$)

Table 5: Performance of different fractions prepared from 2 g of 70% ethanolic extract from *H. courbaril* seeds

| Fractions | Weight (mg) | Yield (%) |
|---|---|---|
| HX | 0 | 0 |
| DCM | 0 | 0 |
| EA | 0 | 0 |
| EAM 70:30 | 140 | 7 |
| EAM 50:50 | 425 | 21.25 |
| EAM 30:70 | 38 | 1.9 |
| MT | 37 | 1.85 |

HX: Hexane, DCM: Dichloromethane, EA: Ethyl acetate, EAM 70:30: Ethyl acetate:methanol (70:30), EAM 50:50: Ethyl acetate:methanol (50:50), EAM 30:70: Ethyl acetate:methanol (30:70), MT: Methanol

**Analysis of flavonoids by HPLC:** Analysis of the fraction EAM 70:30 of hydroethanolic extract from *H. courbaril* seeds by HPLC revealed the presence of four major peaks (Fig. 2). The PAD scanning in the UV-vis region of each peak showed a spectrum with typical absorption bands of flavonoids (Fig. 3), which are recognized by presenting band II, with maximum in the spectral range of 240-290 nm, attributed to A-ring and band I, which peaks in the spectral range of 300-390 nm, attributed to ring-B[50].

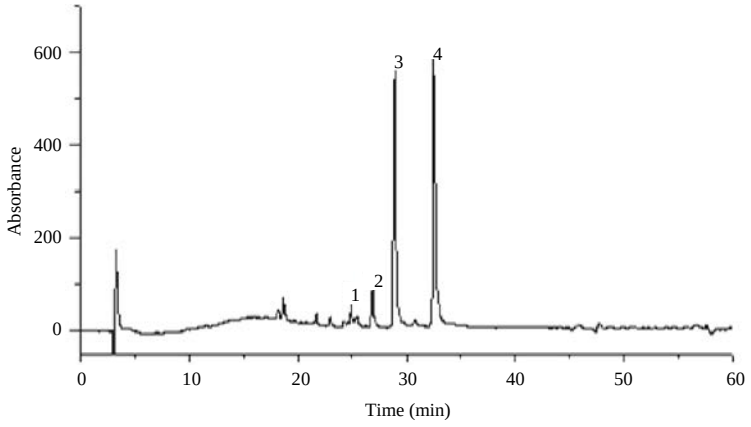


Fig. 2: HPLC-PAD flavonoids profile of the fraction EAM 70:30 from 70% ethanol extract from *H. courbaril* seeds

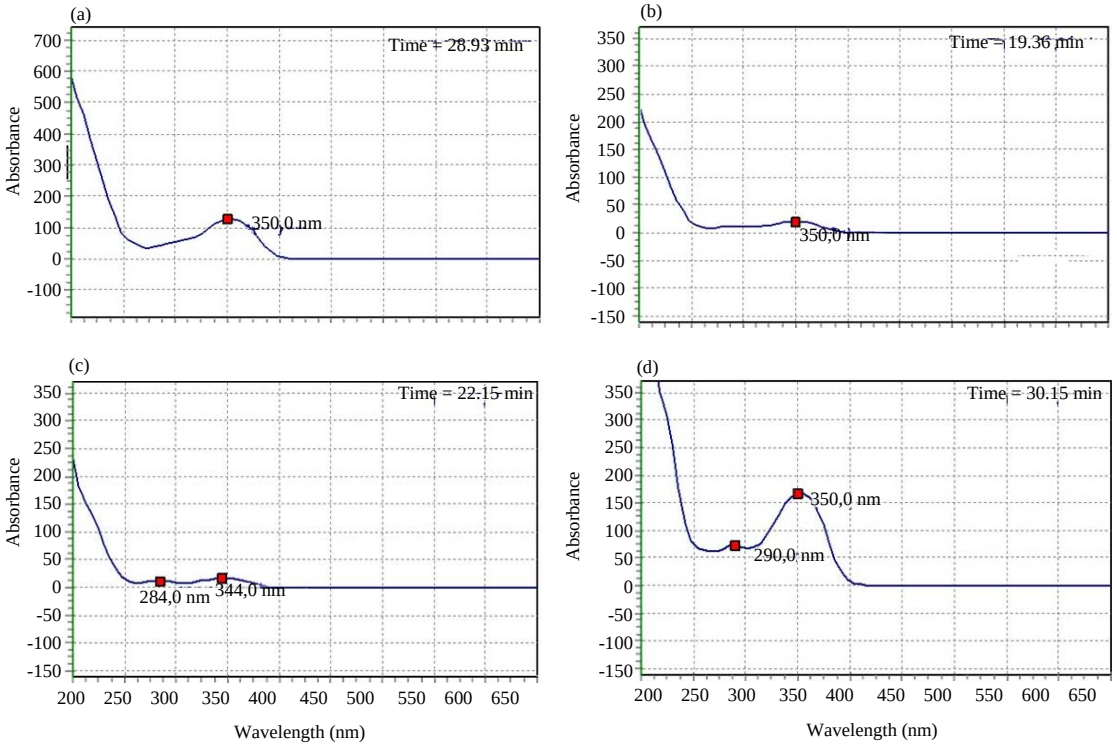


Fig. 3(a-d): Spectra in the UV-vis region obtained by HPLC-PAD of the fraction EAM 70:30 of 70% ethanol extract from *H. courbaril* seed

Table 6: DPPH Radical scavenging activity, ferric reducing antioxidant power (FRAP) and total phenolic content of the fractions of hydroethanolic extract from *H. courbaril* seeds

| | Antioxidant activity | | |
|---|---|---|---|
| Samples | Radical scavenging (%) | FRAP (μM TE $g^{-1}$ dry extract) | Total phenols (mg GAE $g^{-1}$ dry extract) |
| EAM 70:30 | 72.21±1.08[a] | 3029.97±09.78[a] | 491.56±02.62[a] |
| EAM 50:50 | 5.65±2.62[b] | 118.98±50.77[b] | 026.96±07.83[b] |
| EAM 30:70 | 7.24±0.76[b] | 122.67±40.74[b] | 017.53±02.26[b] |
| MT | 4.90±3.02[b] | 277.76±22.85[c] | 028.87±01.74[b] |
| Gallic acid (80 μg $mL^{-1}$) | 79.98±1.60[c] | | |

HX: Hexane fraction, DCM: Dichloromethane fraction, EA: Ethyl acetate fraction, EAM 70:30: Ethyl acetate:methanol (70:30 v/v) fraction, EAM 50:50: Ethyl acetate:methanol (50:50 v/v) fraction, EAM 30:70: Ethyl acetate:methanol (30:70 v/v) fraction, MT: Methanol fraction. Values are Mean±SD. Same subscript letters within the same column indicate no significant diferences among samples by Tukey test ($\alpha \leq 0.05$)

Previous studies have reported the presence of phenolic compounds from the genus *Hymenaea*. Da Cruz Silva *et al*.[51] evaluated the ethanol extract from the stem bark of *Hymenaea martiana* and identified characteristic bands of phenolic compounds, possibly derived from cinnamic acid, flavonolorflavanone. Orsi *et al*.[18] assessing the bark and fruit of *Hymenaea stigonocarpa* found that these portions are constituted mostly of phenolic compounds such as flavonoids, and condensed tannins.

## CONCLUSION

The results of this study indicate a high antioxidant activity in DPPH and FRAP tests by 70% ethanol extract of the *H. courbaril* seed, which thus may be considered a source of phenolic compounds. Among the fractions evaluated, EAM 70:30 showed the best results for DPPH, FRAP and total phenolic content; these results can be employed in a future investigation to isolate and characterize the active principles with biological activity.

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