RESEARCH ARTICLE

# Antioxidant Activity and Determination of Total Phenolics of Natural Products from the Brazilian Flora

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## Abstract

**Introduction:** In the scientific field, several studies have been developed to find new antioxidant substances of plant origin. Free radicals play an important role in the biochemical/physiological reactions of the human body. However, if there is excessive production during pathophysiological processes or due to adverse environmental factors and antioxidants are not available in the medium, profound tissue damage can occur.

**Methods:** This article aimed to identify potential natural products with antioxidant action and with a high content of total phenolics. Seventeen native natural products were evaluated, in the antioxidant tests, the DPPH method was used and, for total phenolics, the Folin-Ciocalteau method was used.

**Results and Discussion:** The extracts that showed higher phenolic contents were *Anadenanthera macrocarpa* and *Triplaris gardneriana*, with values of 2266.84 ± 9.28 µmol $g^{-1}$ and 2526.13 ± 476.90 µmol $g^{-1}$ for antioxidants, and 563.04 ± 4.17 mg gallic acid $g^{-1}$ and 414.82 ± 414.82 ± 3.57 mg gallic acid $g^{-1}$ for phenolics. Therefore, this study contributed to help future works aimed at the development of products from the species studied.

**Keywords:** Natural products; antioxidants; phenolics.

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## INTRODUCTION

Antioxidants are compounds that can reverse or inhibit the oxidation of other substances.[1] Several promising studies have been developed with the proposal to identify potential natural products with antioxidant properties, is widely used as a parameter for medicinal bioactive components.[2,3]

A widely used method to assess the antioxidant capacity of a product is DPPH (2,2-diphenyl-1-picryl-hydrazyl) by Brand-Williams *et al*.[4] Its application is to determine the antioxidant capacity of a compound to sequester free radicals, based on the capture of the DPPH radical by antioxidants, producing a decrease in absorbance at 515 nm.

Phenolic compounds are substances produced in the secondary metabolism of plants, with the purpose of defense in situations of stress, against ultraviolet radiation or aggression by pathogens.[5] In recent years, these compounds have increased interest due to their potential for possible health benefits and the prevention of free radical-induced diseases, due to their ability to scavenge free radicals, which are substances that cause oxidative stress when found in excess.[6-8] Studies show a relationship between phenolic compounds and the antioxidant activity of plants.[9,10] The antioxidant activity of these compounds is mainly due to their reducing properties and their chemical structure, characteristics that play an important role in the neutralization or scavenging of free radicals.[11] Thus, phenolic compounds may be responsible for the antioxidant activities of plant products, providing prevention against cellular damage caused by the imbalance between the levels of free radicals and antioxidants.[12] Plant antioxidants are very varied in nature, but phenolic compounds have been identified as responsible for greater antioxidant capacity, being represented by flavonoids and isoflavonoids, tannins, lignans, xanthones and others that can be found in a diversified way in the Brazilian flora, which encourages researchers to carry out several pharmacological and biological studies, mainly of secondary metabolites of plant species found in Brazil that may have antioxidant activity.[13]

Thus, the present work aimed to determine the antioxidant activity, as well as the phenol content of seventeen natural products of plant origin. The plant

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species and structures used to obtain the extracts were: *Anadenanthera Colubrina*, *Anadenanthera macrocarpa*, *Arrabidaea chica*, *Bauhinia forficata*, *Caryocar brasiliense Cambess*, *Copaifera langsdorffii*, *Croton heliotropiifolius*, *Croton blanchetianus*, *Ipomoea bahiensis*, *Hyptis suaveolens*, *Mauritia flexuosa*, *Myrciaria glazioviana*, *Piptadenia moniliformis Benth*, *Protium heptaphyllum*, *Senecio brasiliensis*, *Sterculia striata* and *Triplaris gardneriana*.

## MATERIAL AND METHODS

### Chemicals

DPPH (2,2-diphenyl-1-picryl-hydrazyl), Quercetin 95% and Gallic Acid 98% were purchased from Sigma-Aldrich (Germany), spectroscopic grade Methanol and Ethanol were purchased from Tedia (USA). Sodium carbonate was purchased from Vetec (Brazil). MiliQ ultrapure water.

### Plant Materials

The plant species collected in the state of Piauí (Brazil) and the structures used to obtain the extracts were: *A. Colubrina* (Angico branco - leaf), *A. macrocarpa* (Angico preto - bark), *A. chica* (Crajiru - leaf), *B. forficata* (Miroró - leaf), *C. brasiliense Cambess* (Pequi - seed), *C. langsdorffii* (Pau d'óleo - leaf), *C. heliotropiifolius* (Velame - leaf), *C. blanchetianus* (Marmeleiro - leaf), *I. bahiensis* (Jetirana - flower), *H. suaveolens* (Bamburral - leaf), *M. flexuosa* (Buriti - bole), *M. glazioviana* (Peludinha - leaf), *P. moniliformis Benth* (Angico de bezerro - flower), *P. heptaphyllum* (Amescla - leaf), *S. brasiliensis* (Maria mole - leaf), *S. striata* (Chichá - leaf, fruit and external part) e *T. gardneriana* (Pajeú - flower).

### Obtaining the Extracts

The dry materials (leaves, fruits or peels) were subjected to cold extraction with 95% ethanol, separately in a glass container. Four extraction processes were carried out with an interval of 8 days between them, to ensure maximum extraction of the chemical constituents. The solution was stirred daily during the extraction period. The extract was filtered through filter paper and the solvent was distilled at a temperature of 60ºC, under reduced pressure, in a rotary evaporator. After this solvent evaporation process, the crude ethanol extract was obtained.[14]

### Evaluation of Antioxidant Activity by the DPPH Method

The evaluation of the antioxidant capacity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method was performed following the methodology of Brand-Williams *et al*. with modifications by Alves *et al*., monitoring the consumption of the free radical DPPH. [15,22]

Following the proposed methodology, the analyzes had quercetin as a standard, and the calibration curve was obtained with the following concentrations: 0.01, 0.05, 0.1, 0.2 and 0.3 mM. All extract solutions were prepared in triplicate to obtain a concentration of 1 g $L^{-1}$ using methanol as solvent. A volume of 0.1 mL of the extract solution or solution containing the standard was used and added to 3.9 mL of the 0.06 mM DPPH solution. The analyzes were performed in a UV-vis spectrophotometer (Model Varian 1E), at a wavelength of 515 nm, with a reaction waiting time of 30 minutes in the absence of light.

The calibration curve was constructed from the average absorbance values of each DPPH solution versus the concentrations used, through linear regression analysis.

The inhibition percentage, that is, the percentage of antioxidant activity (%AA) corresponding to the amount of DPPH consumed, was calculated by the formula:

$$[(A_o - A_t)/A_o] \times 100 \qquad \text{Equation 1}$$

Where: $A_o$ = pure DPPH absorbance; $A_t$ = sample absorbance. The results were expressed in µmol of Quercetin per gram of extract.

### Analysis of Total Phenolics

For the analysis of total phenolics, the colorimetric method of Folin Ciocalteau was used, according to the methodology described by Bonoli *et al*.[16] An aliquot of 100 µL of the methanolic solutions of the 1000 mg.$L^{-1}$ extracts was transferred to test tubes of 10 mL and 500 µL of the Folin-Ciocalteau reagent, 6 mL of distilled water and left under stirring for 1 minute were added. Then, 2 mL of 15% $Na_2CO_3$ was added and stirred for 30 seconds. The volume was made up with distilled water. A blank was prepared in parallel. The absorbance of the samples was measured in a UV-vis spectrophotometer (Model Varian 1E), at a wavelength of 750 nm, after 30 min of reaction. Results were expressed as mg of GA (gallic acid) per gram of extract and analyzes were performed in triplicate.

For the calibration curve, gallic acid standards (10–100 mg.$L^{-1}$) were used.

The total phenolic content was determined by the linear regression equation from the calibration curve constructed with standard solutions of gallic acid.

### Statistical Analysis

All experiments were expressed as mean and standard deviation. Linear regression analysis was performed using GrahpPad Prism 5.0 software.

## RESULTS AND DISCUSSION

The equation obtained from the DPPH calibration curve was $y = 0.2052x + 0.6911$. Table 1 shows the total antioxidant and phenolic content of the tested extracts of the seventeen plant species, through the mean and standard deviation.

The antioxidant result is expressed as µmol of DPPH per gram of extract, and that of phenolics is expressed as mg of GA (gallic acid) per gram of extract.

In the analyzed extracts, the phenolic content ranged from 563.04 to >10 mg GA $g^{-1}$, being them related to *A.*

**Table 1:** Antioxidant Activity and total phenolics of the extracts

| *Extract* | *Antioxidant* | *Total phenolics* |
|---|---|---|
| | *µmol/g ± SD* | *mg GA/g ± SD* |
| *A. colubrina* | 267.67 ± 9.28 | 97.73 ± 4.17 |
| *A. macrocarpa* | 2266.84 ± 41.67 | 563.04 ± 8.33 |
| *A. chica* | 107.36 ± 3.82 | 5.53 ± 1.94 |
| *B. forficata* | 637.98 ± 41.15 | 112.43 ± 1.83 |
| *C. brasiliense* | 8.37 ± 0.12 | 40.89 ± 2.94 |
| *C. langsdorffii* | 1331.56 ± 97.23 | 65.29 ± 4.44 |
| *C. blanchetianus* | 51.20 ± 8.33 | < 10 |
| *C. heliotropiifolius* | 130.24 ± 0.33 | 84.76 ± 4.68 |
| *Hyptis suaveolens* | 166.89 ± 8.07 | 24.52 ± 2.02 |
| *Ipomoea bahiensis* | 76.12 ± 1.04 | 17.32 ± 0.44 |
| *Mauritia flexuosa* | 456.15 ± 18.52 | 55.95 ± 3.21 |
| *Myrciaria glazioviana* | 730.25 ± 33.34 | 44.93 ± 0.79 |
| *Piptadenia moniliformis Benth* | 432.07 ± 22.22 | 31.96 ± 0.67 |
| *Protium heptaphyllum* | 1766.68 ± 94.70 | 46.84 ± 7.86 |
| *S. brasiliensis* | 132.69 ±7.41 | 85.41 ± 4.88 |
| *S. striata* (outer part of the peel) | 577.89 ± 4.43 | 24.04 ± 0.20 |
| *S. striata* (leaf) | 130.61 ± 10.41 | 10.59 ± 1.07 |
| *S. striata* (fruit) | 230.58 ± 4.86 | 24.64 ± 0.32 |
| *T. gardneriana* | 2526.13 ± 476.90 | 414.82 ± 3.57 |

GA: ácido gálico; SD: Standard Deviation

*macrocarpa* (Angico Preto - peel) and *C. blanchetianus* (marmeleiro - leaf), respectively. It is known that the higher the value of gallic acid, the more phenolics there are in the sample. Rufino *et al*. classified the phenol content into three categories: low (<10 mg GA $g^{-1}$), medium (10–50 mg GA $g^{-1}$) and high (>50 mg GA $g^{-1}$) in plant material dry.[17] Thus, it was observed that, in general, the extracts presented medium and high levels of phenols.

For the results obtained on antioxidant activity, it is known that the higher the value of antioxidant activity, the greater the consumption of DPPH and the inhibition of free radicals. That is, the higher the concentration of the sample and the lower the absorbance, the greater the consumption of DPPH.[18] The results in general ranged from 8.37 to 2526.13 µmol.$g^{-1}$.

The extracts that showed the highest percentages of antioxidant activity were *A. macrocarpa* (Angico Preto - peel) and *T. gardneriana* (Pajeú -leaf), with values of 2266.84 and 2526.13 µmol $g^{-1}$, respectively. And those with lower levels were *C. brasiliense Cambess* (Pequi - seed) with 8.37 µmol $g^{-1}$. Phytochemical tests were carried out with the extract of *A. macrocarpa* (Angico Preto - peel), according to the methodology of Matos.[19]

With the tests, it was possible to qualitatively observe the presence of possible substances responsible for the antioxidant properties, they are: flabobenic tannins, chalcones, aurones, flavones, flavonols, flavanones and flavanonols and xanthones. The antioxidant characteristic of the sample can be attributed to these phenolic compounds.[13]

In general, antioxidant activity is associated with the phenolic content present in plants, due to their reducing capabilities.[20,21] A directly proportional relationship between antioxidant activity and total phenols content is expected, which happened in the extracts that showed greater activity, such as, *A. macrocarpa* (Angico Preto - peel) and *T. gardneriana* (Pajeú - leaf). However, some divergences were noticeable, where the extract of *P. heptaphyllum* (Amescla - leaf) showed good antioxidant activity, however it did not show a high content of phenols; *C. langsdorffii* (pau d'óleo - leaf) showed a good phenolic content, but low antioxidant activity; and the *Caryocar brasiliense Cambess* (Pequi - seed) showed moderate phenolic content, but low antioxidant activity. These facts can be attributed to some interferences, resulting in false positive tests, which is the influence of some factors on their results, such as: the absence of tannins, non-significant presence of flavonoids and presence of proteins.[20,22] Another factor is that phenolics may not always be a good indicator of the antioxidant activities of crude extracts, as they also depend on specific chemicals.[8,23]

Studies support the results obtained in this work. According to Palsikowski, *B. forficata* can be considered a good source of phenolic compounds and flavonoids with antioxidant activity.[24] Almeida and Macêdo point to *T. gardneriana* as having a good antioxidant potential as a species rich in phenolic constituents, mainly flavonoids.[6,7]

## CONCLUSION

This research could add knowledge about the bioactivity of seventeen species of Brazilian plants, revealing their antioxidant potential and phenolic content. It is worth mentioning that studies such as this one are of great relevance since in the literature it is not common to find studies that cover a screening of several species such as those listed here, as well as their respective antioxidant and phenolic characteristics. Therefore, this study provides important information to help future works aimed at the development of products from the species studied.

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