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Myroxylum peruiferum: Antioxidant activity and phenolic content

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ABSTRACT

This study aims to evaluate the antioxidant and phenolic content of *Myroxylon peruiferum leaves* aqueous extract (MPAE) and *M. peruiferum* leaves methanolic extract (MPME). The antioxidant activity of these extracts was tested by reducing power assay, hydrogen peroxide and DPPH radical scavenging. Total phenolic content was measured by the Folin-Ciocalteu method. The phenolic content was 237.2 ± 3.12 mg/g GAE for MPME and 228.0 ± 2.09 mg/g GAE for MPAE. These extracts showed a high ability to inhibit tested reactive species. MPME showed the lowest IC_{50} values. The IC_{50} values found were 64.79 µg/mL and 56.60 µg/mL in the DPPH assay; 183.48 µg/mL and 158.96 µg/mL for reducing power; 121.44 µg/mL and 97.01 µg/mL for hydrogen peroxide. This work reports for the first time the antioxidant activity of *M. peruiferum.* The antioxidant compounds of this plant are important for food industry, cosmetics and pharmaceutical preparations.

KEYWORDS: Caatinga, *Myroxylon peruiferum*, free radicals

1. INTRODUCTION

Myroxylon peruiferum L., popularly known as cabreúva, is found in most of the Brazilian territory, and it is widespread in the Caatinga [1, 2]. Its wood is used for construction, furniture and for other purposes [3]. The trunk provides, as a result of injury, tolu balsam, which is used in perfumery and as a herbal anti-inflammatory agent for diseases of the respiratory, reproductive and urinary systems [4]. *M. peruiferum* has, as part of its phytochemical composition, volatile oils, esters, alcohols, triterpenes, alkaloids, phenols, proteins and glycosides [5].

Phenolic compounds are primary antioxidants that donate electrons to free radicals, or react with these radicals to form a lipid-antioxidant complex. Whereas, secondary antioxidants retard the reaction of auto-oxidation by different mechanisms: sequestration of oxygen, hydrogen peroxide decomposition, absorption of ultraviolet radiation, etc. [6].

Free radicals are reactive oxygen species often generated as byproducts of oxidative biochemical reactions or exogenous factors [7]. The irregular use of synthetic antioxidants in high doses can cause damage to the body. So there is a need to identify alternative natural sources [8]. Diverse works have demonstrated the antioxidant potential of plant material [9, 10]. This study aims to evaluate the antioxidant and phenolic content of *Myroxylon peruiferum leaves* aqueous extract (MPAE) and *M. peruiferum* leaves methanolic extract (MPME).

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2. MATERIALS AND METHODS

2.1. Plant material

M. peruiferum leaves were collected from PARNA do Catimbau, Pernambuco, Brazil, during the rainy season (Fig. 1). Botanical identification was made at the Herbarium of the Instituto de Pesquisa Agronômica de Pernambuco (IPA-PE), Brazil, and a voucher specimen (IPA 84.113) was deposited in the herbarium.

2.2. Preparation of *M. peruiferum* extracts

M. peruiferum leaves were dried at room temperature for 7 days, finely powdered and used for extraction. The powder (1 g) was mixed with 10 mL of methanol (MPME) or 10 mL of water (MPAE) by agitation at 3000 rpm for 15 minutes and then the extract was filtered through a Whatman (Nº. 1) filter paper. The supernatants collected were mixed in a round bottom flask and concentrated at 45 °C. The residue was kept at -20°C for future use.

2.3. Determination of total phenol content

The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu procedure [11]. Samples (200 µL) were introduced into test tubes and 1.0 mL of Folin-Ciocalteu's reagent (1:1 v/v) and 2.5 mL of sodium carbonate (20%) were added. The mixture was incubated for 30 min and allowed to stand for 30 min (GeneQuant 1300, GE Healthcare).

The amount of total phenol was calculated as mg Gallic Acid Equivalents (GAE)/g of dry mass from the calibration curve of gallic acid standard solution. For the gallic acid, the curve absorbance versus concentration is described by the equation $y = 1.5221x + 0.0081$ ($r^2 = 0.9712$).

2.4. DPPH radical scavenging activity

The free radical-scavenging activity of the extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich). A solution of DPPH in methanol was prepared, showing absorbance at 517 nm between 0.6 and 0.7. To 250 µL of this solution was added, on a microplate, 40 µL of extract solution in the appropriate solvent at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH radical scavenging free radical percentage (% SFR) was calculated using the following formula:

$$\% \text{ SFR} = \frac{(A_c - A_s) \times 100}{A_c}$$

where, Ac is the Control absorbance (Ascorbic acid absorbance) and As is the Sample absorbance [12].

2.5. Reducing power assay

The reducing power of different fractions was determined by Oyaizu's method [13]. Different

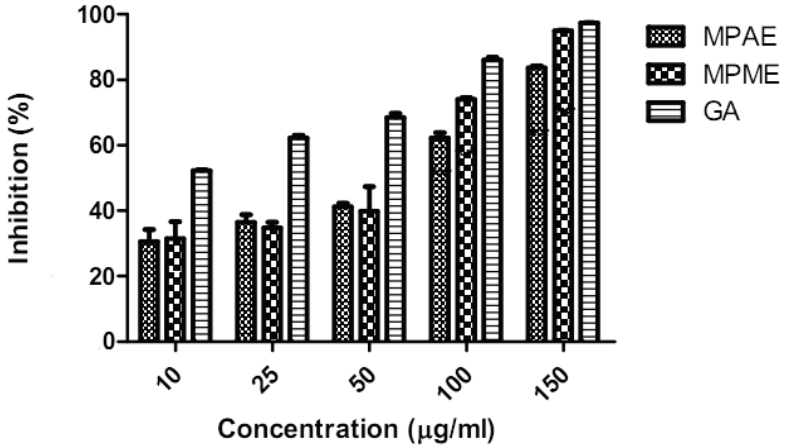

Fig. 1. DPPH radical scavenging of extracts, gallic acid was used as the reference. Absorbance of the reaction was measured at 230 nm. Values are means ± S.D. (n = 3).

extract concentrations (1 mL) were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. A volume of 2.5 mL of 10% trichloroacetic acid was then added and centrifuged at 3000 rpm for 10 min. An aliquot of 2.5 mL of supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%) and the absorbance was measured spectrophotometrically at 700 nm. An increase in absorbance of the reaction mixture was interpreted as an increase in reducing activity of the extract and the results were compared with gallic acid (positive control). The percentage reduction of the sample as compared to the standard (gallic acid) was calculated using the formula:

Reducing Power (%): [1 - (1 - A_S/A_C)] x 100

where, A_C = absorbance of the standard at maximum concentration tested and A_S = absorbance of the sample.

2.6. Hydrogen peroxide radical scavenging assay

Different concentrations of each extract were dissolved in 3.4 mL of phosphate buffer (pH 7.4; 0.1 M) and mixed with 0.6 mL of hydrogen peroxide (43 mM). The absorbance value (at 230 nm) of the reaction mixture was recorded after 10 min. For each concentration, a separate blank sample was used for background subtraction [14]. The scavenging activity was measured by the following formula:

$$\% \text{ scavenged } [H_2O_2] = \frac{(A_c - A_s) \times 100}{A_c}$$

where A_c is the absorbance of the control (blank, phosphate buffer without extract) and A_s is the absorbance in the presence of the extract.

2.7. Statistical analysis

Each experiment was performed at least three times and results are presented as the mean ± SD. Statistical analysis was performed using the Student's t-test. Differences were considered significant at $p < 0.05$. The concentration needed for 50% inhibition (IC_{50}) was estimated graphically by linear regression analysis.

3. RESULTS AND DISCUSSION

3.1. Determination of total phenolic content

The phenolic content of extracts from *M. peruiferum* leaves was determined by Folin-Ciocalteu's method. Both extracts showed a high phenolic content and no statistical difference was found between them. Plant phenols have been shown to inhibit the formation of various free radical agents, such as nitric oxide, superoxide anion, hydroxyl radical, peroxide hydrogen, etc. [15, 16].

3.2. DPPH radical scavenging activity

The pathogenic potential of free radical is related to damage of biological macromolecules, which arises from an imbalance between radical-generating and radical-scavenging systems [17]. DPPH assay is widely used to determine the radical scavenging ability of various samples [18, 19]. The DPPH radical scavenging effects of *M. peruiferum* extracts are shown in Fig. 1. Both extracts had dose-dependent effects. Additionally, the DPPH radical scavenging activity was very similar for both extracts; statistical differences ($p < 0.05$) were observed only at 100 µg/mL and 150 µg/mL. The IC_{50} values were found to be 64.79 µg/mL, 56.60 µg/mL and 21.2 µg/mL for MPAE, MPME and Gallic acid (Table 1).

Table 1. Phenolic contents and free radical scavenging activity of *M. peruiferum* extracts.

Extract	Total phenol content[1]	DPPH IC_{50}[2]	Reducing power IC_{50}[2]	H_2O_2 IC_{50}[2]
MPME	237.2 ± 3.12	56.60	158.96	97.01
MPAE	228.0 ± 2.09	64.79	183.48	121.44
Gallic acid	-	21.2	74.97	16.43

[1]Total phenol content expressed as mg GAE g^{-1}.
[2]IC_{50} expressed as µg/mL.

3.3. Reducing power assay

The *M. peruiferum* leaf extracts exhibited a strong reducing activity (Fig. 2). MPME showed the best results ($p < 0.05$) and the lowest IC_{50} values (158.96 µg/mL), while MPAE had an IC_{50} value of 183.48 µg/mL (GA 74.97 µg/mL). A linear increase in reducing power of extracts was observed (R^2 values of 0.98 for both). The reducing capacity of a compound is linked to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive species [20, 21].

3.4. Hydrogen peroxide quenching assay

The hydrogen peroxide quenching activity of leaf extracts are shown in Fig. 3. Those extracts had high scavenging capacities in this assay and no statistical differences were found between them. The IC_{50} values were 97.01 µg/mL and 121.44 µg/mL for MPME and MPAE, respectively. Although hydrogen peroxide may not be very reactive, it may induce the formation of other, more powerful free radicals, such as hydroxyl radicals, which would result in great damage to cells [22, 23]. Additionally, hydrogen peroxide can cause the oxidation of essential thiol groups (-SH) inactivating a few enzymes directly [24]. Earlier authors have

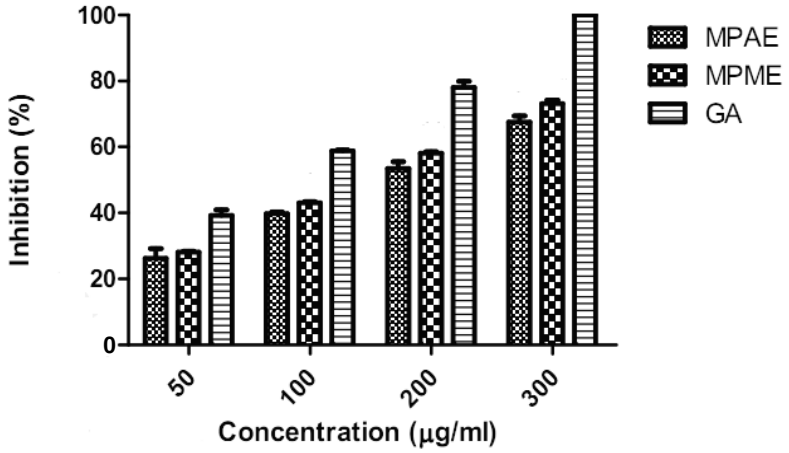

Fig. 2. Reducing power activity of extracts, gallic acid was used as the reference. Absorbance of the reaction was measured at 230 nm. Values are means ± S.D. (n = 3).

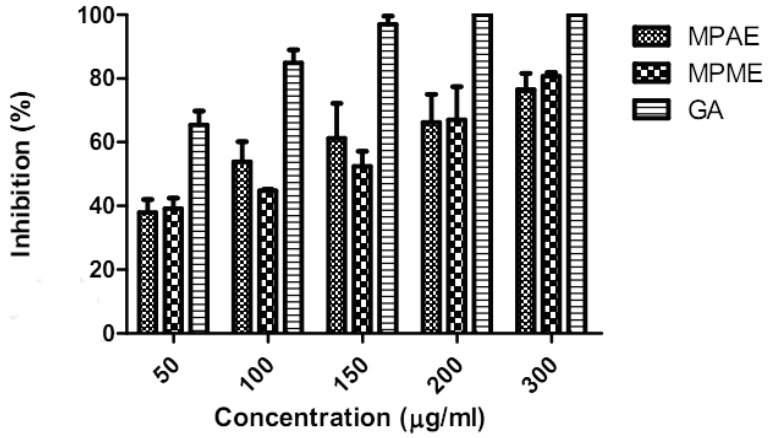

Fig. 3. Hydrogen peroxide quenching ability of extracts, gallic acid was used as the reference. Absorbance of the reaction was measured at 230 nm. Values are means ± S.D. (n = 3).

shown the capacities of polyphenol compounds to inhibit the cytotoxicity induced by hydrogen peroxide [25, 26].

4. CONCLUSION

The results obtained in this study clearly demonstrated for the first time that leaves of *M. peruiferum* exhibit strong antioxidant activity in the *in vitro* assays performed. The bioactive compounds of this plant have great potential for use as natural preservatives for the food and cosmetic industries. In this context, further research is warranted for isolation and identification of individual compounds, and understanding of *in vivo* antioxidant activity and the different antioxidant mechanisms involved.

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