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Antioxidant potential and total phenolic content of leaf extracts from *Parkinsonia aculeata* L. cultivated in Brazilian Caatinga biome

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ABSTRACT

Parkinsonia aculeata L. is a species native to the Brazilian Caatinga and is used in folk medicine as an antihyperglycemic, antimalarial and amebicidal agent. This work evaluated the antioxidant and polyphenol content of *Parkinsonia aculeata* leaf extracts cultivated in the Brazilian Caatinga. *P. aculeata* aqueous and methanolic leaf extracts were investigated for their antioxidant capacities by four *in vitro* methods: DPPH radical scavenging, hydrogen peroxide scavenging, phosphomolybdenum and reducing power assays. The total polyphenol content was measured by the Folin-Ciocalteu method. Both extracts showed high activity, while the methanolic extract had the best results in all antioxidant systems ($p < 0.001$). These results can be explained by higher polyphenol content found in this extract. Our results showed that *P. aculeata* extracts displayed potent antioxidant and radical scavenging properties, supporting the ethnomedical use of this plant for treatment of diseases.

KEYWORDS: antioxidant, Caatinga biome, *Parkinsonia aculeata* L., phenolic content

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1. INTRODUCTION

The Caatinga is a biome whose occurrence is restricted to Brazil, yet despite its socioeconomic importance and being subjected for many decades to intense and predatory exploitation of natural resources, it remains poorly protected and studied. Among the biomes, the Amazon has the largest protected area, with nearly 17% of the total within Federal Protected Areas, 7.9% granted full protection. In contrast, federally protected areas granted full protection account for around 1% of the Caatinga, Pampas, Atlantic Forest and Pantanal biomes, which is well below the world average of 5% [1].

Among the various species of the Caatinga, many plants are popularly exploited for medicinal use. In such cases, their leaves, bark and roots are sold on the sidewalks and streets of major cities, markets and fairs. Foremost among these are *aroeira* and *angico* (astringent), *araticum* and *catingueira* (antidiarrhoeal), *pau-ferro* (asthma and antiseptic), *velame* and *marmeleiro* (antipyretic), *sabiá* (expectorant), *juazeiro* (stomachic), *jericó* (diuretic), among others [2-4].

Parkinsonia aculeata L., commonly known as *cina-cina*, *espinho de Jerusalém*, *acácia de aguijote*, *rosa da Turquia* and *turco*, is a representative species with economic value found in riparian forests in the Caatinga in the state of

Pernambuco, Brazil. The *turco* is a small tree belonging to the family Leguminosae - Caesalpinoideae, used in papermaking, timber [5], in energy [6] and the production of gums such as galactomannans [7]. It is used in folk medicine for the treatment of hyperglycemia [8], as an antimalarial [9], antimicrobial [10], and amebicidal agent [11].

However, there is a large potential for Caatinga species to serve many functions, though this is often unknown or unexplored due to lack of resources and cultural heritage [12]. Since the Caatinga biome is one that has gone through an extensive process of change and environmental deterioration caused by irrational use of natural resources over the years, it is important to understand the biological properties of its members, aimed at their preservation and sustainable exploitation. This study aimed to investigate the phenolic content and to evaluate the antioxidant and antibacterial activity of aqueous and methanol leaf extracts from *P. aculeata* grown in Parque Nacional do Catimbau (PARNA do Catimbau), Pernambuco, Brazil, a preservation area of the Caatinga biome.

2. MATERIALS AND METHODS

2.1. Plant material

P. aculeata leaves were collected from PARNA do Catimbau, Pernambuco, Brazil, during the rainy season (Figure 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 *P. aculeata* extracts

P. aculeata 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 (PAME) or 10 mL of water (PAWE) by agitation at 3000 rpm for 15 minutes, 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 [13]. 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

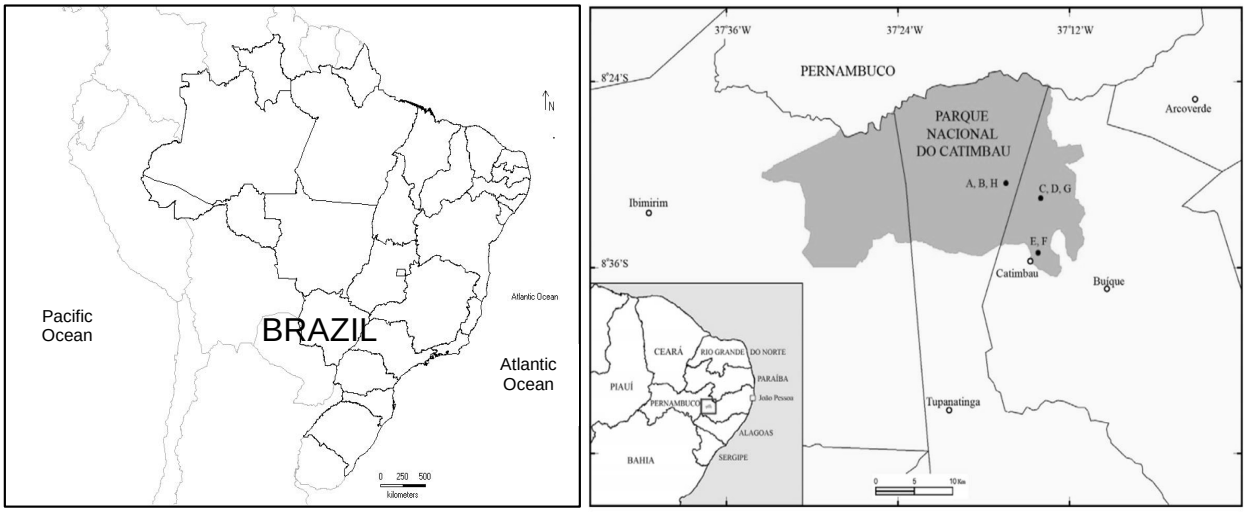

Figure 1. PARNA do Catimbau (Buíque, Tupanatinga and Ibimirim Municipalities) localization, Pernambuco State, Brazil.

versus concentration is described by the equation $y = 1.5221x + 0.0081$ ($r^2 = 0.9712$).

2.4. Total antioxidant capacity by phosphomolybdenum assay (P–Mo)

The total antioxidant capacity (TAC) was evaluated by the method of Prieto *et al*. [14]. An aliquot of 0.1 mL of sample solution (100 µg/mL) was combined with 1 mL of reagent solution (600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. Afterward, the absorbance was measured at 695 nm against a blank (1 mL of reagent and 0.1 mL of the solvent). Total antioxidant activity was expressed in relation to ascorbic acid and calculated by the following formula:

$$\%\ \text{TAC} = \left(\frac{A_s - A_c}{A_{aa} - A_c} \right) \times 100$$

where A_c is the absorbance of the control (blank, without extract), A_s is the absorbance in the presence of the extract and A_{aa} is absorbance of ascorbic acid.

2.5. 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:

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

where, A_C is the Control absorbance (Ascorbic acid absorbance) and A_S is the Sample absorbance [15].

2.6. Reducing power assay

The reducing power of different fractions was determined by Oyaizu's method [16]. Different 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 by using the formula:

Reducing power (%): $[1 - (1 - A_S/A_C)] \times 100$

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

2.7. Hydrogen peroxide 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 [17]. The scavenging activity was measured by the following formula:

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

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.

2.8. 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.

Table 1. Phenolic contents, antioxidant and free radical scavenging activity of *P. aculeata* extracts.

Extract	Total phenol content[1]	% TAC	DPPH IC_{50}[2]	Reducing power IC_{50}[2]	H_2O_2 IC_{50}[2]
PAME	288 ± 5.67	46.94 ± 3.73	35.29 ± 4.95	162.66 ± 0.49	32.11 ± 0.85
PAWE	196 ± 7.2	26.52 ± 2.57	49.38 ± 5.12	200.74 ± 0.68	46.13 ± 1.12
Gallic acid	-	-	10.83 ± 3.12	74.97 ± 1.09	16.43 ± 1.22

[1]Total phenol content expressed as mg GAE g^{-1}.
[2]IC50 expressed as µg/ml.

3. RESULTS

3.1. Determination of total phenol content

The total polyphenol contents of *P. aculeata* are shown in Table 1. PAME had the biggest phenol content, 288 ± 5.67 mg/g GAE, whereas PAWE was found to have 196 ± 7.20 mg/g GAE. The total polyphenol contents showed significant differences ($p < 0.05$) between them.

3.2. Total antioxidant capacity by phosphomolybdenum assay (P–Mo)

In the phosphomolybdenum assay, the total antioxidant capacity of the extracts was related to ascorbic acid antioxidant ability. A higher TAC value corresponded to a higher antioxidant activity. Both extracts exhibited significant activities (Table 1). PAMA had the higher total antioxidant activity (46.94% ± 3.73; $p < 0.001$) in relation to ascorbic acid activity ($p < 0.05$), whereas PAWE had 26.52% (± 2.57) (Table 1).

3.3. DPPH radical scavenging activity

The DPPH radical scavenging effects of *P. aculeata* extracts are shown in Figure 2. Both extracts had significant activities when compared with gallic acid and a dose-response relationship was found in the DPPH radical scavenging activity. The activity increased as the concentration increased for each sample. The IC_{50} values were found to be 35.29 ± 4.95, 49.38 ± 5.12, 10.83 ± 3.12 µg/mL for PAME, PAWE and gallic acid, respectively ($p < 0.05$) (Table 1).

3.4. Reducing power assay

Reducing power properties of *P. aculeata* leaf extracts are exhibited in Figure 3. In this assay, a linear increase was observed in the reducing power of extracts with index of determination (R^2) values of 0.993, 0.983 and 0.977 for PAME, PAWE and GA, respectively. The half inhibitory concentration (IC_{50}) values of the extracts showed significant differences ($p < 0.05$) and were

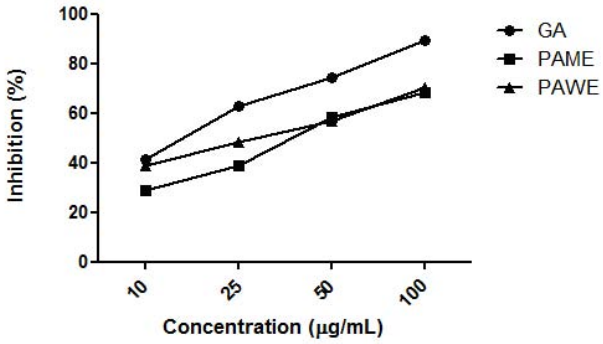

Figure 2. DPPH radical scavenging of extracts, gallic acid was used as the reference. Absorbance of the reaction was measured at 230 nm. Values are means ± S.D. (n = 3).

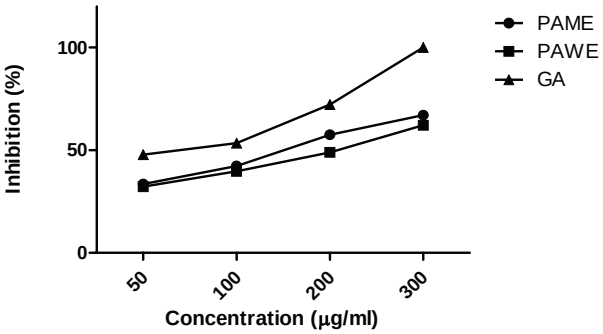

Figure 3. Reducing power activity of extracts, gallic acid was used as the reference. Absorbance of the reaction was measured at 230 nm. Values are means ± S.D. (n = 3).

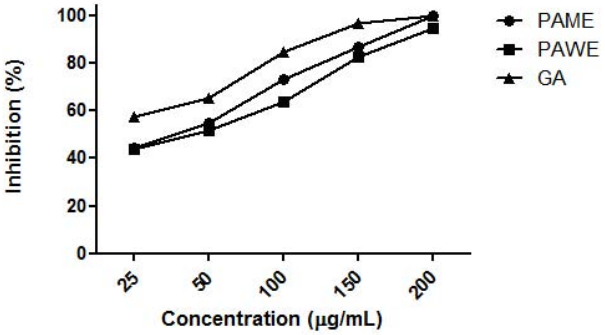

Figure 4. Hydrogen peroxide quenching ability of extracts, gallic acid was used as the reference. Absorbance of the reaction was measured at 230 nm. Values are means ± S.D. (n = 3).

162.66 ± 0.49, 200.74 ± 0.68, 74.97 ± 1.09, for PAME, PAWE and GA, respectively (Table 1).

3.5. Hydrogen peroxide quenching assay

Figure 4 shows the hydrogen peroxide quenching activity of *P. aculeata* extracts. These extracts had high scavenging capacities in this assay. PAME had the lowest IC_{50} at 32.11 ± 0.85 µg/mL, whereas PAWE had 46.13 ± 1.12 µg/mL and GA had 16.43 ± 1.22 µg/mL) (Table 1).

4. DISCUSSION

Recently, several studies have been performed to assess the antioxidant capacity of crude plant extracts [18, 19]. In this work, the antioxidant activity and total phenolic content of the methanol and aqueous extracts of *Parkinsonia aculeata* leaves collected at PARNA Catinbau, a preservation area of Brazilian Caatinga, were evaluated by assessing their effects on phosphomolibdenium assay, reducing power, DPPH radical scavenging activity and hydrogen peroxide quenching ability.

These extracts exhibited a notable phenolic content. Phenolic compounds constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers. They are the most important classes of phytochemicals in plants and there are more than 8000 phenolic phytochemicals [20]. The total phenol content of these compounds was measured using Folin-Ciocalteu reagent, which reduces yellow hetero-polyphosphomolybdatetungstate anions producing a blue color. The intensity of the blue-colored complex is related to the presence of hydrogen-donating groups in the phenolic compounds [21]. Phenolic compounds have been reported to be highly effective free radical scavengers and antioxidants due to their redox properties [22].

The total antioxidant activity of these plant extracts was tested by the phosphomolibdenium assay. This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH which is then determined spectrophotometrically [14]. Ascorbic acid was used for the purpose of comparison and our extracts had significant activities in relation to that of ascorbic acid. As cited above, the antioxidant activity of a plant extract is related to many beneficial effects of medicinal plants [23].

Reducing power is one mechanism for action of antioxidants and may serve as a significant indicator of potential antioxidant activity for antioxidants [24]. For the measurement of the reducing ability, the transformation of Fe^{3+}–Fe^{2+} was investigated in the presence of extract samples using the method followed by Oyaizu [16]. The electron and hydrogen atom donating capacity, reflecting the reducing power of bioactive compounds, is associated with antioxidant activity [25]. In this assay, the extracts showed strong activities in a concentration-dependent manner, indicating the chemicals in this plant performed as good electron donors and therefore should be able to terminate radical chain reactions by converting free radicals and reactive oxygen species to more stable products. The reducing properties are generally associated with the presence of reductones [26], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Our data on the reducing power of the tested extracts suggest that it is likely to contribute significantly towards the observed antioxidant effect.

The DPPH radical is considered to be a model for a lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid autoxidation. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [27]. DPPH radical scavenging methods are common spectrophotometric procedures for determining antioxidant capacities

of components [28]. In this assay, the good antioxidant activity on DPPH radicals of *P. aculeata* extracts may be attributed to a direct role in trapping free radicals by donating hydrogen atoms or electrons.

Furthermore, the extracts were tested for their capacity to inhibit hydrogen peroxide. They showed a very strong ability, with low IC_{50} values considering that they are crude plant extracts. Hydrogen peroxide itself is not very reactive, but it may induce hydroxyl radicals, which would result in great damage to cells. Hydrogen peroxide can inactivate a few enzymes directly by oxidation of essential thiol (-SH) [29]. Thus, it is important to discover some chemicals with good scavenging capacity on these reactive oxygen species. Earlier authors have shown the capacities of polyphenol compounds to inhibit the cytotoxicity induced by hydrogen peroxide [30].

In conclusion, our results indicated that *P. aculeata* leaf extracts possess high antioxidant and free radical scavenging activities and that polyphenol content is related with their activities. These results suggest very important applications of this species for the pharmaceutical and food industries. However, further investigation of individual phenolic compounds and *in vivo* antioxidant activity and mechanisms is warranted.

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