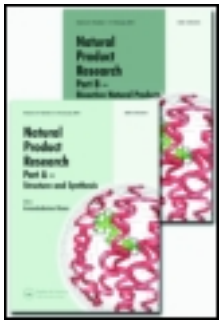

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Redox properties of Abarema cochliacarpos (Gomes) Barneby & Grime (Fabaceae) stem bark ethanol extract and fractions

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SHORT COMMUNICATION

Redox properties of *Abarema cochliacarpos* (Gomes) Barneby & Grime (Fabaceae) stem bark ethanol extract and fractions

A.S. Dias[a], A.C.B. Lima[a], A.L.M.L. Santos[a], T.K. Rabelo[a], M.R. Serafini[a], C.R. Andrade[a], X.A. Fernandes[b], J.C.F. Moreira[c], D.P. Gelain[c], C.S. Estevam[a] and B.S. Araujo[a]*

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The redox properties of the hydroethanol extract (EE) and its ethyl acetate (EAF) and hydromethanol (HMF) fractions obtained from *Abarema cochliacarpos* (Gomes) Barneby & Grimes stem bark were evaluated. EAF had the highest total phenol content ($848.62 \pm 78.18\,mg\,g^{-1}$), while EE showed the highest content of catechin ($71.2\,\mu g\,g^{-1}$). EE, EAF and HMF exhibited the highest levels of antioxidant activity at 100 and $1000\,\mu g\,mL^{-1}$ when the non-enzymatic antioxidant potential was evaluated by the total reactive antioxidant potential, total antioxidant reactivity and nitric oxide scavenging assays. In addition, EAF and HMF showed SOD-like activity. The results for EE, EAF and HMF in this study showed that *A. cochliacarpos* (Gomes) Barneby & Grimes stem bark have redox properties and may be able to help the endogenous enzymatic and non-enzymatic systems to keep the redox balance.

Keywords: *Abarema cochliacarpos*; redox properties; oxidative damage; superoxide dismutase; NO scavenger; TRAP/TAR

1. Introduction

Oxidative damage is related to the continuous production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the organism. These substances can attack membrane lipids by reducing double bonds in a process called peroxidation, which can lead to membrane destabilisation and damage to the cell metabolism. This can establish the conditions needed for diseases such as diabetes, cardiovascular diseases and cancer, as well as pain and inflammation, to occur if they are not effectively scavenged by cell neutralising mechanisms. Hence, the search for new sources of antioxidants, mainly of natural origin such as plants, has increased in the last few years (Gülçin, Elmastas, & Aboul-Enein, 2011; Nikki, 2010).

Abarema cochliacarpos (Gomes) Barneby & Grimes, Fabaceae, is popularly known as 'barbatimão' and frequently found in the Atlantic Forest and the Caatinga biomes in the northeast region of Brazil. Decoctions of its stem bark are used by communities in this

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region to wash external ulcers, while the decoction and a tincture produced by placing the bark in white wine or in an alcoholic beverage called 'cachaça' are drunk in order to treat inflammation and gastric ulcers (Silva, Antoniolli, Batista, & Mota, 2006; Silva et al., 2010). It is known that ROS and RNS are secondary products during the generation of inflammatory mediators and plants with anti-inflammatory properties are frequently found to be effective against oxidative damage to tissues by reducing the effect of these reactive species (Basu & Hazra, 2006). Thus, the aim of this study is to evaluate the redox protective properties of the hydroethanol extract and fractions from the stem bark of *A. cochliacarpos* (Gomes) Barneby & Grimes.

2. Results and discussion

Abarema cochliacarpos (Gomes) Barneby & Grimes stem bark ethanol extract (EE) was fractioned to give hexane (HF), chloroform (CF), ethyl acetate (EAF) and hydromethanol (HMF) fractions. EAF showed the highest total phenol content (848.6 ± 78.2 mg EGA g^{-1}), which was significantly different ($p < 0.001$) from that of EE (253.2 ± 25.61 mg EGA g^{-1}), CF (120.5 ± 65.3 mg EGA g^{-1}), HF (38.5 ± 9.38 mg EGA g^{-1}) and HMF (344.3 ± 26.5 mg EGA g^{-1}). Due to the high total phenol content, EE, EAF and HMF were further evaluated for their secondary metabolites classes by precipitation and colorimetric methods. Several classes of secondary metabolites were found in EE (phenolics such as aurones, catechins, chalcones, flavanols, flavones, flavonols, leucoanthocyanidins, tannins and xanthones, besides saponins and steroids), EAF (EE less leucoanthocyanidins and tannins) and HMF (EE less leucoanthocyanidins and steroids plus triperpenoids). As of general, secondary metabolites in EE were fractionated in FAE and FHM. Therefore, interactions between these metabolites were decreased, which in turn reduced the competition and facilitated the reaction with the Folin–Ciocalteu (FC) reagent as stated by Ainsworth and Gillespie (2007). This allowed the detection of a higher amount of phenolics in the fractions than previously detected for the EE.

HPLC analysis revealed the presence of catechin (RT = 7.89 min) with EE showing the highest catechin content ($71.2\,\mu g\,mg^{-1}$), followed by HMF ($59.0\,\mu g\,mg^{-1}$) and EAF ($46.9\,\mu g\,mg^{-1}$). These results showed that phenolic compounds of several classes were present in EE and its polar fractions (EAF and HMF) as have been previously found by Silva et al. (2009). High total phenol content has been previously determined in another polar fraction (butanol) of this species (Silva et al., 2010). These findings may be similar to what can be found in the plant decoctions, hence the polar nature of water. In addition, the study of Silva et al. (2010) showed the main components of the plant butanol fraction were catechins, which were detected in the HPLC analysis of EE, EAF and HMF. Catechins are abundant in the stem bark of Fabaceae species and are well known for their activity against ROS and RNS, contributing to the antioxidant activity of green tea *in natura* (Santos et al., 2002; Sutherland, Rahman, & Appleton, 2006). Therefore, it can be suggested that catechins in the extract and fractions may contribute to their antioxidant activity, which was later determined.

Chemiluminescence is driven by the production of luminol-derived radicals generated from its reaction with AAPH peroxyl radical and any antioxidant molecule should be able to trap these luminol- and/or AAPH-derived radicals with immediate and lasting effects. These can be measured by the total antioxidant reactivity (TAR) and total reactive antioxidant potential (TRAP) assays, respectively (Melo et al., 2011). EE, EAF and HMF showed immediate effect against AAPH-induced peroxyl radicals when they were used in the non-enzymatic TAR antioxidant assay. Reductions of 39–60, 55–62 and 30–42 folds

between 10 and 1000 μg mL^{-1}, respectively, were observed in light intensity in absence of sample (I_0)/light intensity after sample addition (I) ratios in this assay (Figure 1A). In addition, the activity against peroxyl radicals was still found in the reaction mixture 60 min after the initial addition of EE, EAF and HMF using the TRAP assay. In this case, over 90% of the chemiluminescence was still being reduced by the extract and fractions with no significant differences ($p > 0.05$) at 10, 100 and 1000 μg mL^{-1} (Figure 1B). This finding suggests that EE, EAF and HMF have antioxidant reactivity over an extended period of time and may be able to regulate the steady-state concentration of oxidative species. Nitric oxide (NO) plays an important role as a mediator in inflammatory processes and is the main RNS in the organism (Cury, Picolo, Gutierrez, & Ferreira, 2011). EE, EAF and HMF showed RNS scavenger potential (Figure 1C) by significantly reducing ($p < 0.05$), the NO formation at 100 (29.7%, 34.3% and 36.7%, respectively) and 1000 μg mL^{-1} (52.7%, 18.4% and 30.5%, respectively).

Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) are part of the endogenous system that controls the physiological redox balance (Halliwell & Gutteridge, 1998). Results showed that EE did not have SOD-like activity, while EAF and HMF showed significant ($p < 0.05$) enzyme-like effects. They prevented the formation of superoxide (Figure 1D) at 100 μg mL^{-1} (28.7% and 33.1%, respectively) and 1000 μg mL^{-1} (39.3% and 45.1%, respectively). In addition, no significant CAT-like activity was observed for EE, EAF and HMF. The capacity of EAF and HMF to scavenge superoxide radicals may contribute not only to the reduction of superoxide anion formation in the organism, but may also help to minimise peroxyl radical production from superoxide dismutation.

In conclusion, the ethanol crude extract from *A. cochliacarpos* (Gomes) Barneby & Grimes stem bark and its ethyl acetate and hydromethanol fractions showed *in vitro* protective effects against the oxidative damage caused by ROS and RNS in concentrations ranging from 10 to 1000 μg mL^{-1}. The results suggest the plant may be able to help the endogenous enzymatic and non-enzymatic systems to keep the redox balance.

3. Experimental

3.1. *Plant material, extract preparation and partitioning*

Abarema cochliacarpos (Gomes) Barneby & Grimes stem barks were collected in Caípe Velho, São Cristóvão, Sergipe, Brazil. Herbarium voucher specimens were deposited at the Department of Biology of the Federal University of Sergipe under the registration number 14639. Plant material (2 kg) was dried (37°C), ground into a powder and extracted with ethanol (90%) at room temperature over 5 days. After filtration, the solvent was concentrated by evaporation at 50°C to give EE (400 g, 20% yield), which was re-suspended in methanol : water (2 : 3). This resuspension was then extracted to give 7.2 g (1.80% yield) of CF, 12.5 g (3.13% yield) of HF, 65.68 g (16.42% yield) of EAF and 212.6 g (53.15% yield) of HMF.

3.2. *Phytochemical screening, quantification of total phenol and HPLC analysis*

Extracts and fractions were qualitatively analysed by precipitation and colorimetric methods described by Matos (2009) to detect anthocyanins and anthocyanidins, aurones, chalcones, flavanols, flavones, flavonols and xanthones (pH-related colour variation using 3 mol L^{-1} sodium hydroxide and 1 mol L^{-1} hydrochloric acid), leucoanthocyanidins and catechins (acid-base reactions followed by heating), tannins (1 mol L^{-1} ferric chloride precipitation), steroids and triperpenoids (Lieberman–Buchard reaction), saponnins (Lieberman–Buchard reaction and foam formation) and alkaloids (Dragendorff reaction).

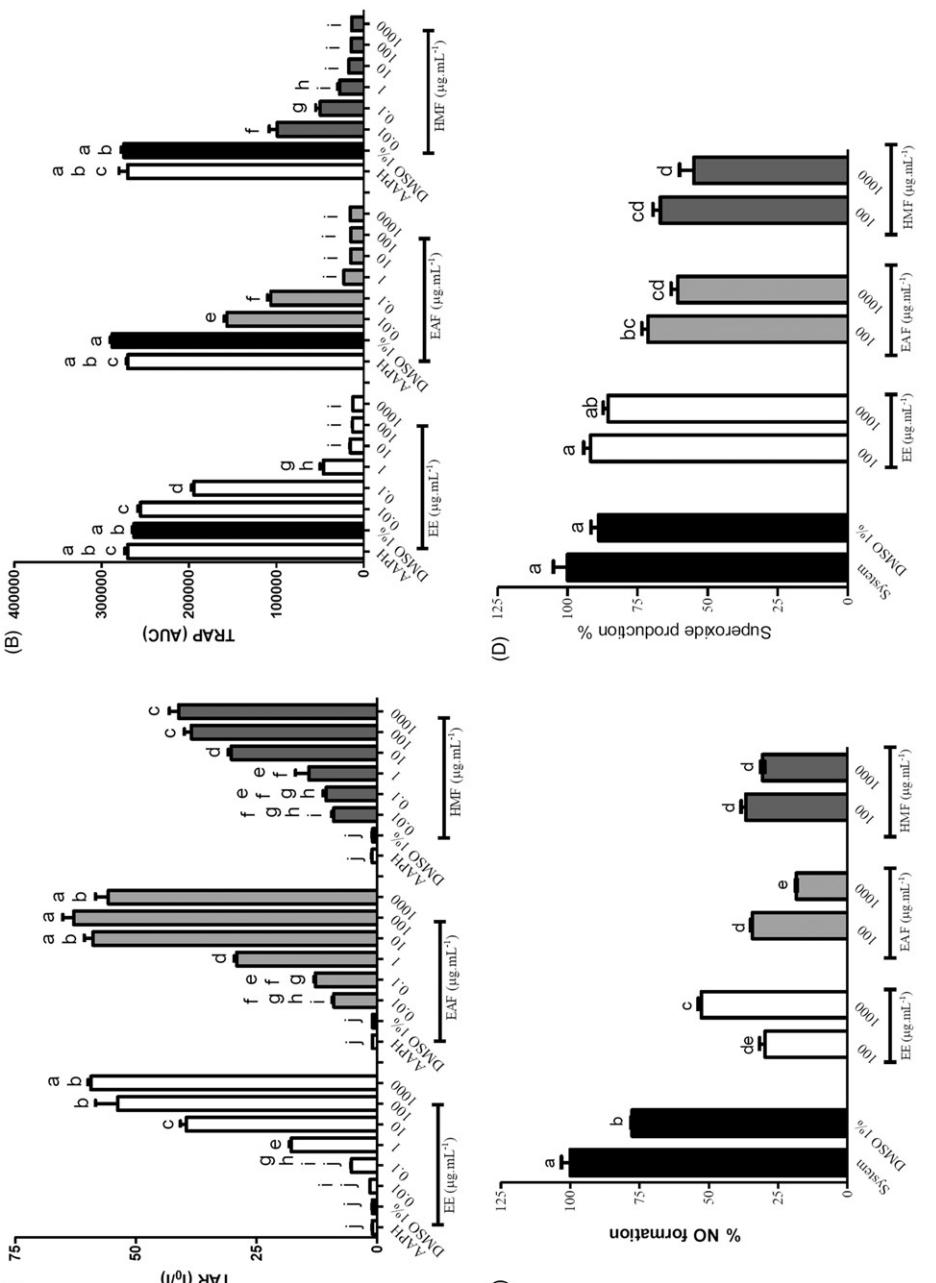

Figure 1. (A) Total antioxidant reactivity. EE, EAF and HMF were able to reduce the light intensity in absence of samples (I_0)/light intensity right after sample addition (I) ratios from 30 to 62-fold between 10 and 1000 μg mL^{-1}. (B) Total reactive antioxidant potential. Activity against peroxyl radicals was found in the reaction mixture 60 min after the initial addition of EE, EAF and HMF. Over 90% of the chemiluminescence was still being reduced by the extract and fractions at 10, 100 and 1000 μg mL^{-1}. (C) NO scavenging activity. EE, EAF and HMF reduced NO formation at 100 (29.7%, 34.3% and 36.7%, respectively) and 1000 μg mL^{-1} (52.7%, 18.4% and 30.5%, respectively). (D) SOD-like activity of EE, EAF and HMF. EAF and HMF prevented the formation of superoxide at 100 μg mL^{-1} (28.7% and 33.1%, respectively) and 1000 μg mL^{-1} (39.3% and 45.1%, respectively). Bars with similar lower case letters indicate the means are not significantly different ($n = 3$; ANOVA followed by Tukey's test at $p < 0.05$).

Extract and fractions 500 $\mu g\,mL^{-1}$ (5 mL) ethanol solutions were treated with the different reagents. Total phenol content was determined using the FC method (Souza et al., 2007). Samples (1 $mg\,mL^{-1}$) were analysed (20 µL) at 280 nm in a Shimadzu chromatographic system using an analytical C_{18} Shim-pack column ($250 \times 4.6\,mm^2$ i.d.; 5 µm particle diameter) under a flow rate of 1.0 $mL\,min^{-1}$ in a gradient elution of 30% methanol : water (0–40 min) and 100% methanol (40–60 min). A standard curve was made using catechin (40–400 $\mu g\,mL^{-1}$) for its quantification in the extract and fractions.

3.3. *Redox properties*

TRAP, TAR, NO scavenger potential, SOD-like and CAT-like activities were measured as described by Melo et al. (2011). All tests were performed in triplicate and data were expressed as mean ± SEM. They were evaluated by a one-way analysis of variance followed by Tukey's test ($p < 0.05$).

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