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Antioxidants, quinone reductase inducers and acetylcholinesterase inhibitors from *Spondias tuberosa* fruits

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ABSTRACT

The methanolic extract of umbu (*Spondias tuberosa*) presented high antioxidant activities in the DPPH, ABTS and ORAC assays, as well as acetylcholinesterase (AChE) inhibition activity. The dichloromethane extract exhibited cancer chemopreventive activity, with a quinone reductase induction in Hepa1c1c7 cells. The localization of the active compounds was performed by HPLC activity-based profiling, and preliminary structural information was obtained by HPLC-PAD-ESI-MS and UHPLC-TOF-HRMS. The main constituents from the methanolic extract were efficiently isolated in a single step by preparative MPLC-UV. Two new natural products were identified, together with five known compounds. The structures of the compounds were elucidated by 2D NMR and ESI-HRMS. The dichloromethane extract was fractionated by SPE and by semi-preparative HPLC-UV-ELSD. Using this approach, one anacardic acid derivative was isolated. However, this compound was not responsible for QR induction. This study highlights the potential of umbu as an active ingredient for functional food formulations.

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Abbreviations: DPPH•, 2.2-diphenyl-1-picrylhydrazyl free radical; ABTS•+, 2.2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); ORAC, oxygen radical absorbance capacity; AChE, acetylcholinesterase; QR, quinone reductase; MEM, Minimum Essential Medium; HPLC, high performance liquid chromatography; MPLC, medium pressure liquid chromatography; SPE, solid phase extraction; UHPLC, ultra-high performance liquid chromatography; TOF, time-of-flight; ESI, electrospray ionization; MS, mass spectrometry; NMR, nuclear magnetic resonance; HMBC, heteronuclear multiple-bond correlation

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1. Introduction

In Brazil, a large variety of edible fruits exists since the country is one of the three largest fruit producers in the world (Maia, Sousa, Lima, Carvalho, & Figueiredo, 2009). Tropical fruits represent an original and valuable source of bioactive compounds, and their consumption is increasing on the national and international markets due to growing recognition of their nutritional and therapeutic value (Steinmetz & Potter, 1996; Viegas et al., 2007; Zeraik et al., 2011). Nevertheless, fruits consumed on the local Brazilian market are poorly studied for their chemical constituents, and their biological activities remain unknown. This is the case of *Spondias tuberosa* Arr. Camara, a native fruit popularly known as "umbu".

S. tuberosa is a tropical Anacardiaceae native to the Northeast of Brazil and plays an important role in the local economy of people living in the Caatinga ecosystem, since it blooms and bears fruits during the dry season, making this plant a valuable source of income for the local population during this period (Braga, 2001). The fruit of this species is appreciated in the North and Northeast of Brazil mainly because of its refreshing and acidic flavour. It can be consumed fresh, as a juice, ice cream, sweet, jam or as the traditional "umbuzada" (fruit pulp boiled with milk and sugar) (Cavalcanti, Resende, & Brito, 2000).

Scientific information on this fruit is scanty. Recent studies have shown that some Brazilian fruits, including umbu, have antioxidant potential that can be attributed to the presence of phenolic compounds in the pulp (Almeida et al., 2011; Goncalves, Lajolo, & Genovese, 2010; Rufino et al., 2010). Previous work reported the acetylcholinesterase (AChE) inhibitory properties of some Brazilian fruits by autobiography, and showed an activity in the umbu seeds (Omena et al., 2012). Vitamins and minerals have also been identified in the pulp (Narain, Bora, Holschuh, & Vasconcelos, 1992). Recently, 37 volatile compounds found in ripe umbu fruit pulp have been described (Galvao, Narain, dos Santos, & Nunes, 2011). However, no phytochemical study has been performed on the polar compounds present in the fruit pulp. The present study describes the isolation and structure elucidation of all major polar constituents from the methanolic and dichloromethane extracts found in the umbu fruit. This led to the identification of compounds responsible for the AChE inhibition, antioxidant and cancer chemopreventive activities of the extracts. Eight compounds were identified and two of them are new natural products.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were measured in a methanolic solution on a Perkin-Elmer 241 polarimeter (Perkin-Elmer, Waltham, MA, USA), using one decimeter tube. UV spectra were measured on a Perkin-Elmer Lambda 20 spectrophotometer (Perkin-Elmer, Waltham, MA, USA). NMR spectroscopic data were recorded on a 500 MHz Varian Inova spectrometer (Varian, Palo Alto, CA, USA). Chemical shifts are reported in parts per million (δ) using the residual DMSO-d_6 signal (δ_H 2.50; δ_C 39.5) as internal standards for ^{1}H and ^{13}C NMR, and coupling constants (J) are reported in Hz. Complete assignment was performed based on 2D experiments (COSY, NOESY, edited-HSQC and HMBC). ESI-HRMS data were obtained on a Micromass LCT Premier time-of-flight (TOF) mass spectrometer from Waters with an electrospray ionization (ESI) interface (Waters, Milford, MA, USA). Analytical HPLC was performed using an HP 1100 system equipped with a photodiode array detector (Agilent Technologies, Santa Clara, CA, USA). Preparative medium pressure liquid chromatography (MPLC) was performed using a modular Buchi MPLC system (Flawil, Switzerland) equipped with 681 pump module C-615, UV–Vis Detector module C-640 and fraction collector module C-660 (Buchi, Flawil, Switzerland). The column (460 × 70 mm i.d.) was loaded with ZEOprep® C_{18} as the stationary phase (ZEOprep® C_{18}, 15–25 μm, Zeochem, Uetikon am See, Switzerland). Semi-preparative HPLC was performed using a Shimadzu LC-8A pump (Shimadzu, Columbia, MD, USA) equipped with a UV detector using an X-Bridge C_{18} column (150 × 21 mm i.d.; 5 μm) (Waters, Milford, MA, USA). HPLC-microfractionation was performed with an Armen modular spot prep II (Saint-Avé, France) with an X-Bridge RP C_{18} column (250 × 10 mm, i.d.; 5 μm) (Waters, Milford, MA, USA).

2.2. Plant material

S. tuberosa Arr. Camara (Anacardiaceae) fruits were collected in João Pessoa, Paraíba, Brazil, in January 2012 by Prof. Marçal de Queiroz Paulo from the Federal University of Paraíba (UFPB). A voucher specimen was deposited at the School of Pharmaceutical Sciences, Phytochemistry and Bioactive Natural Products Research Unit, University of Geneva (n° 2015.001).

2.3. Extraction

The umbu pulp was separated from the seed and homogenized with a mixer. After this step, the pulp was frozen and lyophilised. The powdered pulp was exhaustively extracted by maceration with hexane, followed by dichloromethane and methanol. The dry extracts were obtained after removing the solvent by evaporation under reduced pressure at 40 °C.

2.4. HPLC-UV/PDA-MS analysis

The HPLC-UV/PDA analyses were carried out on an HP 1100 system connected to a photodiode array detector (Agilent Technologies, Santa Clara, CA, USA). The separation was performed using an X-Bridge C_{18} column as the stationary phase (250 × 4.6 mm i.d.; 5.0 μm) supplied by Waters, Milford, MA, USA, preceded by a guard column (20 × 4.0 mm i.d.; 5.0 μm), containing the same stationary phase. The solvent system used was a mixture of H_2O (A) and MeOH (B) both with 0.1% formic acid, gradient mode 5 to 100% of B in 60 min, with linear gradient. The samples (10 μg/mL) were injected automatically (20 μL), with a flow rate of 1 mL/min. The chromatogram was monitored simultaneously at 254, 280 and 366 nm and UV spectra of individual peaks were recorded in the range of 200–400 nm. LC-PDA-MS data were obtained with an Agilent 1100 series system consisting of an auto sampler, high-pressure mixing pump and PAD detector connected to a Finnigan MAT LCQ ion trap mass spectrometer equipped with a Finnigan ESI. The LC effluent was split using a T-splitter to