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Research Paper

X-ray structure of *O*-methyl-acrocol and anti-cancer, anti-parasitic, anti-bacterial and anti-Zika virus evaluations of the Brazilian palm tree *Acrocomia totai*

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ABSTRACT

Acrocomia totai Mart ("macaúba") is a palm tree native from Brazil, whose potential for biodiesel production has been widely explored. In spite of the industrial interest in the oil from the nuts, little is known about the potential applications of other parts of the plant, especially in the pharmacological area. A phytochemical study of the plant thorns led to the identification of a new compound 3-(*R*)-methoxy-21-(*R*)-H-hop-22(29)-en-30-ol 1, two known triterpenes 2–3, four steroids 4–7 and a stilbene, piceatannol 8. The structures were elucidated by spectroscopic analyses, including 1D and 2D NMR and low and high resolution mass spectrometry. Compound 1 was purified as crystals, which allowed the determination of the absolute configuration of the asymmetric carbons by analysis of the X-ray diffraction spectrum. Biological tests were performed with crude extract (CE), fractions and isolated compound. The assays showed activity for CE against lung carcinoma (GI_{50} 59.2 μg mL^{-1}). The ethyl acetate fraction (EAF) showed efficacy against many tumor cell lines, and the tests showed the most prominent activity for breast cancer (GI_{50} 10.4 μg mL^{-1}), glioma (GI_{50} 77.3 μg mL^{-1}), uterine cervix (SiHa) HPV 16 (IC_{50} 39.8 μg mL^{-1}), (HeLa) HPV 18 (IC_{50} 12.0 μg mL^{-1}) and Caco-2 (IC_{50} 40.0 μg mL^{-1}) and showed bacteriostatic action against *Staphylococcus aureus* (MIC 50 μg mL^{-1}). Piceatannol 8 isolated from EAF showed activity against the protozoan which causes leishmaniosis (IC_{50} 58.4 μg mL^{-1}). For *Trypanosoma cruzi*, the methanol fraction (EC_{50} 15.5 μg mL^{-1}), CE (20.5 μg mL^{-1}), and HEF (43.8 μg mL^{-1}) were the most active, being highly selective for the protozoan and less toxic against Vero cells. The compound 8 was further tested against Zika virus MR 766 strain on MOI 2, however the assays showed no inhibition against virus infection.

1. Introduction

Acrocomia is a genus with 42 species of palm plants. It belongs to the Arecaceae family and is native to several South American countries. It is widely distributed throughout the Brazilian savannah (Vaughan, 1960; Abreu et al., 2012). Historical records show that Arecaceae plants were used by ancient human populations since the Holocene period to manufacture artifacts and ropes (Rodriguez and Aschero, 2005).

In Brazil, the production of nuts from *Acrocomia totai* Mart. (popularly known as "macaúba") is performed through family agriculture, and is a highly productive oleaginous plant. Special attention has been devoted to this plant because of the increased use of its fruits for the production of biodiesel, representing an alternative to the growing energy demand of modern society and for creating direct and indirect

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jobs through its chain of production (Carvalho et al., 2013; Basso et al., 2013; Navarro-Díaz et al., 2014; Aguieiras et al., 2014; Iha et al., 2014; Michelin et al., 2015; Prado et al., 2016; Silva et al., 2016).

A. totai is conspicuous due to its large oil productivity (around six tons) per hectare. Besides the biodiesel production, *A. totai* fruits are used in food and cosmetics industries (Silva and Andrade, 2013; Evaristo et al., 2016). However, the palm trunk is usually covered by large thorns, which are a major drawback for manual fruit collection, as it can cause accidents with serious injuries to workers (INPA, 2015). These injuries (from the thorns) often cause extreme pain and skin redness, raising suspicions about the possible presence of biologically active substances in the thorns.

In recent years, studies have shown important chemical and biological properties for *Acrocomia* genus plants. For example, from roots of *A. mexicana* a hypoglycemic compound named coyolosa was isolated (Perez et al., 1997); also, studies with fruits from *A. aculeata* led to the isolation of galactoglucomannan, a immunostimulant polysaccharide that can be used as a vaccine adjuvant (Silva et al., 2009). Moreover, some studies report that the fruit from *A. aculeata* has antioxidant properties and, mainly, chemo-prevention activity against cancer (Traesel et al., 2015; Traesel et al., 2014; Magosso et al., 2016).

Phytochemical studies have revealed the presence of pentacyclic triterpenes, flavonoids, steroids, and saponins from plants of the Arecaceae family (Galotta and Boaventura 2005; Lima et al., 2015; Oliveira et al., 2016). Several palm trees have been used in traditional medicine for treatment of various diseases, especially against parasitic infections, digestive disorders, and depression (Peng et al., 2015; Lima et al., 2015).

Recently, Latin America has been experiencing an outbreak of the Zika virus infection (ZIKV), a vector-borne flavivirus transmitted by *Aedes aegypti* mosquitoes. It is the causative agent of many still unclear pathological disorders, from mild outcomes, to several congenital malformations in fetuses, and neuronal disorders in adults (Melo et al., 2016a,b; Brasil et al., 2016; Sabbir et al., 2016). The virus is detected in different infected tissues and body fluids, such as the placenta, amniotic fluid, and the brain (Garcez et al., 2016; Sarno et al., 2016; Calvet et al., 2016). In Brazil, the ZIKV outbreak has prompted an increase in microcephaly in newborns, which is becoming a major public health problem (Zanluca et al., 2015; Rasmussen et al., 2016 Rasmussen et al., 2016). In addition, neotropical populations also suffer with the incidence of neglected parasitic diseases, such as leishmaniosis and the Chagas disease (Cogo et al., 2015; Kaplum et al., 2016 Kaplum et al., 2016). Thus, efforts that aim for the discovery of biologically-active molecules against these pathogens are a priority.

Plants have been a major source of bioactive compounds for decades, and the present work represents the first phytochemical study of *A. totai* thorn extracts. This contributes to more knowledge concerning Brazilian biodiversity. In this study, thorn extracts are sought after because they may be sources of bioactive compounds with potential against infectious agents, such as *Leishmania amazonensis*, *Trypanosoma cruzi*, bacteria, fungi, Zika virus, and also for the control of cancer cell proliferation. Different compounds were isolated and identified by 1D and 2D NMR spectral analyses, low- and high-resolution mass spectrometry, polarimetry and X-ray diffraction, followed by comparisons with literature data. The chemical study allowed the identification of a new triterpene natural product.

2. Materials and methods

2.1. Plant material

The thorns were collected from a trunk of a native "macaúba" palm tree (*Acrocomia totai* Mart), in Moreira Sales, a city in southern Brazil, (24° 03′ 24.5 ″S/53° 03′ 38.3″ W). An exsicata was authenticated by Dr. N T V Junqueira (EMBRAPA) and deposited in the herbarium of the State University of Maringá (HUEM 29683).

The *A. totai* thorns were dried (35 °C for 36 h) and grounded (Cienlab mill). Methanol (MeOH) 100% was used as extraction solvent (1000.84 g of grounded thorns in 4 L of MeOH) affording 33.07 g of crude extract (CE) after evaporation under reduced pressure. Part of CE (27.81 g) was extracted with organic solvents in polarity gradient, resulting in four fractions with solvents *n*-hexane (HEF 0.53 g), chloroform (CHF 0.62 g), ethyl acetate (EAF 14.56 g), and methanol (MEF 12.09 g).

For column chromatography, glass columns (3–5 cm d) packed with silica gel 60 (0.0063-0.200 mm, Merck) were used, eluted with *n*-hexane, chloroform, ethyl acetate (AcOEt), and methanol in an increasing polarity gradient. The CHF was subjected to column chromatography and at *n*-hexane:$CHCl_3$ polarity (8:2) it was isolated compound **1** (1.6 mg); from *n*-hexane:$CHCl_3$ (1:1) polarity it was isolated compound **3** (5.3 mg). The compounds **4** and **5** (6.6 mg) were isolated in mixture from *n*-hexane:$CHCl_3$ (3:1) followed by recrystallization with AcOEt. Then, the supernatant (35.2 mg) was subjected to column chromatography yielding a mixture of compounds **6** and **7** (15.2 mg), from *n*-hexane:$CHCl_3$ (3:7). From HEF column fractionation, compound **2** was isolated from fractions eluted with *n*-hexane:$CHCl_3$ (9:1) (1.0 mg).

The EAF was also subjected to column chromatography. A fraction eluted with *n*-hexane:$CHCl_3$ (9:1) was obtained and diluted with H_2O for three minutes under ultrasound (50/60 Hz). After phase separation, the precipitate (soluble in MeOH) was fractionated in a Sephadex LH-20 column (3 cm d; sample:Sephadex 1:40) using pure MeOH as eluent, yielding the pure compound **8** (129.9 mg).

2.2. Gas chromatography–mass spectrometry analyses

GC–MS analyses were performed in a Focus GC (Thermo Finnigan) gas chromatograph coupled to a DSQ II (Thermo Finnigan) mass selective detector operating with 70 eV electron impact, fitted with a quadrupole analyzer. Data acquisition was done with Xcalibur software with NISCG-MS Search Version 2.0 library database. Analyses were performed with the injector operating at 250 °C, in splitless injection mode. Helium gas was used at a column flow of 1 mL min^{-1} and the capillary column DB-5 (30 m × 0.25 mm × 0.25 μm) (5% phenyl, 95% methylpolysiloxane) (J & W Scientific) was used. The injection volume was 1.0 μL and the oven temperature program was from 50 to 290 °C at 15 °C min^{-1}. Samples were prepared from 1 mg of each compound dissolved in 1 mL of $CHCl_3$ (HPLC grade).

2.3. High-resolution mass spectrometry analyses

HR-ESI-(+)-MS analyses were performed in a Xevo Q-Tof (Waters™), equipped with a nanoESI type ionization source. Samples were prepared at a concentration of 5 ppm in H_2O:MeCN 1:1, with 0.1% formic acid and injected by direct infusion in a flow of 20 μL min^{-1}. The instrumental parameters used were 3 kV capillary voltage, 20 V cone voltage, 120 °C source temperature, and 0.5 L h^{-1} nebulization gas flow. Before each analysis, the instrument was calibrated with 0.005% H_3PO_4 solution in H_2O:MeCN (1:1) with mass/charge ratio range from *m/z* 100 to 1000.

2.4. Optical rotations

The optical rotations were measured in $CHCl_3$ in a PerkinElmer 343 polarimeter at 20 °C and 589 nm, with an optical cell path of 10 mm.

2.5. Infrared (IR) analyses

Infrared spectra were obtained using KBr pellets in a Thermo Fisher Scientific (Nicolet iZ10), on Smart Omni-Transmission. The resolution was 4 cm^{-1} and the wavenumber range was from 4000 to 400 cm^{-1}.