ARTIGO ORIGINAL

Phytochemical evaluation of *Conocarpus erectus* leaves

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ABSTRACT: *Conocarpus erectus*, popularly known as button mangrove, is one of the most common species found in mangroves and it is widely used in folk medicine for anemia, phlegm and diabetes prevention. The present study aimed to evaluate the methanolic extract of leaves of *Conocarpus erectus* (MELCe) to unravel its phytochemical constituents. The botanical material was collected in Itamaracá,PE, Brazil, mangrove. The extract was obtained by infusion with methanol and characterized by high-performance liquid chromatography (HPLC) and high-performance thin layer chromatography (HPTLC). Analysis on HPTLC showed the presence of phenolic compounds, mainly ellagic acid and *p*-coumaric acid. In addition, to analyze and to quantify the phenolic compounds, it was performed a flavonoid quantification at concentrations of 1000, 500, and 250 µg/ml. HPLC confirmed the presence of ellagic acid and a quercetin-type compound. Flavonoid quantification assays shows 72.52 ± 5.12, 36.54 ± 1.13, and 17.29 ± 1.45 µg/ml, respectively, obtained as quercetin equivalent. Leaves of *C. erectus* contain phenolic compounds, and the presence of the flavonoid quercetin is suggested.

Keywords: *Conocarpus erectus*; chromatography; extract; phenolic compounds.

RESUMO: Avaliação fitoquímica de folhas de *Conocarpus erectus*. *Conocarpus erectus*, popularmente conhecido como mangue de botão, é uma das espécies mais comumente encontradas em manguezais e amplamente utilizada na medicina popular para a prevenção de anemia, catarro e diabetes. O presente estudo teve como objetivo avaliar o extrato metanólico de folhas de *Conocarpus erectus* (MELCe) para desvendar seus constituintes fitoquímicos. O material botânico foi coletado no manguezal de Itamaracá, PE. O extrato foi obtido por infusão com metanol e caracterizado por cromatografia líquida de alta eficiência (HPLC) e cromatografia em camada delgada de alto desempenho (HPTLC). Análises em HPTLC mostraram a presença de compostos fenólicos, principalmente ácido elágico e ácido *p*-cumárico. Além disso, para analisar e quantificar os compostos fenólicos, foi realizada a quantificação dos flavonoides nas concentrações de 1000, 500 e 250 µg/ml. A HPLC confirmou a presença de ácido elágico e de composto flavonoide tipo quercetina. Os ensaios de quantificação de flavonoides mostram 72,52 ± 5,12, 36,54 ± 1,13, e 17,29 ± 1,45 µg/ml, respectivamente, obtidos como equivalentes de quercetina. Folhas de *C. erectus* contêm compostos fenólicos, e a presença do flavonoide quercetina é sugerida.

Palavras-chave: *Conocarpus erectus***;** cromatografia; extrato; compostos fenólicos.

INTRODUCTION

The use of natural products with medicinal purpose features a reality since the dawn of civilization (Firmo et al. 2011). Due to the increasing use of plants for these purposes, it is important and necessary to explore its constituents, its therapeutic mechanism of action and to identify the active principles responsible for the different biological activities. The great demand of plants with therapeutic applications is used based on folk medicine (Lana et al. 2012), showing the lack of scientific knowledge and exploration of its pharmacological and toxicological properties (Firmo et al. 2011).

The genus *Conocarpus* L. is represented by the species *Conocarpus erectus*. This species belongs to the family Combretaceae, popularly known as a mangrove tree, and one of the most present species in mangroves (Galvani and Lima 2010). The species of this family are present in

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Brazil and are rich in a group of biologically active substances called phenolic compounds (Merlin et al. 2017) showing mitogenic and antioxidant activities (Abdel-Hameed et al. 2012). In folk medicine, it has been shown that the species is astringent, hemostatic and effective in preventing anemia, catarrhal infections, conjunctivitis, diabetes, diarrhea, fever, gonorrhea, headache, hemorrhage, orchitis, pruritus, swelling and syphilis (Abdel-Hameed et al. 2012; Abdel-Hameed et al. 2013). However, studies on its benefits are still not enough explored scientifically.

Research in Brazil are of great value because the country is considered one of the world's largest biodiversity reservoirs, in addition to a large territory, being home to many types of ecosystems, with its own peculiarities, which makes it a real source of molecules to be discovered (Ferreira et al. 2012). The Ministry of Health of Brazil has encouraged the use of complementary therapies and, in 2006, it was implemented the National Policy on Integrative and Complementary Practices in the Unified Health System (*Sistema* Único *de Saúde - SUS*), inserting medicinal plants through phytotherapy, acupuncture, and among other therapies in the public health system (Geniole et al. 2011).

However, it is important to uncover new natural compounds used in folk medicine in order to promote their use by the population with restricted access of health care (Cartaxo-Furtado et al. 2015). In this perspective, the aim of the present study was to evaluate phenolic contents of leaves of *Conocarpus erectus* (MELCe) in order to clarify its popular utilization and to improve the therapeutic bioprospection.

MATERIAL AND METHODS

Botanical Material

Conocarpus erectus L. (Combretaceae) leaves were collected in adult stage in flowering period (January 2016) in Itamaracá mangrove, district of Vila Velha, state of Pernambuco – Brazil, with 7° 40' South latitude and 34° 50' West longitude. Green leaves with intact appearance, free of mechanical damage, pests, diseases or altered color were selected. One copy of the voucher was identified by Marlene Barbosa and deposited in the Herbarium of the Federal University of Pernambuco (UFPE) under number 69.655. The The gathering was authorized by Empresa Pernambucana de Controle da Poluição Ambiental e Administração de Recursos Hídricos (CPRH) under authorization CA DFRB N. 120/2014. We also provided a government authorization to access *C. erectus* usage under the protocol Sisgen (nº AC03C8C).

Extract Preparation

MELCe was obtained from the Instituto Nacional do Semiárido (INSA), in Campina Grande, Paraiba, Brazil. Fresh leaves of *C. erectus* (2 kg) were ground in a Pulverisette 14 (Fritsch). The extract was produced through accelerated solvent extraction (ASE 350, Thermo Scientific) using 100 mL stainless steel extraction cells. Thirty grams of ground plant material were filled into a cell and extracted at 40 °*C and approximately 100* bar with 100 ml of methanol. The process was repeated 19 times in order to obtain a total of 7.6 l of extract. The extract was concentrated under reduced pressure at 40 °*C* Genevac Rocket TM (Genevac), applying the preset program for solvents with low boiling point. The concentrated extract was partitioned with metanol in the ratio of 1:1 (v/v).

HPLC Analysis

The extract was analyzed by an Agilent Technologies 1200 Infinity Series liquid chromatography (RP-HPLC) equipped with quartenary pump, auto-sampler, column oven and diode array detector. Separation was performed on a Zorbax 4,6 mm x 250 mm, 5 µm, C-18 reversed phase column. The sample (5 µl) was subjected to the following linear gradient at a flow rate of 2.4 ml/min and 30 ºC with 0.3% acetic acid in water (solvent A) and Acetonitrile (solvent B). The gradient program was as follows: 98% A/2% B - 0 min, 90% A/ 10% B - 10 min, 15% A/ 85% B - 27 min.

HPTLC Analysis

HPTLC modules (CAMAG, Swiss) consisting in an automatic TLC Sampler 4, automatic development chamber ADC2 and integrated software WINCATS (version 1.4.46337) were used for the analysis of extract of *C. erectus* in different samples. The stationary phase was pre-coated silica gel glass plate 60 F_{254} (10 cm x 10 cm, Merck, Germany). The extract of *C. erectus* were loaded on the plate at 9.5 mm wide bands with distance of 8 mm from the bottom and 20 mm from the side, and the space between two spots was 12 mm, with constant application rate of 150 nl/s under a flow of N_2 gas. The first stage was called pre-revelation where the plates remained empty and the second stage was called post-revelation applying NEU reagent commercially acquired (Merck, Germany), where the concentrations were inserted and the bands identified.

Flavonoid contents

Aliquot of 1 ml extract (1 mg/ml), 1 ml aluminum chloride (5%) and 2 ml methanol were run in a test tube. White was prepared. The sample remained for 30 min in the dark and then read in a

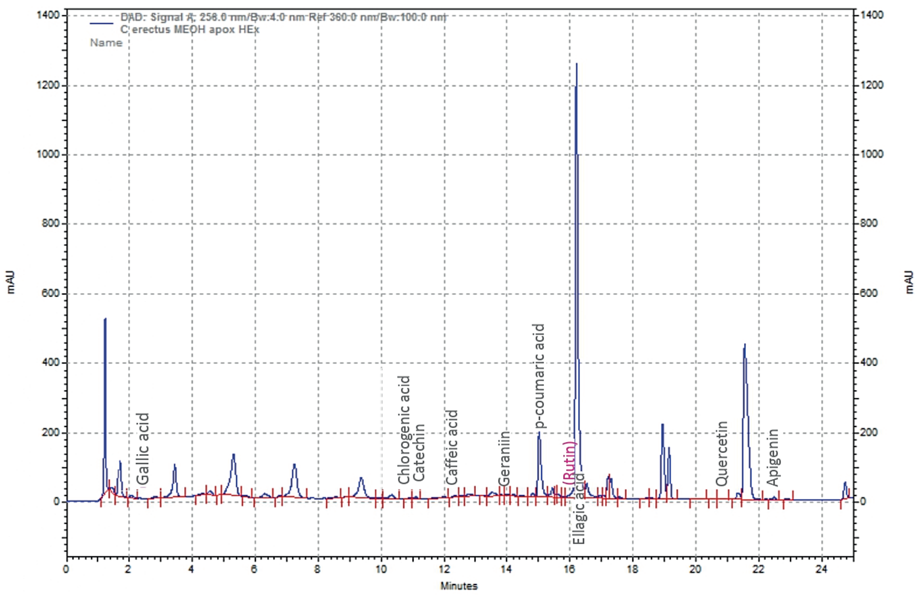

FIGURE 1: Chromatogram by HPLC of phenolic compounds.

spectrophotometer at 425 nm. Quercetin was used as the standard at the concentrations of 1000, 500 and 250 µg/ml for the construction of the calibration curve. From the equation of the line obtained, the calculation of the flavonoid content, expressed as quercetin equivalent of the sample (mg/g of extract) (Woisky and Salatino 1998).

RESULTS AND DISCUSSION

Chromatographic analysis

High Performance Liquid Chromatography (HPLC)

Quercetin, chlorogenic acid, catechin, caffeic acid, geraniin and apigenin were confirmed by HPLC analyzes. The main compounds were ellagic acid and p-coumaric acid in MELCe (Figure 1).

Quercetin is known as a powerful natural antioxidant that already has applications in the therapeutic scope. It is found in different plant species (Aras et al. 2014), showing antimicrobial, antitumor and antioxidant activities. It has been verified its effectiveness in reducing the atherosclerosis, against allergies and it shows antiangiogenic activity against human endothelial cells (Costa 2005; Sepúlveda et al. 2011; Vizzotto et al. 2010).

A phytochemical study of the methanolic extract of *C. erectus* also identified the presence of the compounds: catechin, quercetin, apigenin and ellagic acid (Abdel-Hameed et al. 2012), demonstrating that ellagic acid is a major component of the fruit of this plant. These data reinforce our findings and allow us to suggest that *C. erectus* has phenolic compounds in several parts of the plant.

Ellagic acid is a powerful bioactive compound and anti-carcinogenic with various pharmacological and industrial applications and, therefore, resulted in commercial interest due to its properties and benefits for humans (Sepúlveda et al. 2011; Vizzotto et al. 2010).

High Performance Thin Layer Chromatography (HPTLC)

The HPTLC analysis of MELCe showed the presence of phenolic compounds, confirming the results obtained by HPLC (Figure 2).

Similar fingerprints were observed in seeds of *Eugenia uniflora* applying Thin Layer Chromatography (TLC) methodology (Oliveira et al 2014). Using NEU reagent, they showed the presence of phenolic compounds and TLC was used in order to determine the antioxidant activity.

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The quantitative assays to determine the flavonoid equivalent to quercetin at the concentrations of 1000, 500, and 250 µg/ml were performed and are shown in Table 1. *C. erectus* showed a flavonoid content according the above concentrations of 72.52 ± 5.12, 36.54 ± 1.13, and 17.29 ± 1, 45, respectively.

In order to evaluate the antioxidant potential, significant maximum levels of flavonoids (629.4 ± 1.57) were identified in the dichloromethane extract of the aerial part of *Conocarpus lancifolius* whereas the aqueous root extract showed the lowest flavonoid content of 104.2 ± 0.06 with reference to quercetin,

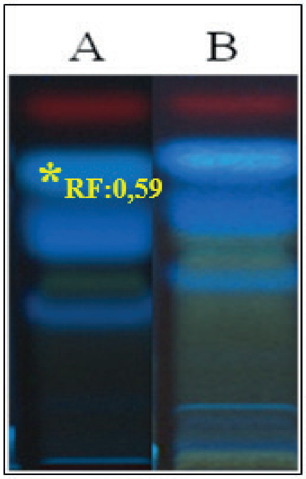

FIGURE 2: Chromatographic plate: (A) Standard with quercetin equivalent and (B) Extract of *C. erectus*. *Rf: retention factor.

in addition to the ethanolic extract of the branches and leaves of *C. erectus* reference to the equivalent of rutin and quercetin, it was verified an amount of 14.04 ± 0.87, 4.34 ± 0.32, respectively (Raza et al. 2016; Saadullah et al. 2016).

In contrast, Hussein (2016) applied primary qualitative analysis to evaluate the presence of phenolic compounds in crude methanolic extract of *C. erectus* leaves and they did not verify the presence of any flavonoids. We suggest that those qualitative tests applied at their study were insufficient to identify the presence of such compounds.

The phytochemical evaluation of *C. erectus* showed the presence of flavonoids, suggesting the presence of quercetin. Thus, the plant contains phenolic compounds, justifying its use in folk medicine. The results suggest the need to continue studies with the plant, including the successive fractionation of the extract in order to confirm and characterize the compounds found in leaves, besides the analysis of other parts of the plant.

In the determination of the phytochemical profile of *C. erectus,* the presence of flavonoids was detected, among them the presence of quercetin is suggested. Thus, we concluded, therefore, that this plant has the presence of phenolic compounds, justifying its use in folk medicine. The results suggest the continuity of studies with the plant, including the successive fractionation of the extract in order to prove the constituents found, besides the analysis of other compounds.

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