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Phenolic Compounds Isolated From *Euphorbia phosphorea*

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Abstract: Euphorbiaceae is one of the largest families among angiosperms, being found in several distinct habitats and vegetation types, mainly in tropical and subtropical áreas of America and Africa. Despite its notable economic importance, as well as its abundant occurrence in northeastern Brazil, few reports have been made about its metabolites and its pharmacological activities. The genus *Euphorbia* L. is globally distributed, but specially abundant in arid and semi-arid regions, where its occurrence is mostly as succulent xerophytes. As reported by previous studies, constituents from species of the genus euphorbia, such as tannins and flavonoids, have shown relevant activity in wound healing, conferring the genus therapeutical potential. This study conducted phytochemical investigations on the species *Euphorbia phosphorea*, obtained from the semi-arid region of Paraíba. Aerial parts of *E. phosphorea* were collected from Serra Branca, Paraíba, Brazil. The fresh material was dried in a circulating air oven, pulverized and macerated with etanol, yielding an ethanol extract. The obtained extract was then partitioned with the organic solvents hexane, dichloromethane, ethyl acetate and n-butanol to obtain the respective soluble fractions. The ethyl acetate fraction was submitted to sephadex LH-20 column chromatography with metanol as eluent. The 88 sufractions obtained were then analysed in thin layer chromatography and gathered according to their retention factor similarity. ^{1}H NMR spectrum of fraction 47-51 revealed characteristics of polyphenolic compounds, being confirmed later by analysing its ^{13}C-APT NMR and LC-MS data. Applying chromatographic and spectroscopic techniques, the study of *Euphorbia phosphorea* led to the obtaining of ellagic acid and corilagin, contributing to the phytochemical knowledge of the genus *Euphorbia*.

Keywords: Euphorbiaceae; *Euphorbia*; Tannins; Polyphenols.

1. Introduction

Euphorbiaceae is one of the largest families among angiosperms, being found in several distinct habitats and vegetation types, mainly in tropical and subtropical areas of America and Africa. Despite its notable economic importance, as well as its abundant occurrence in northeastern Brazil, few reports have been made about its metabolites and its pharmacological activities. The genus *Euphorbia* L. is globally distributed, but specially abundant in arid and semi-arid regions, where its occurrence is mostly as succulent xerophytes. As reported by previous studies, constituents from species of the genus euphorbia, such as tannins and flavonoids, have shown relevant activity in wound healing, conferring the genus therapeutical potential. This study conducted phytochemical investigations on the species *Euphorbia phosphorea*, obtained from the semi-arid region of Paraíba.

2. Results and Discussion

Subfractions 47-51 obtained from CC showed the same profile on TLC, and therefore were gathered. The ^{1}H NMR spectrum of the subfractions 47-51 sample revealed a profile similar to polyphenols, and after comparison to literature data, was coded as “Ep-1”.

On Ep-1 ^{1}H NMR spectrum, four singlets were observed in the region of aromatic hydrogens, in δ_H 7.43, 7.03, 6.56 and 6.49, suggesting that Ep-1 was a phenolic compound. The singlet in δ_H 7.43, integrating for two hydrogens, suggested the presence of ellagic acid. Other singlets observed in the ^{1}H NMR spectrum were attributed to the galoil (δH 7.03) and hexahydroxydiphenol (δ_H 6.56 and 6.49) groups. Characteristic signals of an osidic unit were observed in the region of δ_H 3.7 to 4.6, as well as a characteristic doublet of anomeric hydrogen in δ_H 6.20, associated to one unit of glucose. Therefore, the signals also suggested the presence of tannin on the sample.

The ^{13}C-APT NMR spectrum (100 MHz, DMSO-d 6) confirmed the presence of the ellagic acid in the sample by characteristic shifts at δ_C 159.4, attributed to carbonyls and at δ_C 140.4 and 148.4 assigned to aromatic oxygenated carbons (C-2 / C-7 and C-3 / C-8, respectively). The spectrum of Ep-1 also revealed the presence of characteristic carbonyl ester signals at δ_C 164.9, 166.9 and 167.3,as well as oxygenated aromatic carbons in δ_C 144.1 to 145.7. Chemical shifts in 62.3 to 77.6 reinforced the presence of the galoyl and hexahydroxydiphenolic groups and the glucose unit present in the tannin structure. By comparing ^{1}H and ^{13}C NMR data with those described in the literature, it was possible to suggest that the other substance in the sample was 1-O-galloyl-3,6- (R) -hexydroxydiphenyl-β-glucopyranose (corilagine).

Analyzing the data obtained by low resolution HPLC-ESI-MS operating in the negative mode, it was possible to observe peaks with retention time in approximately 26.2 min and 38.9 min, corresponding respectively to ions with m/z 633 $[M\text{-}H]^-$ for corilagine and m/z 301 $[M - H]^-$ for ellagic acid, confirming the presence of these compounds in the sample.

3. Materials and Methods

Aerial parts of *E. phosphorea* were collected from Serra Branca, Paraíba, Brazil The botanical identification was carried out by Doctor José Iranildo Miranda de Melo from Department of Biology of Universidade Estadual da Paraíba (UEPB).

Column chromatography (CC) was performed using sephadex LH-20 as stationary phase and methanol as mobile phase. Thin layer chromatography (TLC) was performed on silica gel GF_{254}. ^{1}H and 13C-APT NMR spectra were acquired with a Bruker Ascend^{TM} 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer. Chemical shift (δ) values are given in ppm, with TMS as internal standard. HPLC-ESI-MS was performed with a Shimadzu Prominence 20A system consisting of a CBM-20A controller, LC-20 AD parallel-flow pumps, a DGU-20A degasser and a SIL-20A auto sampler. The HPLC system was coupled to an AmazonX Ion-Trap mass spectrometer(Bruker Daltonics, Bremen, Germany). The ESI-MS acquisition parameters were capillary voltage 4.5 kV, negative ESI mode; end-plate offset 500 V; nebulizer gas 35

psi; dry gas (N_2) with a flow rate of 8.0 L ·min^{-1} and temperature of 300 ºC.

The fresh material was dried in a circulating air oven, pulverized and macerated with etanol, yielding an ethanol extract. The obtained extract was then partitioned with the organic solvents hexane, dichloromethane, ethyl acetate and n-butanol to obtain the respective soluble fractions. The ethyl acetate fraction was submitted to sephadex LH-20 column chromatography with methanol as eluent. The 88 subfractions obtained were then analyzed in thin layer chromatography and gathered according to their retention factor similarity.

4. Conclusions

Applying chromatographic and spectroscopic techniques, the phytochemical study of *Euphorbia phosphorea* led to the obtaining of ellagic acid and corilagin, contributing to the phytochemical knowledge of the genus *Euphorbia*.

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Conflicts of Interest

The authors declare no conflict of interest.

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