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Phytochemical investigation of *Erythroxylum rimosum* O. E. Schulz

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Abstract: *Erythroxylum rimosum* O. E. Schulz is a species restricted to the northeastern region of Brazil, found in the states of Ceará, Piauí, Sergipe and Bahia, occurring respectively in Restinga, Cerrado and Carrasco vegetation. The study of the extract of *E. rimosum*, reported the identification of pentacyclic triterpenes, steroid, tropic alkaloid and flavonoids. Thus, a chromatographic study of its crude ethanol extract was carried out. The botanical material of the aerial parts was collected in the municipality of Pirambu, state of Sergipe and identified by Profa. Dra. Ana Paula do Nascimento Prata, Department of Biology, Federal University of Sergipe (FUS). It was then oven dried with circulating air at an average temperature of 40 ° C, ground in a mechanical mill and subjected to steeping with 95% EtOH. The BSE (105 g) was dissolved in a methanol: water (7:3 v/v) solution and partitioned with the following solvents: hexane, dichloromethane and ethyl acetate. The AcOEt phase was subjected to column chromatography, using silica gel 60 as stationary phase and as mobile phase, the Hex, AcOEt and MeOH solvents, pure and in binary mixtures in increasing order of polarity. This yields 30 fractions which after analytical thin layer chromatography (TLC) were pooled according to their respective retention factors (Rfs). The fractions from 23 to 25 were submitted to High Performance Liquid Chromatography Coupled to a Diode Array Detector (HPLC-DAD). Getting yourself 8 fractions. From fractions 4 and 2 the coded substances of Er-1 and Er-2 respectively were obtained. They have had their structures identified by ^{1}H-NMR, ^{13}C, and two-dimensional techniques in comparison with literature data, namely: kaempferol-3-rutinoside and quercetin-3-*O*-β-D-glucopyranoside-α- L-raminoside, two glycosylated flavonoids that are being reported for the first time in the study species.

Keywords: Erythroxylaceae; *E. rimosum;* Kaempferol-3-rutinoside.

1. Introduction

E. rimosum O. E. Schulz is a species restricted to the northeastern region of Brazil, found in the states of Ceará, Piauí, Sergipe and Bahia, occurring respectively in Restinga, Cerrado and Carrasco vegetation.[1] The study of the crude ethanolic extract of *E. Rimosum* leaves carried out by RIBEIRO, (2011), reported the identification of pentacyclic triterpenes (α-amirin, β-amirin), steroid (β-sitosterol), tropic alkaloid and flavonoids.[2]

2. Results and Discussion

The substance encoded as Er-1 was isolated as a brown solid with 20 mg. The ^{13}C-APT spectrum obtained at 100 MHz in CD_3OD showed the presence of 27 signals. Of these 9 were attributed to nonhydrogenated carbons, 16 to methyl carbons, 1 methylene carbon and 1 methyl carbon.163,1; 101.4; 166.3 and 94.9 were assigned to the C-5 aromatic carbons; C-6; C-7 and C-8, respectively, suggesting to treat the A-ring substitution pattern of oxygenated flavones in C-5 and C-7. The signal at δc 132.1 was corresponding to the C-2 'and C-6' carbons and the signal at δc 116.70 was attributed to the H-3 'and H-5' hydrogens; being these characteristic of ring B of flavones. The signals at δc 158.7; 136.4 and 179.6 were assigned to the C-2 carbons; C-3 and C-4 respectively, being compatible with an oxygenated flavone at C-3. According to the ^{13}C data, it was also possible to suggest the presence of two osydic units, being a glucose and a rhamnose, this was due to the signs of the carbonos anomeric in δc 103,6 and δc 102,4 and also to the carbon methylenic in 68,6 and carbon in δc17.8, and, in addition to the set of signals between δc 68.6 and δc 78.2, suggested the presence of rutinose.

In the ^{1}H spectrum, two doublets δ_H 6.38 and δ_H 6.20 (J = 2.0 Hz), characteristic of hydrogen H-8 and H-6 of hydrogen peroxide flavones at positions 5 (J = 8.8 Hz) which was assigned to the hydrogens H-2 'and H-6', a doublet in δ_H 6.93 (J = 8.8 Hz) which was assigned to the H-3 and H-5 hydrogens of the B-ring of flavones in an AA'BB' system. In addition, a doublet in δ_H 5.37 (J = 1.6 Hz) was observed in the spectrum and one in δ_H 4, 57, corresponding to the hydrogen anomeric da glicose and rhamnose respectively. The presence of a doublet in δ_H 4.21 (dd, J = 3.6, 1.6 Hz) and another in δ_H 1.10 (d, J = 6.0 Hz) were attributed to methylene and methyl hydrogels respectively. These data corroborate with an osteoid unit glucose-linked aramenosis. The coupling constant J = 1.6 Hz was consistent with an α-rutinoside unit.

The correlations observed in the Er-1 HMBC contour map of the hydrogen in δ_H 5.37 (H-1 '') with the C-3 carbon in δc 136.4 at three bonds confirmed the insertion of the osydica rutinosidase in C-3. After these analyzes, it was possible to conclude that Er-1 is the camphorol-3-*O*-rutinoside.

The substance encoded as Er-2 was isolated as a brown solid with 15 mg. In the ^{13}C-APT spectrum obtained at 100 MHz in CD_3OD of Er-2 was shown to be quite similar to Er-1 differing only in the chemical shifts at δc 117.8; δc 145.9 and δc 149.9 which were assigned to the C-2 'aromatic carbons; C-3 'and C-4' respectively, when compared to these same carbons in Er-1, it was possible to suggest the insertion of a hydroxyl in C-3 'due to an ortho effect of electron donor group that promoted protection in C -2 'and C-4'.

In the ^{1}H spectrum, it was shown to be quite similar to Er-1, differentiating only in the absence of the signal in δ_H 6.93 (d, J = 8.8 Hz), where a C-3 'group was suggested. It was also observed that the chemical shifts and coupling constants values were δ_H 7.67 (d, J = 2.0 Hz), δ_H 6.89 (d, J = 8.4 Hz) and δ_H 7.63 (dd, J = 8,4; 2,0 Hz), which were attributed to the hydrogen H-2 ', H-5' and H-6 ', respectively, being suggestive of the presence of an ABX system in ring B of flavones. After these analyzes, it was possible to conclude that Er-2 is quercetin-3-*O*-β-D-glucopyranoside-α-L-raminoside.

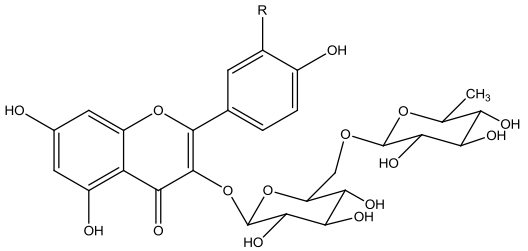
Camphorol-3-*O*-rutinoside (Er-1)

^{1}H NMR (400 MHz, CH_3OD), 6,20 (d, J = 2,0 Hz, H-6), 6,38 (d, J = 2,0 Hz, H-8), 7,78 (d, J = 8,8 Hz, H-2' e H-6'), 6,93 (d, J = 8,8 Hz, H-3' e H-5'), 5,37 (d, J = 1,6 Hz, H-1''), 3,52-4,22 (m, H-2''), 3,52-4,22 (m, H-3''), 3,52-4,22 (m, H-4''), 3,52-4,22 (m, H-5''), 4,21 (dd, J = 3,6; 1,6 Hz, H-6''), 4,57 (s, H-1'''), 3,32-4,22 (m, H-2'''), 3,32-4,22 (m, H-3'''), 3,32-4,22 (m, H-4'''), 3,32-4,22 (m, H-5'''), 1,10 (d, J = 6,0 Hz, H-6'''. APT-^{13}C NMR (100 MHz, CH_3OD), 158,69 (C-2), 136,36 (C-3), 179,63 (C-4), 163,10 (C-5), 101,38 (C-6), 166,29 (C-7), 94,92 (C-8), 159,54 (C-9), 105,60 (C-10), 122,78 (C-1'), 132,05 (C-2'), 116,70 (C-3'), 161,75 (C-4'), 116,70 (C-5'), 132,05 (C-6'), 103,66 (C-1''), 72,20 (C-2''), 77,26 (C-3''), 71,50 (C-4''), 78,20 (C-5''), 68,60 (C-6''), 102,40 (C-1'''), 72,27 (C-2'''), 72,48 (C-3'''), 72,06 (C-4'''), 69,73 (C-5'''), 17,80 (C-6''').

Quercetin-3-*O*-β-D-glucopyranoside-α-L-raminoside (Er-2)

^{1}H NMR (400 MHz, CH_3OD), 6,21 (d, *J* = 2,0 Hz, H-6), 6,41 (d, *J* = 2,0 Hz, H-8), 7,67 (d, *J* = 2,0 Hz, H-2'), 6,89 (d, *J* = 8,4 Hz, H-5'), 7,63 (dd, *J* = 8,4; 2,0 Hz, H-6'), 5,10 (d, *J* = 7,6 Hz, H-1''), 3,25-3,63 (m, H-2''), 3,25-3,63 (m, H-3''), 3,25-3,63 (m, H-4''), 3,25-3,63 (m, H-5''), 3,80 (d, *J* = 9,6; Hz, H-6''), 4,52 (d, *J* = 2,0 H-1'''), 3,52-3,63 (m, H-2'''), 3,25-3,63 (m, H-3'''), 3,25-3,63 (m, H-4'''), 3,25-3,63 (m, H-5'''), 1,13 (d, *J* = 6,0 Hz, H-6'''). APT-^{13}C NMR (100 MHz, CH_3OD), 158,65 (C-2), 135,72 (C-3), 179,56 (C-4), 166,25 (C-5), 100,15 (C-6), 166,28 (C-7), 95,05 (C-8), 159,50 (C-9), 105,74 (C-10), 123,25 (C-1'), 117,82 (C-2'), 145,99 (C-3'), 149,97 (C-4'), 116,22 (C-5'), 123,69 (C-6'), 102,55 (C-1''), 75,85 (C-2''), 77,34 (C-3''), 71,53 (C-4''), 78,30 (C-5''), 68,58 (C-6''), 101,40 (C-1'''), 72,23 (C-2'''), 72,37 (C-3'''), 74,06 (C-4'''), 69,86 (C-5'''), 18,02 (C-6''').

Figure 1. Isolated substances of *E. rimosum*



Er-1: R = H
Er-2: R = OH

3. Materials and Methods

The aerial parts (1.0 kg) were oven dried with circulating air at 40 °C for 72 hours. After drying, the plant material was subjected to a grinding process in a mechanical mill, yielding 480 g of dry powder.

The dried and ground vegetable material was subjected to maceration with 95% ethanol (EtOH). Four extraction processes were performed within 72 hours between them. The ethanolic solution obtained was filtered, followed by evaporation of the solvent with the aid of a rotavaporator at 40 ° C. After this solvent evaporation process, the crude ethanolic extract (BSA) was obtained, which weighed 105 g.

The BSE was dissolved in a methanol: water solution (7:3 v/v) and partitioned with hexane (Hex), dichloromethane (DCM) and ethyl acetate (AcOEt) solvents. Providing the phases, hexane (4.0 g), DCM (2.6 g) and AcOEt (10.1 g). The AcOEt phase was subjected to a column chromatography, using as the silica gel stationary phase, and as mobile phase hex, AcOEt and methanol, pure or in binary mixtures, in increasing polarity order. This gives a total of 30 fractions. These fractions were monitored by analytical thin-layer chromatography (TLC) and pooled according to their retention factors (Rfs) after visualization in ultraviolet light. The fractions were collected in groups. After analytical methodology was developed in CLAE-DAD from the meeting of the fractions from 23 to 25 and then the chromatographic separation in preparative HPLC-DAD, the obtained fractions were analyzed by nuclear magnetic resonance (NMR) of ^{1}H and ^{13}C-APT resulting in two substances known as Er-1 and Er-2.

4. Conclusions

From the phytochemical study of the constituents of the crude ethanolic extract of *Erythroxylum rimosum* O. E. Schulz. Two substances were isolated. Through the analysis of the spectra and comparison with literature data the substances were identified as: camferol-3-O-rutinoside and quercetin-3-*O*-β-D-glucopyranoside-α-L-raminoside. They are reported for the first time in the study species, contributing to the chemotaxonomic study of this species.

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Loiola, M. I. B; Agra, M. F.; Baracho, G. S.; Quiros, R. T. da flora Paraibana Brasil: Erythroxylaceae. Acta Botanica Brasilica, v. 21, p. 473-4887, **2007**.

2. Teses e dissertações: Ribeiro, E. M. O.; Estudo fitoquímico de *Erythroxylum rimosum* O. E.Schulz e *Erythroxylum betulaceum* mart. (Erythroxylaceae). Tese (Doutorado em Química) – Instituto de Química, Universidade Federal da Bahia, Salvador-BA, **2011**.