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Phytochemical research of *Cordia rufescens* A.DC

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Abstract: *Cordia rufescens* A. DC, a small shrub in northeastern Brazil is popularly referred to as "old-growth" or "pigeon-claw". This plant is used in folk medicine as abortive, anti-inflammatory agent and in the treatment of dysmenorrhea and dyspepsia (VALE et al., 2012). Thus, the need for a greater chemical and pharmacological knowledge of *Cordia rufescens* A.DC species was observed, necessitating a more in-depth study of the isolation and characterization of new compounds. The plant material (leaves) was collected in 2002 in the municipality of Cruz do Espírito Santo - PB and identified by Profa. Dr. Maria de Fátima Agra. The material was then oven dried with circulating air at an average temperature of 45 ° C, ground in a mechanical mill and subjected to steeping with 95% EtOH. The crude ethanolic extract (106.10 g) was dissolved in a methanol: water (7: 3 v / v) solution and partitioned with the following solvents: hexane, dichloromethane and ethyl acetate. An aliquot of the dichloromethane phase (7 g) was subjected to the chromatographic column (CC), using as a stationary phase silica gel, and as mobile phase hexane, ethyl acetate and methanol, with elution systems in increasing polarity order, obtaining 34 fractions. All fractions were submitted to analytical thin layer chromatography (ADCC) and analyzed and assembled according to their retention factors (R_fs) and after visualization in ultraviolet light. Fractions 2, 3 and 5 presented different retention factors and therefore were submitted to liquid chromatography of high efficiency in analytical scale, where fraction 5 was chosen to obtain a better resolution of the chromatogram in front of the others, being then submitted to the purification by HPLC on a semi-preparative scale that resulted in the identified substances 4-hydroxy-benzaldeído and ethyl 4 -hydroxy-3,5-dimethoxycinnamate, reported for the first time in the species under study.

Keywords: *Cordia rufescens* A. DC; Boraginaceae; Phytochemical study; 4-hydroxy-benzaldeído; ethyl 4 -hydroxy-3,5-dimethoxycinnamate.

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1. Introduction

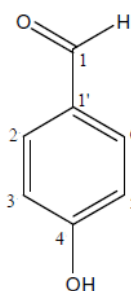
The genus *Cordia* L. has approximately 320 species, which are presented as trees, shrubs or herbs, having as main habituation to South America¹. *Cordia rufescens*, commonly known as "old-growth" or "pigeon-bellied", is a small shrub in northeastern Brazil and, like other species of the *Cordia* genus, is used in folk medicine as an abortifacient, anti-inflammatory agent and in the treatment of dysmenorrhea and

2. Results and Discussion

Purification of fraction 5 by High Resolution Liquid Chromatography (HPLC) led to the isolation of two substances encoded as Crd-1 and Crd-7. The substance encoded as Crd-1 was obtained as an amorphous solid (10 mg). The ¹H NMR spectrum obtained at 400 MHz in CD₃OD showed the presence of 3 signals, these two doublets being around δ_H 7.78 and 6.91 ppm both with J = 8.0 Hz corresponding to the ortho coupling between the hydrogens of the aromatic ring with a characteristic profile of p-disubstituted benzene, and a single with δ_H 9.75 ppm characteristic of hydrogen bound to aldehyde carbonyl. The mass spectrum obtained by EM-AR-IES showed a peak in m / z 121.0301 [M]⁺ compatible with the molecular formula C₇H₆O₂. After analysis of the spectral data and comparisons with data described in the literature it was possible to identify that the compound Crd-1 is 4-hydroxy-benzaldehyde (Figure 1).

The substance encoded as Crd-7 was obtained as an amorphous solid (20 mg). The ¹H NMR spectrum obtained at 400 MHz in CDCl₃ showed the presence of two doublets in δ_H 7.57 and 6.28, with integral for 1H, both with J = 16 Hz attributed to the olefinic hydrogens (H-7 and H-8, respectively) confirming the trans engagement

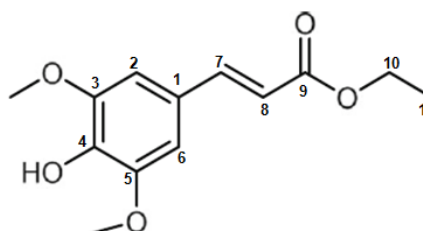
Figure 1. Crd-1: 4-hydroxy-benzaldehyde



dyspesia². Previous chemical studies of *C. rufescens* A.DC identified the presence of saponins, glycosylated steroids, lignan arylnaphthalene and polyphenols^{3,4}. The objective of the present study is to isolate, purify, characterize and identify/ determine fixed chemical constituents of the aerial parts of *Cordia rufescens* A.DC.

between them; a singleton in δ_H 6.75 with integral to 2H, attributed to two aromatic protons (H-2 and H-6); a quartet at δ_H 4.25 with integral for 2H and J = 7.2 Hz attributed to methylene hydrogen (H-10); a triplet in δ_H 1.31 with integral for 3H and J = 7.2 Hz attributed to methyl hydrogens (H-11); and a simpleto in δ_H 3.90 with integral for 6H, corresponding to six methoxylic protons and attributed to two methoxylates (H-3 and H-5). The ¹³C NMR spectrum obtained at 100 MHz in CDCl₃ showed the presence of 10 signals assigned to 13 carbons, being δ_C 14.34 assigned to methyl (C-11); δ_C 56.32 and attributed to methoxylates (C-3 and C-5); δ_C 60.39 assigned to methylene carbon (C-10); δ_C 105.00 assigned to the methinic carbons (C-2 and C-6); δ_C 144.95 and 116.05 assigned to the olefinic carbons (C-7 and C-8, respectively); δ_C 137.05 assigned to non-hydrogenated carbon (C-1); δ_C 147.19 assigned to hydroxyl-bound carbon (C-4); and, δ_C 167.14 assigned to the ester carbonyl. After analysis of the spectral data and comparisons with data described in the literature it was possible to identify the Crd-7 compound as being ethyl 4-hydroxy-3,5-dimethoxy cinnamate (Figure 2).

Figure 2. Crd-7: ethyl 4-hydroxy-3,5 -dimethoxy cinnamate



3. Materials and Methods

The plant material (leaves) was collected in 2002 in the municipality of Cruz do Espírito Santo - PB and identified by Profa. Dr. Maria de Fátima Agra. The material was then oven dried with circulating air at an average temperature of 45°C, ground in a mechanical mill and subjected to steeping with 95% EtOH.

The crude ethanolic extract (106.10 g) was dissolved in a methanol: water (7: 3 v/v) solution and partitioned with the following solvents: hexane, dichloromethane and ethyl acetate. An aliquot of the dichloromethane phase (7 g) was subjected to the chromatographic column (CC), performed on a glass column using Merck silica gel as the stationary phase, and as the mobile phase hexane, ethyl acetate and methanol, with elution systems in order increasing polarity, obtaining 34 fractions.

All fractions were subjected to analytical thin-layer chromatography (ADCC) using chromate plate aluminum-silica gel 60 F254 of Merck, analyzed and pooled according to their retention factors (R_fs) and, after visualization in ultraviolet light under the wave fragments 254 and 366 nm in BOITTON apparatus (model BOIT-LUB01).

Fractions 2, 3 and 5 presented different retention factors and were submitted to analysis

4. Conclusions

The phytochemical study of the constituents of the dichloromethane phase of *Cordia rufescens* A.DC led to the isolation of two substances. Considering the spectral results obtained as well as comparison with the literature data, it was possible to identify Crd-1 as being 4-

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest.

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by high-performance liquid chromatography on an analytical scale, where fraction 5 was chosen to obtain a better resolution of the chromatogram compared to the others, and was then subjected to purification by HPLC on a semi-preparative scale using the Shimadzu system equipped with LC-6AD binary solvent pumping module, SCL-10A injector, SPD-M10A detector with diode arrangement, column composed of reverse phase silica ACE[®] C18, and, mobile phase consisted of (ACN) and acidified water with 0.1% formic acid, in the initial ratio of acidified (5:95) ACN/H₂O. The LC Solution[®] software was used to control the equipment, acquisition and analysis of the data.

After purification, the fractions were subjected to ¹H and ¹³C Nuclear Magnetic Resonance (NMR) analysis, where spectra were recorded on BRUKER spectrometers operating at 400 MHz for hydrogen and 100 MHz for carbon-13 and, by high-resolution mass spectrometry by electron spray ionization where the spectrum was obtained by direct injection using a Trap-amaZonX (Bruker) spectrometer, operating with 3.5 kV capillary voltage, electron spray ionization (ESI) in negative mode.

hydroxy-benzaldehyde and, Crd-7 as ethyl 4-hydroxy-3,5-dimethoxy cinnamate. These are reported for the first time in the study species, contributing significantly to the chemotaxonomic study of this plant.