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ORIGINAL ARTICLE

Apigenin glycoside: an antioxidant isolated from *Alchornea coelophylla* pax & k. Hoffm. (euphorbiaceae) leaf extract

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Abstract

An antioxidant flavonoid has been isolated from methanolic leaf extract of *Alchornea coelophylla* Pax & K. Hoffm. by means of different column chromatography steps with DIAION HP-20 resin and silica gel in combination with analytical high-performance liquid chromatography (HPLC). It was identified as Apigenin-8-*C*-(α-L-rhamnopyranosyl-(1→2)-β-*D*-glucopyranoside) on the basis of spectroscopic analysis and by comparison with related values reported in the literature. This compound exhibited high in vitro antioxidant activity through DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ colorimetric assays with IC_{50} values of 7.528 and 379.7 μg. mL^{-1}, respectively.

Keywords: ABTS$^{\bullet+}$; Column Chromatography; DPPH$^{\bullet}$; Euphorbiaceae; NMR; RP-HPLC.

Introduction

A great variety of molecules exists in nature with the capacity of scavenging free radicals through different mechanisms. Flavonoids are phenolic compounds generally present in plants and fruits. Sometimes, they are responsible for some flower colours such as red, pink, and purple or violet [1] and play biological roles as preventing damages by continuous exposure to UV radiation [2].

Flavonoids display their noteworthy antioxidant activity by three different mechanisms, (*i*) hydrogen transfer to radical type compounds [3], (*ii*) prevention of Fenton type reactions due to metal chelation [4] and (*iii*) synergist effects with other antioxidant compounds [5]. These mechanisms could explain the wide range of biological activities attributed to flavonoids.

A great number of ethnopharmacological applications of species belonging to the *Alchornea* genus is reported in tropical Africa and some regions of central Brazil [6, 7]. The activities are antiulcerogenic, anti-inflammatory, hepatoprotective, antibacterial and cytotoxic, attributed to flavonoids present in *Alchornea castaneafolia* [8], *Alchornea floribunda* [7], *Alchornea glandulosa* [9], *Alchornea laxiflora* [10], *Alchornea triplinervia* [11]

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and *Alchornea cordifolia* [12, 13]. Our aim was to investigate the antioxidant properties of *Alchornea coelophylla*, a wild plant endemic to Colombia with no previous reports on antioxidants and to isolate compounds related to the assessed biological activity.

Materials and Methods

General Methods

Column chromatography was carried out on silica gel (230-400 mesh, Macherey Nagel, Düren, Germany) and macro-porous resin DIAION HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan). Analytical high performance liquid chromatography (HPLC) was carried out on an Agilent 1100 Series liquid chromatograph equipped with an Agilent DAD-G1315A detector for the UV spectrum acquisition (Agilent Technologies, California, United States) and the data acquisition was done using ChemStation (Version B.04.03). Columns RESTEK Ultra AQ C18 (3 µm, 100 x 3.2 mm, RESTEK, Pennsylvania, United States) and Agilent ODS Hypersil (5 µm, 250 x 4 mm, Agilent Technologies, California, United States) were used for analytical purpose. Thin-layer chromatography (TLC) was conducted over pre-coated silica gel $60F_{254}$ plates (Merck, Darmstadt, Germany) and spot detection was performed under UV light (λ = 254 and 366 nm) and then spraying with 1 % $AlCl_3$ in ethanol (EtOH) (Sigma-Aldrich, Missouri, United States). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DRX-400 NMR Spectrometer (Bruker Biospin Gmbh, Rheistetten, Germany) with solvent residual peaks of dimethyl sulfoxide (DMSO)-d_6 at δ_H 2.5 and δ_C 39.52 as references.

Antioxidant colorimetric assays using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ (Sigma-Aldrich, Missouri, United States) and phenolic and flavonoid quantifications, using Folin-Ciocalteu & $AlCl_3$ (Sigma-Aldrich, Missouri, United States), respectively, were assessed on a microplate spectrometer Multiskan GO (Thermoscientific, Massachusetts, Unites States). Infrared Spectroscopic data were acquired in an Agilent Cary 630 FTIR (Agilent Technologies, California, United States) with a Windows compatible version of MicroLab Software (Agilent Technologies, California, United States).

Plant Material

Leaves and twigs of *Alchornea coelophylla* (Euphorbiaceae) were collected in a natural protected zone known as Bremen-La Popa at the coordinates 4º 40' 48.6" North and 75º 37' 32.7" West at 6737 feet above sea level, near to the municipality of Filandia (Quindío, Colombia), this procedure was completed under the contract of access to genetic sources for scientific research without commercial interest number 56 of February 28TH of 2016 granted by the Ministry of Embironment. A voucher specimen was deposited at the Herbarium of the Universidad de Antioquia (Medellín, Colombia), labelled as FJR-3969. The botanical identification was done by Professor Francisco Javier Roldan from Universidad de Antioquia, Medellín, Colombia.

Extraction and Isolation

The collected plant material (leaves and twigs) was dried in a laboratory stove at 50 ºC and further ground with a hammer mill (Nogueira (Ø: 5.1 mm)). Afterwards the dried material (1.212 g) was exhaustively extracted at room temperature allowing it to stand overnight using 10 L of *n*-hexane (HEX), dichloromethane (DCM) and methanol

(MeOH), with an increasing polarity order. The concentration of the respective solutions under vacuum produced the respective dried extracts.

Fifteen grams of methanolic leaf extract were subjected to open column chromatography with DIAION HP-20 resin (200 g, 74.5 x 90 mm) and eluted with MeOH-H_2O mixtures (0:100, 20:80, 40:60, 60:40, 80:20, 100:0 (v/v) with 1.5, 1.25, 5.55, 4.6, 3.7 and 2 L, respectively). After pooling related fractions according to their TLC profiles, 29 fractions (DHP-1 to DHP-29) were finally obtained. Afterwards, a first exclusion step was applied based on the mass availability of each fraction. In this sense, 17 fractions with a mass greater than 300 mg were selected to continue the bio-guided isolation process. Thus, based on the correlation among mass availability and further antioxidant data, fraction DHP-11 (750 mg) were seeded onto a silica gel column (22.5 g, 20 x 150 mm) and then washed with approximately 10 bed volumes (BV) of chloroform ($CHCl_3$) and ethyl acetate (EtOAc) to eliminate low polarity impurities, to finally elute the sample with EtOAc-MeOH-AcOH-H_2O (70:20:5:5) collecting fractions DHP-11-A to DHP-11-G. The presence of the target compound in the fraction DHP-11-C was confirmed by analytical RP-HPLC using a 100 % MeOH system as the mobile phase at a flow rate of 1 mL. min^{-1} at a retention time of 1.010 min. Spectral data were also recorded.

Antioxidant Activity

The antioxidant activity was evaluated along the isolation process to ensure the isolation of a biologically active flavonoid. In this sense, biological activities were assessed on the crude extract and fractions of the first chromatographic purification step following the methodology described by Brand-Williams & Berset [14] and Re [15] for the antioxidant assays of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, respectively, with minor modifications in order to perform the experiments in a 96-well microplate. Measurements were done by triplicate and with two different repetitions in order to obtain statistically homogeneous results. IC_{50} determinations and calibration curves were constructed in the same way and analysed with GraphPad Prism V. 5.01.

Methanolic crude extract and chromatographic fractions were evaluated at concentrations of 1,000 and 500 μg. mL^{-1}, respectively. A 1,000 μg. mL^{-1} methanolic solution of hydroquinone, prepared the same day of the test, was used as positive control. Analogously a photometric blank to each sample extract was employed additionally with the photometric blank (the respective solvent mixture).

$DPPH^{\bullet}$ Radical Assay

100 μL of a 20 μg. mL^{-1} $DPPH^{\bullet}$ solution in MeOH (prepared just before the assay) were thoroughly mixed with 25 μL of the sample. The reaction was allowed to stand for 30 minutes in the absence of light and then the absorbance was measured at a wavelength of 517 nm. The photometric blank for the plant extracts consisted of 25 μL of the sample and 100 μL of MeOH.

$ABTS^{\bullet+}$ Radical Assay

An aqueous 3.5 mM $ABTS^{\bullet+}$ and 1.25 mM potassium persulfate solution was allowed to react 12 hours before the evaluation, then the absorbance of the final solution was adjusted to 0.7 with EtOH. Finally, 294 μL of the adjusted solution was added to each well where 6 μL of the sample extract had been transferred previously. The reaction

was allowed to stand for 30 minutes in the darkness and at that time the absorbance was measured at 732 nm. The photometric blank for plant extract consisted in 6 μL of the sample and 294 μL of EtOH.

In order to be able to compare and report antioxidant activity results as equivalents of the same reference standard, calibration curves of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) were constructed with concentrations ranging from 1 μM to 100 μM, using it as antioxidant reference compound [16].

Additional to the determinations of the biological activity, quantifications of phenolic compounds and flavonoids were considered, in order to determinate which could be the most promising fraction to continue the isolation process.

Quantification of Phenolic Compounds

This determination was completed following the Folin-Ciocalteu method, described by Magalhães [17]. A. coelophylla extracts and fractions were evaluated at 50 μg. mL^{-1} in methanol. The Folin-Ciocalteu reactive was also diluted in a proportion 1:50 with distilled water to adjust the absorbance of the solution into the range of 0.2 to 0.8. Then, 100 μL of NaOH 0.35 M were transferred to the same wells where previously 50 μL of the plant extracts and 50 μL of the diluted Folin-Ciocalteu reactive had been mixed. Afterwards, the reaction was allowed to stand for three minutes in the absence of light and the absorbance was measured at 760 nm in a microplate spectrometer MultiSkan Go.

In this determination the blank for each sample was also used and the photometric blank corresponded to 200 μL of water. Finally, to express the results as equivalents of some comparable parameter [17], a calibration curve of gallic acid was constructed at concentrations of 0, 2, 4, 8 and 16 μg. mL^{-1}.

Quantification of Flavonoid Content

The flavonoid content was assessed following the methodology described by Kim [18]. The sample extracts were evaluated at a concentration of 100 μg. mL^{-1} in methanol. First, 7.5 μL of 5 % aqueous $NaNO_2$ were transferred to each well where the extracts were going to be evaluated. Then, 20 μL of each plant extract and 115 μL of distilled water were then pipetted. After 5 minutes of reaction, 30 μL of 2.5 % $AlCl_3$ were added while shaking; then, once 5 minutes of reaction took place, 50 μL of NaOH 1 M and 50 μL of distilled water were added and thoroughly mixed. Finally, 5 minutes after the NaOH addition the absorbance was measured at 500 nm.

Finally, in the same way of the phenolic compounds determination described above and to obtain these results as equivalents of some comparable parameter, a calibration curve with kaempferol was constructed at concentrations of 0, 0.05, 0.1, 0.2, 0.4 and 0.8 μg. mL^{-1}.

Results

Determination of Antioxidant Activity and Bio-guided Isolation

The dried and grounded plant material of *Alchornea coelophylla* (1.212 g) subjected to successive extraction by maceration with hexane, dichloromethane and methanol, gave 91.0997 g of methanolic dried extract.

A phytochemical screening of the plant extracts revealed that the major flavonoid content occurred in the most polar methanolic leaf extract, which was fractionated in a first chromatographic partition based on polarity and molecular size with DIAION HP-20 resin, through which 29 fractions (DHP-1 to DHP-29) were collected. Mass and yield fractions are shown in **Table 1**.

Antioxidant activities were assessed over the 29 fractions through both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays, in order to determine which fraction could be the most promising for the isolation process. Similarly, total phenolic and flavonoids contents were determined. **Figure 1** summarizes the values of above mentioned evaluations for all the chromatographic fractions.

Table 1. Masses and yields for fractions of the first DIAION HP-20 chromatographic step.

Fraction	Mass [g]	Yield (%)	Fraction	Mass [g]	Yield (%)
DHP-1	1.4575	9.71	DHP-16	0.0694	0.46
DHP-2	0.3108	2.07	DHP-17	0.3963	2.64
DHP-3	0.1995	1.33	DHP-18	0.0363	0.24
DHP-4	0.093	0.62	DHP-19	0.8514	5.68
DHP-5	0.0137	0.09	DHP-20	0.2582	1.72
DHP-6	0.0219	0.15	DHP-21	0.3408	2.27
DHP-7	0.3066	2.04	DHP-22	0.0763	0.51
DHP-8	0.3229	2.15	DHP-23	0.6604	4.40
DHP-9	0.2471	1.65	DHP-24	0.0857	0.57
DHP-10	0.7945	5.30	DHP-25	0.1348	0.90
DHP-11	0.9553	6.37	DHP-26	0.0832	0.55
DHP-12	0.3628	2.42	DHP-27	0.1998	1.33
DHP-13	1.0863	7.24	DHP-28	0.5339	3.56
DHP-14	0.1552	1.03	DHP-29	1.0736	7.16
DHP-15	0.0773	0.52	Total Yield (%)		74.69

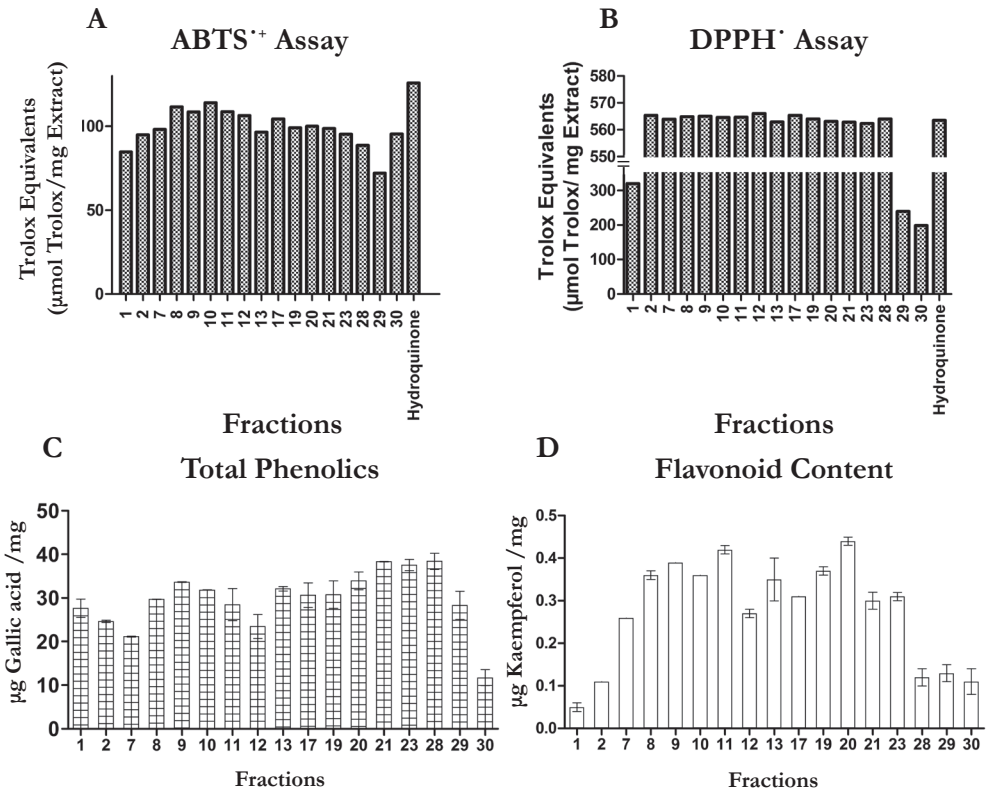

Figure 1. Determinations of the first column chromatographic fractions. **A)** Antioxidant activity through ABTS$^{\bullet+}$; **B)** Antioxidant activity through DPPH$^{\bullet}$; **C)** Total phenolic content and **D)** Total flavonoid content.

After data collection, fraction 11 was selected and subjected to a second Silica gel 0.04-0.063 mm/ 230-400 mesh column to obtain 7 fractions (DHP-11-A to DHP-11-G). Finally the target compound was confirmed to be with a high grade of purity (> 98 % based on HPLC data) in fraction DHP-11-C, fraction that was then used in the spectroscopic data acquisition. Masses and yields of fraction of the second column chromatography are presented in **Table 2**. IC_{50} values of the compound present in fraction DHP-11-C were 7.528 and 379.7 µg. mL^{-1}, through DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays, respectively.

Structural Elucidation of Isolated Compound

Apigenin-8-C-(α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside). Obtained as a yellowish powder. **IR** cm^{-1}: 3400, 2970, 2925, 1700, 1650, 1600, 1560, 1500, 1430, 1350, 1300, 1260, 1200, 1175, 1025, 830, 800. **^{1}H NMR (DMSO-d$_6$, 400 MHz)** δ: 6.74 (1H, *s*, H-3), 6.28 (1H, *s*, H-6), 8.01 (2H, *d, J=8.464* Hz, H-2', H-6'), 6.93 (2H, *d, J=8.475* Hz, H-3', H-5'), 4.77 (1H, *d, J=10.041* Hz, H-1"), 4.95 (1H, s, H-1""), 2.85-4.10 (10H, *m*), 0.46 (3H, *d, J=5.911* Hz, H-6""). **^{13}C NMR (DMSO-d$_6$, 100 MHz)** δ: 164.00 (C-2), 102.43 (C-3), 182.10 (C-4), 161.20 (C-5), 98.29 (C-6), 162.34 (C-7), 104.47 (C-8), 155.83 (C-9), 104.22 (C-10), 121.6 (C-1'), 128.99 (C-2'), 115.89 (C-3'), 160.67 (C-4'), 115.89 (C-5'), 128.99 (C-6'), 70.67 (C-1"), 75.09 (C-2"), 79.90 (C-3"), 70.46 (C-4"), 81.81 (C-5"), 61.17 (C-6"), 100.32 (C-1""), 70.26 (C-2""), 68.22 (C-3""), 71.68 (C-4""), 71.49 (C-5""), 17.71 (C-6"").

Table 2. Masses and yields for fractions of the second Silica gel chromatographic step.

Code	Mass [mg]	Yield (%)
DPH-11-A	283.3	37.7
DPH-11-B	181.4	24.2
DPH-11-C	114.4	15.2
DPH-11-D	24.3	3.2
DPH-11-E	34.5	4.6
DPH-11-F	34.3	4.6
DPH-11-G	12.8	1.7
Total Yield (%)		91.2

Discussion

Determination of Antioxidant Activity and Bio-guided Isolation

The methanolic crude extract obtained by maceration gave a yield of 7.52 %.

The MeOH leaf extract was dissolved in methanol and fractionated by open column chromatography with DIAION HP-20 resin to obtain 29 fractions (DHP1 to DHP29) which then were classified and selected by mass, yielding 17 fractions with an availability greater than 300 mg. Afterwards, the antioxidant activity of the selected fractions was evaluated by the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ methods at 500 μg. mL^{-1}; in addition, the above quantifications were assessed to obtain more information that could suggest the most promising fraction to continue the isolation process.

With all the collected information regarding biological activities, and taking into account the amount available of each fraction, the 11th fraction was selected as the most promising to continue the isolation process with a mass of 955.53 mg, with 51.85 % of antioxidant activity under the $DPPH^{\bullet}$ assay, 99.75 % of antioxidant activity under the $ABTS^{\bullet+}$ test, 28.49 μg. mL^{-1} of gallic acid equivalents and 0.42 μg. mL^{-1} of kaempferol equivalents. As a consequence, this fraction was subjected to a silica gel column fractionation yielding fractions DHP-11-A to DHP-11-G, which finally by analytical HPLC revealed the presence of a flavonoid glycoside in the fraction DHP-11-C.

Thus, based on the antioxidant percentage values obtained for fraction DHP-11-C and using the TEAC calibration curves, total equivalents of Trolox (μmol. mg-1) were found to be 108.7 and 564.67 for the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays, respectively.

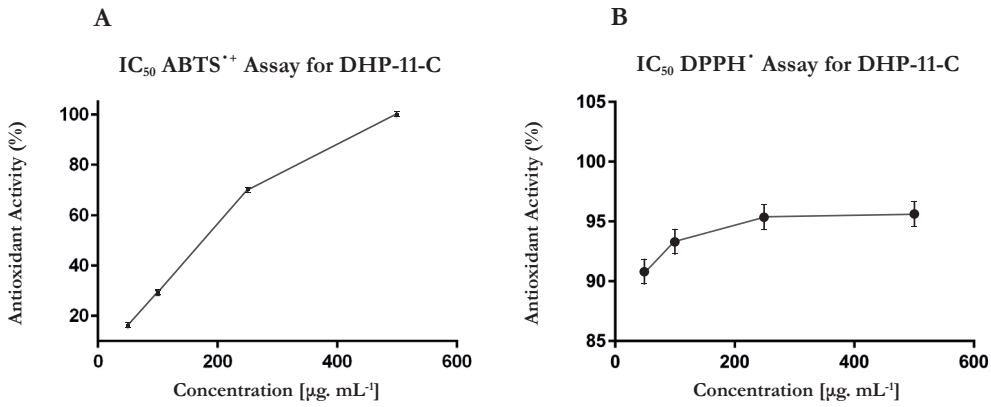

Figure 2. IC_{50} assay with DPH-11-C for **A)** $ABTS^{\bullet+}$ and **B)** $DPPH^{\bullet}$.

Once the purity of the isolated compound was determined, the median inhibitory concentration (IC_{50}) was calculated for both of the above mentioned assays. The biological activity was assessed on the isolated compound at concentrations of 500, 250, 100 and 50 µg. mL^{-1} for both the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ methods. IC_{50} values of 7.528 and 379.7 µg. mL^{-1} were obtained for the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ determinations, respectively. Those results agree with previous investigations in which it was found that the methanolic crude extract of A. coelophylla had an IC_{50} even lower than the reference control (Hydroquinone), which revealed IC_{50} of 41.14 and 151.19 µg. mL^{-1} for both antioxidant colorimetric assays and also that the antioxidant response was notoriously higher when evaluated through the $ABTS^{\bullet+}$ determination than the antioxidant values obtained for the $DPPH^{\bullet}$ determination [19]. Curves constructed for IC_{50} determinations of the isolated compound are in **Figure 2**.

Structural Elucidation of Isolated Compound

The isolated compound, present in fraction DHP-11-C, was obtained as a yellowish powder in which 1H NMR spectrum showed two singlets at δ 6.74 and 6.28 (1H each, H-3 and H-6, respectively), four aromatic protons at δ 6.93 (2H, *d, J=8.475* Hz, H-3', H-5') and 8.01 (2H, *d, J=8.464* Hz, H-2', H-6') indicating *para*-substitution pattern at B aromatic ring, suggesting the presence of the apigenin moiety (See **Figure 3**). Additionally, the spectrum displayed two anomeric protons at δ 4.77 (1H, *d, J=10.041* Hz, H-1") and 4.95 (1H, *s*, H-1"") and one methyl signal at δ 0.46 (3H, *d, J=5.911* Hz, H-6"") indicating the presence of a *β*-glucosyl and *α*-rhamnosyl moieties [20]. The ^{13}C NMR spectrum displayed signals of a carbonyl group at δ 182.10 (C-4), 14 aromatic carbons ranging 98.29 – 162.34, 2 anomeric carbons at δ 70.67 (C-1") and 100.32 (C-1""), 8 oxygenated carbons ranging from δ 68.22 to 79.9, one methyl group at δ 17.71 (C-6"") and one aliphatic methylene at δ 61.17 (C-6") (with negative phase (↓) in DEPT 135º spectrum). These data indicated that the compound correspond to an apigenin diglycoside [21, 22]. Shifting of C-8 signal downfield up to 104.47, absence of a H-8 signal and anomeric shifting of glucose suggest that glycosidic linkage is a C-linkage over such position. Finally, these findings could be supported with literature reported for the structure described and related compounds found in other plant species [23, 24, 25, 26].

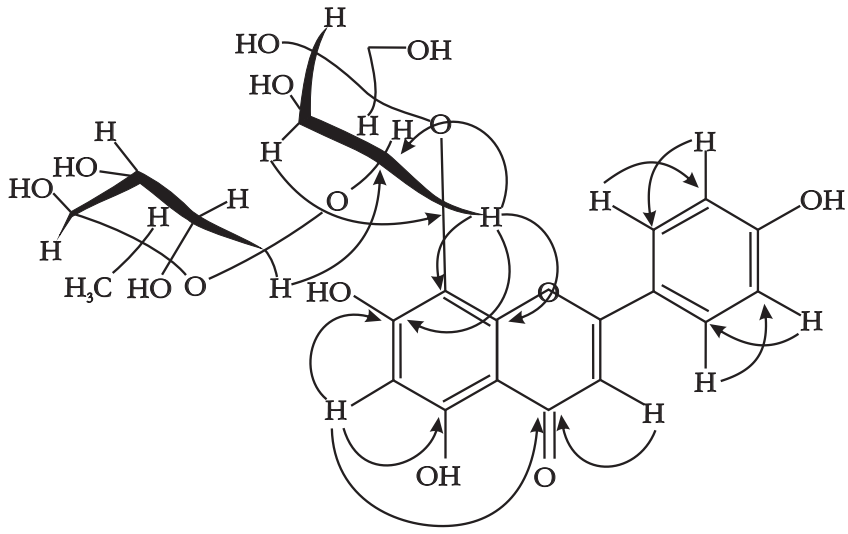

Figure 3. 2D NMR interactions observed in HMBC experiment for the isolated compound.

As displayed in Figure 3, in the heteronuclear multiple-bond correlation (HMBC) spectrum, long range correlations were detected from H-1" (δ 4.77) to C-7 (δ 162.34), C-8 (δ 104.47) and C-9 (δ 155.83), from H-1" (δ 4.77) and H-1"' (δ 4.95) to C-2" (δ 75.09). Correlations involving aromatic protons were also observed. Thus, H-3 (δ 6.74) correlates to carbonyl (δ 182.10) and H-6 (δ 6.28) detected C-7, C-5 (δ 161.20) and C-4. Also symmetric correlations inside the B aromatic para-substituted system H-2',6' (δ 8.01) to H-3',5' (δ 6.93).

Finally, based on the spectroscopic data, bibliographic revisions and comparisons with theoretical predictions of the NMR spectra, the structure of the target compound would be apigenin-8-*C*-(α-L-rhamnopyranosyl-(1→2)-β-*D*-glucopyranoside), illustrated in **Figure 4**. This compound could also receive the name of Apigenin-8-*C*-neohesperoside and vitexin-2"-*O*-rhamnoside.

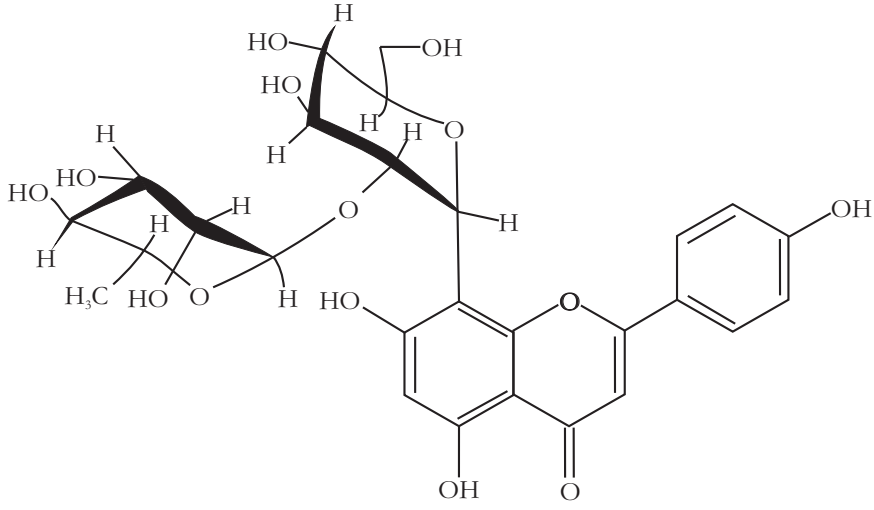

Figure 4. Apigenin-8-*C*-(α-L-rhamnopyranosyl-(1→2)-β-*D*-glucopyranoside) isolated from MeOH extract of *Alchorea coelophylla* (Euphorbiaceae).

Based on the proposed structure upon NMR spectroscopic data, this article is the first report of isolation of this compound from any Euphorbiaceae species [24, 25, 26, 27, 28, 29, 30].

Conclusions

An apigenin glycoside was isolated from the defatted MeOH leaf extract of *Alchornea coelophylla* (Euphorbiaceae). Its structure was elucidated by 1D and 2D NMR spectroscopy experiments. The compound corresponds to Apigenin-8-*C*-(α-L-rhamnopyranosyl-(1→2)-β-*D*-glucopyranoside) which showed moderate to potent antioxidant activity with an IC_{50} of 7.528 and 379.7 μg. mL^{-1} to $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays, respectively.

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Conflicts of Interest

The authors declare no competing financial interests.

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Glicósido de apigenina: un antioxidante aislado del extracto de hoja de *Alchornea coelophylla* Pax & K. Hoffm. (Euphorbiaceae)

Resumen. Se aisló un flavonoide antioxidante del extracto metanólico de hojas de *Alchornea coelophylla* Pax & K. Hoffm. El compuesto se obtuvo por medio de sucesivas cromatografías en columna (con la resina DIAION HP-20 y sílica gel), seguidas de cromatografía líquida de alta eficiencia (HPLC). Con base en el análisis espectroscópico y por comparación con valores relacionados reportados en la literatura, el flavonoide se identificó como Apigenin-8-*C*-(α-L-rhamnopiranosil-(1→2)-β-*D*-glucopiranósido). Este compuesto presentó una alta actividad antioxidante in vitro: ensayos colorimétricos utilizando DPPH$^{\bullet}$ y ABTS$^{\bullet+}$ mostraron valores de IC_{50} de 7.528 y 379.7 μg. mL^{-1}, respectivamente.

Palabras clave: ABTS$^{\bullet+}$; Cromatografía en columna; DPPH$^{\bullet}$; Euphorbiaceae; NMR; RP-HPLC.

Glicosídeo de apigenina: um antioxidante do extrato das folhas de *Alchornea coelophylla* Pax & K. Hoffm. (Euphorbiaceae)

Resumen. Um flavonoide antioxidante foi isolado do extrato metanólico das folhas de *Alchornea coelophylla* Pax & K. Hoffm. por meio de diferentes etapas de cromatografia em coluna usando resina DIAION HP-20 e gel de sílica em combinação com Cromatografia Líquida de Alta Eficiencia (HPLC) analítica. O composto foi identificado como apigenina-8-*C*-(α-L-ramnopiranosil-(1→2)-β-*D*-glicopiranosido) com base em análises espectroscópicas e por comparação com valores descritos na literatura. Este composto exibiu elevada atividade antioxidante in vitro por meio de ensaios colorimétricos por DPPH$^{\bullet}$ e ABTS$^{\bullet+}$ com valores de IC_{50} de 7,528 e 379,7 μg.mL^{-1}, respectivamente.

Palabras clave: ABTS$^{\bullet+}$; cromatografia em coluna; DPPH$^{\bullet}$, Euphorbiaceae, RMN; RP-HPLC.

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