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Phytochemical Study and Antioxidant Evaluation of *Commelina erecta* (Commelinaceae) Stems

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Estudo Fitoquímico e Avaliação Antioxidante dos Caules de *Commelina erecta* (Commelinaceae)

Resumo: O estudo fitoquímico dos extratos em acetato de etila e em etanol dos caules de *Commelina erecta* levou à identificação de luteolina (**1**), isoquercitrina (**7**) quercitrina (**8**), lactona do ácido sacarínico (**2**), três esteroides glicosilados (**3-5**) e ácido chiquímico (**6**). A caracterização química desses compostos foi realizada com base nos dados de EM e RMN, bem como em comparação com a literatura. O presente trabalho é o primeiro relato dessas substâncias em *C. erecta*. Adicionalmente, revelou esta espécie como uma interessante fonte natural de ácido chiquímico. O teor de fenois totais, juntamente com os ensaios de DPPH e ABTS dos extratos foram também avaliados.

Palavras-chave: Isoquercitrina; luteolina; quercitrina; ácido chiquímico; sequestro de radical.

Abstract

Phytochemical study of ethyl acetate and ethanolic extracts from *Commelina erecta* stems led to the identification of luteolin (**1**), isoquercitrin (**7**) quercitrin (**8**), saccharinic acid lactone (**2**), three sterol glycosides (**3-5**), and shikimic acid (**6**). Chemical characterization of these compounds was established based on Mass Spectrometry and Nuclear Magnetic Resonance data, as well as in comparison with the literature. The present work is the first to report such substances in *C. erecta*. Furthermore, it revealed this species as an interesting natural source of shikimic acid. Total phenol content, along with DPPH and ABTS assays of the extracts were also evaluated.

Keywords: Isoquercitrin; luteolin; quercitrin; shikimic acid; radical scavenging.

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Phytochemical Study and Antioxidant Evaluation of *Commelina erecta* (Commelinaceae) Stems

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1. Introduction

The chemotaxonomic study with leaves of 152 species of Commelinaceae showed the presence of *C*-glycosylated flavones and *O*-glycosylated flavonols. The most common *C*-glycosylated flavones were derived from apigenin and luteolin, being the *O*-glycosylated flavonols derivatives of kaempferol, isorhamnetin and quercetin.[1] Previous chemical and pharmacological

investigations of *Commelina* species revealed the presence of bioactive compounds such as alkaloids, phenolics acids, lignins and glycosylated flavonoids, as well as antimicrobial, antioxidant, antiviral and anti-hyperglycemic properties by inhibiting α-glycosidase.[2-6]

Commelina erecta L. (Commelinaceae) is an herbaceous flowering plant, popularly known as "trapoeraba" and "erva-de-santa-luzia". Originally developed in the tropics, *C. erecta* has adapted to subtropical and temperate climates, multiplying by cuttings and seeds which makes it as an invasive species. In folk medicine, *C. erecta* has been used as antiviral, for the treatment of hemorrhage, skin rashes and sores as well as for the treatment of infections. Traditional people from Amazon commonly use *C. erecta* as eye drops for treating ocular infection. Works in the literature have reported *C. erecta* extracts showed inhibition of the growth *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtillis* and *Pseudomonas aeruginosa*.[7-11]

In the SciFinder database no reports were found on chemical composition and antioxidant activity of the ethyl acetate and ethanolic extracts from *C. erecta* stems. In our continuous search for bioactive compounds, this work describes for the first time the phytochemical investigation from the stems of *C. erecta*.

2. Materials and Methods

2.1. Plant material

Stems of *C. erecta* were collected in August 2011 on the North sector Campus of Federal University of Amazonas (UFAM) [GPS coordinates: S 3º06'00.78", W 59º58'28.84", 84 m] Manaus, Amazonas, Brazil. A voucher specimen was identified by Prof. Dr. A. C. Webber and deposited in the UFAM herbarium under the code HUAM 9236.

2.2. Obtaining extracts and fractionation

Dried and pulverized stems (311.9 g) were extracted five times with *n*-hexane, ethyl acetate and ethanolic (1000 mL each solvent) to yield hexane extract (4.7 g), ethyl acetate extract (1.6 g) and ethanolic extract (3.2 g). Part of the ethyl acetate extract (1.3 g) was submitted to silica gel column chromatography (30.0 x 2.0 cm) eluted with hexane and ethyl acetate mixture (from 100:0 to 10:90, v/v) followed by ethyl acetate and methanol mixture (from 100:0 to 20:80 v/v), yielding 35 fractions (30 mL). The fractions were analyzed by TLC and pooled in 10 fractions (1.1-1.10). Fraction 1.4 (69.4 mg) was subjected to Sephadex LH-20 column (20.0 x 1.5 cm) with methanol yielding 7 fractions pooled in 4 sub-fractions (1.4.1-1.4.4). In the sub-fraction 1.4.4 was identified **1** (9.3 mg). Fraction 1.6 (94.7 mg) was submitted to silica gel column chromatography (20.0 x 1.5 cm) eluted with hexane and ethyl acetate mixture (from 100:0 to 0:100, v/v) yielding five sub-fractions (1.6.1-1.6.5). Sub-fraction 1.6.3 (13.6 mg) presented mostly compound **2**. Fraction 1.7 (272.2 mg) showed a white solid which was separated and identified compounds, **3**-**5** (34.2 mg). Part of the ethanolic extract (2.0 g) was submitted to Sephadex LH-20 column (30.0 x 2.0 cm) eluted with methanol affording 16 fractions (30 mL) which were evaluated and pooled according to TLC analysis yielding in six fractions (2.1-2.6). In the fraction 2.4 (447.6 mg) was identified the compound **6**. Fraction 2.5 (369.8 mg) was submitted to silica gel column (30.0 x 2.0 cm) eluted with hexane and ethyl acetate mixture (from 100:0 to 0:100, v/v) yielding 10 fractions pooled in 4 sub-fractions (2.5.1-2.5.4). In the sub-fraction 2.5.2 (177.4 mg), which was once more purified on Sephadex LH-20 column with methanol identified compounds **7** and **8** (15.9 mg).

2.3. Identification of the constituents

All compounds were identified by Mass Spectrometry - MS and/or one and two-dimensional Nuclear Magnetic Resonance - 1D and 2D NMR experiments and in comparison, with the literature. Low-resolution ESI-MS analyses were acquired in the positive or negative ion mode, on the Thermo Scientific an LCQ Fleet or LTQ XL ion trap mass spectrometers. NMR experiments were acquired in $CDCl_3$ or CD_3OD at 303 K on a Bruker AVANCE III 600 NMR spectrometer operating at 14.1 Tesla, observing ^1H and ^{13}C at 600.13 MHz and 150.90 MHz, respectively, equipped with a 5-mm quadrinuclear inverse detection probe with z-gradient. ^1H HR-MAS NMR spectra were acquired at 293 K directly from stems on a Bruker AVANCE 400 NMR spectrometer (9.4 Tesla), observing ^1H nuclei at 400.13 MHz. A 4-mm trinuclear HR-MAS probe with the gradient on magic angle direction was used. For spectra acquisition, stems were frozen in liquid nitrogen and powdered on a mortar and 4 mg inserted in a 50 µL HRMAS rotor followed by the addition of D_2O drops containing 0.1 % TMSP-d_4 and spun at 5 KHz on magic angle.[12] Chemical shifts (δ) were expressed in ppm and coupling constants (J) in Hertz. NMR spectra were referenced by TMS or TMSP-d_4 at 0.00 ppm.

2.4. DPPH and ABTS radical scavenging activity

For DPPH radical scavenging assay was performed as described in the literature with modifications.[13] Brief, 100 µL of methanolic DPPH solution (0.8 mM), 30 µL samples (1 $mg.mL^{-1}$) and 170 µL of ethanol were gently mixed and incubated in dark for 30 min at room temperature (sample abs). Them the absorbance of resulting solutions was measured at 490 nm on a microplate reader (DTX800, Beckman). Values denote percentage of DPPH free radical inhibition. The ABTS radical scavenging assay was performed as described in the literature with modifications.[14] ABTS radical was generated by reacting ABTS (0.7 mM) and potassium persulfate ($K_2S_2O_8$ at 2.4 mM) and then storing in the dark for 16 h at room temperature. On a microplate 30 µL samples (sample abs) were added followed by 270 µL of ABTS solution adjusting for absorbance of 1.000 ± 0.1 at 620 nm. After incubation in the dark for 15 min at room temperature the absorbances was measured. Values denote percentage of ABTS free radical inhibition. For both assays were performed ethanol was used as negative control (control abs) and quercetin as positive control. The Free Radical Scavenging was calculated using the equation: % inhibition = 100 × (1 − sample abs/control abs). The assays were performed in triplicate.

2.5. Total phenolics content

Total phenolic contents of the extracts were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method with modifications.[15] For this, in a microplate 10 µL of extracts at 1 $mg.mL^{-1}$ were added, followed by addition of 50 µL Folin-Ciocalteu's reagent (1:10) and then incubated in the dark for 8 min at room temperature. After, a sodium carbonate solution (0.4 %) was added and newly incubated for three min and the absorbance was measured at 620 nm. The total phenolic content was expressed as gallic acid miliequivalents (mGAE/g extract).

3. Results and Discussions

Phytochemical investigation of ethyl acetate and ethanolic extracts from *C. erecta* stems led to the structural identification of the flavonoids luteolin (**1**), isoquercitrin (**7**) and quercitrin (**8**), shikimic acid (**6**), saccharinic acid lactone (**2**), a mixture of three glucosides steroids, sitosterol (**3**),

stigmasterol (**4**) and campesterol (**5**) that were characterized by spectroscopic methods and according literature data (Figure 1).[16-21] Except for the sitosterol-*β*-D-glucoside mixture all constituents identified here is being reported for the first time in this species. In the family Commelinaceae flavone derivatives are common, mainly in their glycoside forms.[1] The isoquercitrin was previous described in *C. communis*.[2]

Luteolin (1): yellow amorphous solid. ^{1}H NMR (600 MHz, $CDCl_3$, CD_3OD): δ (ppm) = 7.38 (d, *J* = 2.2 Hz, 1H, H-2'), 7.36 (dd, *J* = 8.2 and 2.2 Hz, 1H, H-6'), 6.92 (d, *J* = 8.2 Hz, 1H, H-5'), 6.24 (d, *J* = 2.2 Hz, 1H, H-6), 6.44 (d, *J* = 2.2 Hz, 1H, H-8), 6.50 (s, 1H, H-3); MS: *m/z* 285 $[M-H]^-$ $[C_{15}H_{10}O_6]$.

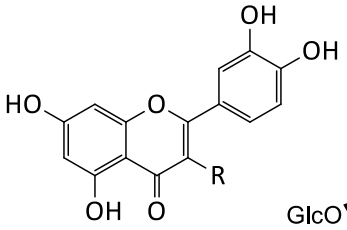
Saccharinic acid lactone (2): ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) = 4.36 (dd, *J* = 10.5 and 3.4 Hz, 1H, H-4a), 4.33 (dd, *J* = 10.5 and 1.0 Hz, 1H, H-4b), 4.15 (dd, *J* = 3.4 and 1.0 Hz, 1H, H-3), 1.47 (s, 3H, CH_3-5). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) = 178.1 (C-1), 73.9 (C-3), 73.1 (C-2), 72.1 (C-4), 21.7 (C-5).

Sterol glucosides, sitosterol (3), stigmasterol (4) campesterol (5): ^{1}H-NMR (600 MHz, CD_3OD): δ (ppm) = 5.37 (m, 1H, H-6), 5.17 (dd, *J* = 15.1 and 8.7 Hz, 1H, H-22), 5.04 (dd, *J* = 15.1 and 8.7 Hz, 1H, H-23), 3.59 (m, 1H, H-3), 4.38 (d, *J* = 7.8 Hz, 1H, H-1'), 3.85 (dd, *J* = 12.0 and 2.2 Hz, 1H, H-6'a), 3.66 (dd, *J* = 12.0 and 5.3 Hz, 1H, H-6'b), 3.36 (m, 1H, H-3'), 3.35 (m, 1H, H-2'), 3.27 (dd, *J* = 5.3 and 2.2 Hz, 1H, H-5'), 3.16 (m, 1H, H-4'). MS: *m/z* 599 $[M+Na]^+$ $[C_{35}H_{60}O_6]$; *m/z* 597 $[M+Na]^+$ $[C_{35}H_{58}O_6]$; *m/z* 585 $[M+Na]^+$ $[C_{34}H_{58}O_6]$.

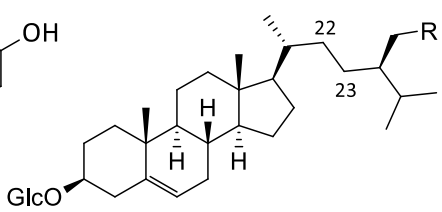
Shikimic acid (6): ^{1}H-NMR (600 MHz, CD_3OD): δ (ppm) = 6.81 (ddd, *J* = 3.7, 1.7 and 1.6 Hz, 1H, H-2), 4.41 (dddd, *J* = 4.7, 3.7, 1.7 and 1.6 Hz, 1H, H-3), 3.72 (dd, *J* = 7.7 and 4.2 Hz, 1H, H-4), 4.01 (ddd, *J* = 7.7, 6.1 and 5.1 Hz, 1H, H-5), 2.72 (dddd, *J* = 18.1, 5.1, 1.6 and 1.6 Hz, 1H, H-6a), 2.20 (dddd, *J* = 18.1, 6.1, 1.7 and 1.7 Hz, 1H, H-6b). ^{13}C-NMR (150 MHz, CD_3OD): δ (ppm) = 170.3 (COOH), 131.3 (C-1), 138.6 (C-2), 67.4 (C-3), 73.1 (C-4), 68.4 (C-5), 32.0 (C-6).

Isoquercitrin (7): ^{1}H-NMR (600 MHz, CD_3OD): δ (ppm) = 6.22 (d, *J* = 2.1 Hz, 1H, H-6), 6.41 (d, *J* = 2.1 Hz, 1H, H-8), 7.70 (d, *J* = 2.2 Hz, 1H, H-2'), 6.87 (d, *J* = 8.5 Hz, 1H, H-5'), 7.59 (dd, *J* = 8.5 and 2.2 Hz, 1H, H-6'), 5.21 (d, *J* = 7.7 Hz, 1H, H-1"), 3.49 (dd, *J* = 9.0 and 7.7 Hz, 1H, H-2"), 3.43 (t, *J* = 9.0 Hz, 1H, H-3"), 3.35 (t, *J* = 9.6 and 9.0 Hz, 1H, H-4"), 3.22 (ddd, *J* = 9.6, 5.3 and 2.5 Hz, 1H, H-1"), 3.72 (d, *J* = 2.5 Hz, 1H, H-6"a), 3.56 (d, *J* = 5.3 Hz, 1H, H-6"b). ^{13}C-NMR (CD_3OD, 150 MHz): δ (ppm) = 159.4 (C-2), 163.1 (C-5), 100.0 (C-6), 166.2 (C-7), 94.8 (C-8), 158.5 (C-9), 105.8 (C-10), 123.1 (C-1'), 117.7 (C-2'), 149.9 (C-3'), 146.2 (C-4'), 116.2 (C-5'), 123.2 (C-6'), 104.5 (C-1"), 75.8 (C-2"), 78.2 (C-3"), 71.4 (C-4"), 78.6 (C-5"), 62.3 (C-6"); MS: *m/z* 463 $[M-H]^-$ $[C_{21}H_{20}O_{12}]$.

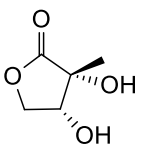
Quercitrin (8): ^{1}H-NMR (600 MHz, CD_3OD): δ (ppm) = 6.21 (d, *J* = 2.1 Hz, 1H, H-6), 6.38 (d, *J* = 2.1 Hz, 1H, H-8), 7.34 (d, *J* = 2.1 Hz, 1H, H-2'), 6.92 (d, *J* = 8.3 Hz, 1H, H-5'), 7.31 (dd, *J* = 8.3 and 2.1 Hz, 1H, H-6'), 5.36 (d, *J* = 1.5 Hz, 1H, H-1"), 0.94 (d, *J* = 6.1 Hz, 1H, H-6"). ^{13}C-NMR (150 MHz, CD_3OD): δ (ppm) 163.0 (C-5), 100.0 (C-6), 94.8 (C-8), 105.5 (C-10), 117.0 (C-2'), 147.7 (C-3'), 150.2 (C-4'), 116.1 (C-5'), 122.9 (C-6'), 103.7 (C-1"), 17,7 (C-6") other overlapping signals; MS: *m/z* 447 $[M-H]^-$ $[C_{21}H_{20}O_{11}]$.



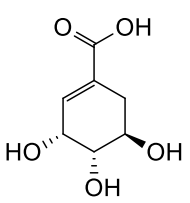
1 R = H
7 R = O-Glc
8 R = O-Rham



3 R = CH_3
4 R = CH_3, $\Delta^{22(23)}$
5 R = H



2



6

Figure 1. Chemical constituents found in *C. erecta* stems

^{1}H HR-MAS NMR spectra obtained directly from the stems of *C. erecta* revealed shikimic acid at high amounts, by the characteristic signals olefinic hydrogen at δ 6.45 and methylenic hydrogens at δ 2.77 and δ 2.20, supporting that *Commelina* species have a strong ability to produce shikimic acid (Figure 2). In *C. benghalensis* stems was found the content of 7.3 % of shikimic acid.[22]

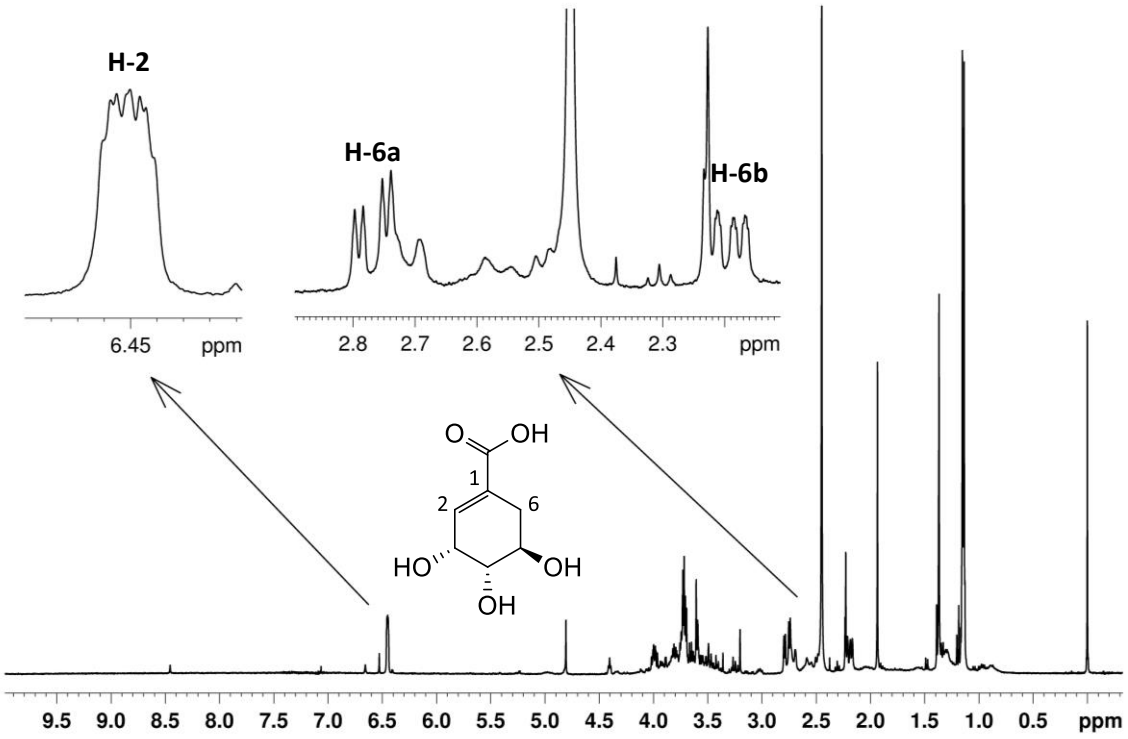


Figure 2. ^{1}H HRMAS NMR spectra from the stems of *C. erecta* evidencing shikimic acid signals

Results of the antioxidant activity from the ethyl acetate and ethanolic extracts of *C. erecta* stems are shown in Figure 3. Both extracts showed weak scavenging activity against DPPH and ABTS with the ethyl acetate extract presenting the highest total phenolics content and radical scavenging activity when compared to the ethanolic extract. This radical scavenging activity can be assigned to the luteolin found in ethyl acetate extract and to the quercitrin and isoquercitrin found in the ethanolic extract. Isoquercitrin has a sequestering capacity of DPPH and superoxide radicals and was previously isolated in *C. communis*.[2] Quercitrin exhibits cytoprotective activity against oxidative stress and activity against DPPH.[23] On the other hand, the ^{1}H NMR analysis of ethyl acetate and ethanolic extracts revealed saccharinic acid lactone and shikimic acid as the main constituents respectively, which can justify the low content of total phenols.

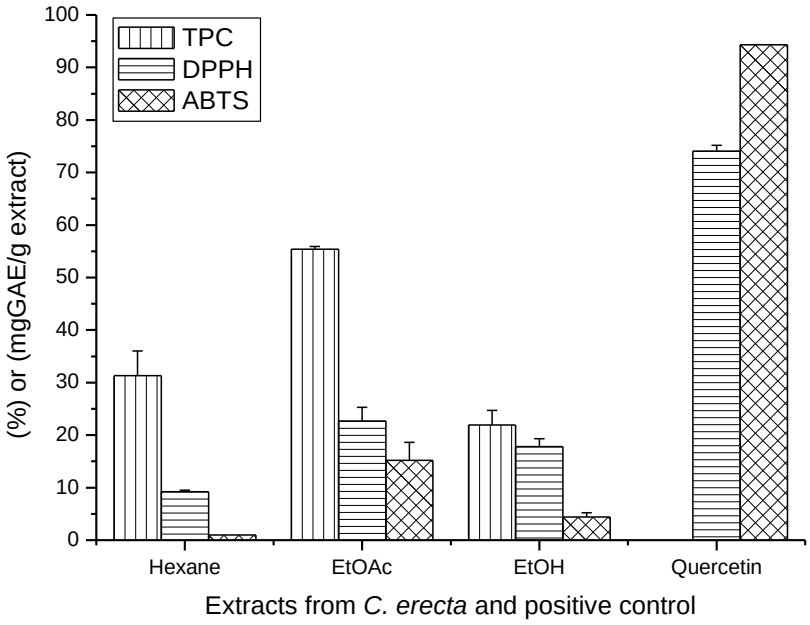


Figure 3. Total phenolic content and free radical scavenging activity of *C. erecta* stems

4. Conclusion

This is the first report on structural identification of the flavonoids luteolin (**1**), isoquercitrin (**7**) and quercitrin (**8**), saccharinic acid lactone (**2**) and shikimic acid (**6**) in the *C. erecta*. The ^{1}H NMR spectra analysis of ethyl acetate and ethanolic extracts revealed saccharinic acid lactone and shikimic acid as the main constituents respectively, which can justify the low content of phenolic compounds and the low DPPH and ABTS radical scavenging activity. However, this species represents an interesting natural source of shikimic acid, a precursor of various classes of secondary metabolites such as flavonoids, lignans and alkaloids and that presents an essential role in the synthesis of antiviral and other biotechnology products.[24] These results stimulate the continuity of the study to quantify the substances of interest from *C. erecta*, since it is a rustic plant and easily accessible by being invasive.

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