Kaempferitrin from *Uncaria guianensis* (Rubiaceae) and its Potential as a Chemical Marker for the Species

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Uncaria tomentosa (Willd.) DC. and *U. guianensis* (Aubl.) Gmel., known as cat’s claw, are large woody vines native to the Amazonian and Central American rainforests. The species contain, in different proportions, indole and oxindole alkaloids, triterpenoid glycosides, sterols and proanthocyanidins. *U. tomentosa* is chemically identified by its oxindole alkaloid profile and content, whereas *U. guianensis* has no satisfactorily established chemical markers. This work describes, for the first time, the isolation of kaempferol-3,7-O-(α)-dirhamnoside (kaempferitrin) in *Uncaria* species. Screening for this compound in leaves, stems or bark of both species through TLC and HPLC-DAD-MS showed the presence of kaempferitrin only in the leaves and stems of *U. guianensis*, at a ratio almost thirty six times greater in the leaves than in the stems. These results reveal the selectivity of *U. guianensis* to produce this bioactive flavonoid glycoside, and suggest this compound as a potential chemical marker for the species.

**Keywords:** *Uncaria guianensis*, *Uncaria tomentosa*, Rubiaceae, kaempferitrin, cat’s claw

Introduction

The genus *Uncaria* (Rubiaceae) contains 34 species distributed among the tropical areas of Southeast Asia, Africa and Central and South America.1 It is represented in Central and South America by two species: *U. tomentosa* and *U. guianensis*.2 These species, known as cat’s claw, unha-de-gato (Brazil), uña de gato, garabato etc., are large woody vines that have been used medicinally by indigenous peoples to treat several diseases for at least two thousand years.3,4

*U. tomentosa* (Willd.) DC. and *U. guianensis* (Aubl.) Gmel., known as cat’s claw, are large woody vines native to the Amazonian and Central American rainforests. The species contain, in different proportions, indole and oxindole alkaloids, triterpenoid glycosides, sterols and proanthocyanidins. *U. tomentosa* can be chemically identified by its oxindole alkaloid profile and content, whereas *U. guianensis* has no satisfactorily established chemical markers. This work describes, for the first time, the isolation of kaempferol-3,7-O-(α)-dirhamnoside (kaempferitrin) in *Uncaria* species. Screening for this compound in leaves, stems or bark of both species through TLC and HPLC-DAD-MS showed the presence of kaempferitrin only in the leaves and stems of *U. guianensis*, at a ratio almost thirty six times greater in the leaves than in the stems. These results reveal the selectivity of *U. guianensis* to produce this bioactive flavonoid glycoside, and suggest this compound as a potential chemical marker for the species.

From the genus *Uncaria* there have so far been isolated over 150 compounds with a predominance of alkaloids. Other classes of isolated compounds include terpenoids and terpenoid glycosides, flavonoids (specially flavanols and flavonols) and coumarins.5,6 The South American species *U. tomentosa* and *U. guianensis* present some morphological differences.2,7 and *U. tomentosa* can be further identified by its oxindole alkaloid profile and content.8 On the other hand, these alkaloids are present in *U. guianensis* at very low concentration9,10 and no satisfactory chemical markers have been established for this species so far.11
Previous chemical studies of *U. guianensis* revealed indole and oxindole alkaloids (whole plant),5 proanthocyanidins (bark),11 flavonols (bark)11 and triterpenoid glycosides (bark).13,14 In vitro and clinical studies using a decoction of *U. guianensis* bark have corroborated its traditional use as an anti-inflammatory and an antioxidant.12,15 Bioassay-guided fractionation of the EtOH extract of *U. guianensis* bark using a yeast-based assay for DNA-damaging agents lead to two weakly but selectively active oxindole alkaloids.16 The EtOH extract of the leaves of *U. guianensis* showed anti-inflammatory and anti-allergic activities.17

The present work describes the isolation of kaempferol-3,7-O-(α)-dirhamnoside (kaempferitrin) from the leaves of *U. guianensis* and the screening for this compound in the EtOH extracts from the leaves, stems or bark of *U. guianensis* and *U. tomentosa* through TLC and HPLC-DAD-MS techniques.

**Experimental**

**General experimental procedures**

NMR spectra were recorded on a Bruker DRX-400 (400 MHz for 1H and 100 MHz for 13C) spectrometer in DMSO-d6/drops D2O with TMS as internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. The TLC analyses were made using pre-coated silica-gel 60F254 (Merck), mobile phase EtOAc/HCOOH/HOAc/H2O 100:11:1:27 and UV irradiation (254 and 326 nm) and NP/PEG reagent followed by UV at 365 nm to visualize the spots.18 The HPLC analyses were performed on a Shimadzu HPLC system (Kyoto, Japan) composed of a system controller SCL-10Avp, SIL-10ADvp auto injector, two LC-10ADvp pumps, DGU-12A degasser, SPD-M10Avp diode array detector and equipped with a reverse-phase C18 column (Lichrocart Lichrospher 5 μm, 250 × 4.6 mm i.d.). Elutions were performed in a gradient elution mode at a 0.8 mL min⁻¹ flow: 10 min 10% solvent B (MeCN) in solvent A (H2O with ca. 0.1% HCOOH, pH 3), 10-23 min 10-40% solvent B in A and finally 12 min 40% solvent B in A. An equilibration period of 10 min was used between the runs. The diode-array detector was set at an acquisition range of 200-600 nm at a spectral acquisition rate of 156 scans s⁻¹ (peak width 0.2 min). Flavonol monitoring was performed at 280 nm and 320 nm. The diode-array detector was coupled to a Micromass ZQ single quadrupole mass spectrometer. The conditions for positive ionization mode in the electrospray probe were: capillary 3.0 kV, cone 50 V, extractor 3 V, RF lens 1 V, source temperature 100 °C and 250 °C desolvation temperature. Acquisition was obtained in the m/z 100-700 range. Retention times, UV spectra and MS ions (and their relative abundances) in comparison to those of the isolated kaempferitrin were evaluated.

**Isolation of kaempferitrin**

Leaves of *U. guianensis* from MT were exhaustedly extracted with EtOH as previously described.17 The ethanol extract (2 g) was partitioned between CH2Cl2/H2O to yield an insoluble yellowish solid (27 mg) from the aqueous fraction.

**Screening for kaempferitrin in *U. tomentosa* and *U. guianensis***

Dried and milled leaves and stems of *U. guianensis* from AM, bark of *U. guianensis* from MT and leaves and bark of *U. tomentosa* from AC (5 g of each sample) were exhaustedly and ultrasonically extracted with EtOH. The solvent was evaporated at low pressure and the dried extracts were partitioned with n-hexane and MeOH/H2O 9:1. The EtOH extract (5 g) previously obtained from the leaves of *U. guianensis* from MT were also partitioned in a similar way. Aliquots of 10 μL of the MeOH solutions (c = 25 mg mL⁻¹) of the n-hexane and MeOH/H2O fractions were compared to the isolated kaempferitrin (c = 1 mg mL⁻¹) by TLC. The MeOH/H2O fractions and the isolated kaempferitrin solutions all at c = 1 mg mL⁻¹ were filtered through 0.45 μm nylon membranes and injected (20 μL) into the HPLC system. MeOH/H2O fractions of bark of *U. guianensis* and leaves and bark of *U. tomentosa* were further subjected to Sephadex LH-20 cc eluted sequentially with n-hexane/CH2Cl2 1:4, CH2Cl2/acetone 3:2 and MeOH, in order to concentrate the polyphenolic compounds and thus better visualize the presence (or not) of kaempferitrin. The MeOH subfractions thus obtained (c = 1 mg mL⁻¹ in MeOH) were submitted to HPLC-DAD-MS analysis using the same conditions as described above.
kaempferol-3,7-O-(α)-dirhamnoside (kaempferitrin)

Amorphous yellowish solid; HPLC-UV \(\lambda_{\text{max}}/\text{nm}: 230, 264 \text{ and } 343\); HPLC-MS: \(m/z\) (rel. int.) [M+1]+ 579 (27), 433 (39), 287 (100); \(^1\)H NMR (DMSO-\(d_6/drops D_2O, 400\) MHz): \(\delta 6.46\) (d, \(J 2.4 \text{ Hz, H-6}\); 6.79 (d, \(J 2.0 \text{ Hz, H-8}\); 7.80 (d, \(J 8.8 \text{ Hz, H-2’ and H-6’}\); 6.93 (d, \(J 8.8 \text{ Hz, H-3’ and H-5’}\); 5.30 (d, \(J 1.6 \text{ Hz, 3-O-Rh-1’}\)); 3.99 (dd, \(J 1.6 \text{ and } 3.2 \text{ Hz, H-2’}\)); 3.47 (dd \(J 3.2 \text{ and } 8.8 \text{ Hz, H-3’}\)); 3.15 (t, \(J 8.8 \text{ Hz, H-4’}\)); 3.13 (m, H-5’)); 5.56 (d, \(J 1.6 \text{ Hz, 7-O-Rh-1’’}\)); 3.84 (dd, \(J 1.6 \text{ and } 3.4 \text{ Hz, H-2’’}\)); 3.64 (dd, \(J 3.4 \text{ and } 9.2 \text{ Hz, H-3’’}\)); 3.31 (t, \(J 9.2 \text{ Hz, H-4’’}\)); 3.43 (m, H-5’’)); 1.13 (d, \(J 6.4 \text{ Hz, 7-O-Rh-CH}_3\)); \(^13\)C NMR (DMSO-\(d_6/drops D_2O, 100\) MHz): \(\delta 157.8\) (C-2), 134.5 (C-3), 178.0 (C-4), 160.9 (C-5), 99.4 (C-6), 161.7 (C-7), 94.6 (C-8), 156.1 (C-9), 105.8 (C-10), 120.7 (C-1’), 130.7 (C-2’ and 6’), 115.4 (C-3’ and 5’), 160.1 (C-6’), 101.9 (3-O-Rh-C-1’), 70.0 (C-2’’), 70.3 (C-3’’), 71.1 (C-4’’), 70.7 (C-5’’), 17.5 (3-O-Rh-CH3), 98.4 (7-O-Rh-C-1’’’), 69.8 (C-2’’’), 70.2 (C-3’’’), 71.6 (C-4’’’), 70.0 (C-5’’’), 17.9 (7-O-Rh-CH3).

Results and Discussion

HPLC-DAD-MS analysis of the isolated solid revealed the presence of a major compound (ca. 90% purity) absorbing at UV \(\lambda_{\text{max}}/\text{nm}: 230, 264 \text{ and } 343\), with a pseudo-molecular ion M+1 at \(m/z\) 579 (27%) and fragment ions of \(m/z\) 433 (loss of one rhamnose, 146 a.m.u.) and \(m/z\) 287 (aglycone) in the MS (relative abundance proportions: 27:39:100, respectively). \(^1\)H and \(^13\)C NMR (400 MHz and 100 MHz respectively, DMSO-\(d_6/drops D_2O\)) analyses with the help of the correlation on the COSY, HMQC and HMB spectra and comparison of their spectral data with those already reported confirmed the structure of kaempferol-3,7-O-(α)-dirhamnoside (kaempferitrin) (Figure 1) for this compound.

Screening for kaempferitrin in leaves, stems or bark of U. guianensis and U. tomentosa through HPLC-DAD-MS techniques (Figures 2 and 3) and TLC revealed the presence of this compound only in the leaves and stems of U. guianensis at a ratio almost thirty six times greater in the

Figure 1. Structure of kaempferitrin.

Figure 2. HPLC-MS fragmentograms (extracted ion \(m/z\) 287) of isolated kaempferitrin (A) and MeOH/H2O fractions from: leaves of Uncaria guianensis from Mato Grosso (B), bark of Uncaria guianensis from Mato Grosso (C), leaves of Uncaria guianensis from Manaus (D), stems of Uncaria guianensis from Manaus (E), leaves of Uncaria tomentosa (F), bark of Uncaria tomentosa (G). The area and retention time of kaempferitrin are indicated at the top of each peak.
leaves than in the stems. Kaempferitrin was not found in the bark of the analyzed specimen of *U. guianensis*. Leaves and bark of *U. tomentosa* did not present kaempferitrin.

In addition to reporting for the first time the isolation of kaempferitrin from the *Uncaria* species and of a flavonoid glycoside from the leaves of *Uncaria guianensis*, the present study describes the selective production of this compound in leaves and stems of *U. guianensis*; a result that contributes to the chemotaxonomy of the *Uncaria* species and also could be a useful chemical tool in the differentiation of the leaves of *U. guianensis* from those of *U. tomentosa*. Furthermore, diverse bioactivities are reported for the isolated kaempferitrin; a fact that may be connected to some of the traditional uses and pharmacological properties of *U. guianensis*.

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Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br, as PDF file.

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*Figure S1. 1H NMR spectrum (400 MHz), in DMSO-d6/drops D2O and TMS as internal standard of kaempferitrin.*

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Figure S2. $^1$H NMR spectrum (400 MHz), in DMSO-d$_6$/drops D$_2$O of the aromatic proton signals of kaempferitrin.

Figure S3. COSY spectrum (400 MHz), in DMSO-d$_6$/drops D$_2$O and TMS as internal standard of kaempferitrin.
Figure S4. $^{13}$C NMR spectrum (100 MHz), in DMSO-d$_6$/drops D$_2$O and TMS as internal standard of kaempferitrin.

Figure S5. TLC profiles of the MeOH/H$_2$O fractions of: (A) *U. tomentosa* leaves (25 mg mL$^{-1}$); (B) *U. tomentosa* barks (25 mg mL$^{-1}$); (C) kaempferitrin (1 mg mL$^{-1}$); (D) *U. guianensis* leaves (25 mg mL$^{-1}$) and (E) *U. guianensis* barks (25 mg mL$^{-1}$); silica gel, mobile phase EtOAc/HCOOH/HOAc/H$_2$O 100:11:11:27. The left plate under UV at 254 nm and the right plate with NP/PEG-UV at 365 nm. Digital photo.
Figure S6. MS of the isolated kaempferitin at retention time = 23.493 min (A) and MS at the same retention time in leaves of Uncaria tomentosa (R_t = 23.511 min) (B).