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HPLC-PDA method for quinovic acid glycosides assay in Cat's claw (*Uncaria tomentosa*) associated with UPLC/Q-TOF–MS analysis

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ABSTRACT

Uncaria tomentosa (Willd.) is a medicinal plant largely used in folk medicine due to its wide range of biological activities, many of which are usually ascribed to the two main classes of secondary metabolites, namely, alkaloids and quinovic acid glycosides. In this work, a reversed phase HPLC-PDA method was developed and validated for the assay of quinovic acid glycosides in crude and dried extracts of *Uncaria tomentosa* (Cat's claw) bark. The validation comprised tests of specificity, accuracy, linearity, intermediate precision, repeatability and limits of detection and of quantification. Alpha-hederin was used as the external standard. High coefficients of determination with lower R.S.D. were achieved for both external standard and crude extract. The structural characterization of the main quinovic acid glycosides presented in the crude extract was carried out through UPLC/Q-TOF–MS. The identities of the compounds were obtained through the comparison of their fragmentation patterns with those reported in the literature. The analytical method was successfully applied for quantifying quinovic acid glycosides in two different dried extracts from *U. tomentosa* and in one quinovic acid glycosides purified fraction.

1. Introduction

Uncaria tomentosa (Willd) DC. is a climbing tree native from Central and South America, where it is commonly known as "uña-de-gato" or "cat's claw". Barks of cat's claw are extensively used in traditional medicine for the treatment of gastritis, asthma and arthritis [1]. Extracts of *U. tomentosa* have been a subject of pharmacological and toxicological studies [2,3] and phytopharmaceuticals derived from its steam bark are currently marked in more than 30 countries as tea, capsules and tablets [4].

Phytochemical investigations on *U. tomentosa* bark revealed the presence of indole and oxindole alkaloids [5–7] along with anthocyanin [8] and acid triterpenes, namely quinovic acid glycosides (Fig. 1) [9–12].

Despite anti-inflammatory and antiviral activities were ascribed to the cat's claw quinovic acid glycosides [10,11], from an analytical point of view there are very few studies regarding the characterization and content assay of triterpenes in *U. tomentosa*. An HPLC-ES/MS method to characterize the quinovic compounds in crude extract was previously described [13]. However, no validated assay method for quinovic acid glycosides in extracts from *U. tomentosa* is available to date. Within that context, aims at the development and validation of an HPLC-PDA assay method for the quinovic acid glycosides content in *U. tomentosa* raw material, supplemented with UPLC/Q-TOF–MS ones designed for identification purposes. Its application is also exemplified with two technological intermediate products obtained through two different drying processes and one quinovic acid glycosides purified fraction (QAPF).

2. Materials and methods

2.1. Chemical and solvents

Previously dried and ground stem barks of *U. tomentosa* were kindly donated by Laboratorios Induquímica S.A., Lima, Peru. Acetonitrile and formic acid had HPLC grade (Merck, Darmstadt, Germany) and water had ultrapure quality (Milli-Q, Millipore, Bedford, MA, USA). Colloidal silicon dioxide (Aerosil® 200) proceeded from Degussa (Germany). Alpha-hederin (purity ≥ 90%, Extrasynthese, France) was employed as the external standard.

2.2. HPLC-PDA analysis

The reversed-phase gradient method was performed through a Shimadzu LC 10 Class (Tokyo, Japan) provided with a FCV-10

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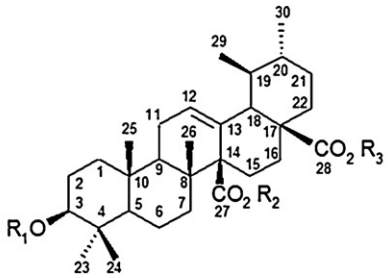

Quinovic acid glycosides	R_1(C-3)	R_2 (C-27)	R_3 (C-28)	Number of sugars at Glycosidic chain	Reference
1	-glc-(3→1)-fuc (Hex→6-des)	-glc (Hex)	-H	3	[9]
2	-glc-(3→1)-fuc (Hex→6-des)	-H	-glc (Hex)	3	[9]
3	-rha-(3→1)-glc (6-des→ Hex)	-glc (Hex)	-H	3	[12]
4	-H	-H	-glc-glc (Hex→Hex)	2	[10]
5	-glc-(3→1)-fuc (Hex→6-des)	-H	-H	2	[9]
6	-qui (6-des)	-H	-glc (Hex)	2	[10]
7	-fuc (6-des)	-H	-glc (Hex)	2	[10]
8	-qui (6-des)	-glc (Hex)	H	2	[11]
9	-fuc (6-des)	-glc (Hex)	-H	2	[11]
10	-rha-(3→1)-glc (6-des→ Hex)	-H	-H	2	[12]
11	-qui-(3→1)-glc (6-des→ Hex)	-H	-H	2	[12]
12	-qui-(3→1)-gal (6-des→ Hex)	-H	-H	2	[12]
13	-H	-glc (Hex)	-H	1	[12]
14	-rha (6-des)	-H	-H	1	[12]

Fig. 1. Quinovic acid glycosides isolated from *Unacria tomentosa.* Note: glc: β-D-glucopyranosyl; fuc: β-D-fucopyranosyl; gal: β-D-galactopyranosyl; qui: β-D-quinovose; rha: α-L-rhamnopyranosyl; hex: hexose; 6-des: 6-desoxy-hexose.

AL system controller, a LC-10 AD pump system, a SIL-10 A automatic injector (20 μL-loop) and an SPD-M20A (Tokyo, Japan). The data were processed by LC-Solution Multi-PDA software. A column Sinergy Fusion RP-18 150 mm × 3.9 mm i.d., 5 μm (Phenomenex, Torrance, CA, USA) protected by a RP-18 guard column (4.0 mm × 3.0 mm i.d.) (Phenomenex, USA) were used. The detection was at 205 nm. The mobile phase consisted of formic acid 0.01% (solvent A) and acetonitrile:formic acid 0.01% (90:10, v/v) (solvent B). A gradient elution program was developed: 29.0% B (0–3 min), 29.0–38.0% B (3–14 min), 38.0% B (14–15 min), 38.0–57.0% B (15–30 min) and 57.0% B (30–37 min). After each analysis the column was washed up with 70.0% B (5 min) followed by an equilibrium step with 29.0% B (5 min). The flow rate was kept constant at 1.0 mL/min and the analyses were conducted at 35 ± 1 °C. The injection volume was 20 μL.

2.2.1. Alpha-hederin standard solutions

Stock solutions of alpha-hederin were prepared through the dissolution of an accurately weighed 20.0 mg-sample in 100.0 mL of acetonitrile:water (50:50, v/v). Appropriate dilutions were prepared to yield concentrations of 2.0, 4.0, 6.0, 8.0 and 10.0 μg/mL. The samples were filtered through a 0.45 μm membrane (Millipore, Bedford, USA) prior to injection. Each analysis was repeated three times, over a 3-day period. The slope and other statistical parameters of the regression curves were calculated by linear regression (Minitab 14®, USA).

2.2.2. Samples preparation and regression curve

A 1000 g -sample of ground stem barks of *U. tomentosa* was extracted through a 4-day maceration, using 10 L of a 40% (v/v) hydroethanolic solution. The mixture was pressed, filtered and concentrated under vacuum to half of their original weight. A 300 mL