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Evaluation of the chemical composition of the alcoholic extract of *Gynoxys cuicochensis* Cuatrec: Identification of metabolites and exploration of their pharmacological properties

Evaluación de la composición química del extracto alcohólico de *Gynoxys cuicochensis* Cuatrec: Identificación de metabolitos y exploración de sus propiedades farmacológicas

Omar Malagón[1], Patricio Cartuche[1],Gianluca Gilardoni[1], Sandra Espinosa[1], Nixon Cumbicus[2] and Angel Montaño[1]

[1]*Departamento de Química, Universidad Técnica Particular de Loja, Calle M. Champagnat s/n,1101608, Loja Ecuador.*
[2]*Departamento de Ciencias Biológicas y Agropecuarias, Universidad Técnica Particular de Loja, Calle M. Champagnat s/n, 1101608, Loja Ecuador.*

*Corresponding author: aamontano1@utpl.edu.ec

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Abstract

Gynoxys cuicochensis Cuatrec., a member of the Asteraceae family, inhabits the Fierro Urco moor in the province of Loja. Despite not being used in traditional medicine, it possesses significant landscape value due to its striking yellow inflorescence. For investigating its chemical composition and potential pharmacological properties, a phytochemical characterization of the plant's ethanolic extract was conducted. The extract was obtained through static maceration for three days for the initial filtration, followed by one day for the second and third filtrations. Chlorophyll was separated using Diaion HP-20 resin as a solid phase packed in separation funnels, and an ethanol:water liquid phase with a concentration gradient ranging from 6:4 to 9:1 for elution. The dechlorophyllized extract was then freeze-dried and fractionated using gravity column chromatography. The obtained fractions were further purified through microcolumn and preparative chromatography. To elucidate the chemical structure of the molecules, nuclear magnetic resonance spectroscopy and electrospray ionization mass spectrometry were employed. Two metabolites were isolated for this study. The first one is a known glycosylated flavonoid called Nicotiflorin, while the second one is a phenolic derivative named 1,3-di-O-trans-feruloylquinic acid, which lacks a precise pharmacological description. This discovery represents an interesting and unique finding for this species, suggesting its potential medicinal use.

Keywords: *Gynoxys cuicochensis Cuatrec*, NMR, ESI, flavonoid, nicotiflorin, feruloylquinic acid.

Resumen

La especie *Gynoxys cuicochensis Cuatrec.*, es una Asterácea que habita el páramo de Fierro Urco de la provincia de Loja. Si bien no es utilizada dentro de la medicina tradicional, posee un alto valor paisajístico por su llamativa inflorescencia amarilla. Con el objetivo de conocer su composición química y posibles propiedades farmacológicas, se efectuó una caracterización fitoquímica del extracto etanólico de la planta, obtenido mediante maceración estática por el lapso de 3 días para la primera filtración, y un día para la segunda y tercera filtración. La clorofila fue separada utilizando una fase sólida de resina Diaon Hp 20, la misma que fue empacada en embudos de decantación, y una fase líquida de EtOH:H_2O en gradiente de concentración desde 6:4 hasta 9:1 para la elución. El extracto desclorofilado se liofilizó y posteriormente se fraccionó utilizando cromatografía en columna por gravedad. Las fracciones obtenidas fueron purificadas mediante microcolumna y cromatografía preparativa. Para elucidar la estructura química de las moléculas, se recurrió a la espectrometría de resonancia magnética nuclear y espectrometría de masas de ionización por electrospray. Como resultado, se aislaron dos metabolitos: el primero es un flavonoide glicosilado conocido como Nicotiflorina, mientras que el segundo es un derivado fenólico, denominado ácido 1,3-di-O-trans-feruloilquinico que aún no cuenta con una descripción farmacológica precisa. Este descubrimiento representa un hallazgo interesante y único para esta especie en particular, lo que sugiere un posible uso medicinal.

Palabras clave: *Gynoxys cuicochensis Cuatrec*, RMN, ESI, flavonoide, nicotiflorina, ácido feruloilquinico.

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Orcid IDs:

Omar Malagón: https://orcid.org/0000-0001-7946-7858
Patricio Cartuche: https://orcid.org/0009-0008-2835-0922
Gianluca Gilardoni: https://orcid.org/0000-0003-0915-9416
Sandra Espinosa: https://orcid.org/0000-0002-2697-8897
Nixon Cumbicus: https://orcid.org/0000-0002-4880-6607
Angel Montaño: https://orcid.org/0009-0007-2929-5737

1 Introduction

The Asteraceae family is known for its diversity with around 24,000 species worldwide, making it the largest plant family after the orchids (Del Vitto and Petenatti, 2009). They are found in a wide variety of habitats, from the tropics to temperate regions. In Ecuador, this plant family represents approximately 10% of the country's flora.

Ecologically, the Asteraceae have a significant impact as they are important food sources for various animals, including insects, birds, and mammals (Flann et al., 2010). Regarding the chemical composition, the Asteraceae are known for their richness and variety of secondary metabolites, including iso- and chlorogenic acids, sesquiterpene lactones, pentacyclic triterpene alcohols, essential oils, alkaloids, and various acetylenic derivatives. These compounds have a great medical interest due to their cytotoxic and anticancer properties, among others (Figueroa, 2016).

A member of this plant family is the species *Gynoxys cuicochesis Cuatrec.*, which features a flexible, striated stem with finely tomentose nodes. Its leaves are opposite, ovate-oblong, with striated petioles and slightly curved edges. The upper surface is glabrous and smooth green, except for the main vein, which is tomentulose, while the underside is densely tomentose-ochraceous, and the main vein is prominent, surrounded by 10-11 secondary veins on each side (Robinson and Cuatrecasas, 1992). The inflorescences are terminal, cymose-paniculate, and tomentose, with lanceolate, short, tomentose bracts. The heads are heterogeneous, with a conical-campanulate involucre, consisting of 7-8 scarious and glabrous bracts. The female flowers are marginal, with ligulate corollas, and the hermaphroditic flowers are grouped in 8-9, with tubular corollas and deeply dentate campanulate limbs (León-Yánez et al., 2011). These morphological characteristics provide a basis for identifying the species and are important for its botanical description. In the Ecuadorian highlands, this plant is commonly known as "piquiles" or "tunash". It is used both as firewood and to provide structural support in the construction of temporary shelters (Lojan, 1992).

Figure 1. *Gynoxys cuicochensis Cuatrec.* in its habitat.

Although this species is not used in traditional medicine, this plant serves as an excellent pollinator attractor due to its abundant inflorescence during the early months of the year, making it an interesting candidate for phytochemical studies. To date, there are no records of scientific studies on

the fixed chemical composition of this plant species. For this reason, this research focuses on the isolation of secondary metabolites from the plant using microcolumn gravity liquid chromatography techniques. The structure of the metabolites was elucidated through Nuclear Magnetic Resonance (NMR) and electrospray ionization mass spectrometry (ESI). The objective of this paper is to chemically characterize the species *Gynoxys cuicochesis Cuatrec*. to contribute to its ethnobotanical description.

2 Materials and Methods

2.1 General Information about the Sample

The collection of 25 kg of the aerial parts (leaves) of the plant species *Gynoxys cuicochensis Cuatrec.* was carried out in the Sebadal - Fierro Urco sector of the San Isidro neighborhood, San Pablo de Tenta parish, Saraguro, Loja province, located at an altitude of 2 990 meters above sea level (m.a.s.l.). The exact coordinates of the collection were S 3°40'47.4816 and W 79°1838.5056, under the collection permit for plant species MAE-DNB-2016-0048 granted by the Ministry of the Environment. For subsequent processing, the plant material was dried for 10 days at a temperature of 40°C, using the dehydrator located in the Chemistry laboratory at Universidad Técnica Particular de Loja (UTPL). As a result, 5 kg of dry plant material was obtained, ready to be used in research.

2.2 Extraction of the Plant Extract

The dry plant sample was manually ground and then macerated in individual containers containing 2 kg of plant material and 20 liters of an ethanol: water (EtOH:H_2O) solution in a 7:3 ratio, respectively. Three static maceration processes were conducted, the first for 3 days and the subsequent two for one day each. After filtering the extract, the ethanol was removed using a rotary evaporator. Then, the dry extract was lyophilized using a Labconco Corporation model 7754047 freeze-drier. The 100 ml samples were stored in Boeco®jars, and the experimental process lasted 5 days for each sample.

For separating the chlorophyll, two separation funnels of 500 ml and 1,000 ml were used. In the 1,000 ml funnel, 400 g of Diaion®HP-20 resin was packed in an EtOH:H_2O solution with a 6:4 gradient, and 50 g of the extract diluted in the previously mentioned EtOH:H_2O solution was added. The same procedure was applied in the 500 ml funnel, but with 240 g of Diaion®HP-20 resin and 35 g of the extract. To obtain the solvent-free extract (EtOH), a BUCHI R-220 Pro rotary evaporator was used under the following conditions: cooling temperature of -10°C, rotation of 40 rpm, bath water temperature of 30°C, steam temperature of 27°C respectively, and vacuum pressure starting at 120 mbar and gradually increasing to 30 mbar.

Finally, to facilitate the use of the dechlorophyllized extract, it was lyophilized using the Labconco Corporation model 7754047 equipment. The sample was placed in a 100 ml Boeco®jar with a 400 ml volume, and the lyophilization process was conducted over 5 days.

2.3 Fractionation and purification of metabolites

The preparation of a sugar-free extract was carried out using a 1000 ml Butanol:Water (ButOH:H_2O) solution, dividing the solution into equal parts and constantly shaking for half an hour to achieve complete homogenization. After one hour of resting, the water was removed, and 15 grams of lyophilized extract were diluted in 700 ml of butanolic solution, adding an equal volume of distilled water, and set aside for an hour to allow the separation of compounds according to their polarity. This process was repeated twice to obtain the organic phase.

The organic phase was subjected to liquid chromatography on a microcolumn by gravity, using a polarity of Ethyl Acetate, Methanol, and Water (AcOEt, MeOH, and H_2O) in an 8:1:1 ratio, with a load of 800 mg of organic phase extract and 3 grams of direct phase silica gel. The collected fractions were subjected to continuous flow column chromatography (CCF), using Dichloromethane (DCM) as the solvent, and applying a polarity of DCM, AcOEt, MeOH in a 6:2:2, 5:3:2, and 4:5:1 ratio, respectively, adding 5 drops of Acetic Acid (AcOH) to each concentration. Preparative thin layer chromatography was performed on direct phase silica gel plates of 20cm x 20cm, using the same DCM, AcOEt, MeOH solution in a 6:2:2 ratio and 5 drops of AcOH.

The elution process was conducted in a glass chamber, and with the aid of UV light at 254 nm, the compounds to be separated were identified. The silica gel impregnated with the compounds was washed with the solution (DCM, AcOEt, MeOH 6:2:2 and 5 drops of AcOH) which was eluted in the CCF and placed in 10 ml vials for drying using nitrogen.

2.4 Characterization of Secondary Metabolites

The samples containing the separated metabolites were taken to the BRUKER NMR equipment, model MSC 500 MHz, to perform the necessary analyses and obtain spectra of ^{1}H, ^{13}C, DEPT, COSY, HMBC, HSQC, and TOCSY. The equipment operated at a frequency of 500 MHz, and deuterated chloroform ($CDCl_3$) was used as the solvent. Additionally, an ESI experiment was conducted through direct injection on a Bruker amaZon speed device, using nitrogen during the process.

3 Results and Discussion

Previous studies on various species of the genus Gynoxys, such as *Gynoxys Sancto-antonii Cuatrec*, *Gynoxys psilophylla Klatt*. (Bohlmann et al., 1986), *Gynoxys dielsiana Domke* (Zdero et al., 1980), *Gynoxys acostae Cuatr.*, *Gynoxys nitida Mushcl.* and *Gynoxys buxifolia Cass.* (Keriko et al., 1995), have facilitated the isolation of various 3β, 6β 10β-H diacetyloxy furanoeremophilanes and 1β, 6β 10β-H diacetyloxy furanoeremophilanes, primarily modified with tigloyloxy, angeloyloxy, acetyloxy, and senecioyloxy substituents, which have been proposed as potential chemotaxonomic markers of the genus. However, a study on *Gynoxys oleifolia Muschl*. reports the existence of substituted ent-kaurane diterpenes, generating a debate about the presence of this metabolite in the genus Gynoxys (Beltrán et al., 2006).

It should be noted that, as minority components, 6-acetyl-2,2-dimethyl-chroman-4-one compounds have been isolated in *Gynoxys psilophylla Klatt* (Bohlmann et al., 1986) Germacrene D, bicyogermacrene, spatulenol, oleanolic acid in *Gynoxys nítida* Muschl. and piceol in *Gynoxys buxifolia* (HBK) Cass (Keriko et al., 1995) Betulin in *Gynoxys cf. pulchella* (Kunth) Cass (Rodriguez, 2016). After conducting various phytochemical analysis tests on ethanolic fractions of *Gynoxys hirsuta* Wedd., the presence of pyrrolizidine alkaloids, furanoeremophilanes, and coumarins has been suggested (Ramírez, 2011).

Similarly, tests carried out on the volatile fraction of the leaves of *Gynoxys meridiana* Cuatrec, obtained through hydrodistillation, show γ-curcumene (31.9%), fukinanolide (22.3%), β-pinene (9.5%), α-phellandrene (7.1%), and α-pinene (5.7%) as main compounds. The most recent study on the chemical composition of the essential oil of *Gynoxys miniphylla* Cuatrec. reports that its major components are α-phellandrene (∼ 17%), α-pinene (∼ 15%), germacrene D (∼ 13,5%), trans-myrtanol acetate (8.8%), δ-cadinene (∼4.5%), β-phellandrene (∼3,5%), (E)-β-caryophyllene (∼2,5%), o-cymene (2.4%), α-cadinol (2.5%), and α-humulene (2%) (Malagón et al., 2022).

In this research, for the first identified fraction, in the proton analysis (Table 1), a doublet at 6.47 ppm and another at 6.73 ppm were detected, corresponding to H-6 and H-8 respectively. Both signals displayed a meta coupling constant J6,8= 2 Hz. Additionally, a doublet at 7.80 ppm integrating for 2H was identified, related to the equivalent positions H-2′ and H-6′. Shifted to higher fields due to the influence of a hydroxyl group, there is a doublet at 6.94 ppm that integrates for 2H, corresponding to the equivalent protons H-3′ and H-5′, which have an ortho coupling constant J2′,3′= 8.8 Hz. Furthermore, a singlet at 5.56 ppm corresponding to the anomeric position 1″ of glucose and a doublet at 5.38 ppm related to the anomeric position 1‴ of rhamnose were observed, suggesting the presence of the disaccharide rutinose.

The combination of data derived from proton analysis and ESI mass spectrometry provides significant information about the molecule. The ESI analysis reveals a molecular ion $[M+H]^{+}$=595.13, which indicates the presence of nicotiflorin, chemically known as Kaempferol-3-O-rutinoside.

Table 1. ^{1}H RMN of Nicotiflorin (Kaempferol-3-O-rutinoside).

13C (500 MHz), CD_3OD	δ (ppm)	DEPT	1H (500 MHz), CD_3OD	δ (ppm)	Mult.	∫	J (H)	COSY	HMBC	HMQC
C-2			-	-	-	-	-	-		
C-3			-	-	-	-	-	-		
C-4			-	-	-	-	-	-		
C-5	162.2		-	-	-	-	-	-		
C-6	99.30	CH	H-6	6.47	d	1H	2	H-6 / H-8	162; 94.27; 106.23	H-6 / C-6
C-7			-	-	-	-	-	-		-
C-8	94.27	CH	H-8	6.73	d	1H	2	H-8 / H-6	156.69; 162.2; 99.30; 106.23	H-8 / C-8
C-9	156.69		-	-	-	-	-	-		
C-10	106.23		-	-	-	-	-	-		
C-1'	120.98		-	-	-	-	-	-		
C-2'	130.63	CH	H-2'	7.80	d	2H	8.8	H-2' / H-3'	160.38 130.63	H-2' / C-2'
C-3'	115.32	CH	H-3'	6.94	d	2H	8.8	H-3' / H-2'	160.38; 115.32; 120.8	H-3' / C-3'
C-4'	160.33	C	-	-	-	-	-	-	-	
C-5'	115.32	CH	H-5'	6.94	d	2H	8.8	H-5' / H-6'	160.38 115.32 120.98	H-5' / C-5'
C-6'	130.63	CH	H-6'	7.80	d	2H	8.8	H-6' / H-5'	160.38 130.63	H-6' / C-6'
C-1"	98.4	CH	H-1"	5.56	s	1H	-			H-1" / C-1"
C-2"			H-2"							
C-3"			H-3"							
C-4"			H-4"							
C-5"			H-5"							
C-6"	69.7	CH_2	H-6"	3.83	dd	2H	3.3; 9.5			
C-1"'	102.08	CH	H-1"'	5.38	d	1H	1.3		70.75	H-1"' / C-1"'
C-2"'	70.75	CH	H-2"'	3.67	m	1H	-	-	69.65; 72.11	
C-3"'	72.11	CH	H-3"'							
C-4"'	-	CH	H-4"'	3.33	m	1H	-	-	70.75	
C-5"'	69.65	CH	H-5"'	3.48	m	1H	-	-	16.71; 70.75	
C-6"'	16.71	CH	H-6"'	1.25	m	1H	-	-		

Nicotiflorin (Figure 1) is a flavonoid composed of a glucose molecule and a rutinose molecule. Glucose is a monosaccharide with the chemical formula $C_6H_{12}O_6$, while rutinose is a flavonoid consisting of a flavone structure attached to a glucuronic acid molecule. Furthermore, nicotiflorin possesses a nicotinyl group attached to the C-6 position of glucose, distinguishing it from other flavonoids. The chemical structure of nicotiflorin is complex and features various functional groups, such as hydroxyls, ketones, and ethers.

Glycosylated flavonoids, also known as flavonoid glycosides, are widely distributed in plant species and are known for their diverse pharmacological effects. Most of these compounds contain glucose as the sugar present, although galactose, rhamnose, xylose, and the disaccharide rutinose have also been found. In plants, flavonol glycosides and aglycones have important functions, such as protection against UV radiation, internal physiological regulation, and reproduction. They also act as antioxidants, scavenging free radicals and sup-

porting the plant's immune system (Slámová et al., 2018).

Addressing pharmacodynamics, it is known that glycosylation significantly enhances the water solubility of flavonoids, thereby increasing the bioavailability of the corresponding flavonoid aglycone, depending on the nature of the sugar. Importantly, glycosides are absorbed more rapidly than rhamnosides and rhamnoglucosides due to the availability of hydrolyzing enzymes in the human gastrointestinal tract. These enzymes, such as intestinal lactase-phlorizin hydrolase or β-glucosidase present in the epithelial cells of the small intestine can metabolize glycosides. Conversely, there are no α-l-rhamnosidase or rutinosidase enzymes in humans, which means that the bioavailability of flavonoids containing rhamnose depends entirely on their cleavage by the intestinal microbiota (Slámová et al., 2018; Khodzhaieva et al., 2021).

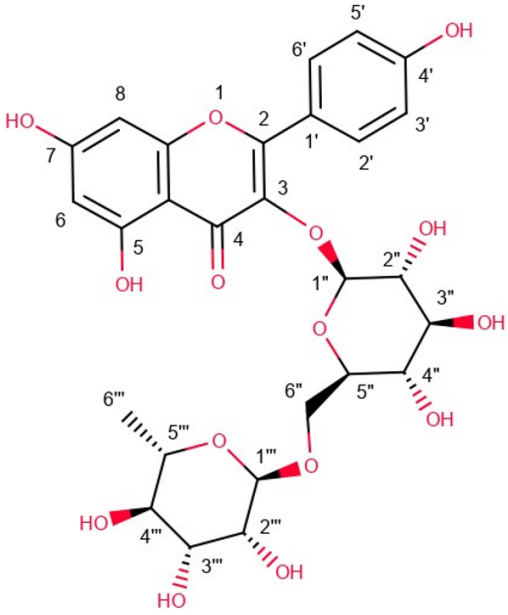

Figure 2. Chemical Structure of Nicotiflorin (Kaempferol-3-O-rutinoside).

The addition of rhamnose to flavonoids can enhance their solubility and stability, as well as confer specific pharmacological properties and selectivity. The sugar used in glycosylation is typically a disaccharide composed of L-rhamnose (also known as 6-deoxy-mannose) and D-glucose, which are linked by an α-(1-2) or α-(1-6) glycosidic bond. While glucosylation or galactosylation increases the water solubility of the flavonoid, the presence of rhamnosyl residues may slightly decrease it. Thus, the presence of a sugar residue in a flavonoid appears to play a significant role in solubility, bioavailability, and biological activity. For example, flavonoid glycosides can act as "prodrugs" to release aglycones into the gastrointestinal tract (Slámová et al., 2018).

Kaempferol is a flavonoid commonly found in the Asteraceae family, and it has been shown to have anticancer and anti-inflammatory effects. Furthermore, kaempferol and its associated compounds have also been found to possess antibacterial, antifungal, and antiprotozoal activities (Adebayo et al., 2010). Recently, kaempferol has been reported to have neuroprotective action in the brain, inhibiting pro-inflammatory cytotoxicity and the activity of significant inflammatory pathways (Silva et al., 2021). Chronic inflammation and free radicals are known risk factors for cancer development. Therefore, active principles capable of inhibiting these factors are useful for inducing cytotoxicity in cancer cells. The anticancer activity observed in so-

me kaempferol derivatives is noteworthy, making plant species that contain it ideal candidates for the search for treatments against various types of cancer.

Numerous derivatives of kaempferol have demonstrated anticancer activity. For instance, trifolin (kaempferol 3-O-D-galactoside) induces apoptosis in lung cancer cells through both intrinsic and extrinsic pathways (Kim et al., 2016). Azfelin (kaempferol-3-O-rhamnoside) has been suggested as a chemotherapeutic therapy for breast cancer due to its antioxidant properties and its ability to prevent oxidative damage to biomolecules (Vellosa et al., 2015). Meanwhile, nicotiflorin (kaempferol-3-O-rutinoside) has been shown to be hepatoprotective, reducing cytokine and serum enzyme levels and restoring antioxidant indicators in liver homogenate (Zhao et al., 2017).

Furthermore, Li et al. (2006) have demonstrated that nicotiflorin significantly reduces neuronal damage and cell death in models of permanent focal cerebral ischemia and in neuronal cultures subjected to oxidative stress. It also decreases the production of reactive oxygen species (ROS) and the activation of inflammatory factors in these models, suggesting a neuroprotective action of kaempferol. Therefore, kaempferol derivatives hold significant potential as therapies against cancer and neurodegenerative diseases.

According to Patel (2022), nicotiflorin has pharmacological properties such as antioxidant, anti-inflammatory, cardioprotective, and antidiabetic effects. This compound has traditionally been used in Chinese medicine to treat various conditions, including gastrointestinal disorders, respiratory infections, and cardiovascular diseases. To date, glycosylated flavonoids have not been reported in the literature as present in species of the genus Gynoxys. Therefore, the isolation of nicotiflorin in *Gynoxys cuicochensis Cuatrec.* represents a valuable contribution to the available chemotaxonomic information. For the second fraction, a structural elucidation has been proposed that suggests the presence of a derivative of quinic acid known as 1,3-di-O-trans-feruloylquinic acid.

NMR analysis reveals distinctive features in the proton signals (Table 2). Firstly, there is an evident aromatic contribution due to signals corresponding to positions H2 (7.07 ppm, d, J=8Hz, 2H), H5 (6.82 ppm, d, J=8Hz, 2H), and H6 (7.18 ppm, d, J=6Hz, 2H), indicating the presence of a trisubstituted system. Additionally, two signals are highlighted for H7 (7.64 ppm, d, J=16Hz, 2H) and H8 (6.40 ppm, d, J=16Hz, 2H), suggesting the existence of a double bond in a trans configuration. It is important to note that the presence of methoxy groups in the aromatic structure is also observed by the signals for OCH_3 (3.88 ppm, s, 6H).

The 1,3-di-O-trans-feruloylquinic acid (Figure 2) is an ester derivative of dihydroxycinnamoylquinic acid, characterized by the presence of two feruloyl groups attached at positions 1 and 3 of the quinone unit in the central ring. Wenzl et al. (2000) also reported the adoption of a chair conformation with the carboxylic group in an axial position in this molecule, due to steric interactions between the functional groups in its structure.

The axial position of the carboxylic group is attributed to the need to minimize such steric interactions among the present functional groups. In this context, the hydroxyl group at position 5 and the feruloyl groups at positions 1 and 3 are oriented in the opposite direction to the carboxyl group, leading to the minimization of steric interactions.

Additionally, it is important to note that the chair conformation is stable for molecules with six-membered rings, such as the central ring of 1,3-di-O-trans-feruloylquinic acid. In this conformation, the substituent groups on the carbon atoms of the ring are oriented in opposite directions, which contributes to the minimization of steric interactions and, thereby, enhances the overall stability of the molecule.

It has been observed that this metabolite accumulates in the more mature regions of the root system, excluding both root tips and shoots. This metabolite has been attributed with antioxidant activity that regulates blood lipids in rats. Moreover, *in vitro* studies have demonstrated its anti-inflammatory activity, suggesting its potential as a therapeutic agent in inflammatory diseases. Various additional biological activities have been described for this compound, including antimicrobial, antitumor, antidiabetic, and neuroprotective properties (Wenzl et al., 2000).

Table 2. ^{1}H RMN of 1,3-di-O-trans-feruloylquinic Acid.

^{1}H (500 MHz), CD_3OD	δ (ppm)	m	J (Hz)	∫	δ (ppm)	m	J (Hz)	∫
7”	7.54	d	16	2.3	7.52	d	16	1
7’	7.44	d	16	3.5	7.37	d	16	1
2”	7.30	s ancho	-	1.2				
2’	7.27	s ancho	-	1.6	7.24	s	-	2
6”	7.10	d	8	1.4				
6’	7.05	d	8	2.8	7.03	d	8.1	2
	7.01 - 6.90	m	-	2.7				
5’ 5”	6.79	d	8	2.9	6.75	d	7.8	1
					6.73	d	7.8	1
	6.78 - 6.67	m	-	4.0				
8”	6.44	d	16	1.5	6.39	d	16	1
8’	6.36	d	16	1.5	6.36	d	16	1
	6.28 - 6.13	m	-	2.3				
	5.74	m	-	0.5				
	5.51	m	-	0.7				
3	5.19 - 5.38	m	-	2.1	5.18	dt	10/4	1
	4.89	m	-	0.8				
	4.11	s ancho	-	0.8				
	4.01	s ancho	-	1.3				
	3.83	s	-	3	3.78	s	-	3
	3.82	s	-	3	3.75	s	-	3
4.5	3.76	m	-	2.2	3.69	m	-	1
					3.64	m	-	1
2	2.35	m	-	1.3	2.28	t	13	3
	2.24 - 2.14	m	-	2.1				
	2.11 - 1.95	m	-	2.1				
6	1.92 - 1.63	m	-	4.7	2.00	dd	12/3	1
					1.85	d	12	1
	1.48	m	-	1.4				
	1.24	s ancha	-	3.48				

Given that the bibliographic information available on this metabolite is limited and does not allow for a comprehensive comparison among sources, the biological activity described for its precursor, quinic acid, has also been considered as a reference point.

Quinic acid is a natural hydroxycarboxylic acid with a simple phenolic chemical structure. It consists of a six-carbon ring bearing two hydroxyl groups and one carboxyl group. In plants, it plays several important roles, including regulating growth and development, protecting against environmental stress, and defending against pathogens. Moreover, quinic acid is involved in the synthesis of other phenolic compounds, such as flavonoids. It has been shown to induce the production of phytoalexins, which are antimicrobial compounds produced by plants in response to pathogen infection (Ma and Ma, 2015).

Although the direct relationship between the metabolite and quinic acid still requires more detailed investigation. The known biological activity of quinic acid provides a solid foundation for exploring the therapeutic potential and biological properties of this understudied metabolite. A comparative approach with quinic acid may aid in gaining a deeper understanding of its biological activity and potential applications.

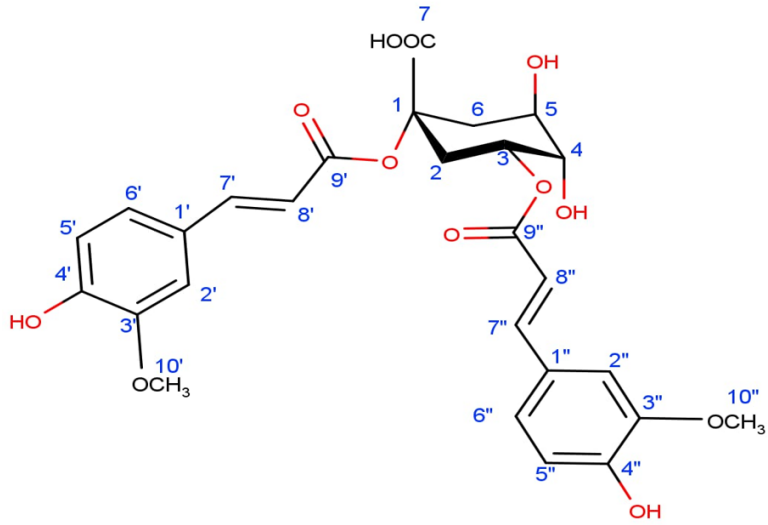

Figure 3. Chemical Structure of 1,3-di-O-trans-feruloylquinic Acid.

Quinic acid has been extensively researched due to its remarkable biological activity, spanning various areas of interest. Properties such as antioxidant, anti-inflammatory, antihypertensive, antidiabetic, and neuroprotective activities have been related with this compound (Pero et al., 2009; El-Askary et al., 2022). Additionally, it has been demonstrated to exert beneficial effects on cardiovascular health, glucose metabolism, obesity, and cellular damage.

Studies have revealed that quinic acid can regulate gene expression, modify enzymatic activity, and modulate cellular signaling pathways. These actions shape its biological impact on various diseases and pathological conditions. Its antioxidant capacity enables it to neutralize reactive oxygen species, thus protecting cells from oxidative damage. Likewise, its anti-inflammatory activity helps to reduce excessive inflammatory responses in different tissues and biological systems (Heikkilä et al., 2019).

Quinic acid has also shown positive effects on blood pressure regulation, making it a potential agent for controlling hypertension. Additionally, its ability to enhance glucose metabolism is interesting in the context of diabetes and insulin resistance (Singh et al., 2021; Lee et al., 2022). Regarding its neuroprotective action, quinic acid has demonstrated the capacity to protect nerve cells against oxidative stress and other harmful events, suggesting its potential in the prevention and treatment of neurodegenerative disorders (Zuo et al., 2015; Clifford et al., 2017).

4 Conclusions

The alcoholic extract of *Gynoxys cuicochensis Cuatrec.*, was successfully obtained from a sample collected in the Sebadal-Fierro Urco area in Loja. Using microcolumn liquid chromatography, NMR, and ESI techniques, the fractionation and chemical characterization of the extract were achieved.

Phytochemical screening of the alcoholic extract from the leaves of *Gynoxys cuicochensis Cuatrec.* revealed the presence of a flavonoid compound and a quinic acid derivative, both known for their pharmacological properties. However, it is important to note that this species is not used in traditional medicine.

In the experimental phytochemical study of Gynoxys cuicochensis Cuatrec., the analysis of proton and carbon nuclear magnetic resonance spectra (^{1}H and ^{13}C), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), distortionless enhancement by polarization transfer (DEPT), correlated spectroscopy (COSY), and total correlation spectroscopy (TOCSY) enabled the identification of the first me-

tabolite as nicotiflorin (kaempferol-3-O-rutinoside). It is noteworthy that the presence of this compound in the Gynoxys genus has not been previously reported, opening new doors for further research on this species and contributing to scientific advancement in the region.

Regarding the second isolated metabolite, 1,3-di-O-trans-feruloylquinic acid, the available scientific information is limited. However, a possible antioxidant and anti-inflammatory activity can be proposed due to its derivation from quinic acid, as phenolic compounds are generally known to have these properties. To fully understand the properties and therapeutic potential of this metabolite, further research and more detailed studies are necessary. These results contribute to the scientific knowledge of the region and lay the groundwork for exploring new pharmacological and therapeutic applications in the field of phytotherapy.

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Author Contribution

Conceptualization: A.M.; Data curation: O.M, G.G., S.E., N.C. and A.M.; Formal analysis: O.M., P.C., G.G., S.E. and A.M.; Acquisition Financing: O.M.; Research: O.M., G.G., S.E. and A.M.; Methodology: A.M.; Project administration: O.M.; Resources: O.M.; Supervision: O.M. and G.G.; Validation: O.M. and G.G.; Visualization: O.M. and A.M.; Writing-original draft, review and editing: A.M.

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