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Research article

Gochnatia glutinosa (D.Don) D.Don ex Hook. & Arn.: A plant with medicinal value against inflammatory disorders and infections

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ABSTRACT

Gochnatia glutinosa is a shrub that grown in the Argentinean semiarid region (Monte region) used in the ancestral medicine as an antiseptic and anti-inflammatory agent.

This study was aimed to examine the morpho-anatomical characteristics of *G. glutinosa* aerial parts, identify the chemical composition of traditionally used preparations to assess its pharmacobotanical characterization and evaluate its activity as antiseptic and anti-inflammatory to give scientific support to its traditional uses. *G. glutinosa* morpho-anatomical description was performed following standard histological techniques. Tincture and infusion of its aerial parts were prepared and were subjected to phytochemical analysis. Xanthine oxidase (XOD) and lipoxygenase (LOX) inhibition experiments, as well as $ABTS^{\bullet+}$, superoxide radical, and hydrogen peroxide scavenging activity, were carried out. The growth inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA) strains was also determined.

The morpho-anatomical traits of *G. glutinosa* leaves and stems were reported for the first time. The medicinal preparations exhibited a large amount of phenolic chemicals mainly flavonoids such as rhamnetin, arcapillin, rhamnacin, hesperetin, isorhamnetin, centaureidin, europetin 7-O-mehylmyricetin, cirsiliol, sakuranetin, genkwanin and eupatorine and also phenolic acids and diterpenoid derivatives. Both preparations had free radical scavenging activity and were able to reduce both XOD and LOX activity, indicating their anti-inflammatory properties. Besides, tincture was effective against all MRSA strains (MIC values ranging from 60 to 240 g DW/mL).

The results obtained in this work scientifically support the medicinal popular use of *G. glutinosa* as an antiseptic and anti-inflammatory. The identification of bioactive compounds and their

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morpho-anatomical description contribute to the quality control of this medicinal plant from Argentine Calchaquí Valley.

1. Introduction

Gochnatia Kunth (Asteraceae family) includes about seventy species found in North and South America, as well as Asia. *Gochnatia cardenasii* S·F.Blake, *G. curviflora* (Griseb.) O.Hoffm. *(Syn. Moquinia curviflora)*, *G. foliolosa* (D.Don) D.Don ex Hook. & Arn., *G. glutinosa* (D.Don) D.Don ex Hook. & Arn., and *G. palosanto* Cabrera are distributed in Argentina [1].

Based on morphology, geographical distribution and molecular data [2–4] was proposed to resurrect and use the Genus *Pentaphorus* [5] formerly placed as a section of *Gochnatia* S.L. [6], but this genus is not widely used and many times it is pointed out as unresolved or synonym. Either treated as genus or section *Penthaporus* includes two species endemics from Chile and Argentina, *Pentaphorus foliolosus* D. Don, formerly *G. foliolosa* and *Pentaphorus glutinosus* D. Don formerly *G. glutinosa* [7,8]. The plant list database cites *G. glutinosa* as the accepted species and *P. glutinosus*, *G. glutinosa* var *media* Kuntze; *G. glutinosa* var *puberula* Kuntze; *G. glutinosa* var. *viscosissima* (Kuntze) and *G. viscosissima* (Kuntze) S.F. Blake as synonyms [9].

Gochnatia glutinosa (D. Don) Hook. & Arn. (Sect. Pentaphorus (D. Don) DC.; sensu Cabrera, 1971) commonly named "jarillilla", "jarilla sacancia", "jarilla sacanza", "sacancia", "sacanza", "acancio", "tola", "tola muña", is a resinous - sticky to the touch, aromatic, monoecious shrub (0.60–2 m high) [10] with white-lilaceous flowers (Fig. 1). It grows between 500 until 4000 m above sea level in the provinces of Catamarca, Jujuy, La Pampa, La Rioja, Mendoza, Neuquén, Salta, San Juan, San Luis, and Tucumán, and was included in the Red List of Endemic Plants of Argentina in category 1 [11].

Although there is some data on *Gochnatia* species morphology and anatomy [3,12–19], to date, there is no reports on *G. glutinosa*.

The Calchaquíes communities use "jarilla sacanza" aqueous and ethanolic preparations to treat infectious and inflammatory processes. Because it is a shrub that has traditionally been used by the locals as a rubefascient, antiseptic, pedic deodorant (antifungal), anti-inflammatory, and antioxidant [20], it is of utmost importance to perform pharmacognostic, morpho-anatomical, chemical and functional characterization of this species that allow validate its ancestral use and a future quality control of the plant drug and its derivate products.

Some secondary metabolites in the Subtribe Gochnatiinae were previously reported [21]. Previous research on a *G. glutinosa* collection from Argentina's Mendoza province yielded the separation of two ent-pimaradienes [22]. Meanwhile, a linear sesquiterpene lactone and two homosesquiterpene lactones were discovered from a Tucumán province (Argentina) collection. Genkwanin, sakuranetin, 7'-methoxy-5,3',4'-trihydroxyflavanone, 5,7,4'-trihydroxy-3,3'-dimethoxyflavone, and 5,3',4'-trihydroxy-3,7-dimethoxyflavone were also discovered, as well as minor levels of pinoresinol and scopoletin [23,24].

Calderón et al. (2009) [25] found that methanolic extracts of *G. glutinosa* have antiparasitic action against *Trypanosoma cruzi*, *Plasmodium falciparum*, and *Leishmania*. Ethanolic extracts showed antifungal activity against *Candida tropicalis*, *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264, *Microsporum gypseum*, and *Trichophyton rubrum* [26]. Calderón et al. (2006)

Fig. 1. Photography of *Gochnatia glutinosa,* at the collection site (Amaicha del Valle, Tucumán, Argentina). A) Adult plants of *Gochnatia glutinosa* with flowers B) Magnification of flowers of *G. glutinosa*.

[27] also described cytotoxic activity.

This study was aimed to examine the morpho-anatomical characteristics of *G. glutinosa* aerial parts, identify the chemical composition of traditionally used preparations to assess its pharmacobotanical characterization and evaluate its activity as antiseptic and anti-inflammatory to give scientific support to its traditional uses.

2. Material and methods

2.1. Plant material

Aerial parts from *Gochnatia glutinosa* were collected in February of 2020 at Amaicha del Valle, Department Tafí del Valle, Province Tucumán (latitude 26.593997, longitude −65.857492). Voucher specimens of each collection were deposited at the Herbarium of Fundación Miguel Lillo (LIL), Tucumán, Argentina (Herbarium numbers 707950/LIL).

2.2. Histological analysis

Segments of stem from the third and fifth internode and six leaves (third to fifth leaf from the apex) of five specimens were used fresh to perform histochemical tests or were fixed in acetic acid, formalin, 50% ethanol (5:5:90 v/v/v) for anatomical characterization [28,29]. Direct scraping was used to obtain leaf epidermis [30] and then, were diaphanized [31], and dyed with cresyl violet. The foliar venation was described according to Hickey (1974, 1979) and Ellis et al. (2009) [32–34]. Stomata types were described according to Dilcher (1974) [35].

An optic microscope with polarizing light filter (Zeiss Axiolab) was used. The Axio Vision software version 4.8.2 (Carl Zeiss Ltd, Herts, UK) was used to measure the tissues (n = 30). To acquire statistical summary measures, the Infostat (Version 1.1) program (Di Rienzo et al., 2018) was used.

2.3. Histochemical analysis

Solutions of Ferric chloride, Toluidine blue O, Iodine potassium iodide (IKI), Sudan IV, Draggendorf reagent, Nadi reagent (nafthol-α-dimetil-*p*-fenilenediamina) and acid fuchsine were used to visualize phenolic compounds, polysaccharides, starch, lipids, alkaloids, terpenoids and proteins respectively [28,36–38] in fresh and rehydrated leaf. Sections of leaf (25–30 μm) treated and without treatment were observed under light microscope.

2.4. Phytochemical analysis

2.4.1. Extracts preparation

G. glutinosa aerial parts were dried in a forced air stove at 40 °C, then ground to fine powder and were used to obtain ethanolic (tincture) and aqueous (infusion) preparations.

Tincture: The powder was extracted by maceration in ethanol 80° (5 g/100 mL) during 1 h with temperature (40 °C) in ultrasonic bath with cycles of 10 min. To obtain the dry extracts, the extracts were filtered, dried in a rotatory evaporator, and freeze dried. The extracts were then vacuum-packed in oxygen barrier bags (Multivac, D-8941, Germany).

Infusions: The powder was extracted in distilled water (5 g/100 mL) at 100 °C for 10 min and then was filtered. Infusions were freeze dried to afford the dry extracts that were vacuum-packed in oxygen barrier bags until used.

2.4.2. Phytochemical characterization

The extractives solutions (tincture and infusions) were characterized by the quantification of total phenolic compound content through Folin–Ciocalteu method [39]. Total flavonoids content was estimated using $AlCl_3$ [40].

The chemical profile of the extracts was analyzed by liquid chromatography. HPLC system (Waters 1525, Binary HPLC Pump system with a 1500 Series Column Heater, Waters 2998 photodiode array detector, XBridgeTM C18 column (4.6 × 150 mm, 5 μm) and EmpowerTM 2 software) was used to analyze the extracts. The solvent system used for the component separation from *G. glutinosa* infusion and tincture was composed of solvent A (9% acetic acid in water) and solvent B (methanol) (25–45% B from 0 to 10 min and kept at 45% B from 10 to 20 min; 45–70% B from 20 to 40 min; 70–75% B from 40 to 50 min; 75–100% B from 50 to 55 min). The detection of the components was achieved in comparison with commercial compounds and through co-injection of commercial compounds with the *G. glutinosa* infusion and tincture.

2.4.3. UHPLC-q-TOF-ESI-MSn

The separation and identification of secondary metabolites from *G. glutinosa* were carried out on a UHPLC-ESI-QTOF-MS system, equipped with UHPLC Ultimate 3000 RS with Chromeleon 6.8 software (Dionex GmbH, Idstein, Germany), and a Bruker maXis ESI-QTOF-MS. The chromatographic equipment consisted of a quaternary pump, an autosampler, a thermostated column compartment, and a photodiode array detector. The elution was performed using a binary gradient system with eluent (A) 0.1% formic acid in the water, eluent (B) 0.1% formic acid in acetonitrile, and the gradient: isocratic 1% B (0–2 min), 1–5% B (2–3 min), isocratic 5% B (3–5 min), 5–10% B (5–8 min), 10–30% B (8–30 min), 30–95% B (319–38 min), and 1% B isocratic (39–50 min). The separation was carried out with an acclaim Thermo 5 μm C18 80 Å (150 mm × 4.6 mm) column at a flow rate of 1.0 mL/min. ESI-QTOF-MS experiments in

negative ion mode were recorded and the scanning range was between 100 and 1200 *m/z*. Electrospray ionization (ESI) conditions included capillary temperature of 200 °C, a capillary voltage of 2.0 kV, dry gas flow of 8 L/min, and a pressure of 2 bars for the nebulizer. The experiments were performed in automatic MS/MS mode. The structural characterization of compounds was based on HR full MS, fragmentation patterns, and similarity with literature data. For the analysis, 5 mg of each extract was dissolved in 2 mL of methanol, passed through a polytetrafluoroethylene (PTFE) filter, and 10 μL were injected into the apparatus. MS data was analyzed using Bruker Data Analysis 4.0 (Bruker Daltonik GmbH, Bremen, Germany) and ACD lab spectrum processor (New York, USA) software.

2.5. Biological assays

2.5.1. Antimicrobial activities

2.5.1.1. Microorganisms. Methicillin-resistant *Staphylococcus aureus* strains (INBIOFIV–S1–S10) were obtained from clinical samples of skin and soft tissue infections from Nestor Kirchner Hospital, San Miguel de Tucumán, Tucumán, Argentina. *S. aureus* ATCC 29213 and ATCC 43300 were used as controls. All organisms were preserved in brain–heart infusion containing glycerol 30% at −80 °C. The strains were transferred to Mueller Hinton agar (MHA) and incubated at 35 °C during 12 h. Individual colonies were suspended in 5 mL of 0.9% NaCl solution. The inocula were prepared by adjusting turbidity to 0.08 at 560 nm (10^8 CFU/mL). The cell number in cation-adjusted Mueller–Hinton broth (CAMHB) was estimated using a serial dilution technique according to CLSI (2015) [41] for each assay.

2.5.1.2. Bioautographic assay. Plates of silica gel 60 F-254 (0.2 mm, Merck) were seeded by duplicate with the different extracts of *G. glutinosa*. The plates were developed with toluene: acetone: chloroform (4.5; 3.5:2.5 v/v), air-dried and one set of the plates were revealed with UV light at 365 nm and with Neu's reagent. The corresponding duplicate remained for the bioautographic assay. Bioautographic assay was performed using 2 mL of soft medium (BHI with 0.6% agar) containing 10^5 CFU of methicillin-resistant *S. aureus* (INBIOFIV–S1) and ATCC 29213. The plates were covered with soft medium with inocula, were maintained at 37 °C for 16–20 h and revealed with MTT solution (2.5 mg/mL) in PBS [42]. The growth inhibition areas, yellow colored, were compared with the Rf of the related spots on the TLC plate revealed with Neu's reagent [43].

2.5.1.3. Minimum inhibitory concentration (MIC). The antibacterial activity of extracts was evaluated with the agar macrodilution method by two-fold serial dilution [41]. The test was performed in MHA medium. Plates containing MHA and different dilutions of extracts (0.03–0.24 mg DW/mL) were inoculated in spots of 2 μL with each bacterial cell suspension (10^4 CFU) and aerobically incubated for 18 h at 35 °C. Solvent controls (ethanol 80° and distilled water) were included.

The MIC was defined as the lowest concentration of soluble principle or reference antibiotics where no colony was observed after incubation (CLSI, 2006). The MIC values were also determined for different commercial antibiotics: Vancomycin (Van), Ampicillin (Amp), Gentamycin (Gen), Methicillin (Met), Oxacillin (Oxa), Ciprofloxacina (CIP), Levofloxacina (LEV), Moxifloxacina (MOX), Eritromicina (ERI), Clindamicina (CLI), Quinupristina (QD), Linezoline (LIN), Teicoplanina (TEI), Minociclina (MIN), Tetraciclina (TET), Rifampin (RFA), Trimetropina sulfa (TMS).

2.6. Antioxidant activity

2.6.1. $ABTS^{\bullet+}$ scavenging activity

The antioxidant capacity was determined by the modified $ABTS^{\bullet+}$ method as described by Cattaneo et al., 2016 [44]. One mL $ABTS^{\bullet+}$ (absorbance of 0.70 ± 0.02 at 734 nm) was put in contact with samples dilutions (0.1–7.6 μg GAE/mL). Absorbance was recorded at 734 nm during 6 min. Results are shown as SC_{50} values (μg GAE/mL required to scavenge 50% $ABTS^{\bullet+}$).

2.6.2. Superoxide radical scavenging assay

The capacity of infusion and tincture (1–36 μg GAE/mL) to scavenging superoxide radicals was measured with the phenazin methosulfate method [45]. The SC_{50} values were measured as μg GAE/mL necessary to inhibit the 50% of superoxide radicals. Quercetin was used as antioxidant reference compound.

2.6.3. H_2O_2 scavenging assay

The H_2O_2 scavenging assay was assayed according to Fernando and Soysa method [46] with a few modifications. The extracts (1–36 μg GAE/mL) and H_2O_2 (0.7 mM) were pre-incubated for 3 min at 37 °C. Then, phenol (12 mM) and 4-aminoantipyrine (0.5 mM) and horseradish peroxidase were added (0,1 U/mL) to the mixture and incubated at 37 °C during 30 min. The formation of colored quinone was measured at 504 nm. The concentration necessary to scavenge 50% of H_2O_2 (μg GAE/mL) was determined. Quercetin and ascorbic acid were used as controls.

2.7. Antiinflammatory activity

2.7.1. Xanthine oxidase assay

The effect of different aliquots of extracts (9–117 μg GAE/mL) on the activity of xanthine oxidase (0.003 U) was determined

spectrophotometrically at 290 nm (Microplate Reader Thermo Scientific Multiskan GO) by measuring the synthesis of uric acid from xanthine as substrate (60 μL, 1 mM). The reaction mixture (120 μL) was incubated at 25 °C during 30 min [47]. Indomethacin (20–100 μg/mL), acetylsalicylic acid (2–40 μg/mL) and allopurinol (2–100 μg/mL) were used as positive controls.

2.7.2. Inhibition of lipoxygenase

LOX (EC 1.13.11.12) inhibition was determined spectrophotometrically [48]. The method is based on the enzymatic oxidation of linoleic acid to its hydroperoxide. The reaction mixture containing soybean 1-LOX (948 U/mL. 0.9 mM) was exposed to different concentrations of the samples (2–13 μg GAE/mL) or vehicle (0.1% DMSO final concentration) and its substrate, linoleic acid (50 μM). Caffeic acid (up to 100 μg/mL) and naproxen (up to 25 μg/mL) were used as positive controls. The mixture was incubated at 25 °C for 5 min. The quantity of hydroperoxides produced from linoleic acid was determined by measuring the absorbance at 234 nm and the kinetics of the enzymatic reaction was followed by measuring product formation every 30 seg. Dose-response curves were plotted for each sample, and the result was expressed as IC_{50} (concentration that inhibits 50% of LOX's activity).

2.8. Statistical analysis

All assays were performed at least three times with three different sample preparations. Each experimental value was expressed as the mean ± standard deviation (SD). The scientific statistic software InfoStat was used to evaluate the significance of differences between groups (Tukey's test) [49]. Correlations between the antioxidant methods were calculated.

3. Results

3.1. Morpho-anatomical characteristics of G. glutinosa aerial parts

Leaves were alternate, sessile, subcoriaceous. The lamina appeared linear-ovate to linear-obovate, medially symmetrical with resinous deposits on its surface. The leaves were classified as microphylls 20.55 ± 2.55 mm length by 2.55 ± 0.75 mm latitude; with acute attenuated and slightly sheeted base, entire margins, and acute apex (Fig. 2A). The primary venation was characterized as

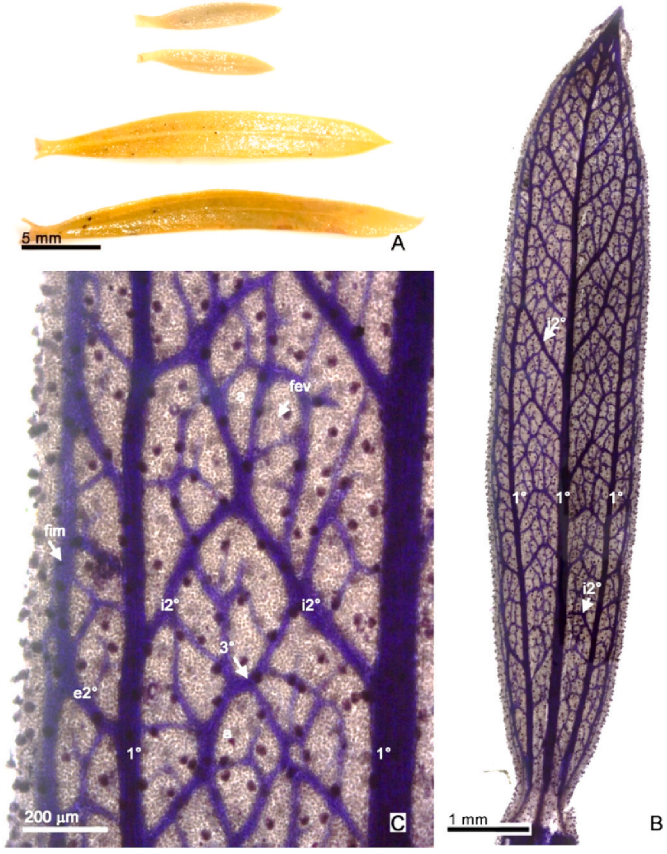

Fig. 2. *Gochnatia glutinosa.* A. Third node and fully expanded leaves. B. Venation pattern. C. Venation pattern detail. References: 1°, primary vein; 3°, tertiary vein; a, areole; e2°, exterior secondary; fev, freely ending veinlet; fim, fimbrial vein; i2°, interior secondary.

paralelodromous three-veined, instead of actinodromous [2,15]. The three primary veins run in convergent arches toward the leaf apex and are originated beside each other at the leaf base (Fig. 2B). Secondary veins were excurrent, alternate to sub opposite, diverging in acute angles from the primary veins with irregular spacing forming interior secondaries. In the basal and middle section, interior secondary veins connect with other secondary in prominent arches while at the apical third of the leaf they connect directly with the lateral primary veins (Fig. 2B). Sometimes fimbrial marginal vein presented external incomplete loops form by third order veins (Fig. 2C). Tertiary veins constitute the minor veins order, they diverge from the primary and secondary veins at variable angles forming a randomly reticulated frame anastomosing with other tertiary or secondary veins to form an irregular polygonal net and areoles with no veinlet or with one rarely branched freely ending veinlet (Fig. 2 C).

The leaves were amphistomatic, with smooth to slightly striated cuticle and anomocytic stomata (36.31 ± 2.54 μm long. by 29.83 ± 3.31 μm lat. and 40.17 ± 4.93 μm long. by 32.25 ± 2.52 μm lat. for the adaxial and abaxial surface respectively). The stomata were

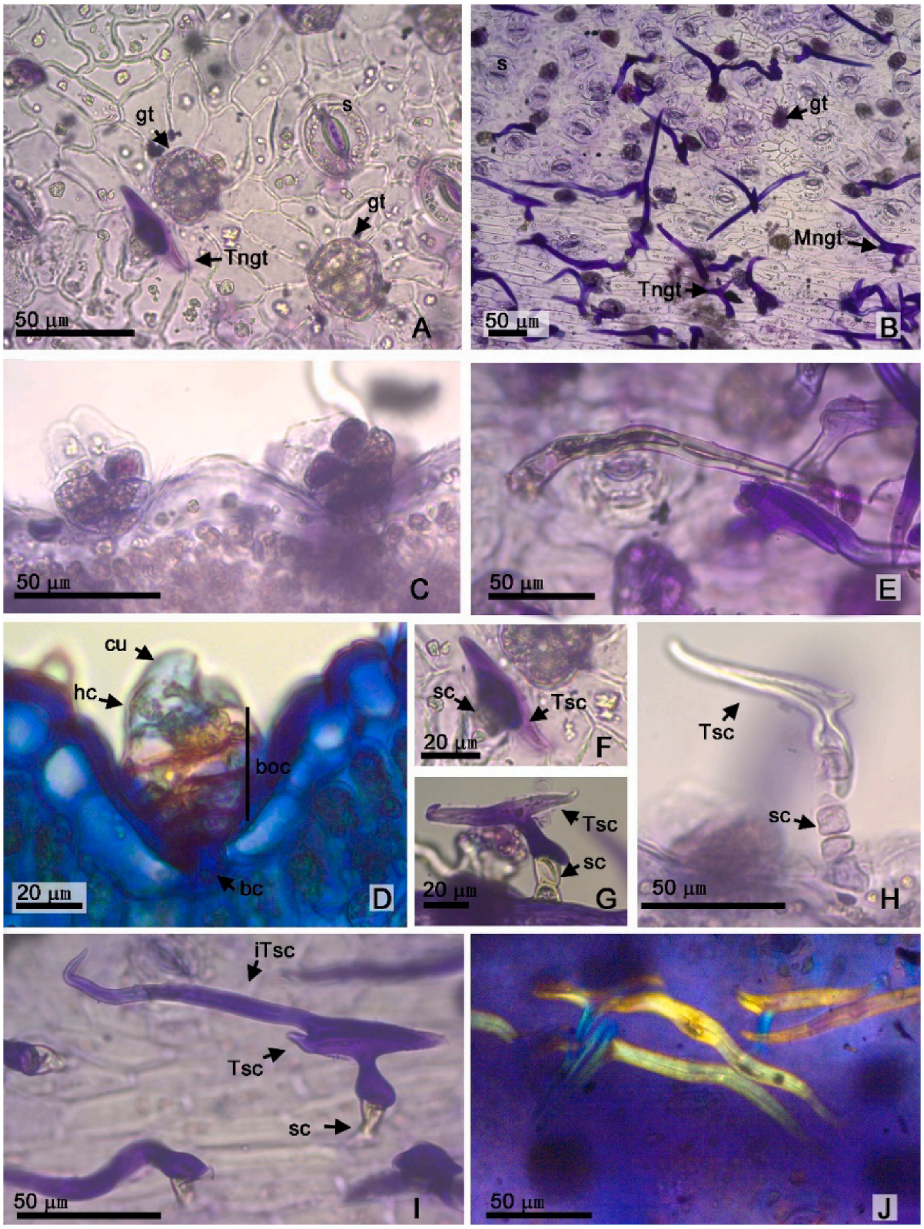

Fig. 3. *Gochnatia glutinosa*. Leaf superficial view A-B. Adaxial and abaxial epidermis respectively. C-D. Biseriated glandular trichome in frontal and lateral view respectively. E. Flagellate unicellular non-glandular trichome. F–H. T-shaped non glandular trichome with one, two and four stalk cells respectively. I. Multistoried T-shaped non glandular trichome. J. Non glandular trichomes refrigent apical cell under polarized light. References: bc, basal cells; boc, body cells; cu, cuticle; gt, glandular trichome; hc, head cells; iTsc, invented T-shaped cell; Mngt, multistoried non glandular trichome; s, stoma; sc, stalk cell; Tngt, T shaped non glandular trichome; Tsc, T-shaped cell.

raised above the level of the epidermal cells; at a density of 59.21 ± 17.50 and 72.08 ± 9.70 $mm^{2\ -1}$ for the superior and inferior epidermis respectively. Both surfaces presented polygonal epidermal cells with slightly curved anticlinal walls (Fig. 3A–B).

Upper and lower surfaces presented sunken biseriates glandular trichomes comprised by a two celled foot, 3 to 4 rows of body cells and a two celled head enclosed by a persistent or collapsed cuticular vesicle, not always evident, in fresh tissues the secretion products appeared spilled on the top of the trichome and on the surface of the lamina. The glandular trichomes presented approximately 43.43 ± 3.76 μm long. by 40.91 ± 4.33 μm lat. the major semi axis of the heat (Fig. 3C–D) and were present in a density of 72.07 ± 20.94 and 69.58 ± 23.28 $mm^{2\ -1}$ for the adaxial and abaxial epidermis respectively. This type of glandular hairs is widespread in many Asteraceae and especially in *Gochnatia*. In *Pentaphorus* they cover almost the entire surface of the leaf [12,13,15,19]. Glandular trichomes, seems to be a common trait shared by many species of the genus *Gochnatia*, these structures may allow an adaptive defense against herbivores and other biotic and abiotic factors [18]. Three types of non-glandular trichomes were also observed, i) flagellate unicellular trichomes with 132.24 ± 39.48 μm length, rarely observed (Fig. 3E) previously cited by others authors as restricted to the margins [3,15]; ii) two-armed hairs or T-shaped, malpighiaceous dolabriform hairs with one to five short stalk cells, uniseriate, and a unicellular apical head cell T-shaped with symmetrical or asymmetrical arms (Fig. 3F–H); and iii) multistoried T-shaped hairs similar to the 2-armed hairs but comprising a second apical inverted T-shaped cell with a prolongation (Fig. 3I). The last two types of trichomes presented highly variable dimensions according to the number of cells at the base and the length of the apical cells. Trichomes type ii and iii were randomly distributed on the leaf blade more frequently in the lower epidermis, they presented apical cells with thickened walls refringent under polarized light (Fig. 3J). In mature leaves non glandular trichomes were rare, possibly lost due to the fragility of the basal cells. T-shaped trichomes were previously cited for *Gochnatia* sect. *Moquiniastrum*, *G. cordata* Less., *Cyclolepis*, and *Hyalis argentea* D. Don ex Hook. & Arn. among the *Gochnatia* complex and many other Asteraceae [12,14,15,19], whereas multistoried T-shaped trichome are not very common in Asteraceae and only have been reported in the tribe Senecioneae (Robinson, 1989) and in *Ianthopappus* genus [15].

G. glutinosa leaves in transversal section showed heterogenous isolateral mesophyll with dense content, formed by three to four layers of short adaxial palisade chlorenchyma, three to four layers of compact spongy tissues with rounded cells and one or two layers of palisade abaxial parenchyma (Fig. 4A). This is an interesting character since *G. polymorpha* (Less.) Cabrera and *G. barosii* Cabrera exhibited dorsiventral leaves [17–19]. The cuticles thick, uniseriate epidermis with stomata raised above the epidermal cells were

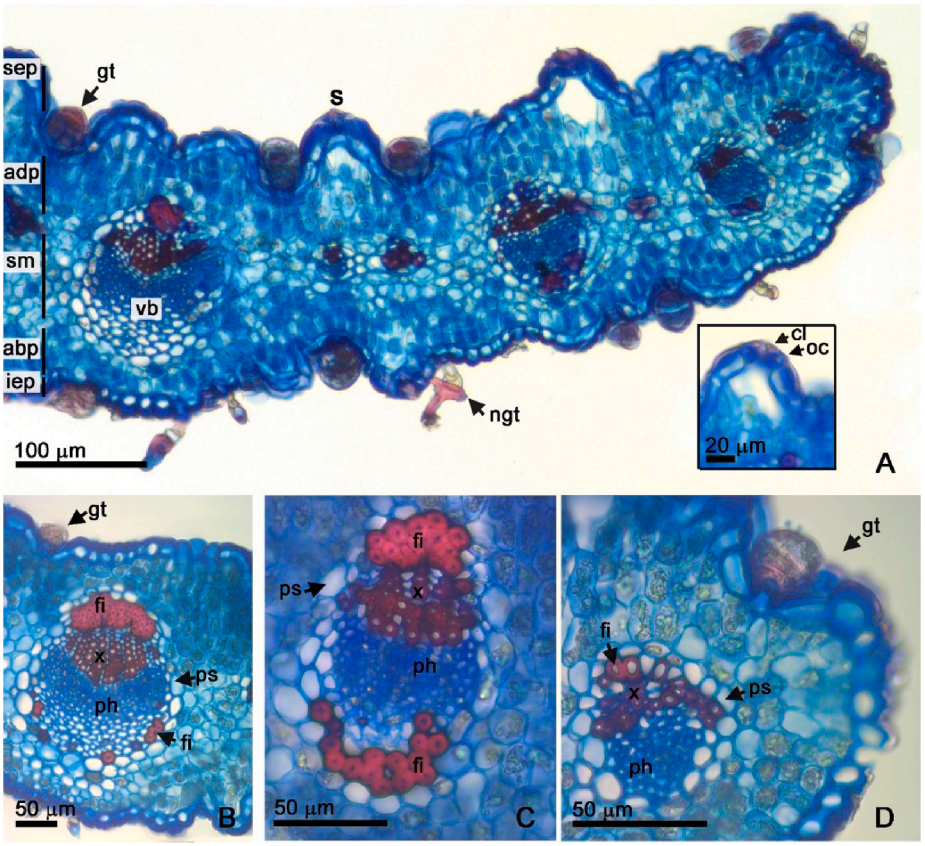

Fig. 4. *Gochnatia glutinosa*. Leaf section. A. Leaf transversal section. Detail of raised stomata with outer cuticular ledge. B-D. Vascular bundles with development of different fiber reinforcements as cups or isolated fibers. References: abp, abaxial palisade; adp, adaxial palisade; cl, cuticular ledge; fi, fibers; gt, glandular trichome; iep, inferior epidermis; ngt, non-glandular trichome; oc, occlusive cell; ph, phloem; ps, parenchyma sheath; s, stoma; sep, superior epidermis; sm; spongy mesophyll; vb, vascular bundle; x, xylem.

found. The guard cells exhibited a remarkable outer cuticular ledge (Fig. 4A). Unlike what it was reported for *G. pulchra* Cabrera [13], idioblast were not observed in the mesophyll. Minor collateral vascular bundles were embedded in the central spongy mesophyll and enclosed by a parenchyma sheath or alternating parenchyma cells and fibers toward the phloem and xylem poles being more or less developed as caps in the higher order bundles or as isolated fibers in the minor bundles (Fig. 4B–D). In transection the midrib showed a slightly biconvex to concave-convex shape, uniseriate epidermis, with continuous adaxial palisade at the level of the vascular bundle. The vascular system exhibited a single collateral vascular bundle surrounded by a parenchymatous sheath reinforced by sclerenchyma cups or isolated fibers toward the xylem pole or toward the xylem and phloem poles (Fig. 4A–B).

In superficial view, the stem presented quadrangular epidermal cells with straight anticlinal walls, raised anomocityc stomata, glandular and eglandular trichomes identical to those described for the leaves (Fig. 5A). In transection it showed circular shape with incipient secondary growth, a single layer of epidermal cells, a cortex conformed by 5–6 layers of small thick-walled parenchyma cells

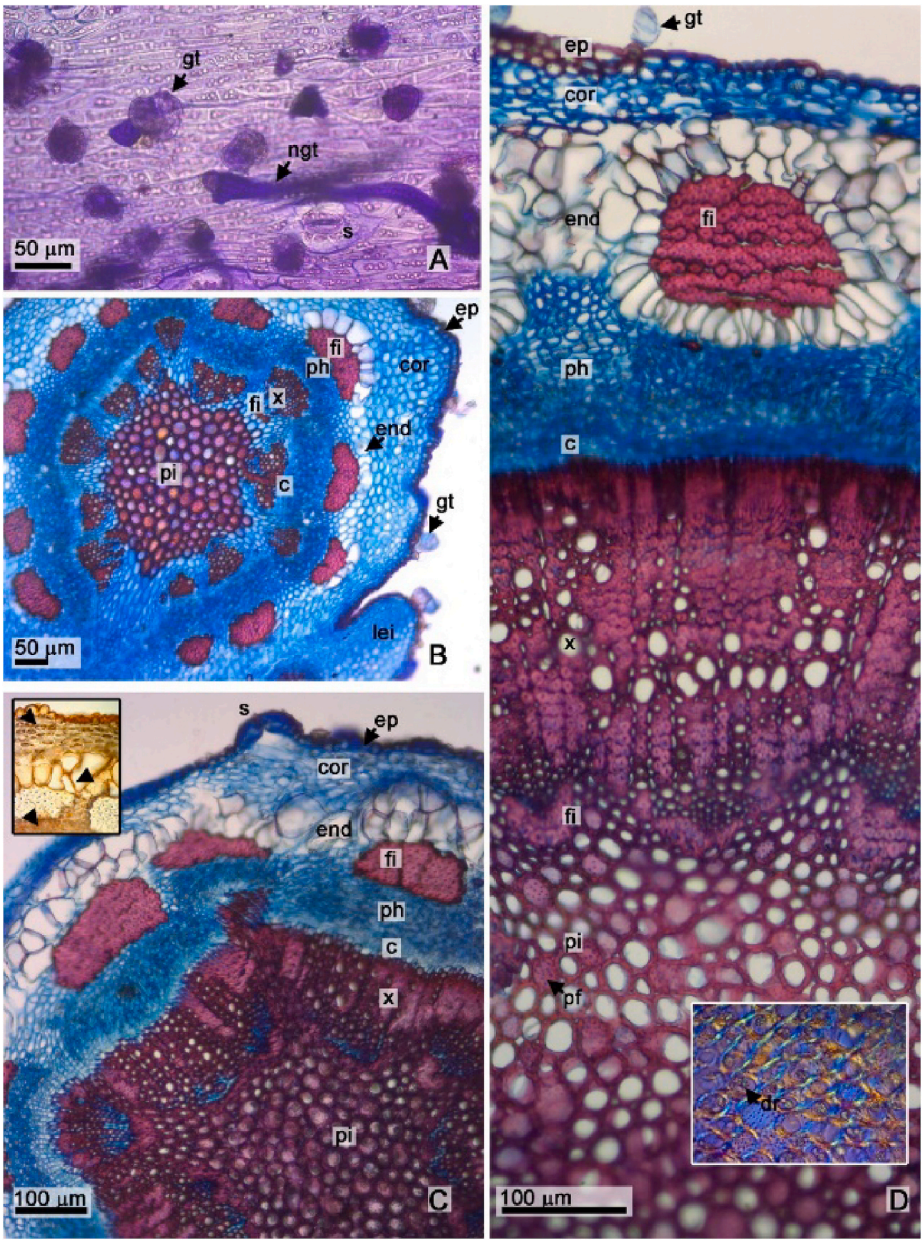

Fig. 5. *Gochnatia glutinosa*. Stem A. Stem epidemis. B-D. Stem transversal sections in incipient and more advanced secondary growth. C. Detail with suberinized endodermoid layer stained with sudan IV which also revealed the presence of lipophilic substances (arrow) in the cuticle of the epidermal cells, cortex and phloem cells. D. Detail of calcium oxalate crystal in the pith under polarized ligth. References: c, cambium; dr, druse; end, endodermoid layer; ep, epidermis; fi, fiber; lei, leaf insertion; pf, punctuation field; pi, pith; ph; phloem; x, xylem.

and a vascular cylinder delimited by a suberinized endo-dermoid layer with thin walls and cells larger than the ones observed at the cortex. The vascular cylinder shows a typical eustele structure with open collateral vascular bundles and perivascular fiber caps toward the phloem and less developed toward the xylem. The pith was stellate with lignified cells with calcium oxalate crystals as druses and with evident punctuation fields (Fig. 5B).

In a more advanced stage of secondary growth, the endo-dermoid tissues appeared multilayered surrounding perivascular fibers caps, and the secondary xylem develops abundant fibers (Fig. 5C–D).

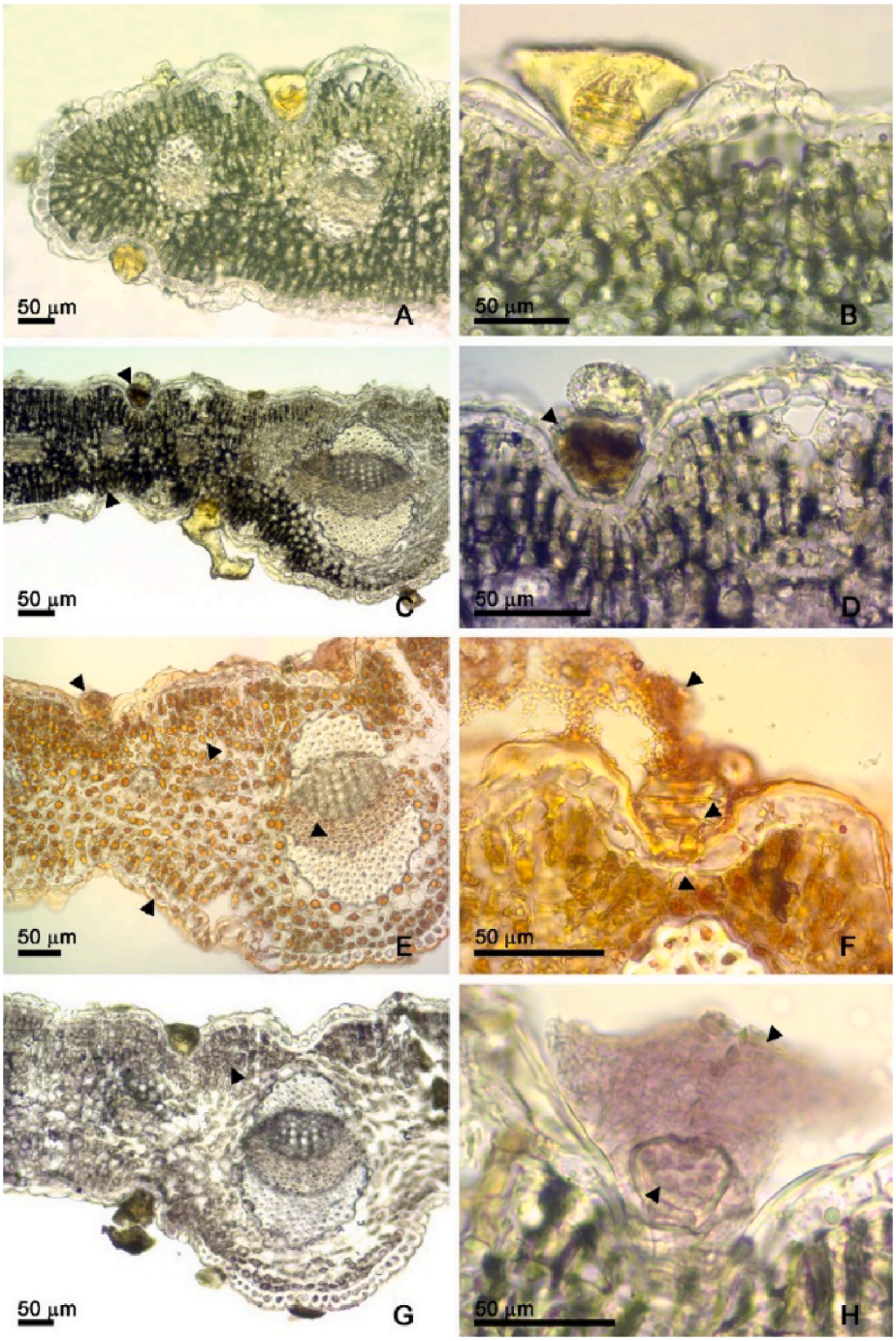

Fig. 6. *Gochnatia glutinosa*. Leaf histochemistry. A-B. Control fresh sections. A, C, E, G. Leaf mesophyll and mid vein B, D, F, H. Glandular trichome. C-D. $FeCl_3$ reactive for phenolic. E-F. Sudan IV staining for lipophilic substances. G-H. NADI reagent for terpenoids. References: arrow heads indicated positive staining or reactions.

Endodermis or endordermoid layers were previously described for the stems of *G. polymorpha* [19], *G. glutinosa* [16] and other Asteraceae [50]; notwithstanding its subsequent development in a multilayer tissue in *G. glutinosa*, is described for the first time in the present work.

The morpho-anatomical description of the aerial parts of *G. glutinosa* will contribute to the quality control of this medicinal plant from Argentine Calchaquí Valley.

3.2. Histochemical analysis

In fresh sections, the mesophyll cells presented refringent content and chlorophylls whereas the glandular trichomes and the products secreted by them presented amber color (Fig. 6A–B). $FeCl_3$ reactive revealed the presence for phenolic compounds (black-grey) in part of the mesophyll content and into the body cell and head cells of the glandular trichomes (Fig. 6C–D), coinciding this type of compound with flavone, flavonol and coumarins previously reported for the species [23,24,51].

Similarly, Sudan IV staining for lipophilic substances (red) gave positive coloration in the refringent droplets of the mesophyll cells and phloem, the cuticles, part of the content of the head cells of the glandular trichomes and part of the compounds spilled by them (Fig. 6E–F). More over NADI reagent for terpenoids (purple) positively colored part of the content of the mesophyll, the apical cells of the glandular trichome and part of the product secreted by them (Fig. 6G–H), probably revealing the presence of compounds such as sesquiterpenes and diterpenes; 8-hydroxy-3-7-11-trimethy dodeca-cis-2-trans-6-10-trien-13,1-olide; ent pimara-8(14)-15-diene-3-β-18-diol [22,23,51].

Head cells of the glandular trichomes and the product secret by them also stained positive to polysaccharides different than starch with toluidine blue O (blue) (Fig. 7A). The presence of proteins was revealed by acid fuchsine (pink) for part the content of the mesophyll, mainly in the palisade parenchyma (Fig. 7B). Draggendorf and lugol tests to detect alkaloids and starch gave negative results (not shown).

3.3. Phytochemical composition of G. glutinosa extracts

3.3.1. Quantitative analysis

In this work was analyzed the phytochemical composition of two traditional preparations of *G. glutinosa* aerial parts (tincture and infusions). Extracts exhibited a large amount of phenolic chemicals; the results are shown in Table 1. The tincture shown 23.30 ± 1.0 g GAE/100 g dry extract of total phenolic compounds and the infusion has 19.25 ± 1.5 g GAE/100 g dry extract. The flavonoids contents were also high, 78.00 ± 1.00 mg QE/100 g dry extract for the tincture, and 15.00 ± 2.00 mg QE/100 g dry extract for the infusion. The extractive yields were higher for the tincture than for the infusion (34.85 and 14.83 g dry extract/100 g plant material, respectively) (Table 1).

3.3.2. Metabolomic in G. glutinosa extracts

Twenty-five peaks (Fig. 8) were tentatively identified for the first time in an aqueous extract (Fig. 8 a) and ethanolic extract (Fig. 8 b) of *G. glutinosa* using UHPLC/ESI/MS/MS in negative mode. The metabolites identified in this species were mainly flavonoids, phenolic acids and diterpenoid derivatives (Table 2).

3.3.2.1. Phenolic acids. Peaks 2 and 3 with daughter ion characteristic of chlorogenic acids, were identified as isomers of di coumaroyl quinic acid (3,5 di C-QA and 4,5 di C-QA, $C_{25}H_{23}O_{12}$), respectively, while peak 4 was assigned as diapocynin ($C_{18}H_{17}O_6$) and peak 14 as sclerketide D ($C_{18}H_{27}O_5$), finally, peak 19 with an anion at *m/z*: 321.1707 identified as 2,4 zearalenone ($C_{18}H_{24}O_5$). Diapocynin has been proven to be scavenger of superoxide anions, antitumoral, and antiinflammatory [52–54]. Antiinflammatory activity of Sclerketide D and antioxidant activity of 4,5 di C-QA were also reported [55,56].

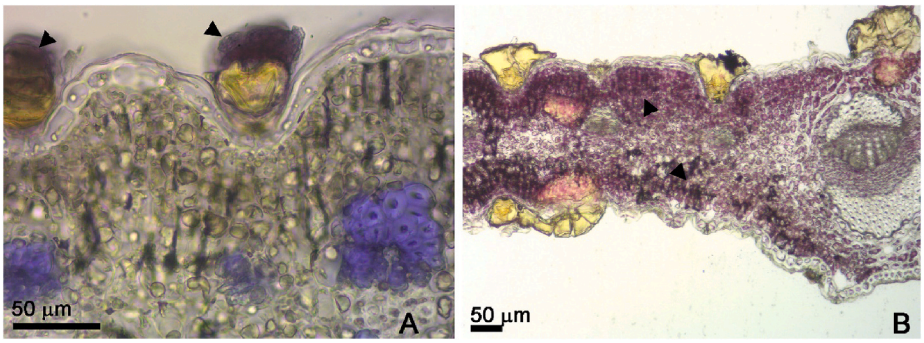

Fig. 7. *Gochnatia glutinosa*. Leaf histochemistry. A. Toluidine blue O stain to detection of polysaccharides different than starch. B. Acid fuchsine for proteins. References: arrow heads indicated positive staining or reactions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Phytochemical content of *G. glutinosa* extracts.

Phytochemical content	A	B
Total phenolics (g GAE/100 g DW)	19.25 ± 0.02[a]	23.3 ± 0.03[b]
Flavones and flavonols (g QE/100 g)	0.015 ± 0.001[a]	0.078 ± 0.001[b]
%Yield (g DW/100 g plant material)	14.83 ± 0.01[a]	34.85 ± 0.02[b]
%Yield (g DW/100 mL extract)	0.74 ± 0.01[a]	1.74 ± 0.01[b]

A: Infusion¸ B: Tincture. Different letters (a, b) in the same line show significant differences in the phytochemical content among each extract, according to Tukey's multiple comparison ($p \leq 0.05$). ND: no detected. GAE: Gallic acid equivalent; QE: Quercetin equivalent.

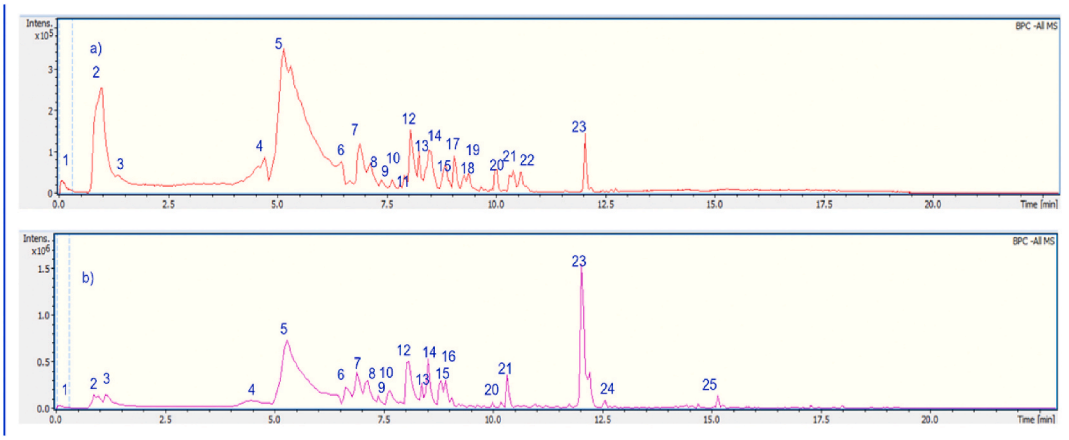

Fig. 8. UHPLC/ESI/MS/MS Chromatograms a) *Gochnatia glutinosa* aqueous extract, b) *Gochnatia glutinosa* ethanolic extract.

3.3.2.2. Flavonoids. Several flavonoids were identified, some of them with co spiking with authentic standards. Peak 1 with pseudomolecular ion at 345.0615 was identified as axillarin ($C_{17}H_{13}O_8$), in the same manner peaks 6–13, as rhamnetin, arcapillin, rhamnacin, hesperetin, isorhamnetin, centaureidin, europetin 7-O-mehylmyricetin, and cirsiliol, respectively, peak 15 and 16 as sakuranetin ($C_{16}H_{13}O_5$) and genkwanin respectively, and finally peak 20 as eupatorine. Among these compounds, genkwanin has been proven as a multi-functional pharmaceutical agent (antibacterial, antiplasmodial, antioxidant, chemopreventive, antiinflammatory, inhibitor for dehydrogenase type 1 in the 17 β-hydroxysteroid pathway), [57]. It was shown that methylation of free hydroxyl groups in apigenin and naringenin increases their metabolic stability and enhances their membrane transport, facilitating absorption and greater oral bioavailability [57]. Sakuranetin has many demonstrated biological activities such as antiinflammatory, antimutation, anti-*Helicobacter pylori*, antidiabetic, antiviral, and anticonvulsant properties. It can also exert protective effect on the brain and can be used to treat Alzheimer's disease [58]. Hesperetin has been proven to have anti-inflammatory, antifungal, antiviral, antioxidant, and anticancer properties [59].

Rhamnetin has demonstrated biological activities such as antioxidant, anticancer, anti-inflammatory, antiviral, and antibacterial activity [60]. Isorhamnetin is well known for its potential as a natural antioxidant, anti-adipogenic, anti-proliferative, and anti-tumor activities, it significantly suppressed LPS induced secretion of pro-inflammatory mediators, including nitric oxide (NO) and prostaglandin E2, without exhibiting significant cytotoxicity [61]. Cirsiliol exhibits activities such as antibacterial, antiproliferative and anti-inflammatory functions [62]. Eupatorin has anti-inflammatory activity, and it has a potential role in various cancerous disorders, such as human uterus carcinoma, human gastric adenocarcinoma, breast carcinoma, melanoma, and colon carcinoma [63].

Axillarin has been proven to have antioxidant [64], antiviral [65], antibacterial, antifungal and antiquorum sensing activity [66]. It was reported that arcapillin possesses antispasmodic activity [67] and it is a potent inhibitor of α-glucosidase and protein tyrosine phosphatase 1B [68], which could potentially be used to treat gastrointestinal disorders and as a preventive agent in the treatment of diabetes respectively. Centaureidin was reported to have anti-inflammatory activity as it inhibits pro-inflammatory cytokines [69], and the enzyme xanthine oxidase [70].

3.3.2.3. Coumarins. Peak 21 was identified as ferujol ($C_{19}H_{23}O_4$), a coumarin with estrogenic activity [71].

3.3.2.4. Fatty acids. Peak 22 was assigned as aleuritic acid methyl ester ($C_{17}H_{33}O_5$), peak 18 as Cibaric acid ($C_{18}H_{27}O_5$).

3.3.2.5. Terpenes. Peaks 23 and 24 with pseudomolecular anions at *m/z*: 317.2117 and 359.2227 were identified as Ent-8(14),15-pimaradien-1,2-epoxy-3 beta,18-diol, and Ent-8(14),15-pimaradien-1,2-epoxy-18-acetoxy, 3beta-ol, respectively, which are epoxidated derivatives of a compound previously isolated from this plant (Fig. 9), peak 17 as Cinnamodial ($C_{17}H_{23}O_5$), peak 25 as (−)

Table 2
Chemical composition of *Gochnatia glutinosa*.

Peak	Tentative identification	[M − H]⁻	Retention time (min.)	Theoretical mass (*m/z*)	Measured mass (*m/z*)	Accuracy (ppm)	Metabolite type	MS ions (ppm)
1	Na formiate (internal standard)	$C_4H_2O_4$	0.22	112.9829	112.9856	3.1	Standard	588.8964, 656.8829,724.8745
2	Di coumaroyl quinic acid (3,5 di C-QA)	$C_{25}H_{23}O_{12}$	0.72	515.1192	515.1195	−0.46	Phenolic acid	353.0877, (CA) 191.0571
3	Di coumaroyl quinic acid (4,5 di C-QA)	$C_{25}H_{23}O_{12}$	1.21	515.1192	515.1190	−0.36	Phenolic acid	353.0872, 191.0571 (QA)
4	Diapocynin	$C_{18}H_{17}O_6$	4.5	329.1089	329.1064	−7.7	Phenolic acid	303.0514, 179.0729
5	Axillarin	$C_{17}H_{13}O_8$	5.31	345.06068	345.0615	−2.6	Flavonol	315.0507, 691.13021 (2M − H), 179.9921, 151.00349
6	Rhamnetin[a]	$C_{16}H_{11}O_7$	6.54	315.0511	315.0505	−2.6	Flavonol	299.05616 (M-CH3), 279.1235, 255.0314
7	Arcapillin	$C_{18}H_{15}O_8$	6.95	359.0775	359.0761	−3.19	Flavone	277.1078, 112.9847, 179.0761,
8	Rhamnacin[a]	$C_{17}H_{13}O_7$	7.32	329.0657	329.0666	−2.91	Flavonol	315.0462, (M-CH3), 277.1075, 300.05554, 151.0020, 256.03405
9	Hesperetin[a]	$C_{16}H_{13}O_6$	7.65	301.0714	301.0716	−0.42	Flavonone	263.12825, 201.05187
10	Isorhamnetin[a]	$C_{16}H_{11}O_7$	7.51	315.0511	315.0515	−2.6	Flavonol	299.05616 (M-CH3), 287.0555
11	Centaureidin	$C_{18}H_{15}O_8$	7.72	359.07724	359.07665	−1.63	Flavonol	341.06717, 317.0666, 299.0643, 112.9847, 179.0761,
12	Europetin 7-O-methylmyricetin	$C_{16}H_{11}O_8$	8.07	331.0457	331.0457		Flavone	329.0656, 315.0513, (M-CH3), 301.0351 (M-2CH3)
13	Cirsiliol	$C_{17}H_{13}O_7$	8.12	329.0666	329.0669	0.92	Flavone	315.0465, (M-CH3), 271.0260, 300.05554, 151.0020, 256.03405
14	Sclerketide D	$C_{18}H_{27}O_5$	8.25	319.1707	309.1701	−2.09	Phenolic acid	179.0717, 97.0296, 619.3466 (2M − H)
15	Sakuranetin[a]	$C_{16}H_{13}O_5$	8.56	285.0768	285.0759	−2.96	Flavonone	247.0972,
16	Genkwanin[a]	$C_{16}H_{12}O_5$	8.81	283.0612	283.0602	−2.92	Flavone	247.1515, 269.0407, 201.1487
17	Cinnamodial	$C_{17}H_{23}O_5$	8.90	307.1548	307.1551	−0.72	terpene	283.09470, 615.31601 (2M − H)
18	Cibaric acid	$C_{18}H_{27}O_5$	9.09	323.1864	323.18557	−2.57	Fatty acid	271.0255, 165.0193
19	2,4 Zearalanone	$C_{18}H_{24}O_5$	9.27	321.1700	321.1707	−2.71	Phenolic acid	643.3434
20	Eupatorin	$C_{18}H_{15}O_7$	10.11	343.0823	343.0823	0.03	Flavone	321.1702
21	Ferujol	$C_{19}H_{23}O_4$	10.81	315.1601	315.1601	−0.15	Coumarin	261.1498, 243.0977
22	Aleuritic acid methyl ester	$C_{17}H_{33}O_5$	11.25	317.23335	317.23725	12.2	Fatty acid	271.2244, 112.9822
23	*Ent*-8(14),15-pimaradien-1,2-epoxy-3β,18-diol	$C_{20}H_{29}O_3$	12.58	317.2122	317.2117	−1.59	terpene	112.9850, 635.43097 (2M − H)
24	*Ent*-8(14),15-pimaradien-1,2-epoxy-18-acetoxy, 3β-ol	$C_{22}H_{31}O_4$	14.23	359.2227	359.2227	−0.12	terpene	317.2122 (M-AC), 299.19795
25	(−) Helvolic Acid	$C_{34}H_{47}O_7$	15.26	567.3327	567.33243		terpene	515.38619

[a] Identified by spiking experiments with an authentic compound.

Helvolic Acid ($C_{34}H_{47}O_7$). Cinnamodial has been proven as a larvicidal and antimicrobial agent [72,73]. Helvolic acid is a representative fusidane-type antibiotic, which exhibits potent activity against Gram-positive bacteria (Methicillin-resistant *Staphylococcus aureus* DMST 20654, *Staphylococcus aureus* ATCC 25923, *Staphylococcus saprophyticus* ATCC 15305, *Streptococcus pneumonia* DMST 15319, *Enterococus faecalis* ATCC 29212 and *Bacillus subtilis* ATCC 6633) and Gram-negative plant pathogenic bacteria (*Ralstonia solanacearum*, *Xanthomonas campestris pv. vesicatoria*) [74].

3.4. Antibacterial activity

Staphylococcus aureus resistance is a global concern. Therapeutic options for Methicillin-resistant *Staphylococcus aureus* (MRSA) infection are limited because most strains are resistant to multiple antimicrobial agents. These strains may be considered as first-class MultiDrug Resistant (MDR) pathogens [75] are often responsible for chronic, persistent, and recurrent infections, which pose a challenge for healthcare practitioners [75]. Although the antimicrobial constituents (multi-drug) in plant extracts may occur in lower concentrations, they may be a better source of anti- *Staphylococcus* compounds than synthetic drugs because they can act on different

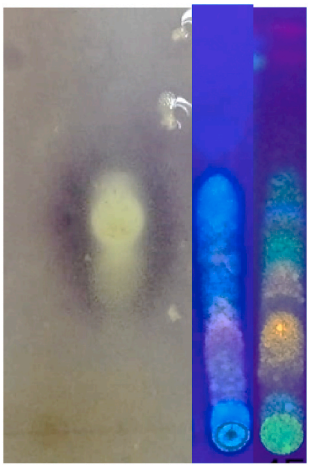

Fig. 9. A) Phenolic compounds profile on Sílica gel F254 plates of *G. glutinosa* extracts reveled with Neu's reagent y visualized at UV_{365nm}. **B)** Bioautographic assay on methicillin resistant *Staphylococcus aureus* (INBIOFIV–1S) of *G. glutinosa* extracts. **1)** Infusion. **2)** Tincture.

targets [76]. Extracts of *G. glutinosa* were tested against *S. aureus* strains (10 clinical isolations) which were selected because of their clinical importance. The resistance profile of each strain was obtained from the determination of the MIC values which were compared with those proposed by the CLSI and it was found that all *S. aureus* strains were resistant to methicillin and oxacillin, while 6% presented resistance to gentamicin, 5% to erythromycin, 2% to levofloxacin and moxifloxacin and only 1% had resistance to ciprofloxacin. None of the strains studied showed resistance to the rest of the antibiotics tested (Table 3). The tincture was active against all tested MRSA. MIC values varied between 120 and 240 μg DW/mL (Table 3). MRSA is included in the group II of priority of the list of resistant bacteria, published by the World Health Organization, for which new antibiotics are urgently needed [77]. In this work it is reported for the first time, antibacterial activity of *G. glutinosa* extracts against MRSA. *Zuccagnia punctata*, a species that also grows in the same eco-region (2200–3000 m.a.s.l.) showed similar MIC values against MRSA [78]. The antimicrobial activity found should be regarded as strong for *S. aureus* according to Rıos and Recio classification (2005) [79]. This antibacterial activity explains, at least in part, the traditional use of the species in the highlands medicine as an antibiotic.

Bioautographic assays (Fig. 9 A1 and A2) revealed that the infusion was inactive against Gram-positive microorganisms in the range of concentrations tested (Fig. 9 A1). Furthermore, at least two bands with antibacterial activity (*Rf* 0.61 and 0.78) were showed to tincture (Fig. 9 A2). These results would indicate that not only the phytocomplex is an efficient antibiotic, but also that the isolated metabolites are active components (Fig. 9B1 and B2).

Some authors have already reported antimicrobial activity of sakuranetin against strains of *S. aureus* sensitive and resistant to

Table 3
MIC values of *G. glutinosa* tincture against clinical isolated of *S. aureus* and *S. aureus* ATTC strains and resistance profiles of recommended antibiotics.

Strain	MIC mg DW/mL	Fenotype of clinical isolate
INBIOFIV-S1	0.24 ± 0.03	MET^R, OXA^R, GEN^R, ERI^R, VAN^S, CIP^S, LEV^S, MOX^S, CLI^S, QD^S, LIN^S, TEI^S, MIN^S, TET^S, RFA^S, TMS^S
INBIOFIV-S2	0.12 ± 0.03	MET^R, OXA^R, GEN^R, ERI^R, VAN^S, CIP^S, LEV^S, MOX^S, CLI^S, QD^S, LIN^S, TEI^S, MIN^S, TET^S, RFA^S, TMS^S
INBIOFIV-S3	0.24 ± 0.03	MET^R, OXA^R, GEN^S, ERI^S, VAN^S, CIP^S, LEV^S, MOX^S, CLI^S, QD^S, LIN^S, TEI^S, MIN^S, TET^S, RFA^S, TMS^S
INBIOFIV-S4	0.12 ± 0.03	MET^R, OXA^R, GEN^R, ERI^S, VAN^S, CIP^S, LEV^S, MOX^S, CLI^S, QD^S, LIN^S, TEI^S, MIN^S, TET^S, RFA^S, TMS^S
INBIOFIV-S5	0.24 ± 0.03	MET^R, OXA^R, GEN^R, ERI^R, CIP^S, LEV^R, MOX^R, VAN^S, CLI^S, QD^S, LIN^S, TEI^S, MIN^S, TET^S, RFA^S, TMS^S
INBIOFIV-S6	0.24 ± 0.03	MET^R, OXA^R, ERI^R, GEN^S, VAN^S, CIP^S, LEV^S, MOX^S, CLI^S, QD^S, LIN^S, TEI^S, MIN^S, TET^S, RFA^S, TMS^S
INBIOFIV-S7	0.24 ± 0.03	MET^R, OXA^R, GEN^S, ERI^S, VAN^S, CIP^S, LEV^S, MOX^S, CLI^S, QD^S, LIN^S, TEI^S, MIN^S, TET^S, RFA^S, TMS^S
INBIOFIV-S8	0.24 ± 0.03	MET^R, OXA^R, GEN^R, ERI^S, VAN^S, CIP^S, LEV^S, MOX^S, CLI^S, QD^S, LIN^S, TEI^S, MIN^S, TET^S, RFA^S, TMS^S
INBIOFIV-S9	0.24 ± 0.03	MET^R, OXA^R, CIP^R, GEN^R, ERI^R, LEV^R, CLI^R, MOX^R, VAN^S, QD^S, LIN^S, TEI^S, MIN^S, TET^S, RFA^S, TMS^S
INBIOFIV-S10	0.24 ± 0.03	MET^R, OXA^R, GEN^S, ERI^S, VAN^S, CIP^S, LEV^S, MOX^S, CLI^S, QD^S, LIN^S, TEI^S, MIN^S, TET^S, RFA^S, TMS^S
ATCC 43300	0.24 ± 0.03	
ATCC 29213	0.12 ± 0.03	

Vancomycin (VAN), Gentamycin (GEN), Methicillin (MET), Oxacillin (OXA), Ciprofloxacina (CIP), Levofloxacina (LEV), Moxifloxacina (MOX), Eritromicina (ERI), Clindamicina (CLI), Quinupristina (QD), Linezoline (LIN), Teicoplanina (TEI), Minociclina (MIN), Tetraciclina (TET), Rifampin (RFA), Trimetropina sulfa (TMS).

methicillin and against the reference strains ATCC 25923 and 29213 [80,81]. On the other hand, several authors have reported that some of the compounds identified by UHPLC/ESI/MS/MS of the extracts of *G. glutinosa,* such as axillarin, rhamnetin, cirsiliol, cinnamodial, and helvolic acid, presented antibacterial activity against *S. aureus* sensitive and resistant to methicillin [66,73,74,82,83].

3.5. Antioxidant capacity

$ABTS^{\bullet+}$, hydrogen peroxide and superoxide anion scavenging effects.

Both preparations (infusions and tincture) were able to transfer hydrogen ions to $ABTS^{\bullet+}$. A dose-response relationship between phenolic compounds content and antioxidant activity was demonstrated. SC_{50} values of 1.8 and 3.6 μg GAE/mL were found for infusions and tincture, respectively (Table 4).

The herbal preparations showed a high scavenger effect on $O_2^{\bullet-}$ and H_2O_2 (Table 4). Although hydrogen peroxide itself is not very harmful, it can generate other highly reactive species, like $HO^{\bullet}$, responsible of diseases linked to oxidant, inflammatory and infectious processes [84]. The effect demonstrated on the superoxide anion and hydrogen peroxide to phytoformulations makes them an important medicine to improve the oxidative state. This is the first report of antioxidant activity of *G. glutinosa* preparations. As previously described in section 3.3.2, other studies reported antioxidant activity of some compounds identified in *Gochnatia* extracts, such as genkwanin, hesperetin, rhamnetin, isorhamnetin, and axillarin [57,59–61,64]. These compounds could be deserve to be highlighted as responsible for their antioxidant capacity.

The Pearson correlation coefficient between the antioxidant methods was calculated for *G. glutinosa* extracts (Table 5). Although the correlation coefficients between $ABTS^{\cdot+}$ and $O2^{\cdot-}$ and between $ABTS^{\cdot+}$ and H_2O_2 for extract A were lower than those of extract B, the three techniques used to determine the antioxidant activity of the extracts presented a high positive correlation for all the relationships.

3.6. Antiinflammatory activity

Xanthine oxidase (XOD) and lipoxygenase (LOX) are enzymes related to inflammatory processes, both acute and chronic. XOD upregulates processes such as acute ischemia, reperfusion, and inflammation [85]. In addition, the activity of this enzyme generates chronic inflammation processes that result in one of the main factors in the etiology of colon cancer [86]. Recently, attention has been focused on the search for new XOD inhibitors instead of reducing urate concentrations. Therapy with XOD inhibitors to control patients' uricemia and antioxidants to reduce the levels of reactive oxygen species (ROS), result in a decrease in the risk of develop oxidative stress and chronic inflammation [87]. On the other hand, mediators of the arachidonic acid cascade such as LOX, a proinflammatory enzyme, are responsible of the production of leukotrienes, molecules related to acute inflammatory processes [88]. This enzyme is also involved in the inflammatory pathological mechanism of adenomyosis, a type of chronic immune inflammatory disease [89]. In addition, it has been proven that the deregulation of some enzymes belonging to the LOX family can induce oxidative stress, tissue damage and cell death [90].

3.6.1. Effect of G. glutinosa extracts on xanthine oxidase activity

XOD catalyzes the oxidation of xanthine and hypoxanthine into uric acid and superoxide anion, a reactive oxygen species. Its overproduction produces an oxidative stress-associated inflammation process called gout with tissue damage by precipitation of urate. For these reasons, the antioxidants could be used as XOD inhibitors. In this sense, the extracts of *G. glutinosa* are interesting because, in addition to presenting antioxidant activity, as shown before, they were able to inhibit XOD activity (Table 4) with IC_{50} values of 48.1 to 60.2 μg GAE/mL for infusions and tincture, respectively. The infusion has a similar activity to allopurinol, a specific inhibitor of XOD (Table 4). Many studies have discovered higher xanthine oxidase inhibitory activity of aglycones than that of glycosides. Therefore, to increase the effect of plant extracts, it is crucial to remove the glycosides from the extracts by hydrolysis process. Flavones, flavanone and flavonols shared some characteristics for high inhibition such as having hydroxyl moieties at C7 and C5, double bond between C3

Table 4
Antioxidant activity and inhibitory activity of proinflammatory enzymes by *G. glutinosa* extracts and reference compounds.

	Antioxidant activity SC_{50} (μg GAE/mL)			Proinflammatory enzyme inhibition IC_{50} (μg GAE/mL)	
	$ABTS^{\cdot+}$	$O_2^{\cdot-}$	H_2O_2	LOX	XOD
A	1.80 ± 0.02^{a}	11.0 ± 0.02^{a}	16.0 ± 0.04^{a}	9.22 ± 0.03^{a}	48.1 ± 0.3^{c}
B	3.60 ± 0.02^{b}	14.0 ± 0.03^{a}	14.0 ± 0.06^{a}	9.87 ± 0.03^{a}	60.2 ± 0.5^{d}
Reference compound					
Quercetin	1.40 ± 0.03^{b}	60.50 ± 4.70^{b}	17.30 ± 0.50^{b}		
Naproxen				14.0 ± 0.70^{b}	
Allopurinol					50.0 ± 2.0^{c}
Indomethacin					40.0 ± 2.0^{b}
Acetyl salicylic acid					6.1 ± 0.3^{a}

A: Infusion¸ B: Tincture. SC_{50}: Concentration of extracts necessary to scavenge 50% of ABTS, $O_2^{\cdot-}$, H_2O_2.
IC_{50}: inhibitory concentration of 50% of enzyme activity.
GAE: Gallic acid equivalent. Values are reported as mean ± standard deviation of triplicates. Different letters in the same column for each extract indicated significant differences according to Tukey's test ($p \leq 0.05$).

Table 5
Pearson's correlation coefficient between antioxidant activities for *G. glutinosa* extracts.

	A		B	
	$ABTS^{\cdot+}$	$O_2^{\cdot-}$	$ABTS^{\cdot+}$	$O_2^{\cdot-}$
$ABTS^{\cdot+}$	–	–	–	–
$O_2^{\cdot-}$	0.77[a]	–	0.99[a]	–
H_2O_2	0.77[a]	1.00[a]	1.00[a]	0.99[a]

[a] Significant at $P < 0.01$.

and C2, and carbonyl moiety at C4 [91]. It has been reported that some of the compounds that were identified in the extracts of *G. glutinosa* mentioned in section 3.3.2 such as axillarin [92], crisiliol [93], hesperetin [62], and centaureidin [70] exhibited anti-inflammatory properties since they had XOD inhibitory activity. Based on the obtained results, *G. glutinosa* might are used as rheumatic agent by inhibition of the XOD activity.

3.6.2. Effect of G. glutinosa extracts on Lipooxigenase activity

Lipoxygenases family are enzymes present in the human body that play an important role in stimulating inflammatory reactions as they catalyze the oxidation of polyunsaturated fatty acids, inducing the formation of inflammatory substances (hydroperoxides) that can lead to multiple serious diseases. In the work was assayed the effect of *G. glutinosa* extracts as inhibitor of activity of LOX. Table 4 shows the IC_{50} values obtained with extracts, tincture, and infusion, of *G. glutinosa* (IC_{50} = 9.2 and 9.8 μg GAE/mL, respectively). The inhibitory activity on LOX of both extracts was higher than naproxen and caffeic acid, two compounds used as reference. There is a very close link between the antioxidant capacity of polyphenols and their antiinflammatory activity [94]. High concentrations of ROS induce an inflammatory process that can lead to the stimulation of cytokine release leading to the subsequent activation of LOX [95]. Thus, the scavenging of ROS by the active components of plants attenuates the activation of certain inflammatory mechanisms. On the other hand, flavonoids are attributed the ability to inhibit the activity of proinflammatory enzymes (such as LOX) or affect the transcription of prooxidant enzymes [96]. The inhibitory activity of *G. glutinosa* extracts on LOX could be attributed to flavonone such as sakuranetin and flavone such as genkwanin, two components of these extracts; and other flavone and flavonone also present in the extracts. According with previous reports, sakuranetin may be considered as a selective inhibitor of 5-LOX [97]. Furthermore, genkwanin was an effective anti-inflammatory,against anti-rheumatoid arthritis (anti-RA) through inhibiting the phosphorylation of NF-κB pathway and down-regulating the expression of iNOS, COX-2 and IL-6 mRNA, and also by inhibiting the abnormal proliferation of FLSs and its NO and IL-6 secretion levels [98,99]. Previous studies concluded that the vital features for the inhibition of LOX, are the presence of orthodihydroxyl groups (a catechol moiety) in the A-ring and in the B ring, the presence of the 2,3-double bond, a 4-oxo group in the C-ring and the hydroxylations at C-5 and C-7 since these features are involved in important interactions and hydrogen bond formations with LOX active site, for these, genkwanin and sakuretin could be a good anti-LOX [100] (Ribeiro et al., 2014). These results would support the ethnomedicinal use of *G. glutinosa* as an antiinflammatory agent.

4. Conclusions

In this work, it was demonstrated that the ethanolic extract of *G. glutinosa* rich in phenolic compounds presented antimicrobial activities against clinical isolates of methicillin resistant *Staphylococcus aureus* and ATCC strains, also displaying inhibitory effect on enzymes related to inflammatory processes thus providing scientific support for its traditional medicinal use as an antiseptic and anti-inflammatory. These results verify and highlights the ethnomedicinal importance of this species for the communities that use it. Furthermore, the identification of the major bioactive compounds and the morpho-anatomical characterization of *G. glutinosa* are presented for the first time, contributing to pharmacobotanical description necessary to assure the botanical identity of this medicinal plant from Argentine Calchaquí Valley in quality control assays.

Declarations

Author contribution statement

Maria Inés Isla, Mariana Leal, María Inés Mercado, María Alejandra Moreno, José Javier Martínez Chamas, Iris Catiana Zampini, Graciela Inés Ponessa, Mario J Simirgiotis: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

No data was used for the research described in the article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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