Antioxidant activity and phenolic acid constituents of two andean Hypericum L. species from Colombia

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**Abstract**

Antioxidant capacity of extracts of different polarity obtained from two Hypericum L. species (H. juniperinum and H. mexicanum) was assessed by means of total polyphenolic content (TPC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, ferric reducing antioxidant power (FRAP) assay and oxygen radical absorbance capacity (ORAC) assay. Their phenolic acid composition was also determined by HPLC. The ethyl acetate extract of H. juniperinum was the most active in the ABTS, FRAP and TPC assays with 10867.48 μmol TEAC/g, 242.80 mg AAE/g and 491.08 mg GAE/g respectively. On the other hand, the methanol extract obtained from H. mexicanum appeared as the most active extract in the DPPH assay (3714.23 μmol TEAC/g). Similarly, the butanol fraction coming from the methanolic extract of H. mexicanum showed the highest activity in the ORAC assay (12910.06 μmol TEAC/g). HPLC analysis of the extracts revealed the presence of phenolic acid compounds, such as chlorogenic (50.09 mg/g) and p-coumaric acids (63.36 mg/g) in H. mexicanum and p-coumaric acid (8.45 mg/g) in H. juniperinum. A high correlation between antioxidant activity and total polyphenol content was established. Specifically, H. mexicanum exhibited the highest ORAC capacity, which may be associated with the high content of chlorogenic and p-coumaric acids present in medium to polar extracts. Our results constitute a significant contribution to the study of antioxidant activity and the determination of the phenolic acid profile in both species. The analysed extracts showed promising antioxidant activity that could be useful in the pharmaceutical, cosmetic and food industries.

**Keywords**

Hypericum juniperinum, Hypericum mexicanum, antioxidant activity, phenolic acids, radical scavenging

**Introduction**

Organisms are continually exposed to reactive oxygen species (ROS); when there is an imbalance between ROS and antioxidant defences in the human body, oxidative stress is produced (1). This phenomenon affects biological macromolecules, causes negative impacts on many cellular functions, and is associated with the pathogenesis of many conditions (2). Antioxidants play a vital role in preventing diseases, as they counteract the deleterious action of ROS (3).
Several research reports have suggested that antioxidants from plants are helpful in mitigating the harmful effects of ROS (4). Plants synthesize a great array of secondary metabolites, such as phenolic compounds (flavonoids, coumarins, lignans etc.), alkaloids and terpenes (5). The crude extracts of plants, rich in phenolic compounds, have been recognized to have medicinal properties and are effective scavengers of oxidants and inhibitors of lipid oxidation (6).

The species belonging to the genus Hypericum L. are naturally occurring or have been introduced to all continents, except Antarctica. It represents one of the 100 largest genera of angiosperms in the world. These species are mainly found as herbs, shrubs and sometimes as trees. They are distributed in temperate regions and high tropical mountains, encompassing different habitats (7). The genus Hypericum has 469 species reported worldwide. Hypericum is native to Europe and Asia and subsequently introduced in the United States (8).

In the catalogue of plants and lichens of Colombia, 54 species belonging to the genus Hypericum are described (9). Most of the native species of Central and South American mountain regions are an integral part of the paramo and sub-paramo vegetation types (10). Particularly, Hypericum mexicanum L., commonly known as chite or lunaria, is distributed in Colombia in the departments of Antioquia, Boyacá, Cundinamarca and Santander (11). In the rural areas of Bogotá, decoctions obtained from the leaves of the H. mexicanum species are used to treat kidney problems and disinfect wounds. In addition, roots mixed with other species of the same genus, such as H. juniperinum and H. myricariifolium are consumed to relieve pain (12). On the other hand, the flowers of H. juniperinum Kunth, commonly known as chite or guardarocio are traditionally used to treat coughs and the branches are used to make brooms or fire (13). It occurs in the form of a bush, with leaves arranged in a rosette shape, and is distributed in the paramo along the Colombian eastern mountain range (14).

Little information is known about the chemical constituents and biological and pharmacological activities of both of the Hypericum species, H. juniperinum and H. mexicanum. Briefly, evidence is the methanolic extract of H. juniperinum has antidepressant effects on animal models (15). In another study, the acetone extract from a Colombian H. juniperinum specimen displayed a high total polyphenolic content (TPC) value and the presence of anthocyanins (16). In the case of H. mexicanum, liquid and solid soap formulations of this species have shown antibacterial activity against different strains (11). Likewise, the methanolic extract showed antibacterial activity against S. aureus, E. coli and S. epidermidis and the total extract and the methanolic and chloroform fractions displayed a low minimum inhibitory concentration value in comparison with the H. perforatum extract against S. aureus (17). Moreover, dimeric acylphloroglucinols isolated from the chloroform fraction of leaves from H. mexicanum displayed strong anti-candidial activity (18). Recently, the essential oils (EOs) of these 2 Hypericum species were chemically characterized and evaluated against the maize weevil. H. mexicanum EO showed a promising fumigant toxicity and repellent action (19).

The antioxidant capacity of a huge spectrum of other Hypericum species, mainly from Europe, have been studied. H. androsaemum, H. ericoides, H. moserianum, H. olympicum and H. triquetrum have been evaluated for TPC, flavonoid content and radical scavenging activity (20). Taking into account that H. juniperinum and H. mexicanum belong to the same genus of H. perforatum, the extracts obtained are expected to contain metabolites with considerable antioxidant properties (21). Therefore, the purpose of this study was to investigate the total polyphenolic content, evaluate the antioxidant activity and determine the phenolic acid content of different polarity solvent extracts obtained from these 2 native Colombian species of Hypericum.

Materials and Methods

Chemicals and reagents

Milli-Q water (Millipore, Bedford, MA) was used; HPLC grade acetonitrile, methanol, phosphoric acid, and acetic acid (Merck, Darmstadt, Germany) were used after filtration through a 0.45 µm pore size membrane filter. Stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, 2,2,6,6-tetramethylpiperidinyl-(2-oxo-1)trimethylammonium (TPTZ), 2,2’-azo-bis(2-aminopropane)dihydrochloride (AAPH), fluorescein, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and ascorbic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Chlorogenic, caffeic, ferulic, and p-coumaric acids, potassium persulfate, FolinCiocalteu reagent, gallic acid and ascorbic acid were obtained from Merck® (Germany). All spectrophotometric experiments were performed on a Multiskan Spectrum UV-Vis plate reader (Thermo Scientific®, Finland). The decrease in fluorescence intensity measured in the oxygen radical absorbance capacity (ORAC) assay was performed on a Perkin-Elmer® LS-55 spectrophuorometer (Beaconfield, UK). The chromatographic studies by HPLC were carried out on a Shimadzu® liquid chromatograph from the Prominence UFLC series (Japan).

Plant material

Fresh plant material (leaves, stems, and flowers of both species) was obtained from the vereda Arbolocos belonging to the town of Cuítiva in the department of Boyacá, Colombia, at about 3300 m.a.s.l. H. juniperinum Kunth and H. mexicanum L. were identified by D.A. Fonseca (Herbario UFLC series (Japan). Materials and Methods.

Preparation of extracts

The dried plant material was moistened with 80% methanol. Three percolation extraction procedures were carried out until exhaustion. The extracts obtained were reduced in volume under reduced pressure using a rotary evaporator.
at a temperature of 40 °C and 60 rpm. The extract was heated in a water bath at 50 °C until complete dryness. Finally, a portion of the dry extract was taken and redissolved in 80% methanol and a liquid-liquid fractionation was carried out following the methodology described by Kupchan, adapted from (22), to obtain hexane, chloroform, ethyl acetate, butanol and aqueous fractions.

**Polyphenolic content and antioxidant activity assays**

**Folin-Ciocalteu assay**

TPC was measured by using the Folin-Ciocalteu colorimetric method, according to the standard methodology (23). The standard was gallic acid, and the absorbance was read at 760 nm. Results were expressed as mg of gallic acid equivalent (GAE)/g of extract.

**2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity**

The cationic radical ABTS+ was generated through an oxidation reaction of ABTS with potassium persulfate. The ability of the samples to trap the ABTS radical was evaluated by means of the decrease in the absorbance after 30 min of reaction at a wavelength of 732 nm (24). Results were expressed as µmol of Trolox equivalent antioxidant capacity (TEAC)/g of extract by constructing a standard curve using Trolox as an antioxidant.

**DPPH radical scavenging activity**

The ability of the samples to trap the DPPH radical was evaluated by means of the decrease in the absorbance after 30 min of reaction at a wavelength of 517 nm, following the method of Cao et al. with some modifications (25). For each sample studied, the percentage of radical inhibition was calculated, and the results are expressed as µmol of TEAC/g of extract by constructing a standard curve using Trolox as an antioxidant.

**Ferric reducing antioxidant power (FRAP) assay**

The test was carried out in an acetic acid-sodium acetate buffer (pH 3.4) containing TPTZ and FeCl3. 900 µl of this solution, 50 µl of sample and 50 µl of distilled water were used. After 60 min of reaction time, the absorbance was determined at a wavelength of 593 nm. For each sample, the reading of the absorbance of the blank without chromophore was taken into account. The reference curve was constructed using ascorbic acid as the primary standard. The activities of the samples were expressed as mg of ascorbic acid equivalent (AAE)/g of extract (26).

**ORAC assay**

The ORAC assay was determined by the following methodology. In a quartz cell, 3 ml were prepared from the following solution: 21 µl of a 10 µM solution of fluorescein, 2899 µl of 75 mM phosphate buffer (pH 7.4), 50 µl of 600 mM AAPH, and 30 µl of extract (27). Fluorescence was recorded on a Perkin-Elmer LS-55 spectrophotometer with a thermostatted multicell. The ORAC value (µmol TEAC/g of extract) was calculated by a calibration curve using different concentrations of Trolox and the differences in areas under the fluorescence decay curve between the blank and the sample, which was compared against the Trolox curve, according to Equation 1:

\[
ORAC = \frac{(AUC - AUC^0)}{(AUC_{Trolox} - AUC^0)} f[Trolox] \\
(\text{Eqn. 1})
\]

Where AUC is the area under the curve of the samples, AUC^0 is the area under the curve for the control, AUC_{Trolox} is the area under the curve for the Trolox and f is the dilution factor of the extracts.

**Determination of phenolic acids by HPLC**

The extracts were filtered (pore size 0.45 µm) and dilutions were made using Milli-Q water. The phenolic compounds were eluted at the following conditions: mobile phase acetonitrile/acidified water (40/60 v/v); flow of 1 ml/min, 25 °C and isocratic conditions. The UV-Vis spectrum ranged from 200 to 600 nm for all peaks; the identification and quantification of the compounds was done with calibration curves for each of the phenolic acids (chlorogenic, caffeic, ferulic and p-coumaric acid). The results were expressed as mg of phenolic acid/g of extract.

**Statistical analysis**

All experiments were carried out in triplicate. Regressions were calculated with a significance level of 95% (P < 0.05) using the Statgraphics Plus version 5.0 program (Statistical Graphics Corp., Rockville, MD). Correlation coefficients (Pearson’s correlation coefficient, r) were determined using Excel software (Microsoft, 2010 version).

**Results**

In this study, *in vitro* antioxidant activities and determination of the main phenolic acids were established in different polarity solvent extracts obtained from two *Hypericum* species.

**Phenolic content and antioxidant activity assays**

Folin-Ciocalteau assay is not a specific reagent for polyphenolic compounds, since other compounds can reduce it. Despite that, the assay is reproducible, easy to implement and accessible, since the reagent is commercially available (28). The TPC and antioxidant activity results for *H. mexicanum* and *H. juniperinum* extracts are presented in Table 1.

The TPC values for the butanol and ethyl acetate fractions of *H. mexicanum* and the methanolic and butanolic fractions of *H. juniperinum* were very similar and oscillated between 211.87 and 269.84 mg GAE/g of extract; however, the ethyl acetate extract of *H. juniperinum* showed the highest value for TPC in this assay (491.08 mg GAE/g of extract). The aqueous extract of both species showed the lowest TPC values, 1.70 for *H. juniperinum* and 44.53 mg GAE/g of extract for *H. mexicanum*.

DPPH is a purple, stable radical with a maximum absorption in the visible spectrum ranging from 515 to 528 nm. When the radical traps an electron or a radical species, it reduces the intensity of the purple colour until it turns yellow. This assay has been extensively used for determining the antioxidant activity of diverse plant natural ex-
tracts, including those from fruits and vegetables because it allows analysis of a high number of samples in a short period of time and is sensitive enough to detect antioxidant compounds at low concentrations (28). In the DPPH assay, the values ranged from 6.94 to 3,714.23 μmol TEAC/g of extract. The aqueous fractions showed lower values in both species, 409.11 μmol TEAC/g of extract for H. mexicanum and 6.94 μmol TEAC/g of extract corresponding to H. juniperinum. The methanol extract of H. mexicanum had the highest radical scavenger effect (3,714.23 μmol TEAC/g of extract), followed closely by the ethyl acetate extract of H. juniperinum (1,987.87 μmol TEAC/g of extract).

The ABTS scavenging assay is applicable for both hydrophilic and lipophilic compounds because the ABTS' radical cation is soluble in water and methanol, and it is not affected by ionic strength, therefore it can be carried out in different media to determine the antioxidant activity (28). Antioxidant activity is measured when the blue-green ABTS' radical cation that is formed gradually loses its colour. In this assay, the ethyl acetate and butanol extracts from both species displayed the highest antioxidant activity. The ethyl acetate extract of H. juniperinum exhibited the highest scavenging capacity of the ABTS' radical cation with a value of 10,867.48 μmol TEAC/g of extract followed by the butanol extract (4,672.20 μmol TEAC/g of extract). For H. mexicanum, the most active sample was the butanol extract with 8,500.86 μmol TEAC/g of extract followed by the ethyl acetate extract (7,557.33 μmol TEAC/g of extract). The less active samples on this assay were the aqueous fractions (19.20 μmol TEAC/g of extract for H. juniperinum and 1,987.87 μmol TEAC/g of extract for H. mexicanum).

The FRAP assay consists of the electron transfer and the power to reduce iron to its intensely blue-coloured ferrous ion. The FRAP mechanism is through a single electron transfer (SET), so it is not valid to compare this method with those where a radical scavenging mechanism is involved (29). Similar to the previous test, the most active sample in the FRAP assay was the ethyl acetate fraction of H. juniperinum with 242.80 mg AAE/g of extract followed by the methanol (99.21 mg AAE/g of extract) and butanol (97.32 mg AAE/g of extract) fractions. Again, the fraction with the lowest antioxidant activity was the aqueous fraction from H. juniperinum. Furthermore, the FRAP values for H. mexicanum ranged from 11.59 to 80.97 mg AAE/g of extract, corresponding to the hexane and the ethyl acetate fraction respectively.

In the ORAC assay, the ROO' reacts with a fluorescent probe to produce a non-fluorescent species, which can be quantitated by fluorescence decay; as the product formed decreases, the radical scavenging capacity seems to be higher (30). The two species had similar values on the ORAC test in each solvent. The butanol extract of H. mexicanum and the ethyl acetate extract of H. juniperinum possessed the highest values of antioxidant activity, 12,910.06 and 12,204.40 μmol TEAC/g of extract, respectively. Similar to the previous antioxidant tests, the less active samples were the aqueous fractions of both plants, showing ORAC values of 14.53 and 1,866.45 μmol TEAC/g of extract for H. juniperinum and H. mexicanum respectively.

Antioxidant and chemical studies of these species are limited; however, some comparisons are possible. The results obtained in the TPC assay agree with the data reported earlier (16), who described high polyphenol content (100.37 mg GAE/g plant dry material) in H. juniperinum. Moreover, in studies with different Hypericum species, the ethyl acetate extract showed the highest content of phenolic compounds, while the hexane and chloroform fractions were relatively poor in this kind of compounds (20). Studies on other Hypericum species showed that less polar solvents than water tend to perform better scavenging activity on DPPH and ABTS assays. The ethyl acetate fraction of Hypericum hyssoptilolum also showed the highest value for the DPPH test. Also, flavonoids like quercetin and glycosyl quercetin derivatives were isolated from these fractions (31). Additionally, a study with Hypericum ascyron extracts showed higher ABTS scavenging capacity in the ethyl acetate extract than in the methanolic one, but in the FRAP assay, the reducing power was better for the

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Table 1. Total polyphenolic content (TPC) and antioxidant activity results for H. mexicanum and Hypericum juniperinum extracts. Values are expressed as: TPC: mg GAE/g of extract, ABTS: μmol TEAC/g of extract; DPPH: μmol TEAC/g of extract; FRAP: mg AAE/g of extract and ORAC: μmol TEAC/g of extract.

| Plant          | Extract          | TPC     | ABTS       | DPPH       | FRAP         | ORAC         |
|---------------|------------------|---------|------------|------------|--------------|--------------|
| Hypericum     | Methanol crude   | 82.09±2.9 | 3082.19±7.3 | 3714.23±282.3 | 31.15±1.1 | 7619.61±576.5 |
| mexicanum     | Aqueous          | 44.53±1.5 | 1065.43±416.9 | 409.11±5.8  | 13.84±0.6  | 1866.45±171.0 |
| Butanol       | 235.34±23.8      | 8500.86±732.9 | 1187.20±34.8 | 70.09±3.6   | 12910.06±877.7 |
| Ethyl acetate | 268.78±6.0       | 7557.33±387.3 | 1513.76±43.9 | 80.97±5.1   | 5447.49±355.6 |
| Chloroform    | 133.44±9.0       | 4498.31±237.4 | 1326.48±68.3 | 77.25±7.7   | 5447.15±337.6 |
| n-hexane      | 86.91±2.4        | 3172.11±159.1 | 1072.40±79.2 | 11.59±0.7   | 2164.08±256.8 |
| Methanol      | 211.87±4.5       | 2115.32±106.3 | 1528.41±2.0  | 99.21±4.1   | 159.17±220.7 |
| crude extract | Aqueous          | 1.70±0.06  | 19.20±1.1   | 6.94±0.2    | 0.51±0.05   | 14.53±1.8    |
| Butanol       | 269.84±17.6      | 4672.20±260.6 | 1615.76±46.3 | 97.32±4.1   | 3990.45±252.7 |
| Ethyl acetate | 491.08±33.3      | 10867.48±992.6 | 3196.87±184.0 | 242.80±14.3 | 12204.40±487.2 |
| Chloroform    | 157.62±6.0       | 3590.72±349.2 | 896.59±44.2 | 39.93±2.2   | 2754.12±60.0 |
| n-hexane      | 102.72±2.8       | 2995.60±34.4 | 548.87±23.6 | 19.13±0.1   | 1384.91±45.2 |

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methanol extract (483.32 μmol Trolox/g sample). Nevertheless, quercetin-3-O-β-D-glucoside, quercetin-3-O-β-D-galactoside and kaempferol were isolated from the ethyl acetate extract and provided better results on these assays (32), providing evidence of the relation between the presence of those metabolites and their antioxidant power. The results obtained for the ORAC test in both species were similar to those reported for other Hypericum species, where the highest ORAC values of the crude methanol extract were for H. caprifolium (820 μmol Trolox/g of extract) and H. carinatum (347 μmol Trolox/g of extract) and the lowest was for H. polyanthemum (240 μmol Trolox/g of extract) (33).

Relationships between antioxidant assays and TPC results have been described for other species. The ethanol extract of Hypericum lymajohioides, H. triquetfolium and H. scabroideae showed high scavenging ability in the DPPH assay and a TPC of 307, 267 and 333 mg GAE/g sample respectively (34, 35). Those values were higher than the value of the methanol extract from H. mexicanum (82.02 mg GAE/g sample) but similar to the ethyl acetate fraction (268.78 mg GAE/g sample) of the same species. In comparison with H. juniperinum values, the ethyl acetate extract had better results for TPC (491.08 mg GAE/g sample). This accounts for the different behaviour of the extracts in each solvent due to the extract composition.

According to our results, there is a direct correlation between the concentration of phenolic compounds and the antioxidant potential. The TPC of the H. juniperinum extracts showed high correlations with the antioxidant activity values. Pearson’s correlation coefficient (r) for DPPH, ABTS, FRAP and ORAC were 1.00, 0.98, 0.98 and 0.97 respectively. High correlations between these variables were observed with determination coefficients (r²) greater than 95%. In H. mexicanum extracts, the correlations between TPC and antioxidant activity were lower than those obtained in H. juniperinum, with Pearson’s correlation coefficients of 0.97, 0.83, 0.079 and 0.72 for ABTS, FRAP, DPPH and ORAC respectively. It is important to note that only ABTS had a high correlation with TPC (r² = 94%).

The presence of phenolic compounds had been previously described for both H. mexicanum and H. juniperinum and for other Hypericum species. Preliminary phytochemical studies with H. mexicanum and H. juniperinum have reported the presence of terpenes/steroids, phenolics, flavonoids, quinones, tannins, saponins and coumarins (15, 17). In addition, Mejia-Agudelo et al. isolated the flavonoid quercetin and chlorogenic acid methyl ester (5-O-caffeoylquinic acid methyl ester) from an ethyl acetate fraction of H. juniperinum (36). Furthermore, the presence of quercitin and rutin was also established in the ethyl acetate fraction of H. juniperinum by means of HPLC (15). More recently, a complete characterization of the phenolic profile from stems, roots and leaves of H. mexicanum has been described (18).

Concerning our study, in general, the most active antioxidant extracts, in both plants, were the ethyl acetate and butanol. Polyphenolic compounds may have a wide range of polarities, thus both organic and aqueous extracts showed a significant antiradical and antioxidant activity. The fact that most of the assays presented the best results with the ethyl acetate fraction indicates that the responsible compounds for the antioxidant activity correspond to medium to high polarity metabolites, such as flavonols (i.e., quercetin and kaempferol), which show a high ability to scavenge radicals (37). Indeed, the composition of the phenolics in Hypericum species are mainly quercetin and kaempferol derivatives (38). This is important since these kinds of compounds are well known for their good antioxidant potential (39). Their antioxidant capacity depends on different structural features, such as the hydroxyl and carbonyl groups arrangement around the molecule, which mainly determines the metal-chelating potential, the presence of hydrogen or electron-donating substituents in order to reduce free radical and the structural ability of the flavonoid to delocalized unpaired electrons, leading to the formation of a phenoxy radical (40). For flavonols, such as quercetin and kaempferol, it is widely suggested that the C3 free hydroxyl group is responsible for the high oxidation inhibition; as well as the pattern of substitution of the other phenolic hydroxyls in the A and B rings contributes to the activity (41). Furthermore, the butanol extract was the second most active in the tests, suggesting the presence of more polar compounds, such as quercetin and kaempferol glycosyl derivatives (38). Likewise, the most polar extract (aqueous) was the least active in most of the assays, which might be because this extract could present a low content of reactive antioxidants, such as glycosyl flavonoids like rutin, hyperoside, isoquercitrin and quercitrin, which are recognized compounds in H. perforatum, rutin and quercitin in H. juniperinum and isorhamnetin-3-O-glycoside and quercetin-3-O-glucoside in H. mexicanum (15, 18, 39).

Although the mechanism of action of phenolic compounds is not completely elucidated yet, for definition, the role of the hydroxyl group attached to the aromatic ring is to interrupt the radical chain reaction. Due to the structural substitution pattern of glycosyl compounds, antioxidant and antiradical potential might decrease when compared to the aglycone due to the masking of phenolic groups (38). There are 2 antioxidant mechanisms proposed, the hydrogen atom transfer (HAT) and SET. ABTS and DPPH are considered mixed methods (SET- and HAT-based), while the FRAP test is based on the SET reaction, which is pH dependent and the ORAC test is based on the HAT mechanism (28). The mechanism is relevant when it is associated with the antioxidant capacity detection methods. Most of the assays were done at non-physiological pH values; therefore, it is advisable to perform assays that occur through different mechanisms of action. In the same line, more studies to estimate the antioxidant action under in vivo models are necessary.

**Phenolic acids detection and quantification**

Phenolic acids can be found in almost all plants, and they have become important because of their possible protective action against diseases where oxidative damage is present (42). Phenolic acids can be classified as hydroxybenzoic or hydroxycinnamic; the latter exhibits a great antioxidant activity due to the conjugation effects of...
the carbonyl with the double bond. Hence, in this study, only hydroxycinnamic acids (chlorogenic, caffeic, p-coumaric and ferulic acid) were quantified (Table 2). As an example of the HPLC analysis, the chromatographic profiles of the *Hypericum* methanolic extracts are shown in Fig. 1.

Concerning the chlorogenic acid concentration, *H. mexicanum* values ranged from 1.53 to 50.09 mg/g. The aqueous fraction had the highest concentration (50.09 mg/g) followed by the butanol extract (14.40 mg/g). No chlorogenic acid concentration was detected in the n-hexane fraction for *H. mexicanum*. On the other hand, *H. juniperinum* presented significantly lower chlorogenic acid concentration ranging from 0.85 to 2.47 mg/g, with no detection in the chloroform extract. Neither *H. mexicanum* extracts nor *H. juniperinum* extracts reported the presence of caffeic acid.

*p*-Coumaric acid concentration ranged from 1.48 to 63.36 mg/g in *H. mexicanum*, with the aqueous (63.36 mg/g) and butanol (44.06 mg/g) extracts having the highest concentration, similar to the behaviour for chlorogenic acid determination. In the n-hexane extract of *H. mexicanum*, *p*-coumaric acid was not detected. For *H. juniperinum*, only the methanol and butanol extracts presented *p*-coumaric acid with lower values than *H. mexicanum*, ranging from 4.82 to 8.45 mg/g. Finally, ferulic acid was not detected in any of the *H. juniperinum* extracts. In *H. mexicanum*, it was only detected in the ethyl acetate (6.92 mg/g), methanol (1.71 mg/g) and chloroform (1.14 mg/g) fractions.

To the best of our knowledge, this is the first time that hydroxycinnamic acids are determined and quantified for *H. juniperinum*. For *H. mexicanum*, it was reported that the presence of caffeic and cinnamic acids in leaves but not in stems and roots. They also did not detect chlorogenic acid in any of the organs analysed (18). In comparison with our results, we did not detect caffeic acid, but we could determine the presence of *p*-coumaric, chlorogenic and ferulic acids in aerial parts of *H. mexicanum*. Other species of *Hypericum* have also confirmed the presence of phenolic acids. In *H. humifusum*, among other phenols, caffeic (0.056 mg/g sample) and chlorogenic acids (0.064 mg/g sample) were identified (43). Those acids were also quantified in the aqueous extracts of *H. perforatum*, *H. androsaemum*, *H. undulatum* and *H. foliosum*, obtaining chlorogenic acid values of 4.34, 34.18, 6.45 and 29.89 respectively (44). Additionally, *p*-coumaric acid was found in *H. monoatum* (10 mg/kg dried plant material) (45).

The phenolic acids in *H. juniperinum* and *H. mexicanum* can also contribute to the antioxidant activity of the extracts, including the 5-O-caffeoylquinic acid methyl ester, already isolated from *H. juniperinum*. The hydrogen donating capacity of phenolic acids depends on the pres-

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**Table 2.** Phenolic acids occurring in the different solvent polarity fractions obtained from *H. mexicanum* and *H. juniperinum*. Concentrations are expressed as mg acid/g of extract.

| Plant          | Extract | Chlorogenic acid | Caffeic acid | *p*-Coumaric acid | Ferulic acid |
|----------------|---------|------------------|--------------|-------------------|--------------|
| *Hypericum mexicanum* | Aqueous | 50.09 ± 4.51    | n.d.         | 63.36 ± 5.41      | n.d.         |
|                 | Methanol| 1.53 ± 0.04     | n.d.         | 2.56 ± 0.08       | 1.71 ± 0.07  |
|                 | Butanol | 14.40 ± 1.12    | n.d.         | 44.06 ± 3.21      | n.d.         |
|                 | Ethyl acetate | 9.64 ± 0.78 | n.d.         | 17.93 ± 0.12      | 6.92 ± 0.32  |
|                 | Chloroform | 2.39 ± 0.12  | n.d.         | 1.48 ± 0.08       | 1.14 ± 0.07  |
|                 | n-Hexane  | n.d.            | n.d.         | n.d.              | n.d.         |
| *Hypericum juniperinum* | Aqueous | 1.83 ± 0.02     | n.d.         | n.d.              | n.d.         |
|                 | Methanol | 2.47 ± 0.14     | n.d.         | 4.82 ± 0.28       | n.d.         |
|                 | Butanol  | 1.18 ± 0.09     | n.d.         | 8.45 ± 0.61       | n.d.         |
|                 | Ethyl acetate | 0.85 ± 0.02 | n.d.         | n.d.              | n.d.         |
|                 | Chloroform| n.d.            | n.d.         | n.d.              | n.d.         |
|                 | n-Hexane | 1.26 ± 0.07     | n.d.         | 5.09 ± 0.40       | n.d.         |

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**Fig. 1.** HPLC profile of methanolic extract of *H. juniperinum* (A) and *H. mexicanum* (B). Phenolic acids retention time (min): Chlorogenic acid (13.2), *p*-coumaric acid (18.2) and ferulic acid (22.1).
ence of OH substituents in ortho- and para- positions and the unsaturated bonds. These characteristics also determine the capacity of these compounds to donate electrons, similar to flavonoids. Several studies have been conducted to determine the antioxidant capacity of these compounds, suggesting that the more OH substituents at the aromatic ring are present, the more antioxidant capacity they have (46). Therefore, since caffeic acid was not detected, we could suggest that chlorogenic acid would be more active as an antioxidant in the methanolic extract of *H. juniperinum*. Additionally, the amount of ferulic acid in the ethyl acetate fraction of *H. mexicanum* and *p*-coumaric acid in the butanol fraction of *H. juniperinum* might have contributed to the good results in the antioxidant activity.

**Conclusion**

The information available about the biological activities of extracts and fractions obtained from both *Hypericum* L. species is scarce or in some cases absent. There are some preliminary studies with these species, but none of them have focused on the study of antioxidant activity and the profile of organic acids. Our results provide new insights into the antioxidant activities of both *H. mexicanum* and *H. juniperinum* species. For the first time, results of the antioxidant activity of hydrophilic and hydrophobic extracts of both species are reported; the antioxidant tests allowed us to discriminate the oxidative mechanisms by transfer of hydrogen (ORAC) and by electron transfer (FRAP). Furthermore, phenolic acids were determined and quantified for these Colombian Andean species; interestingly, this is the first time the presence of phenolic acids is reported in *H. juniperinum* and in addition, some phenolic acids that had not been previously identified in *H. mexicanum* were found. Chemical constituents of these extracts can produce health benefits and possible applications in the pharmaceutical, cosmetic and food industries. Nevertheless, a more complete chemical characterization would be necessary. In addition, further investigation on the mechanism of action and safety is crucial for the evaluation of their potential as prophylactic agents.

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**Authors contributions**

ACP and AFA performed the experiments. BR, JHG and JCML analyzed and interpreted the data. MR and JCML conceived and designed the experiments. JHG and JCML drafted the manuscript. All authors revised and approved the final manuscript.

**Compliance with ethical standards**

**Conflict of interest:** Authors do not have any conflict of interests to declare.

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**References**

1. Anraku M, Gebicki JM, Iohara D, Tomida H, Uekama K, Maruyama T, et al. Antioxidant activities of chitosans and its derivatives in *in vitro* and *in vivo* studies. Carbohydr Polym. 2018;199:141-49. [https://doi.org/10.1016/j.carbpol.2018.07.016](https://doi.org/10.1016/j.carbpol.2018.07.016)

2. Gioti EM, Fiamengos YC, Skalkos DC, Stilikas CD. Improved method for the *in vitro* assessment of antioxidant activity of plant extracts by headspace solid-phase microextraction and gas chromatography–electron capture detection. J Chromatogr A. 2007;1152(1):150-55. [https://doi.org/10.1016/j.chroma.2007.07.124](https://doi.org/10.1016/j.chroma.2007.07.124)

3. Sindhi V, Vartika G, Kameshwar S, Sonal B, Reeta K, Neeti D. Potential applications of antioxidants – A review. J Pharm Res. 2013;7(9):828-35. [https://doi.org/10.3496/jjpr.2013.10.001](https://doi.org/10.3496/jjpr.2013.10.001)

4. Dumanović J, Nepovimova E, Natić M, Kuća K, Jaćević, V. The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. Front Plant Sci. 2021;11:552969. [https://doi.org/10.3389/fpls.2020.552969](https://doi.org/10.3389/fpls.2020.552969)

5. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects - A review. J Funct Foods. 2015;18(part B):820-97. [https://doi.org/10.1016/j.jff.2015.06.018](https://doi.org/10.1016/j.jff.2015.06.018)

6. Muhammad HA, Rababah T, Alhamad MN, Al-Mahasneh MA, Almajwal A, Gammoh S et al. A review of phenolic compounds in oil-bearing plants: Distribution, identification and occurrence of phenolic compounds. Food Chem. 2017;218:99-106. [https://doi.org/10.1016/j.foodchem.2016.09.057](https://doi.org/10.1016/j.foodchem.2016.09.057)

7. Crockett S, Robson V. Taxonomy and chemotaxonomy of the genus *Hypericum*. Med. Aromat Plant Sci Biotechnol. 2011;5 (Special Issue 1):1-13.

8. Ernst E. *Hypericum*: The genus *Hypericum*. New York (USA): CRC Press; 1st Edition.

9. Robson NKB. *Hypericum*. In: Bernal R, Graddstein SR, Celis M (Editors). Catálogo de plantas y líquenes de Colombia [internet]. Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá. 2014 December 20. [cited 2022 February 24]. Available from: http://catalogoplantasdecolombia.unal.edu.co.

10. Crockett S, Mariann E, Olaf K, Wolfgang S. *Hypericum* species in the paramos of central and south america: A special focus upon *H. irazuense* Kunz et ex N. Robson. Phytochem Rev. 2010;9(2):255-69. [https://doi.org/10.1007/s11101-009-9148-2](https://doi.org/10.1007/s11101-009-9148-2)

11. Corzo-Barragán DC, Gaitán-Vaca DM. Evaluación de la eficacia de distintas formulaciones de jabón con extracto de *Hypericum mexicanum* L. Rev Investig Agrar Ambient. 2017;8 (1):131-38. [https://doi.org/10.22490/21456453.1844](https://doi.org/10.22490/21456453.1844)

12. Gutiérrez M, Pineda M, García AX. Las maticas de mi región. (e-book). 2nd Ed. Bogotá (Colombia): Jardín Botánico de Bogotá José Celestino Mutis; 2014 [cited 2022 February 24]. Available from: [https://isbn.cloud/9789585876190/las-maticas-de-mi-region/](https://isbn.cloud/9789585876190/las-maticas-de-mi-region/)

13. Abadía B, Parra SL. Plantas del páramo y sus usos para el buen vivir: Páramos de Guerrero y Rabanal. In: Menjura-Morales T, Vásquez C (Editors). Buen vivir y usos de biodi-
versidad vegetal en comunidades campesinas de los páramos de Guerrero y Rabanal [e-book]. Bogotá (Colombia): Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, 2016 [cited 2022 February 24]: 25. Available from: http://hdl.handle.net/10.500.11761/9277

14. Marquίn-Casas X, Mejίa-Agudelo LA, Marίn-Lοaiza JC. Morfoanatomίa e histοquímica de Hypericum juniperinum (Hypericaceae). Rev Biol Trop. 2019;67(6):1160-69, http://dx.doi.org/10.15517/rbt.v67i6.36072

15. Mejίa-Agudelo LA, Rojas MA, Guerrero-Pabόn MF, Ramos FA, Castellanos L, Marίn-Lοaiza JC. Antidepressant-like effects of methanol extract and fractions of Hypericum juniperinum Kunth in the forced swimming test. Phcog Mag. 2019;15 (61):226-31. https://doi.org/10.4103/pm.pm_341_18

16. Mejίa-Giraldo JC, Henao-Zuluaga K, Gallardo C, Atehortuεa L, Puertas-Mejίa M.A. Novel in vitro antioxidant and photoprotection capacity of plants from high altitude ecosystems of Colombia. Photochem Photobiol. 2016;92(1):150-57. https://doi.org/10.1111/tpb.12543

17. Plazas-González EA. Phytochemical screening and in vitro antibacterial activity of extracts and fractions from three Colombian species of the genus Hypericum. Rev Cubana Plant Med. 2017;22(1):1-14.

18. Tocci N, Weil T, Perenzoni D, Moretto M, Nürk N, Madriñάn S et al. Potent antifungal properties of dimeric acylphloroglucinols from Hypericum mexicanum and mechanism of action of a highly active 3’-prenyl Uliginosin B. Metabolites. 2020;10(11):459. https://doi.org/10.3390/metabo10110459

19. Patίfο-Bayona WR, Plazas E, Bustos-Cortes J.J, Prieto-Rοdrίguez J.A, Patίfο-Ladino O.J. Essential oils of three Hypericum species from Colombia: chemical composition, insecticidal and repellent activity against Sitophilus zeamais Motsch.(Coleoptera: Curculionidae). Rec. Nat. Prod. 2021;15:111-121. http://doi.org/10.25135/rnp.192.20.05.1665

20. Saddiğe Z, Ismat N, Claire H, Asmita VP, Ghulam A. Phytochemical profile, antioxidant and antibacterial activity of four Hypericum species from the UK. S Afr J Bot. 2020;133:45-53. https://doi.org/10.1016/j.sajb.2020.05.018

21. Seyrekoglu F, Temiz H, Eser F, Yildirim C. Comparison of the antioxidant activities and major constituents of three Hypericum species (H. perforatum, H. scabrum and H. origanifolium) from Turkey. S African J Bot. 2022;146:723-27. https://doi.org/10.1016/j.sajb.2021.12.012

22. Gbate M, Ashamo OM, Kayode AL. Bioprosthetic effect of partitioned extracts of Zanthoxylum zanthoxyloides (Lam.) Zepernick & Timler on Callosobruchus maculatus (Fab.). J Agric Stud. 2021;9(3):215-227. https://doi.org/10.5296/jas.v9i3.18867

23. Lucas BN, Dalla-Nora FM, Boeira CP, Verruck S, da Rosa CS. Determination of total phenolic compounds in plant extracts via Folin-CiŚlacis’s method adapted to the usage of digital images. Food Sci Technol Campinas. 2022;42(e35122),1-6. https://doi.org/10.1590/fsst.35122

24. Alfίaño-González MJ, Barea-Sepúlveda M, Espada-Bellido E, Ferreiro-González M, Lόpez-Castillo JG, Palma M et al. Ultrasound-assisted extraction of total phenolic compounds and antioxidant activity in mushrooms. Agronomy. 2022;12 (8):1812. https://doi.org/10.3390/agronomy12081812.

25. Cao QQ, Fu YQ, Zhang CB, Zhu Y, Yin JF, Granato D et al. Effect of brewing water on the antioxidant capacity of green tea infusion with DPPH assay. J Chem. 2022; 7736117, 8 pages. https://doi.org/10.1155/2022/7736117

26. Meganathan B, Palanisamy CP, Panagal M. Antioxidant, antimicrobial and cytotoxicity potential of n-hexane extract of Cayratia trifolia L. Bioinformation. 2021;17(3):452-59. https://doi.org/10.6026/97320630017452.

27. Giordano A, Morales-Tapia P, Moncada-Basulto M, Pozo-Martίnez J, Olea-Azar C, Nesic A et al. Polyphenolic composition and antioxidant activity (ORAC, EPR and Cellular) of different extracts of Argyia radiata vitropotals and natural roots. Molecules. 2022;27(3):610. https://doi.org/10.3390/molecules27030610

28. Munteanu IG, Aretrei C. Analytical methods used in determining antioxidant activity: A review. Int J Mol Sci. 2021;22 (7):3380. https://doi.org/10.3390/ijms22073380

29. Somogyi A, Rosta K, Pusztai P, Tulasay Z, Nagy G. Antioxidant measurements. Physiol Meas. 2007;28(4):41-55. https://doi.org/10.1088/0967-3334/28/4/R01

30. Gulcin I. Antioxidants and antioxidant methods: An updated overview. Arch. Toxicol. 2020;94(3):651-715. https://doi.org/10.1007/s00204-020-02689-3

31. Cakir A, Mavi A, Yildrim A, Duru ME, Harmandar M, Kazaz C. Isolation and characterization of antioxidant phenolic compounds from the aerial parts of Hypericum hyssopifolium L. by activity-guided fractionation. J Ethnopharmacol. 2003;87 (1):73-83. https://doi.org/10.1016/s0378-8741(03)00112-0

32. Wen-Yi K, Yan-Li S, Zhang L. α-Glucosidase inhibitory and antioxidant properties and antiabetic activity of Hypericum ascyron L. Med Chem Res. 2011;20(7):809-16. https://doi.org/10.1007/s00440-010-9391-5

33. Bernardi APM, López-Alarcόn C, Aspεe A, Rech VB, Von Poser GL, Bridi R et al. Antioxidant activity in southern Brazil Hypericum species. J Chil Chem Soc. 2008;53(4):1658-62. http://dx.doi.org/10.4067/S0717-97072008000400004

34. Hakimoglu F, Gόksel K, Zεki K, Murat K, Hilmi I. The effect of ethanol extract of Hypericum lysimachoides on lipid profile in hypercholesterolemic rabbits and its in vitro antioxidant activity. Atherosclerosis. 2007;192(1):113-22. https://doi.org/10.1016/j.atherosclerosis.2006.07.013

35. Gόksel K, Murat K, Murat Y, Sevil E, Fidan H. Antioxidant activities of ethanol extracts of Hypericum triquetrum and Hypericum scabroids. Pharm. Biol. 2008;46(4):231-42. https://doi.org/10.1080/02698830801739363

36. Mejίa Agudelo LA. Estudio morfo-anatómico, fitoquímico y evaluación de la actividad sobre sistema nervioso central en modelo murino de Hypericum juniperinum (Kunth). Msc [dissertation]. Colombia: Universidad Nacional de Colombia-Sede Bogotά. 2017. Available from: https://repositorio.unal.edu.co/handle/unal/63371

37. Burda S, Oleszεk W. Antioxidant and antiradical activities of flavonoids. J Agric Food Chem. 2001;49(6):2774-79. https://doi.org/10.1021/jf01001413m.

38. Silva BA, Ferreres F, Malоa JD, Dias ACP. Phytochemical and antioxidant characterization of Hypericum perforatum alcohοlic extracts. Food Chem. 2005;90(1-2):157-67. https://doi.org/10.1016/j.foodchem.2004.03.049

39. Pier-Giorgio P. Flavonoids as antioxidants. J Nat Prod. 2000;63(7):1035-42. https://doi.org/10.1021/np9904509.

40. Gulίnί C, Fevί T, Ramazan Č, Mine B, Ahmet G, Umgulsum E. Pomological features, nutritional quality, polyphenol content analysis and antioxidant properties of domesticated and wild ecotype forms of raspberries (Rubus idaeus L.). J Food Sci. 2011;76(4):C585-93. https://doi.org/10.1111/j.1750-3841.2011.02142.x

41. Samsonowicz M, Ewa R. Spectroscopic study of molecular structure, antioxidant activity and biological effects of metal hydroxyflavonol complexes. Spectrochim Acta A Mol Biomol Spectrosc. 2017;173:757-71. https://doi.org/10.1016/j.saa.2016.10.031
42. Gulcin I, Oktay M, Küfrevioğlu Öl, Aslan A. Determinations of antioxidant activity of lichen *Cetraria islandica* (L.) Ach. J Ethnopharmacol. 2020;79(3):325-29. https://doi.org/10.1016/s0378-8741(01)00396-8

43. Toiu A, Vlase L, Dragoi CM, Vodnar D, Oniga I. Phytochemical analysis, antioxidant and antibacterial activities of *Hypericum humifusum* L. (Hypericaceae). Farmacia. 2016;64(5):663-67. Available from: https://farmacijournal.com/wp-content/uploads/2016-05-art-03-Toiu_Vlase_Dragoi_Oniga_663-667.pdf

44. Rainha N, Lima E, Baptista J. Comparison of the endemic azorean *Hypericum foliosum* with other *Hypericum* species: Antioxidant activity and phenolic profile. Nat Prod Res. 2011;25(2):123-35. https://doi.org/10.1080/14786419.2010.512560

45. Napoli E, Siracusa L, Ruberto G, Carrubba A, Lazzara S, Speciale A, et al. Phytochemical profiles, phototoxic and antioxidant properties of eleven *Hypericum* species– A comparative study. Phytochemistry. 2018;152:162-73. https://doi.org/10.1016/j.phytochem.2018.05.003

46. Sroka Z, Cisowski W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem Toxicol. 2003;41(6):753-58. https://doi.org/10.1016/S0278-6915(02)00329-0

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