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Phytochemical Profile and Antioxidant Activity of Hydroalcoholic Extracts of *Corynaea crassa* Hook. f (Balanophoraceae)

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ABSTRACT

Corynaea crassa Hook is a hemiparasitic plant distributed in many regions of America. It has traditionally been ascribed to have aphrodisiac and antimicrobial activities. The objective of this research is to analyze the chemical composition and antioxidant activity of hydroalcoholic extracts of *C. crassa* from Ecuador and Peru.
The whole plant was used for the preparation of the extract. The extracts were made by percolation with 80% ethanol, and the chemical composition was evaluated by ultraviolet visible (UV/VIS), infrared (IR) spectroscopy and Liquid Chromatography-Mass Spectrometry (LC-MS). The quantification of phenols was done by using the Folin-Ciocalteu method, the flavonoids were quantified by using the aluminum chloride method. The antioxidant activity was tested by Ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. UV/VIS spectroscopy of the extracts revealed similar spectral appearance, which denoted the presence of flavonoids and an IR profile characteristic of phenolic compounds, with coincident bands between the extracts but different in intensity and width. Phenols and flavonoids were higher for the Ecuadorian plant–extract. The LC-MS study detected catechin, quercetin glycoside, and a flavanone glycoside, for the first time. It was shown that the antioxidant capacity of extracts was similar or superior to the tested substances (Vitamin C and Trolox), with the activity of the Ecuadorian extract being higher. The study of the chemical composition and antioxidant properties of *C. crassa*, has given credence to the use of the plant in traditional medicine.

***Keywords*:** *Corynaea crassa*, Phytochemical, Antioxidant, Ecuador, Peru.

Introduction

Balanophoraceae is a family of non-photosynthetic flowering holoparasitic plants. They are perennial herbs that live for more than two years with total loss of chlorophyll.[1,2] The family consists of 18 genera and 45 species that are distributed mainly in the tropical and subtropical forests of the world, of which seven genera are exclusively from South America. These plants parasitize on the roots of others, and most of their life takes place underground.[1,3]
Helosis is the only Central America genus, along with *Corynaea*, that present similar characteristics, but they are distinguished because *Helosis* only grows at sea level, while *Corynaea* grows at 2000 meters above sea level.[4]
Corynaea crassa is found in the high Andean forests, with an altitude range of 1,300 - 3,000 meters above sea level.[5] Its harvesting in Ecuador occurs in tropical forests of Azuay, Carchi, Chimborazo, Cotopaxi, Imbabura, Loja, Morona Santiago, Napo, Pichincha, Sucumbíos, Tungurahua, and Zamora Chinchipe, at an approximate altitude of 1050 to 3000 meters above sea level. In Peru, harvesting takes place in the Amazon, Cajamarca, Cusco, La Libertad, Lambayeque, and Pasco, at an elevation of 2,600 to 3,300 meters above sea level.[6]
C. crassa Hook. f., is called *huanarpo macho*, since it is traditionally used as a male aphrodisiac.[7] In Peru, it is known as "male Viagra" and is traditionally used for the treatment of inflammation, viral infection, diabetes, cancer, Chagas disease, and for sexual enhancement as an aphrodisiac.[8]
Few scientific reviews are found in the literature about *C. crassa*; Malca *et al*[9], reported the presence of steroids, triterpenes, flavonoids, tannins, and anthocyanins, and also managed to identify *β*-sitosterol, lupenone, *β*-amirone, lupeol and *β*-amyrin, through phytochemical studies by chromatographic methods and spectroscopic tests performed on the methanol and hexane extracts from the roots. Other preliminary studies revealed the presence of alkaloids, phenolic compounds, steroids, flavonoids, lactones, saponins, and tannins in the ethanol extracts of the roots of the species.[8]
López *et al*,[10] in a study with the species from Ecuador and Peru, reported the chemical composition of an ethyl acetate extract from the entire plant, finding a total of 20 types of species that grow in Ecuador, where safrole and squalene, some sesquiterpenoids, fatty acids and ergosterol stood out. While in the Peruvian species, they did not find sesquiterpenes, and quantitative differences were found between the compounds found in both extracts.
Regarding the pharmacological activity, Bussmann *et al*,[11] showed that the species had antimicrobial activity. They also noted that the aqueous extracts were not toxic at doses lower than 10,000 g/mL. López *et al*,[10] demonstrated that the hydroalcoholic and aqueous extracts of the species from Peru and Ecuador presented anti-inflammatory activity.

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This work aims to analyze the chemical composition and antioxidant activity of hydroalcoholic extracts of *C. crassa* whole plant from Ecuador and Peru.

Materials and Methods

Plant material

In the present work, plants of *C. crassa*, collected in August 2018, from two different countries were studied.

The first one was collected in La Libertad province, Santiago de Chuco Department Agasmarca, Peru (08°07′53″S, 78°03′23″E, 2900 m elevation) labeled as sample Pe and the second one from Yanachoca Reservation in North of Pichincha province, Ecuador (00°05′S, 78°33′E, 3700 m elevation) and labeled as Ec. Specimen of each harvest was identified at the herbarium of Natural Science Faculty, Guayaquil University and deposited under the voucher specimen of 13.115 and 13.116, respectively. In each case, the whole plant was used, which was washed with water. The samples were dried in an AISET model VLD-6000 (China) oven with controlled temperature, at 40°C ± 2°C, over seven days, and subsequently crushed in a Pulvex mill to a particle size of 2 mm for processing and analysis.

Preparation of extracts

A hydroalcoholic extract was prepared by percolation, for the plants of each origin. Dry and ground drug (20 g) previously moistened with 100 mL of 80% ethanol solution (Sigma Aldrich), allowed to stand for 15 minutes and later transferred to the percolator and the extraction process was carried out with an 80% of hydroalcoholic mixture to obtain a fluid extract, according to the procedure proposed by Miranda and Cuellar.[12] The extracts were refrigerated for four days at 10-15°C and then filtered for their corresponding analysis. They were labeled as Ecuador percolation extract (EPE) and Peru percolation extract (EPP), respectively.

Chemical analysis

Ultraviolet spectroscopic analysis

One milliliter of the EPP and EPE hydroalcoholic extracts were concentrated to dryness, redissolved in 10 mL of methanol (Merck) and subsequently analyzed in a UV-Visible spectrophotometer (Analytik Jena brand, model SPECORD-200 plus Germany). A scan was made from 200 to 800 nm.

Infrared spectroscopic analysis

Five milliliters of the EPP and EPE extracts were concentrated to dryness and the solids obtained were added to KBr (Sigma Aldrich) tablets. The measurements were made in Jasco infrared equipment, model FT / IR-460 plus Japan. The infrared spectrum was obtained in the region of 4000 to 600 cm^{-1}.

Determination of total phenols and total flavonoids

Determination of total phenols

Total phenols were determined by the Folin-Ciocalteu method with some modifications.[13] The hydroalcoholic extracts of the species of both origins (EPP and EPE) and gallic acid (reference substance) at concentrations of 10, 20, 30, 40 and 50 mg/mL were used. The reaction mixture was composed of 200 µL of the extracts or reference substance, 10 mL of Folin-Ciocalteu reagent (Sigma-Aldrich) 1:10, 1.8 mL of distilled water and 8 mL of sodium carbonate 7.5% (Sigma Aldrich), after 2 h, the absorbances were read at 765 nm on a spectrophotometer (Rayleigh UV-1601, China). The total phenolic content was expressed in terms of gallic acid (Sigma Aldrich) equivalent (mg/mL).

Determination of total flavonoids

The content of total flavonoids was carried out by the colorimetric method using aluminum chloride.[13,14] The hydroalcoholic extracts and quercetin (Sigma Aldrich) (reference substance) at concentrations of 5, 20, 50, 60, 80 µg/mL were used. The test sample contained 500 µL of the extracts or reference substance, 1.5 mL of ethanol, 0.1 mL of 10% aluminum chloride (sigma-Aldrich), 0.1 mL of 1 M potassium acetate (Sigma Aldrich) and 2.8 mL of distilled water. Samples and standards were incubated at room temperature for 30 minutes and the absorbance was measured at 415 nm with a spectrophotometer (Rayleigh UV-1601, China). The concentration of flavonoids was expressed in terms of quercetin equivalent (mg/mL).

Liquid Chromatography-Mass Spectrometry (LC-MS) analysis

The samples were prepared by dissolving 10 mg of dry extract (hydroalcoholic from both sources) in 1 mL of HPLC grade methanol (Merck) in an ultrasonic bath and subsequently filtered (filter Titan 3 of 0.20 µm). The analysis by liquid chromatograph-diode array-detector-mass spectrometry (LC-DAD-MS) was performed with an ultra high-pressure liquid chromatography (UPLC) Thermo Scientific system equipped with a two-quaternary pump (Dionex ultimate 3000 RS LC), an autosampler (Dionex ultimate 3000 RS), a diode array detector (Dionex ultimate 3000 RS) and linear ion trap mass spectrometer LTQ XL and operated under Xcalibur 3.1 version software. The separation was performed using an Accucore RP-MS column (100 mm x 2.1 mm, 2.6 µm, Thermo scientific) protected by a prepacked column (4 mm x 2 mm). The mobile phase consisted of acetonitrile (A) and 0.1 % formic acid (B). The gradient used was 0-2 min, 10-90% B; 2-6 min 27-73% B; 6-9 min, 30-70% B; 9-12 min, 33-67% B; 12-18 min, 95-5% B; 18-21 min. 10-90% B. The solvent flow rate was 0.4 mL/min and the injection volume was 2 µL. Ultraviolet spectra were monitored from 200-600 nm.

Mass spectra with electrospray ionization (ESI) interface was used. Mass spectra data were recorded on a full scan mode for a mass range of m/z 100-1800. Other mass spectrometer conditions were as follows: capillary temperature 225°C; capillary voltage -50 V; spray voltage 5 Kv; gas flow 34 (nitrogen gas, arbitrary units); auxiliary gas flow 5 and scanning gas 3. The tuning file was optimized with a direct infusion of quercetin (10 µg/mL concentration). Compounds were assigned by comparing the ultraviolet and mass spectra obtained with those of the equipment library and the literature.

Antioxidant activity

For the development of antioxidant activity by the three *in vitro* methods, hydroalcoholic extracts from both sources were used, from which, taking into account the percentage of total solids, the different concentrations to be tested in each method were calculated and prepared.

Ferric reducing antioxidant power (FRAP) assay

The reducing capacity of hydroalcoholic extracts of the species collected in Peru and Ecuador were measured.[15] A UV spectrophotometer (Rayleigh UV-1601, Shanghai, China) was used at an absorbance of 593 nm. All reagents used were from Merck (2,4,6-tripyridyl-s-triazine (TPTZ), sodium acetate anhydrous, acetic acid (99.7 %), hydrochloric acid (37%), $FeCl_3$) and reference substances ascorbic acid (99% purity) and $FeSO_4.6H_2O$.

The extracts were evaluated at concentrations of 0.75, 2, 6, 10 and 15 µg/mL. The results were expressed as µmol equivalents of ascorbic acid and as µmol equivalents of $FeSO_4$ from the calculation, by interpolating the optical density (OD) of the samples in the calibration curves of both reference substances at concentrations of 100, 200, 400, 800 and 1200 µM. The readings were made in triplicate.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity.

For the quantitative determination, the DPPH free radical method was used.[16,17] A UV spectrophotometer (Rayleigh UV-1601, Shanghai, China) was used, and the determinations were measured at 517 nm after 30 minutes. The extracts of the species collected in Peru and Ecuador, and reference substances Vitamin C (99% pur) and trolox (Merck), were tested at concentrations of 2, 6, 10, 15 and 20 µg/mL. The percentage of inhibition of DPPH radical was calculated according to the following formula:

$$\%\ inhibition\ DPPH\ = \frac{Absorbance\ control - Absorbance\ sample}{Absorbance\ control} \times 100$$

The half maximal inhibitory concentration (IC_{50}) was determined with the help of the GraphPad prism 5.0 statistical program.

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2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity

The assay was based on the ability of different substances to sequester the cationic radical $ABTS^{\bullet+}$.[18,19] A UV-visible spectrophotometer (Rayleigh UV-1601, China) was used, and absorbance was taken at 734 nm. Reagents used were of analytical grade (Merck), and they included [ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline) -6-sulfonic acid), potassium persulfate, and ethanol 96%]. The extracts and reference substances Vitamin C (99%) and trolox were tested at concentrations of 100, 200, 300, 500 and 600 µg/mL. The percentage of inhibition of the ABTS radical was calculated as:

$$\%\ Absorbance\ ABTS = \frac{Absorbance\ ABTS - Absorbance\ antioxidants}{Absorbance\ ABTS} \times 100$$

The half maximal inhibitory concentration (IC_{50}) was determined using the GraphPad prism 5.0 statistical program.

Statistical analysis

The experiments were carried out in triplicates and the results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine the differences between multiple groups, followed by Duncan test with $p \leq 0.05$. A Student-t test was used to evaluate the differences in the quantitative determination of phenols and flavonoids and the FRAP assay between two extracts. All data were processed by the statistical program SPSS for Windows version 8.0.

Results and Discussion

Chemical analysis

Ultraviolet and infrared spectroscopy

The ultraviolet visible spectroscopy of the hydroalcoholic extract from Ecuador showed two fundamental absorptions, one at 281.5 nm and the other at 319.5 nm. This behavior could be related to the presence of phenolic compounds, especially flavonoids, which exhibit two bands in the ultraviolet/visible region, where band I appears in a range of 300 to 400 nm and band II is located between 200 and 285 nm. For its part, the extract from Peru presented an important band around 280 nm, which is typical of all-natural organic compounds that have an aromatic ring; It is also related to the presence of unsaturated carbonyl groups or phenols.[20,21]

The UV-Visible analysis showed differences in the spectral appearance of the extracts in relation to the place of origin. It is important to note that this technique, although useful for phytochemical characterization, only offers an orientation regarding the possible presence of functional groups.

When analyzing the hydroalcoholic extracts by infrared spectroscopy, a broad and intense band between 3500 and 3200 cm^{-1} was observed, indicative of the valence vibrations of alcohols or associated phenols. Between 3000 and 2800 cm^{-1}, bands indicative of C-H valence vibrations were observed. A band around 1600-1500 cm^{-1} was also visualized that could be related to the C–C valence vibrations of compounds with double bonds, including compounds with aromatic grouping. A band between 1200 and 1000 cm^{-1}, approximately, indicative of the presence of C-O bonds, mainly phenols, was found. Other bands in the region of 900-690 cm^{-1} were found, and they can be related to substitutions in the aromatic grouping.[22,23] Infrared analysis showed differences in the width and intensity of the bands, which could be associated with the concentration and type of metabolites present in the extracts.

Determination of total phenols and total flavonoids

The quantification of total phenols was determined by the Folin-Ciocalteau method, which measures the amount of extract necessary to inhibit the oxidation of the reagent.[24,25] Total flavonoids were determined by the aluminum chloride colorimetric method, where these compounds react with the reagent, producing a yellow complex that has a light absorption peak at 415 nm. The results are presented in Table 1.

The content of both metabolites; phenols and flavonoids were higher for the species from Ecuador.

On the other hand, it is noteworthy that the content of total phenols was higher than that of flavonoids in the species from both sources, although it must be pointed out that flavonoids are considered within the group of phenolic compounds. There are no reports on the quantification of phenols and flavonoids in the species or in the family, so it is not possible to establish a comparison regarding this.

Phenolic compounds are formed as intermediate or end products of the secondary metabolism of the plant and are considered to play a role in various processes, including growth and reproduction, as well as in adaptation and survival in stressful or environmental conditions, such as geographic location, ultraviolet radiation, high temperatures, attacks by pathogens,[26] soil composition, storage conditions, etc.[27] These factors can respond to the quantitative changes found in the species from different origins, if taken into account that the geographical location and the type of soil are different.

LC-MS analysis

The LC-MS analysis was performed on the hydroalcoholic extracts from the two sources. Figure 1 shows the chromatogram. The main differences were seen in 2-8 minutes.

At 3.68 minutes, a compound eluted was identified as catechin or epicatechin, two diastereoisomers frequently found in the plant kingdom. Due to their structural similarity, it was not possible to differentiate them, but the MS/MS spectrum showed total agreement with that reported for epicatechin with a $[MH]^-$ at m/z 289 and the three main ions at m/z 245 (100%), 205 and 179.[28] This compound was only detected in the extract from Ecuador.

Figure 2A shows the mass spectrum and 2B the MS/MS spectrum of m/z 289, as well as the chemical structure of (epi) catechin. A peak was observed at 7.79 minutes (Figure 1) that showed an ion with m/z 463 and was detected in the extracts from the two sources. The deduced mass (464 Da) coincides with that of a quercetin glycoside, particularly a hexoside (301+162 Da). In the MS/MS spectrum of both extracts that is visualized in figure 3A an ion with m/z 301 can be seen, which can be assigned to the $[MH]^-$ expected for quercetin and which is caused by the loss of a fragment of 162 Da, typical of hexoses; in this compound it would be $[MH-162]^-$. In the same MS/MS spectrum, m/z 151 and 179 are appreciated. These fragment ions coincide with that reported for quercetin fragmentation, where m/z 151 corresponds to the ^{1,2}A-CO ion and m/z 179 with the ^{1,2}A-29 ion. Their low intensity is expected, since it is the MS/MS spectrum of the glycoside and not of quercetin.

Figure 3B shows the fragment ion originated by the loss of the sugar residue (m/z 301) and fragment ions originated from it.[29] other compounds with flavonoid glycoside characteristics eluted at 8.59 and 8.60 minutes for the extracts from Peru and Ecuador, respectively (Figure 1). They showed the same m/z 449 and in turn a mass difference of 14 Da with the previous proposal of quercetin glycoside.

If it is assumed that these compounds are also flavonoids, and the difference of 14 Da could be accounted for by the combination of one less oxygen atom (-16 Da) and 2 more hydrogen atoms (+2 Da); which can be related to a flavonoid nucleus with a single bond between the C-2 and C-3 positions, instead of the double bond that characterizes the flavonol quercetin. The m/z 287 observed in the MS/MS spectrum could be assigned to $[MH-162]^-$ and this value coincides with the $[MH]^-$ of a tetrahydroxyflavanone such as eriodictyol.[29]

Table 1: Content of total phenols and total flavonoids in hydroalcoholic extracts of *C. crassa*

Extracts	Total phenols (mg/mL)	Total flavonoids (mg/mL)
Ecuador	$43.52 \pm 0.10^{a*}$	$5.81 \pm 0.07^{c*}$
Peru	43.28 ± 0.16^{b}	$4.02 \pm 0.04^{d*}$

Values represent Mean ± Standard deviation

*Different letters in a column show significant differences ($p < 0.05$) according to student t-test.

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The mass spectrum is shown in Figure 4A and 4B and MS/MS spectrum of m/z 449, respectively, as well as the chemical structure of the possible aglycon (tetrahydroxyflavanone) of the supposed hexoside that originates m/z 449.

In the area of the highest retention time, some chromatographic signals of interest between 12 and 17 minutes were appreciated for both extracts, of appreciable abundance, which could not be identified, so it is suggested that preparative chromatography be performed with the objective of isolate these compounds and subsequently characterize them by nuclear magnetic resonance.

Only three flavonoids are suggested for the Ecuadorian species, (epi) catechin, quercetina glycoside, and a flavanone glycoside. In the Peruvian species, except for catechin, the two flavonoids mentioned above were also suggested. The lack of detection of catechin in the extract from Peru is perhaps due to its low concentration in the sample.

As this species parasitizes the roots of other plants, its chemical composition can vary according to the host they parasitize,[30] which may to some extent justify the differences found between the species that grows in Ecuador, with respect to that of Peru.

The presence of flavonoids found for the species is in aggreement with studies carried out by Nina *et al*,[31] who, in an HPLC-MS study, were able to identify in the parasitic species *Ombrophytum subterraneum* of the Balanophoraceae family, flavonoids such as flavanones and flavonols, mainly in the form of glycosides. As for this, Bracci *et al*,[32] identified catechin in the methanol extract of *Lophophytum leandri*, another parasitic species of the Balanophoraceae family, in agreement with the investigation carried out on *C. crassa*.

Antioxidant activity

Ferric reducing antioxidant power (FRAP) assay

All the extracts at the five concentrations tested showed, from the qualitative point of view, a colour change from amber to intense blue. This behaviour is due to the presence of antioxidant substances that reduced the ferric ion of the Fe^{3+}-TPTZ complex to a ferrous ion, forming the Fe^{2+}-TPTZ complex.

The results expressed the reducing capacity of the Fe^{3+} cation of each extract, as µM equivalent of ascorbic acid and µM equivalent of $FeSO_4$ (reference substances used and recognized as having a high antioxidant activity).

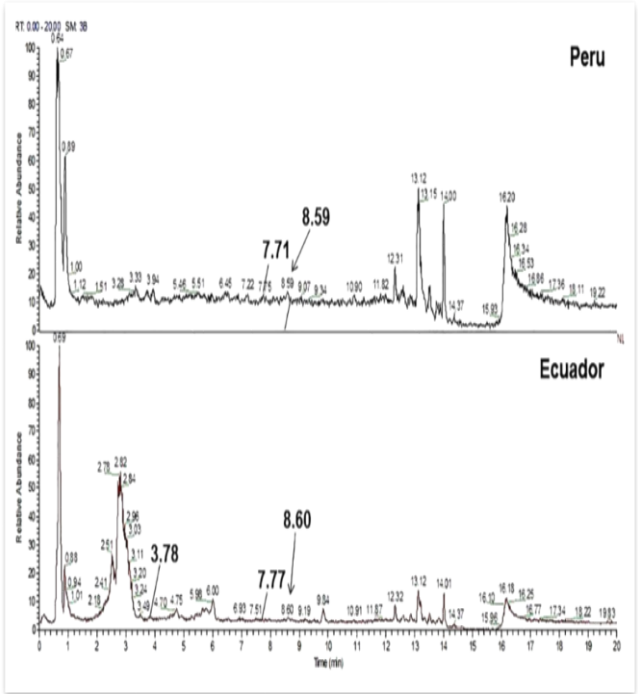

Figure 1: TIC Chromatograms of the hydroalcoholic extracts of *C. crassa*

Tables 2A and 2B show the results expressed as µM equivalents of ascorbic acid and as µM equivalents of iron sulfate, respectively. Ferro-reducing activity was evidenced in a concentration-dependent manner, reaching the highest antioxidant activity at a concentration of 15 µg/mL for both extracts. At the highest concentration tested, the Ecuadorian extract showed the highest antioxidant activity equivalent to vitamin C, while the one from Peru showed the highest activity equivalent to $FeSO_4$.

An analysis of the results in relation to reports from the literature[33] allowed suggesting a good iron-reducing power of the extracts when compared with plants such as *Cornus officinalis* (970.81 ± 65.79), *Davallia mariesii* (957.63 ± 96.67), *Pogostemon cablin* (919.59 ± 8.63), *Psoralea corylifolia* (841.81 ± 15.30), among others, values which is in µM equivalent were lower at a much higher concentration (10 mg/mL) than the maximum concentration tested (15 µg/mL) in this research.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity

As can be seen in table 3, in general terms there were significant differences between the samples at the same concentration. The extracts showed at the lowest concentrations (2 and 6 µg/mL) of percentages of inhibitions greater than 60%, significantly higher than the two standards tested. The extract of the species from Ecuador showed a higher percentage of sequestration of the radical DPPH than that of Peruvian origin.

The lowest IC_{50} value (concentration value at which 50% inhibition of the maximum sequestration effect of DPPH is reached) and therefore the highest antioxidant activity, was obtained for the extract from Ecuador with 1.53 µg/mL, followed by the Peruvian sample with 1.62 µg/mL.

According to this technique, samples that at a concentration close to 20 µg/mL inhibit 50% of the discoloration of DPPH are considered of interest, so it can be considered that in these working conditions, the extracts have a good antioxidant power.

The high sequestering activity of the radical DPPH of the evaluated extracts agrees with the studies carried out by Nina *et al*,[31] on the species *Ombrophytum subterraneum* (Balanophoraceae), who obtained IC_{50} of 1.56 µg/mL, for extracts of tubers. However, when comparing the IC_{50} results of the present investigation with those of the *Rhopalocnemis phalloides* species (IC_{50} = 32.1 µg/mL) of the

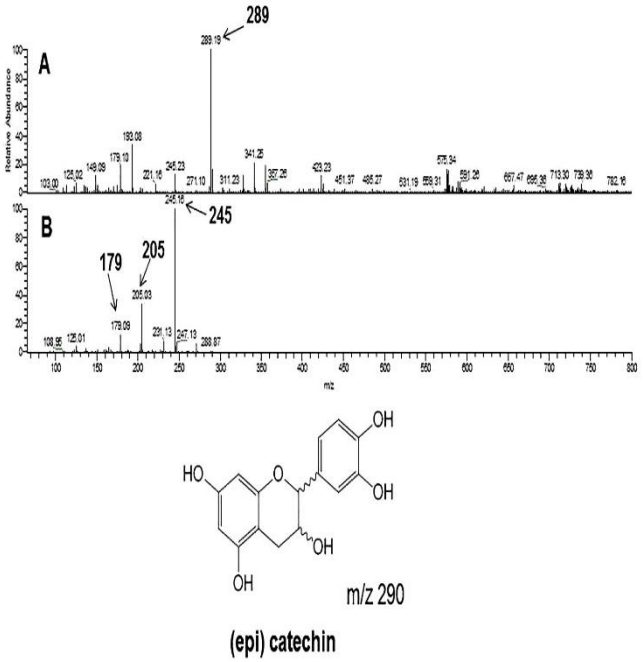

Figure 2: Mass spectrum (**A**) and MS/MS spectrum (**B**) of the compound with m/z 289 present in the hydroalcoholic extract from Ecuador

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same family (Balanophoraceae),[34] a considerably higher antiradical activity could be verified for *C. crassa* (IC_{50} = 1.53 and 1.59 µg/mL for the hydroalcoholic extracts from Ecuador and Peru, respectively).

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity During the development of this method, a discoloration of the cationic radical $ABTS^{\bullet+}$ was observed at all the concentrations evaluated, due to the ability of the samples to neutralize the radical, and this is reflected in a decrease in absorbance and a decrease in the blue colour to intense green until discoloration.

Table 4 shows the ABTS radical inhibition percentages, achieving a sequestration capacity greater than 65% from the lowest evaluated concentration of 100 µg/mL. At the maximum concentration (600 µg/mL) the two extracts showed the highest percentage of similar radical sequestration and with higher values than vitamin C and trolox. Of the evaluated samples, those with the lowest IC_{50} were the two extracts and reference substance Vitamin C, with values of 76.62 µg/mL, 85.86 µg/mL and 105.83 µg/mL, respectively, indicating the best antioxidant effects, which translates to a good ability to sequester the radical ABTS.

When comparing the results of IC_{50} in this research with those obtained for an aqueous extract of the *Cynomorium coccineum* species (IC_{50} = 110 µg/mL) of the Balanophoraceae family,[35] the good sequestering power of the radical ABTS was confirmed by the tested extracts.

The results obtained for the concentration of phenolic compounds and flavonoids justify the antioxidant capacity found for the extracts studied. The higher antioxidant activity shown for the Ecuador extract agrees with the higher content of total phenols.

The results are in agreement with that proposed by Li *et al*,[33] where with lower concentrations of total phenols and total flavonoids, a good reducing power was achieved by some extracts, even higher than in the present research. Such is the case of *Alisma plantagoaquatica* Linn [1119.59 ± 27.44 µM/mL Fe (FRAP value), 35.10 ± 2.41 mg/mL (phenols) and 2.02 ± 0.43 mg/mL (flavonoids)]; *Melia toosendan* [1424.67 ± 53.02 µM/mL Fe (FRAP value), 39.79 ± 0.36 mg/mL (phenols), 3.13 ± 0.06 mg/mL (flavonoids)]; *Phryma leptostachya* L [1414.31 ± 35.77 µM/mL Fe (FRAP value), 25.88 ± 1.81 mg/mL (phenols), 3.48 ± 0.02 mg/mL (flavonoids) and others.

On the other hand, quercetin glycoside and flavanone glycoside were identified in both extracts, which is a polyphenolic antioxidant, and catechin, for the extract of the species from Ecuador, which is a flavonol widely present in plants and exhibits a variety of biological activities and has a potent free radical scavenging capacity. These compounds could contribute to the demonstrated antioxidant activity, since they present hydroxyl groups that can directly contribute to this property.[36-40]

In other phytochemical studies of the plant carried out by López *et al*,[10] the presence of terpenoids, fatty acids, sterols, among other compounds with recognized antioxidant activity, were detected. From this analysis, it could be suggested that the combined action of all these secondary metabolites favours the antioxidant effect observed in our study. However, future experiments should be carried out to study the structure-activity relationship of the metabolites present. Plant extracts contain numerous biologically active compounds with antioxidant activity that act through different reaction mechanisms. The antioxidant capacity of an extract is not only given by the concentration and sum of the antioxidant capacities of each of its components, but it also depends on the microenvironment in which they are found, which may produce synergistic or inhibitory effects.[41]

Antioxidants are capable of stabilizing or deactivating free radicals before they attack cells, and can protect cellular components against oxidative damage, and therefore limit the risk of various degenerative diseases associated with oxidative stress.[42-43] Antioxidants manifest various pharmacological properties of interest, among which are anti-inflammatory properties, antiallergic, antithrombotic, antimicrobial, antineoplastic, and anticancer activities,[44-45] which could justify some of the properties attributed to the species.

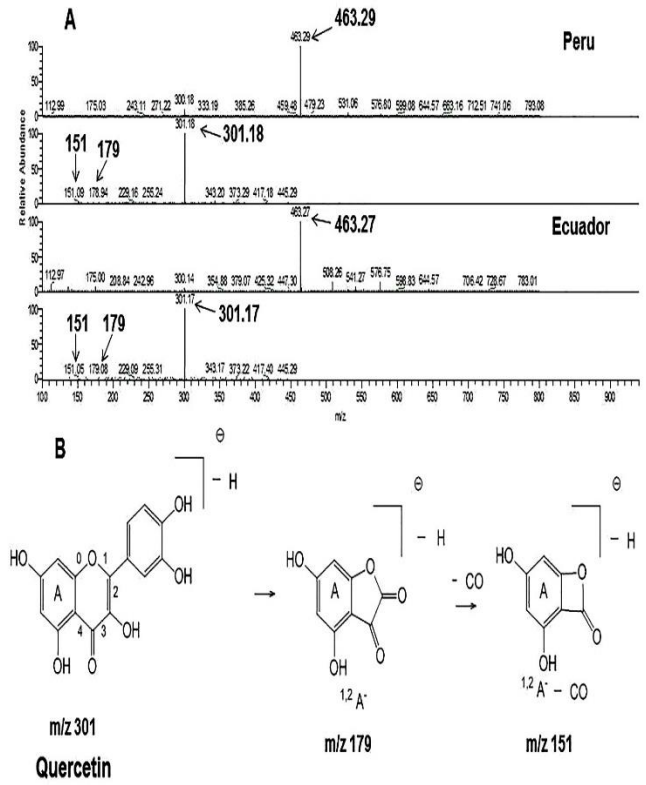

Figure 3: Mass spectrum and MS/MS spectrum (**A**) of the compound with m/z 463 present in the hydroalcoholic extracts of Peru and Ecuador and fragment ion originated by the loss of the sugar residue (m/z 301) and fragment ions originated from it (**B**).

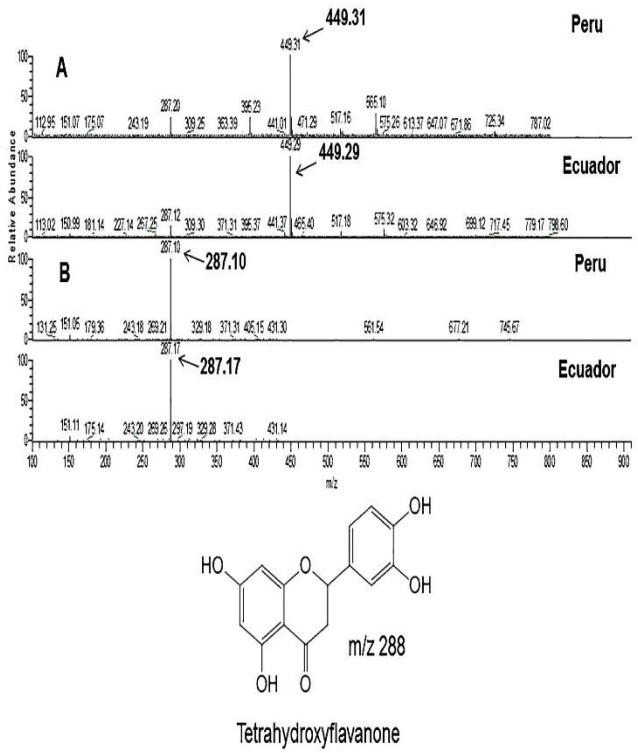

Figure 4: Mass Spectrum (**A**) and MS/MS spectrum (**B**) of the compound with m/z 449 present in the hydroalcoholic extracts of Peru and Ecuador.

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Table 2: Ferro-reducing activity of the extracts of *C. crassa*

	µM equivalents of Vitamin C Concentrations (µg/mL)				
Extracts	**0.75**	**2**	**6**	**10**	**15**
EPE	441.68 ± 13.76^{a*}	588.35 ± 30.13^{b*}	813.35 ± 33.50^{d*}	950.02 ± 94.10^{f*}	1206.76 ± 25.04^{g*}
EPP	429.19 ± 26.26^{a}	467.59 ± 28.52^{c*}	635.02 ± 12.99^{e*}	903.35 ± 26.25^{f*}	1105.85 ± 34.49^{h*}
	µM equivalents of $FeSO_4$ Concentrations (µg/mL)				
Extracts	**0.75**	**2**	**6**	**10**	**15**
EPE	372.03 ± 23.87^{i*}	415.97 ± 20.45^{j*}	559.15 ± 11.80^{l*}	803.09 ± 23.87^{n}*	987.18 ± 31.35^{p*}
EPP	383.40 ± 12.51^{i*}	516.73 ± 27.40^{k*}	721.27 ± 30.69^{m*}	887.94 ± 29.31^{o}*	1078.85 ± 22.88^{q*}

Values are Mean ± Standard deviation. (n=3).
Legend: EPE = hydroalcoholic extract from Ecuador, EPP = hydroalcoholic extract from Peru
*Different letters in a column show significant differences (p <0.05) according to t-student

Table 3: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts of *C. crassa*

	% Inhibition of DPPH Concentrations (µg/mL)					
Samples	**2**	**6**	**10**	**15**	**20**	**IC_{50}**
EPE	65.97 ± 0.33^{a*}	74.48 ± 0.29^{e*}	76.79 ± 0.35^{i*}	77.81 ± 0.43^{k*}	79.90 ± 0.50^{o*}	1.53
EPP	64.67 ± 0.33^{b*}	71.83 ± 0.06^{f*}	74.99 ± 0.27^{j*}	75.32 ± 0.26^{l*}	77.41 ± 0.38^{p*}	1.62
Trolox	60.29 ± 0.38^{c}	69.37 ± 0.21^{g*}	75.82 ± 0.33^{k*}	80.19 ± 0.27^{m*}	82.04 ± 0.99^{q*}	1.85
Vitamin C	48.45 ± 0.23^{d*}	64.85 ± 0.16^{h*}	74.26 ± 0.39^{j*}	76.03 ± 0.23^{n*}	78.60 ± 0.28^{r*}	2.10

Values are Mean ± Standard deviation. (n=3).
Legend: Legend: EPE = hydroalcoholic extract from Ecuador, EPP = hydroalcoholic extract from Peru, IC_{50}: mean inhibitory concentration
*Different letters in a column show significant differences (p <0.05) according to Duncan test

Table 4: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity of the extracts of *C. crassa*

	% Inhibition of the $ABTS^{\bullet+}$ Concentrations (µg/mL)					
Samples	**100**	**300**	**400**	**500**	**600**	**IC_{50}**
EPE	66.80 ± 1.90^{a*}	77.63 ± 1.28^{d*}	87.93 ± 2.38^{g*}	93.87 ± 0.81^{j*}	96.04 ± 1.13^{m*}	76.62
EPP	65.25 ± 0.86^{a}	76.50 ± 1.55^{d*}	87.42 ± 1.35^{g*}	92.08 ± 1.46^{j*}	96.04 ± 0.88^{m*}	85.86
Vitamin C	48.16 ± 1.71^{b*}	70.52 ± 1.14^{e*}	90.25 ± 0.92^{h*}	94.30 ± 0.35^{k*}	95.24 / 0.72^{n*}	105.83
Trolox	42.04 ± 1.47^{c*}	63.74 ± 0.66^{f*}	68.59 ± 1.23^{i*}	93.07 ± 0.14^{l*}	94.72 ± 0.57^{n*}	138.52

Values are Mean ± Standard deviation. (n=3).
Legend: EPE= hydroalcoholic extract from Ecuador, EPP = hydroalcoholic extract from Peru IC_{50}: mean inhibitory concentration
*Different letters in a column show significant differences (p <0.05) according to Duncan test

Conclusion

The species from both sources presented phenolic and flavonoid compounds, which were corroborated by their UV and IR spectra, by quantitative analysis and the LC-MS study, being able to detect for the first time the presence of nicoloside, catechin and a quercetin glycoside in the plant. All the extracts showed a high antioxidant capacity by the three evaluated methods, similar or superior to the reference substances tested, the activity being higher for the samples from Ecuador.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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