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In vitro anthelmintic activity of *Polygonum acre* (Linnaeus, 1754) extracts against the ruminant nematode *Haemonchus contortus* (Rudolphi, 1803)

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ABSTRACT

The genus *Polygonum* exhibits a variety of biological and therapeutic properties. The goal of this study was to determine the *in vitro* anthelmintic activity of *Polygonum acre* extracts against the nematode *Haemonchus contortus*, characterizing the phenolic profile of these extracts. Aerial parts of *P. acre* were subjected to hot aqueous and hydroethanolic extractions obtaining EHW-PA (aqueous) and ETOH-P, (hydroethanolic), respectively. The phenolic content of the extracts was analyzed by using high-performance liquid chromatography with electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) and colorimetric assays. The egg hatch test (EHT) and larval migration test (LMT) were used for anthelmintic activity assays. For comparison, chlorogenic acid and rutin were selected for the EHT. The HPLC-ESI-MS/MS analysis revealed the presence of isoquercitrin, myricitrin, quercetin, rosmarinic acid, rutin, and taxifolin in both extracts. EHW-PA and ETOH-PA showed an egg hatch inhibitory effect. EHW-PA had an IC_{50} of 442.24 µg/mL on the EHT, which was statistically lower ($P \leq 0.05$) than ETOH-PA (IC_{50} = 703.60 µg/mL). Chlorogenic acid had an IC_{50} of 8.66 µg/mL. Rutin presented no effect over parasite eggs. EHW-PA and ETOH-PA did not show any larvicidal effect. Even though, there is a need of new tests, this study has determined the possibility to develop biofriendly compounds based on *Polygonum* spp. constituents against *H. contortus*.

1. Introduction

Haemonchus contortus is one of the most important gastrointestinal nematode parasites of ruminants. This hematophagous parasite can cause severe anemia, which contribute to the reduction of weight, and in more severe infections can cause the death of sheep and goats (Kumarasingha et al. 2016). Currently, conventional parasite control depends almost exclusively on the regular use of large-spectrum anthelmintic products. However, such protocol presents major disadvantages, such as the development of resistant parasite populations, the risk of environmental pollution, and high farming costs (Fortes and Molento 2013). Thus, the search for alternatives for the control of gastrointestinal nematodes in small ruminants is extremely important (Molento et al. 2013). *In vitro* tests have been used to evaluate the antiparasitic activity of plant extracts, acting as a preliminary step to characterize the potential of bioactive plant compounds, and to measure control strategies in the field (Jackson and Hoste 2010; Molento et al. 2016; Jayawardene et al. 2021).

Flavonoids are considered the most common components found in the genus *Polygonum* and have previously been used as chemotaxonomic markers of the genus.

These components play an important role in the systematics of Polygonaceae species (Datta et al. 2002). Plants with high concentrations of phenolic compounds, such as phenolic acids, flavonoids, and condensed tannins are of interest owing their anthelmintic properties in the ethnoveterinary field (Molan et al. 2002; Hoste et al. 2015).

The aim of this study was to analyze the *in vitro* anthelmintic activity of the extracts obtained from the aerial parts of *P. acre* against the nematode *H. contortus*, characterizing the phenolic profile of these extracts.

2. Materials and Methods

2.1. Biological material

2.1.1. Fecal samples and larval identification

Fecal samples were obtained from naturally infected sheep with gastrointestinal nematodes. The animals were housed at the Sheep and Goats Research Unit, located at the Canguiri Experimental Farm, in the city of Pinhais, south of Brazil. Feces were collected directly from from the rectum of the animals and maintained under 4º C. Samples were used to determine the fecal egg count per gram of feces (Gordon and Whitlock 1939). Fecal culture analysis was performed from a pool of feces. The data revealed that *H. contortus* (strain HcUFPR2015) was the most abundant (95%) nematode species, followed by *Trichostrongylus* spp. (4%).

2.1.2. Egg recovery

Eggs were cleaned from the fecal material according to an adaptation of a method proposed by Coles et al. (2006). In summary, 20 g of feces were macerated and homogenized in warm water 30 °C. Then, the samples were washed and filtered through 1 mm, 250, 150, 75, 56, 38 and 25 µm aperture sieves to recover the eggs. The eggs that were retained in the final sieve were collected and centrifuged for 2 min at 140 × g. The supernatant was removed, and saturated sucrose solution was added. In this condition, the eggs floated, allowing their collection. The eggs obtained from this process were transferred to a 12-mL Falcon tube and were, again washed with ultra-purified water at 30 ºC and re-suspended in ultra-purified water. The eggs were recovered and counted using a SM-LUX, Ernst Leitz GmbH microscope (Wetzlar, Germany).

2.1.3. Third stage larvae (L3) preparation

Feces were collected as mentioned above and maintained in a biological oxygen demand (BOD) incubator (Eletrolab, São Paulo, Brazil) at 27 ˚C for 10 days. L3 were collected and placed in a decantation flask. To remove the cuticle, fresh L3 were incubated with 0.3 % (v/v) sodium hypochlorite for 1 h at 25 °C. After that, L3 were washed three times with ultra-purified water and were quantified under an optical microscope and used in the assays.

2.2. Chemicals and reagents

Phenolic standards were obtained from Sigma-Aldrich (St. Louis, USA). Methanol-grade HPLC was obtained from JT Baker (Xalostoc, Mexico). Water was purified using an ultra-purifier MS 2000 model from Gehaka (São Paulo, Brazil). Folin-Ciocalteu reagent was obtained from Chromate (São Paulo, Brazil). Ivermectin (Ivomec 1% w/v) was obtained from Merial Limited (Duluth, USA) and albendazole sulfoxide (Ricoben 13.6% w/v) was obtained from Noxon Limited (Cravinho, Brazil). All other reagents were of analytical grade.

2.3. Plant extracts from the aerial parts of P. acre

The dry aerial parts of *P. acre* were obtained from Chá & Cia - Ervas Medicinais (São José dos Campos, Brazil) certificate analysis #43 from Quimer Comercial Ltda (São Paulo, Brazil) and the *National System of Genetic Resource Management* and *Associated Traditional Knowledge* - SISGEN #AE95785. The plant material was submitted to a hot aqueous extraction (6.7% m/v, 1.5 L) in a water-bath (70 ºC). The extraction was carried out for 1 h with constant mechanical stirring. The second extraction was hydroalcoholic maceration (70% v/v) performed at room temperature (25 ºC) for 10 days. The flask was kept protected from light with aluminum foil. Both extracts were vacuum filtered, concentrated in a rotary evaporator under reduced pressure at 40 °C, freeze-dried, stored at -4 °C in the dark, and designated as EHW-PA, ETOH-PA, respectively.

2.4. Chemical characterization of P. acre extracts

2.4.1. High-performance liquid chromatography with electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) analysis

The analysis of phenolic compounds was made by HPLC-ESI-MS/MS. EHW-PA, and ETOH-PA (30 mg each) were separately submitted to an Agilent Technologies Inc. (Cary, USA) solid phase extraction (SPE) using C18 cartridges. The resultant extracts *were* concentrated in a rotary evaporator under reduced pressure at 40 °C, freeze-dried, stored at - 4 °C in the dark. The extracts (1 mg/mL) were separately analyzed using HPLC-ESI-MS/MS, which was performed at LabEC/INCT-Catalise at Federal University of Santa Catarina. The analyses were conducted using an Agilent 1200 chromatography instrument (St. Clara, USA) with a Phenomenex Synergi Polar-RP 80Å column (150 mm × 2 mm ID, particle size: 4 µm) at 30 ºC. The eluent was formed by mixing solvents A (MeOH/H_2O in ratio of 95:5, v/v) and B (H_2O/formic acid 0.1%, v/v) as follows: 1st stage, 10 % solvent A and 90% B (isocratic mode) for 5 min. A 2nd stage, was made of a linear gradient of solvents A and B (from 10 to 90 % of A) for 2 min; 3rd stage, 90 % solvent A and 10 % B (isocratic mode) for 3 min; and 4th stage, linear gradient of solvents A and B (from 90 to 10% of A) for 7 min with a mobile phase flow rate of 250 µL/min. In all analyses, the injected volume was 5 µL. The liquid chromatography system

was coupled to a mass spectrometry system consisting of a hybrid triple quadrupole/linear ion trap Applied Biosystems/MDS mass spectrometer Qtrap 3200 (SCIEX, USA) with TurboIonSpray as the ionization source. The following source parameters were used: negative ionization mode; ion spray interface temperature of 400 °C; ion spray voltage of 4500 V; curtain gas at 10 psi; nebulizer gas at 45 psi; auxiliary gas at 45 psi; and collision gas, medium. The Analyst 1.5.1 software was used to record and process data. Pairs of ions were monitored by using the multiple-reaction monitoring (MRM) mode by comparing the signals of the selected standard analytes. For the identification and quantification of compounds, 46 standard phenolic compounds (listed by alphabetic order) (4-aminobenzoic acid, 4-hydroxymethylbenzoic acid, apigenin, aromadendrin, caffeic acid, carnosol, catechin, chlorogenic acid, chrysin, cinnamic acid, coniferaldehyde, ellagic acid, epicatechin, epigallocatechin, epigallocatechin gallate, eriodictyol, ferulic acid, fustin, galangin, gallic acid, hispudulin, isoquercetin, kaempferol, mandelic acid, methoxyphenylacetic acid, myricetrin, naringerin, naringin, *p*-anisic acid, *p*-coumaric acid, pinocembrin, protocatechuic acid, quercetin, resveratrol, rosmarinic acid, rutin, salicylic acid, scopoletin, sinapaldehyde, sinapic acid, syringaldehyde, syringic acid, taxifolin, umbelliferone, vanillic acid, and vanillin), obtained from Sigma-Aldrich (St. Louis, MO, USA), dissolved in methanol (0.02 to 6 mg/L), and analyzed under the same conditions as described above.

2.4.2. Qualitative phytochemical screening

The extracts were qualitatively analyzed, following the methodologies described by Matos (2009) for the presence of saponins (permanent lather formation with pH change), cyanogenic heterosides (picrate-impregnated *paper*, amino groups (ninhydrin tests), tannins and polyphenols (test for ferric chloride, gelatin and lead acetate), flavonoids (Shinoda reaction, reaction with aluminum chloride and Pew reaction), steroids and/or triterpenes (Lieberman-Burchard's test), coumarins (reaction with potassium hydroxide and ultraviolet light), anthraquinones, or anthraquinone heterosides (reaction with potassium hydroxide and with hydrogen peroxide).

2.4.3. Colorimetric methods

The total phenol contents were determined by the Folin-Ciocalteu reagent microassay that was adapted from Singleton and Rossi Jr. (1965) utilizing gallic acid as standard. The flavonoid content was measured by aluminum chloride complexation described by Woisky and Salatino (1998), and the results were compared with a rutin standard curve. All tested samples were read on a microplate reader (EPOCH model, BioTek, Winooski, USA) using a TPP Techno Plastic 96-well flat bottom microplate (Trasadingen, CH).

2.5. *In vitro* anthelmintic activity

P. acre extracts (EHW-PA, ETOH-PA), chlorogenic acid (phenolic acid) and rutin (glycosylated flavonoid) were used for the anthelmintic activity assays.

2.5.1. Egg hatch test (EHT)

The EHT was performed using the methodology described by Bizimenyera et al. (2006). The egg suspension was distributed in 24-well plates (n=100 eggs/well) together with EHW-PA or ETOH-PA at concentrations of 50, 100, 250, 500, 1000 µg/mL or with the commercial phenolic standards of rutin or chlorogenic acid at concentration of 1, 5, 10, 25, 100, 200 µg/mL in a total volume of 1 mL. Test samples for the negative control were made with ultra-purified H_2O and a positive control was treated with Albendazole sulfoxide at 60 µg/mL. The plates were maintained in a BOD at 25 °C for 24 h. The egg hatching was blocked by the addition of Lugol iodine solution and the readings were made on INV 100 Bel Engineering inverted microscope (Monza, Italy). The results were expressed as percentages of inhibition of egg hatch determined by the formula: [number of eggs / (number of eggs + number of L1)] × 100, considering (L1) = L1 larvae; (eggs) = eggs not hatched.

2.5.2. Larval migration test (LMT)

The LMT was determined using the method described by Demeler et al. (2010) with modifications. Aliquots of fresh L3 (without cuticle) were distributed into 24-well plates (n=200 larvae/well). The extracts EHW-PA or ETOH-PA were added to a final concentration of 50, 100, 250 and 500 µg/mL. The plates were incubated in a BOD at 25 ºC for 6 h. Tests using a negative control with ultra-purified water and a positive control with ivermectin at 100 µg/mL were performed in parallel. After the first incubation, the material was transferred to plates with an apparatus containing a 22 µm nylon membrane and incubated at the same conditions, but with a source of light (150 Watts) to stimulate L3 migration. After 18 h, the apparatus was removed and the L3 that migrated were counted using an inverted microscope (10x). The L3 that did not migrate were also counted. The average number of L3 that migrated for each tested concentration was transformed into the percentage of migration.

2.6. Statistical Analysis

Results from the chemical analysis were calculated as mean ± standard deviation (SD) (n=3) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Prism 5. The differences were considered statistically significant at $P \leq 0.05$. The results of the EHT and LMT tests (n=3) were expressed as average of inhibition and analyzed by ANOVA and Tukey's multiple comparison test. The values were considered statistically significant if the level of significance was $P \leq 0.05$. The concentration of inhibition (IC) of the extract were

expressed as IC_{20}, IC_{50} and IC_{90}, for 20, 50 and 90% of egg hatch inhibition, respectively, and were calculated using linear equations ($R^2 \geq 0.98$) obtained by linearizing the activity curves using GraphPad Prism 5.

3. Results and Discussion

3.1. Chemical characterization of the extracts obtained from aerial parts of *P. acre*

EHW-PA and ETOH-PA were evaluated by HPLC-ESI-MS/MS using the MRM (Multiple-Reaction Monitoring) mode, which shows the signal of the selected analytes in a list according to the number of standards used in the method. Out of the 46 standards of phenolic tested 13 were identified in the extracts (Table 1).

Standard phenolic compounds*		EHW-PA		ETOH-PA	
	Pubchem CID codes	Qualitative	*Quantifying*	Qualitative	*Quantifying*
Epicatechin	72276	✓	0.792 ± 0.043	-	-
Epicatechin gallate	107905	-	-	✓	0.486 ± 0.211
Eriodictyol	440735	✓	< LQ	✓	0.053 ± 0.008
Ferulic acid	445858	✓	0.152 ± 0.070	✓	-
Galangin	5281616	-	-	✓	< LQ
Isoquercitrin	5280804	✓	0.941 ± 0.419	✓	2.183 ± 0.647
Myricitrin	5281673	✓	0.142 ± 0.050	✓	0.162 ± 0.019
p-Coumaric acid	637542	✓	< LQ	✓	< LQ
Quercetin	5280343	✓	2.160 ± 1.566	✓	1.948 ± 0.217
Rosmarinic acid	5281792	✓	0.055 ± 0.008	✓	0.024 ± 0.008
Rutin	5280805	✓	0.314 ± 0.114	✓	0.583 ± 0.179
Sinapic Acid	637775	✓	0.039 ± 0.022	-	-
Taxifolin	439533	✓	0.044 ± 0.011	✓	0.21 0.012

** Listed by alphabetic order. Results expressed as mg per g of dry extract. ✓ = detected; LQ = below the quantification limit.*
***Table 1** – Qualitative and quantitative analysis of polyphenol compounds present in the extracts EHW-PA and ETOH-PA, obtained from Polygonum acre.*

The structure of these compounds is represented in the Figure 1. Phenolic acids, including ferulic acid, *p*-coumaric acid, and rosmarinic acid were present in the two extracts, while synapic acid was detected only in EHW-PA. Ferulic acid was the major component in the EHW-PA (0.152 ± 0.070 mg/g dry extract), but it was not quantifiable in the ETOH-PA. In this extract, rosmarinic acid was predominant, though at a low concentration (0.024 ± 0.008 mg/g dry extract) (Table 1). We found forms of aglycone (quercetin, taxifolin, eriodictyol), monoglycoside of myricetin (myricitrin), monoglycoside of quercetin (isoquercitrin), and diglycoside of quercetin (rutin) among the flavonoids in both extracts (Table 1).

Four flavonoids: isoquercitrin, quercetin, rutin, and taxifolin were found in the aerial parts of *P. acre*. The hydromethanolic extracts of other *Polygonum* species are reported to exhibit different profiles of phenolic acids. In *P. hydropiper*, *P. aviculare*, *P. mite*, *P. convolvulus*, *P. lapathifolium* spp *tomentosa,* and *P. amphibium* species, the major compound similar with this study is *p*-coumaric acid. For *P. bistoria,* it is ferulic acid; for *P. lapa,* it is protocatechuic acid; and for *P. persicaria*, it is sinapic acid (Smolarz 2000). Flavonoids from *P. lapathifolium* ssp. *tomentosum* were identified, including: taxifolin, quercetin, kaempferol, astragalin, isoquercitrin, hyperin, quercetrin, rutin, and acetylated glycosides and glucuronides forms of quercetin (Smolarz 2002).

Additionally, epicatechin (flavan-3-ol-type flavonoid) was identified in EHW-PA only, while epicatechin gallate (flavan-3-ol-type flavonoid) and galangin (aglycone flavonoid) and were found only in ETOH-PA. Quercetin was the major flavonoid in EHW-PA (2.160 ± 1.566 mg/g dry extract), followed by isoquercitrin (0.941 ± 0.419 mg/g dry extract) and rutin (0.314 ± 0.114 mg/g dry extract). This pattern was different from that found in the ETOH-PA, where isoquercitrin was the prevailing flavonoid (2.183 ± 0.647 mg/g dry extract), followed by quercetin (1.948 ± 0.217 mg/g dry extract) and rutin (0.583 ± 0.179 mg/g dry extract) (Table 1).

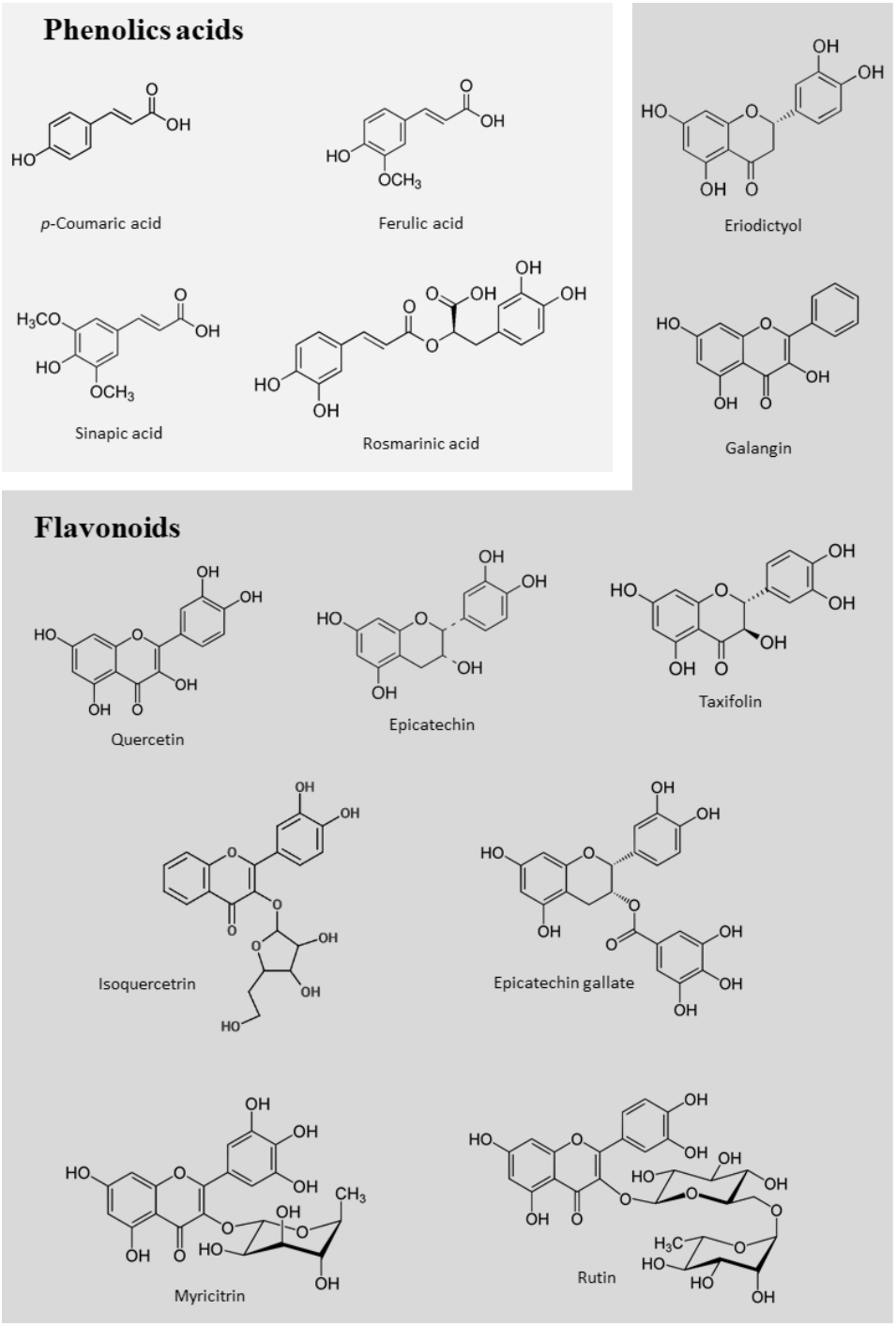

Figure 1 *– Structures of phenolic (Phenolic acids and Flavonoids) compounds identified by HPLC-ESI-MS/MS.*

The phytochemical screening using a qualitative analysis of the extracts of *P. acre* showed the presence of saponins, amino groups, tannins, flavonoids, coumarins and quinones in both EHW-PA and ETOH-PA (Table 2). The presence of cyanogenic heterosides was not verified in both extracts and steroids and/or triterpenes was detected only in the ETOH-PA. The presence of this class of terpenoids is known in the genus *Polygonum*, because the polygodial, a sesquiterpene dialdehyde which has been searched due to its antibacterial and antifungal activity (Castelli et al. 2005, Just et al. 2015). The presence of flavonoids was expected since these compounds are considered as the most common components found in members of the genus *Polygonum* (Datta et al. 2002).

	Phenols[1]	*Flavonoids*[2]
EHW-PA	208.43 ± 11.92 [a]	30.83 ± 2.46 [a]
ETOH-PA	294.77 ± 12.48 [b]	69.25 ± 2.45 [b]

[1] Determined according to the method of Singleton and Rossi Jr (1965); expressed as mg equivalent of gallic acid per gram of dry extract. [2] Determined according to the method of Woiski and Salatino (1998); expressed as mg equivalent of rutin. Different letters in each column represent significant differences by the Tukey test ($p \leq 0.05$). EHW-PA - extract of aerial parts of P. acre at 6.7% (w/v) obtained by aqueous extraction at 70 ºC, for 1 h; ETOH-PA - extract of aerial parts of P. acre at 12% (w/v) obtained by hydro-alcoholic maceration (70% v/v) at 25 ºC for 10 days.

Table 3 – *Total phenol and flavonoid contents of the extracts obtained from the aerial parts of Polygonum acre.*

Class compounds	EHW-PA	ETOH-PA
Saponins	+	+
Cyanogenic heterosides	-	-
Amino groups	+	+
Tannins	+	+
Flavonoids	+	+
Steroids and/or triterpenes	-	+
Coumarins	+	+
Quinones	+	+

Quinone = anthraquinones or anthraquinone heterosides; (+) Positive result; (-) Negative result.

Table 2. *Qualitative chemical characterization of the extracts obtained from the aerial parts of Polygonum acre.*

The quantitative analysis of total phenol and flavonoid contents of EHW-PA and ETOH-PA is described in Table 3. The total phenol content was statistically higher ($P = 0.0036$) in ETOH-PA (294.77 ± 2.48 mg of gallic acid equivalent/g dry extract) than in EHW-PA (208.43 ± 11.92 mg of gallic acid equivalent/g dry extract). The content of flavonoids was statistically different ($P = 0.0001$) between the extracts (EHW-PA = 30.83 ± 2.46 mg equivalent of rutin/g dry extract and ETOH-PA = 69.25 ± 2.45 mg equivalent of rutin/g dry extract).

3.2. *In vitro* egg and larval activity

EHW-PA and ETOH-PA showed a concentration-dependent inhibitory effect (Figure 2). At the highest concentration (1000 µg/mL) the EHW-PA inhibited 91.29% of *egg hatching*, while the ethanolic fraction, in the same concentration, inhibited about 62.0%. The negative control group used distilled water showing no effect (7.4%). The positive control with albendazole sulfoxide (60 µg/mL) presented 97% efficacy for egg hatch (Figure 2). The concentrations of EHW-PA that could inhibit the hatching of *H. contortus* eggs were 103.25, 442.24 and 894.21 µg/mL, considering IC_{20}, IC_{50} and IC_{90}, respectively (Figure 2A and B). ETOH-PA presented an IC_{20}, IC_{50} and IC_{90} of 207.73, 703.60 and 1364.76 µg/mL, respectively (Figure 2C and D). EHW-PA had an IC_{50} of 442.24 µg/mL for inhibiting egg hatch, which was statistically lower ($P \leq 0.05$) than presented by ETOH-PA (IC_{50} of 703.60 µg/mL).

For comparison purposes of EHW-PA and ETOH-PA, chlorogenic acid (phenolic acid) and rutin (glycosylated flavonoid) were selected to be tested in the EHT assay (Figure 3). The concentrations of chlorogenic acid that could inhibit the egg hatch of *H. contortus* were 1.81, 8.66, and 69.70 µg/mL corresponding to IC_{20}, IC_{50}, and IC_{90}, respectively. In contrast, rutin did not show any effect on the EHT. Despite the difference among the IC_{50} values, the results obtained in our study presented similar results as described by the study with the methanolic extract of the aerial parts of *T. filifolia* against the eggs of *H. contortus* (Díaz et al. 2017). The chemical analysis of *T. filifolia* extract demonstrated the presence of quercetagitrin – a glycosylated flavonoid, methyl clorogenate, chlorogenic acid and an analog of the unidentified chlorogenic acid. Among the tested compounds, only chlorogenic acid presented significant activity, showing 100% egg hatch inhibition at 500 µg/mL after 48 h of incubation with an IC_{50} of 248 µg/mL. Like quercetagitrin, another glycosylated flavonoid, rutin, had no considerable activity over egg hatching (Díaz et al. 2017).

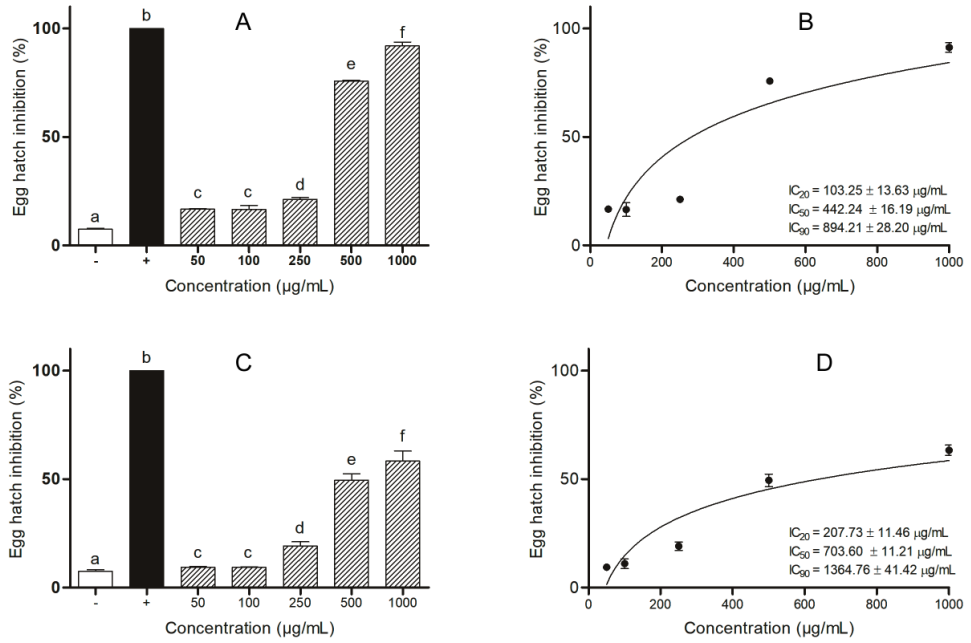

Figure 2 *– Nematode egg hatch inhibition* and IC determination of the aqueous EHW-PA (A, B) and hydroalcoholic ETOH-PA (C, D) extracts from Polygonum acre.*
** Different letters in each column represent significant difference compared to negative control. (-) negative control: ultra-purified H_2O, (+) positive control: albendazole sulfoxide (60 µg/mL).*

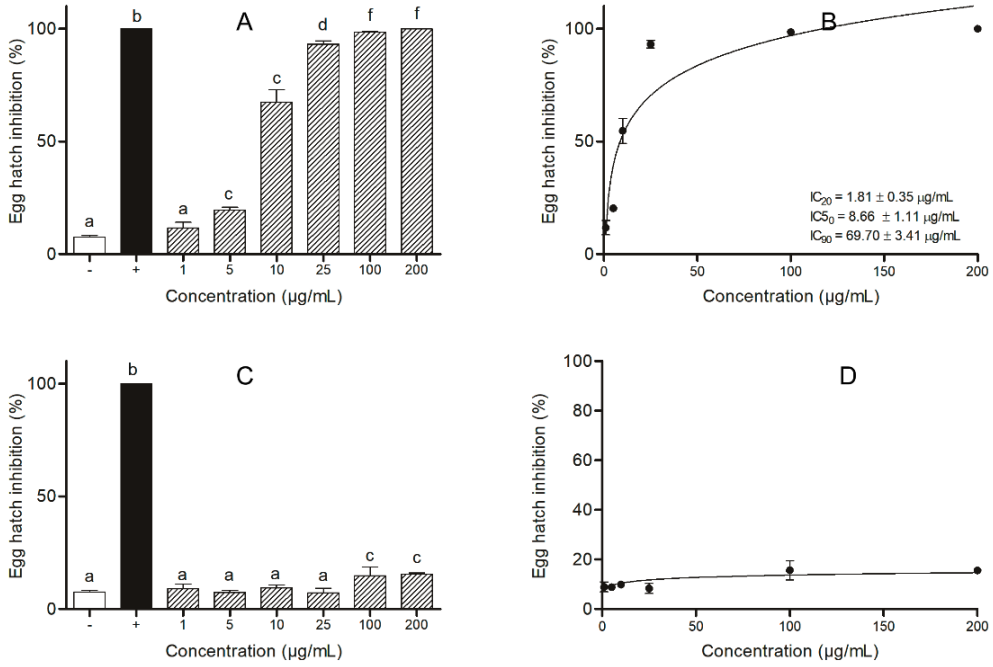

Figure 3 *– Inhibition* of the nematode egg hatch and the concentration of inhibition (IC) determination of chlorogenic acid (A, B) and rutin (C, D).*
** Different letters in each column represent significant difference compared with the negative control.*
(-) negative control with ultra-purified H_2O; (+) positive control with albendazole sulfoxide (60 µg/mL).

EHW-PA and ETOH-PA showed no significant effects against *H. contortus* larval migration (Figure 4). In contrast, 81.6% of larvae treated with 100 µg/mL of ivermectin (positive control) did not migrate through the apparatus. In the case of the LMT, despite of the phenolic content, including phenolic acids, flavonoids and tannins, EHW-PA and ETOH-PA did not affect L3 motility at the conditions tested. Additional studies using different models of anthelmintic activity, such as the larval development could be used to explore the bioactivity of *P. acre* extracts.

3.3. Relationship between of the anthelmintic activity and the phenolic content of *P. acre* extracts

The phenolic content differences between EHW-PA and ETOH-PA influenced their observed ovicidal activity. It can be speculated that the difference found on the content of phenolic acids and flavonoids contributed to a superior the ovicidal activity observed for EHW-PA. Polyphenol compounds such as phenolic acids, flavonoids, and condensed tannins, tested in isolated or as mixture form, have been associated with anthelmintic activity using different experimental models (Molan et al. 2000, Molan et al. 2002, Lasisi and Kareem 2011, Akkari et al. 2014, Klongsiriwet et al. 2015, Díaz et al. 2017, Mancilla-Montelongo et al. 2019, Castañeda-Ramírez et al. 2019, Jayawardene et al., 2021).

Considering the polyphenol-rich plants, it has been demonstrated that the anthelmintic activity of the tested extracts might be the result of the sum of the bioactivity of the phenolic components, which can act in a synergistic or antagonistic effect (Klongsiriwet et al. 2015, Mancilla-Montelongo et al., 2019). Klongsiriwet et al. (2015) demonstrated the synergistic effects between condensed tannins and quercetin and luteolin in the inhibition of *H. contortus* L3 larval development. In another study with phenolic acids, it has been demonstrated that all mixtures of cinnamic acid-analogue compounds were less active than ferulic and chlorogenic acids, which were reciprocally antagonistic, while caffeic acid acted as a synergistic agent (Mancilla-Montelongo et al., 2019). The results obtained in this study corroborate with the literature, especially in relation to our ovicidal data.

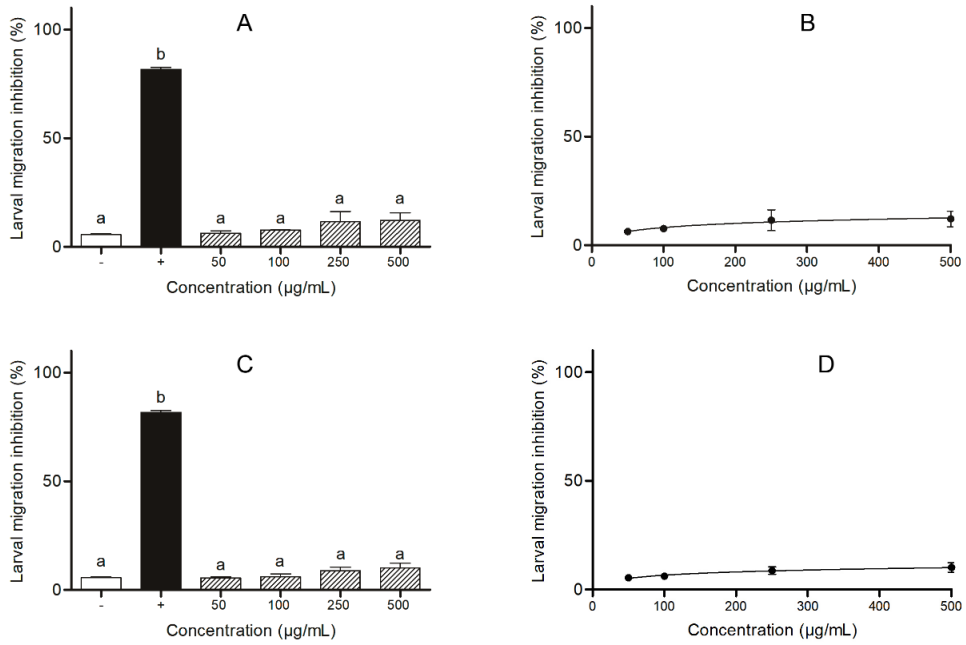

Figure 4 *– Inhibition* of nematode larval migration using the aqueous EHW-PA (A, B) and hydroalcoholic ETOH-PA (C, D) extracts from Polygonum acre.*
** Different letters in each column represent significant difference compared with negative control. (-) negative control - ultra-purified H_2O; (+) positive control - ivermectin (100 µg/mL).*

4. Conclusion

Considering the chemical analysis and the EHT results using the EHW-PA and ETOH-PA; it can be suggested that despite the ETOH-PA presenting higher values of phenolics and flavonoids, the phenolic profile of EHW-PA was significantly higher for egg hatch inhibition. We suggest that the profile of phenolic acids (ferulic acid, rosmarinic acid and sinapic acid) and aglycone flavonoid (quercetin) were relevant for influencing the observed egg hatch activity. The present data reinforces the ethnopharmacological relevance of the genus *Polygonum*. The aerial parts of *P. acre* can be explored for the development of a biofriendly plant-based anthelmintic drug for the veterinary field, helping to keep good welfare conditions to billions of animals worldwide.

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