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# Anti-inflammatory, Antioedema and Gastroprotective Activities of *Aristotelia chilensis* Extracts, Part 2[**].

[Anti-inflammatory Activity of *Aristotelia chilensis* Mol. (Stuntz) (Elaeocarpaceae).]

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## Abstract

**Context:** "Chilean black-berry" *Aristotelia chilensis* is a wild fruit that grows in Southern Chile. This fruit possesses a strong antioxidant activity and is commonly used in foods and beverages in Chile. **Objective:** The gastroprotective and anti-inflammatory activities of the extracts, fractions and subfractions as carrageenan- induced paw oedema in rats are reported here for the first time. **Materials and methods:** Extracts, fractions, subfractions and several compounds were used for measuring the effects in carrageenan-induced paw oedema inflammation of rat model and the gastroprotective activity was analyzed. **Results:** The results showed that extract **B**, fraction **F-4**, and ovatifolin, quercetin, myricetin, luteolin and diosmetin used as pattern compounds were the most active samples together with those subfractions rich in aglycone and phenolic compounds. Thus, **$SF_{11}$-$SF_{15}$**, **$SF_{16}$-$SF_{20}$**, and **$SF_{21}$-$SF_{25}$** showed the best subfractions inhibitors in similar form to indomethacin, a known selective COX inhibitor. Results demonstrated that these samples strongly inhibited the carrageenan-induced inflammation in paw of the rat oedema model. **Discussion and conclusion:** These findings demonstrate that the fruits and their constituents of *A. chilensis* are anti-inflammatory and gastroprotective and thus have great potential as nutraceuticals.

**Keywords:** *Aristotelia chilensis*, anti-inflammatory, gastroprotective.

## Resumen

**Contexto: "**Chilean Black-berry" *Aristotelia chilensis* es un fruto silvestre que crece en el sur de Chile. Este fruto posee una fuerte actividad antioxidante y comunmente es usado en alimentos y bebidas en Chile. **Objetivo:** Se investigo la actividad anti-inflamatoria y gastroprotectora de los extractos, fracciones y subfracciones de este fruto y son informados aqui por primera vez. **Materiales y métodos:** Los extractos, fracciones y subfracciones fueron analizados por su efecto sobre la inflamación en pata de rata a través de la inducción con carragenina en dosis sencillas. Ademas se investigo la actividad gastroprotectora sobre la mucosa del estomago de rata. **Resultados:** Los resultados muestran que el extracto **B**, la fraccion **F-3, F-4**, y ovatifolina, quercetina, myricetina, luteolina y diosmetina, que se usaron como muestras patrones, fueron las mas activas junto con aquellas subfracciones ricas en compuestos fenolicos. Asi, **$SF_{11}$-$SF_{15}$**, **$SF_{16}$-$SF_{20}$**, y **$SF_{21}$-$SF_{25}$** mostraron ser las mejores subfracciones inhibitorias en una forma similar a indometacina un conocido inhibidor selectivo de COX. Los resultados demuestran que estas muestras inhiben fuertemente la inflamacion inducida en el modelo del edema en pata de rata. **Discusión y conclusión:** Estos hallazgos demuestran que los frutos y sus constituyentes de *A. chilensis* poseen una excelente actividad anti-inflamatoria y gastroprotectora, y asi tienen un gran potencial como una fuente de productos naturales saludables. Adicionalmente, estos hallazgos muestran que los flavonoides, acidos fenolicos y antocianinas presentes en este fruto podrian ser los responsables de la actividad nutraceutica.

**Palabras Clave:** *Aristotelia chilensis*, anti-inflammatory activity, gastroprotective.

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## INTRODUCTION

The use of traditional medicine is widespread and plants still present a large source of novel active biological compounds with different activities, including anti-inflammatory, anti-cancer, anti-viral, anti-bacterial and cardioprotective activities (Seigler 1998; Schinella et al., 2002; Yan et al., 2002; Bremmer and Heinrich, 2005).

Berries from South America constitute a rich dietary source of phenolic antioxidant and bioactive properties (Cespedes et al., 2010a; Schreckinger et al., 2010a). Chilean wild black-berry *Aristotelia chilensis* (Mol) Stuntz (Elaeocarpaceae), an edible black-colored fruit, which reach its ripeness between December to March, has a popular and very high consume during these months in Central and South Chile and western of Argentina. Previously, we reported the alkaloid composition of the leaves of *A. chilensis* (Cespedes et al., 1990; Cespedes et al., 1993; Cespedes, 1995; Silva et al., 1997). The botanical characteristics were reported previously (Cespedes et al., 1995; 2008; 2010a).

This plant has enjoyed popularity as an ethno-medicine for many years, used particularly as an anti-inflammatory agent, kidneys pains, stomach ulcers; diverse digestive ailments (tumors and ulcers), fever and cicatrisation injuries (Bhakuni et al., 1976), and the berries have traditionally been consumed as treatment for diarrhea and dysentery, and the Araucanian people prepare a liquor with an ethanolic macerated solution that is used in religious ritual know as "machitun" or "nguillatun" and in daily beverages (Muñoz-Pizarro, 1966).

Up-to-date some studies report that extracts from fruits of *A. chilensis* have good antioxidant activity (Pool-Zobel et al., 1999; Miranda-Rottmann et al., 2002), cardioprotective activity (Cespedes et al., 2008). Other studies report composition of extract constituents (Escribano-Bailon et al., 2006; Cespedes et al., 2010a), and recently it was reported the inhibitory activity against aldose reductase, adipogenesis and the inhibition of expression of LPS-induced iNOS/NO and COX-2/PGE pathways in RAW 264.7 macrophages by an EtOH extract rich in anthocyanins of this fruit (Kraft et al., 2007; Grace et al., 2009; Schreckinger et al., 2010a). Subsequently, we have some recent reported about the effects of EtOH extract from ripe fruits of *A. chilensis* on isquemic/reperfusion system, several antioxidant activities of that extract and its relationship between total phenolic levels and the cardioprotective effect (Cespedes et al., 2008; 2010a), the presence of 3-hydroxyindole in this fruit (Cespedes et al., 2009), and the anti-inflammatory activity against TPA (Cespedes et al., 2010b).

In the continuation of our general screening program of anti-inflammatory evaluation of *A. chilensis* (Cespedes et al., 2010b), a re-examination of the extracts of fruits of *A. chilensis* (Elaeocarpaceae) has been continued. Thus, in the present work, we investigated the anti-inflammatory activity in the carrageenan-induced paw oedema inflammation in mice model of the EtOH, acetone extracts, fractions, and subfractions, the occurrence of phenolic compounds (Cespedes et al., 2010a; 2009), and its correlationship between its phytochemical contents and the gastroprotective effect of these extracts, fractions and sub-fractions from ripe fruits of *A. chilensis*.

The aim of this work was to evaluate the gastroprotective effect in stomach mucose of rats and anti-inflammatory activity of EtOH, acetone, ethyl acetate and MeOH/$H_2O$ extracts from ripe fruits and subfractions from $\mathbf{SF}_4$ to $\mathbf{SF}_{37}$ isolated from **F-3** and **F-4** fractions (Cespedes et al., 2010b) on the carrageenan-induced paw oedema inflammation in rat model (Morikawa et al., 2003; Petrovic et al., 2008; Tadić et al., 2008), as an indirect measurement of release of NO and COX 1 and 2.

Continuously, we are working in a more complete metabolomic profile of the fruits and leaves of this plant and in the dissection of biological activities of leaves.

## MATERIAL AND METHODS

### Plant material

Detail about fruit collection can be found in Cespedes et al., 2010b. The collected fruits were air-dried and prepared for extraction.

### Chemicals and solvents

All reagents used were either analytical grade or chromatographic grade, carrageenan, quercetin, Folin-Ciocalteu reagent, quercetin (3,3',4',5,7-pentahydroxyflavone), myricetin (3,3',4',5,5',7-hexahydroxyflavone), were purchased from Sigma-Aldrich Química, S.A. de C.V., Toluca, Mexico, or Sigma, St. Louis, MO. Glycosides of anthocyanidins (cyanidin 3,5-diglucoside, delphinidin 3,5-

diglucoside, cyanidin, delphinidin) were purchased from Fluka-Sigma-Aldrich Química, Toluca, Mexico), samples of luteolin, diosmetin and proanthocyanidins were a gift from Prof. Dr. David Seigler, University of Illinois at Urbana-Champaign.

Methanol, $CH_2Cl_2$, $CHCl_3$, silica gel $GF_{254}$ analytical chromatoplates, Sephadex LH-20, silica gel grade 60, (70-230, 60A°) for column chromatography, n-hexane, and ethyl acetate were purchased from Merck-Mexico, S.A., Mexico. Indomethacin, quercetin, myricetin, luteolin, diosmetin and ovatifolin were used as pattern samples.

**Apparatus**

A UV Spectronic model Genesys 5 spectrophotometer was used for biological and spectrophotometric analyses. Fluorimetric measurements were determined with TURNER Barnstead-Thermolyne, model Quantech S5 Fluorometer, with 420, 440, 470, 550, and 650 Turner filters. HPLC Hewlett-Packard, Series 1050, with diode array detector, and UV detector at 254, 280, 365 and 520 nm, column YMC C18-Pack ODS-AM-303, AM12S05-2546 WT, 250 x 4.6 mm, ID S-5um, 12nm; mobil phase water/methanol/acetonitrile (50:35:15), isocratic, pressure 212 bar; prepared in 300 µL of each sample in amber vials and injected 20 µL of each sample.

**Obtention of extracts, fractions, subfractions and sample preparation**

All extracts, fractions and subfractions were obtained as described in Cespedes et al., 2010a. The composition of each subfraction was reported in Cespedes et al. 2010b.

**Test animals**

All experiments in this study were performed in accordance with guidelines for animal research from the National Institutes of Health and were approved by the local committee on Animal Research (NIH, 1985; NOM, 1999). Adult male Wistar rats, (at 4 – 5 weeks old weighing 200-250 g) were used in both the carrageenan-induced rat paw oedema and the indomethacin-induced gastric mucosa damage tests. Prior to the experiments the rats were fed with standard food and water *ad libitum*. Experimental groups consisted of 5 animals each.

**Anti-inflammatory activity**

The assay of carrageenan-induced paw oedema in rats was based on the described method (Tadić et al., 2008; Petrović et al., 2008). Rats were divided randomly into five groups and were anaesthetized with Imalgen®. Briefly, the paw oedema of the rat was induced by injecting 50 mL of 1% (w/v) carrageenan in saline. The treatment groups received intraperitoneally (i.p.) 10, 20, 40, 100, and 200 mg/Kg of extract or 10, 25, 50, and 100 mg/Kg compounds samples or 10mg/Kg of indomethacin (a standard anti-inflammatory drug) 1 h before carrageenan injection. Animals in the control group received only the vehicle (saline) in a dose of 1 mL/Kg p.o. One hour after the oral administration of the extracts, compounds or indomethacin; carrageenan-saline solution (0.5%, w/v) and saline were injected in a volume of 0.1 mL into the plantar surface of the right and left hind paw, respectively. The left served as the control (non-inflamed) paw. The animals were killed 3 h after the carrageenan and saline injection and paws were cut off for weighing. The difference in weight between the right and left paw, active drug-treated versus vehicle-treated (control) rats, served as an indicator of the anti-inflammatory activity of drugs tested (the extracts, fraction, compounds and indomethacin). The paw volume of each rat was measured with a plethysmometer 7150 (UGO Basil, Italy) 3 h after injection of the irritant. The anti-inflammatory effect was calculated using the equation:

$$AE\ (\%) = K - e / K \times 100$$

Where AE=anti-inflammatory effect, $K$ is difference in the paw weight in the control group, and $e$ is difference in the paw weight in the treatment group.

**Gastroprotective activity**

In this study the method reported by Petrovic et al., 2008 was used. Briefly, the extracts, fractions and compounds, dissolved in EtOH/water (10:90), was administered p.o. at dose of 200 mg/Kg, immediately after indomethacin (8 mg/Kg p.o.). The animals were killed after 4 h and their stomachs were removed and opened along the greater curvature. The lesions were examined under an illuminated magnifier (3x). The intensity of gastric lesions was assessed according to a modified scoring system of Adami et al., 1964: 0, no lesions; 0.5, slight hyperaemia or ≤5 petechiae; 1, ≤5 erosions ≤5mm in length; 1.5, ≤5 erosions ≤5 mm

in length and many petechiae; 2, 6-10 erosion ≤5 mm in length; 2.5, 1-5 erosions <5 mm in length; 3, >5-10 erosions >5 mm in length; 3.5, >10 erosions >5 mm in length; 4, 1-3 erosions ≤5 mm in lemgth and 0.5-1 mm in width; 4.5, 4-5 erosions ≤5 mm in length and 0.5-1 mm in width; 5, 1-3 erosions >5 mm in length and 0.5-1 mm in width; 6, 4- or 5-grade 5 lesions; 7, ≥6-grade 5 lesions; 8, complete lesion of the mucosa with hemorrhage (Adami et al., 1964).

### Statistical analysis

Data shown in table 1 is the mean results obtained with means of five animals and are presented as mean ± standard errors of the mean (SEM). Data were subjected to analysis of variance (ANOVA) with significant differences between means identified by GLM Procedures. The results are given in the text as probability values, with $p < 0.05$ adopted as the criterion of significance, differences between treatments means were established with a Dunnett's test.

The $EC_{50}$ values for each activity were calculated by Probit analysis on the basis of the percentage of inhibition obtained at each concentration of the samples. $EC_{50}$ is the concentration producing 50% inhibition. Complete statistical analysis was performed by means of the MicroCal Origin 8.0 statistical and graphs PC program.

## RESULTS AND DISCUSSION

### Anti-inflammatory activity.

The results of anti-inflammatory activities of extracts **A, B, C, D,** fractions **F-1** to **F-4**, and subfractions **$SF_4$** to **$SF_{37}$** are outlined in Table 1. These findings show that the carrageenan-induced rat paw oedema inflammation method was well inhibited mainly by extracts **A, B, F-3, F-4**, **$SF_{11}$-$SF_{15}$**, **$SF_{16}$-$SF_{20}$**, **$SF_{21}$-$SF_{25}$**, **$SF_{26}$–$SF_{30}$**, **$SF_{31}$-$SF_{37}$** and in a dose-dependent manner. The obtained anti-inflammatory effect had an $OI_{50}$ of 8.5, 6.1, 1.8, 2.3, 1.15, 1.98, 3.2, 6.7, and 9.2 mg/Kg p.o., respectively. Additionally, quercetin, ovatifolin, diosmetin, luteolin, myricetin and indomethacin showed $OI_{50}$ 1.2, 0.67, 1.89, 0.77, 1.47 and 1.85 mg/Kg p.o., respectively. The bioassay was carried out between 0.5 and 200.0 mg/Kg p.o with all samples being extracts A, **B**, C, D, fraction F-3, **F-4**, subfractions **$SF_{11}$-$SF_{15}$**, **$SF_{16}$-$SF_{20}$**, **$SF_{21}$-$SF_{25}$**, **$SF_{26}$–$SF_{30}$**, and **$SF_{31}$-$SF_{37}$**, quercetin, ovatifolin, diosmetin, luteolin and myricetin the most active samples, therefore with these substances a curve of

**Table 1**. Amounts of extracts, fractions and several compounds (mg/Kg p.o.) of *A. chilensis* and indomethacin needed for inhibitory effect on the carrageenan-induced rat paw oedema inflammation in rat model[a]. OI=Oedema Inhibition. AE=Antiinflammatory Effect (%).

| Samples | Dose | AE(%)[c] | $OI_{50}$ [b] |
|---|---|---|---|
| Indomethacin | 2.0 | 55.8 ± 5.2a | 1.85 |
| | 4.0 | 60.0 ± 7.3b | |
| | 8.0 | 75.5 ± 15.5c | |
| **A**[e] | 10.0 | 55.1 ± 3.6a | 8.5 |
| | 20.0 | 80.0 ± 12.2c | |
| **B**[e] | 10.0 | 67.9 ± 8.8b | 6.1 |
| | 20.0 | 84.5 ± 14.5c | |
| **C**[e] | 40.0 | 45.9 ± 4.8a | 57.9 |
| | 100.0 | 70.0 ± 12.4d | |
| **D**[e] | 100.0 | 45.0 ± 5.5a | 155.0 |
| | 200.0 | 65.0 ± 7.7b | |
| **F-1** | 200.0 | n. d. | >200 [d] |
| **F-2** | 200.0 | n. d. | >200 [d] |
| **F-3** | 2.0 | 55.9 ± 4.9a | 1.8 |
| | 4.0 | 85.2 ± 13.9c | |
| **F-4** | 2.0 | 49.8 ± 8.5a | 2.3 |
| | 4.0 | 67.9 ± 10.2c | |
| **$SF_4$-$SF_6$** | 200.0 | n. d. | >200 [d] |
| **$SF_7$** | 200.0 | n. d. | >200 [d] |
| **$SF_8$-$SF_{10}$** | 200.0 | n. d. | >200 [d] |
| **$SF_{11}$-$SF_{15}$** | 1.0 | 45.0 ± 2.5a | 1.15 |
| | 2.0[c] | 59.9 ± 7.3b | |
| | 4.0 | 89.0 ± 9.8e | |
| **$SF_{16}$-$SF_{20}$** | 1.0 | 40.9 ± 6.8f | 1.98 |
| | 2.0 | 52.8 ± 4.2a | |
| | 4.0 | 71.2 ± 9.8d | |
| **$SF_{21}$-$SF_{25}$** | 2.0 | 39.8 ± 2.2f | 3.2 |
| | 4.0 | 70.1 ± 10.1b | |
| **$SF_{26}$-$SF_{30}$** | 4.0 | 45.5 ± 5.0a | 6.7 |
| | 8.0 | 55.7 ± 6.7b | |
| **$SF_{31}$-$SF_{37}$** | 8.0 | 49.8 ± 5.9a | 9.2 |
| **Quercetin** | 1.0 | 49.8 ± 5.8a | 1.2 |
| | 2.0 | 59.9 ± 5.9b | |
| **Ovatifolin** | 0.5 | 49.9 ± 4.8a | 0.67 |
| | 1.0 | 67.1 ± 9.9d | |
| | 2.0 | 81.0 ± 15.2c | |
| **Diosmetin** | 0.5 | 37.8 ± 1.8f | 1.89 |
| | 1.0 | 40.7 ± 2.2f | |
| | 2.0 | 55.5 ± 7.1b | |
| **Luteolin** | 0.5 | 44.4 ± 2.8a | 0.77 |
| | 1.0 | 58.8 ± 8.8b | |
| | 2.0 | 58.9 ± 7.5b | |
| **Myricetin** | 0.5 | 42.4 ± 4.2f | 1.47 |
| | 1.0 | 49.0 ± 5.8a | |
| | 2.0 | 58.9 ± 8.5b | |

[a]Means of five animals in independent experiments. Data shown here are with the largest effects and expressed as % of the mean ± SD of weight of plant surface. All data was analyzed with t-student test. [b]Each value corresponds to the concentration that inhibits 50% of oedema developed during bioassay stage (mg/kg). [c]$P < 0.05$, values followed by the same letter are not significantly different. [d]$OI_{50}$ was not determined. [e]**A**: Methanol/water (6:4) extract. **B**: Acetone extract. **C**: Ethyl acetate extract. **D**: MeOH/$H_2O$ Residue. (Cespedes *et al*., 2010a).

dose-response was made, obtaining the $OI_{50}$ showed in Table 1. All samples used in this study showed a dose-dependent anti-inflammatory activity.

These effects were compared with those obtained by the commercially available anti-inflammatory drug indomethacin and ovatifolin (Cespedes et al., 2000), together with luteolin and diosmetin as pattern natural compounds (Dominguez et al., 2010), and quercetin and myricetin the major flavonoids that occur in this fruit. All of these compounds assayed inhibited the inflammation. Interestingly, fractions **F-3, F-4**, and subfractions **$SF_{11}$-$SF_{15}$**, **$SF_{16}$-$SF_{20}$** were as active as indomethacin, a known selective cyclo-oxygenase (COX) inhibitor (Table 1).

It is important to mention that **$SF_{11}$-$SF_{15}$**, **$SF_{16}$-$SF_{20}$** showed very good anti-inflammatory activities. Subsequently, this action could be attributed to a synergic effect proportionate by the phenolic rich composition observed in these sub-fractions.

On the other hand, a decrease in the anti-inflammatory activity was observed with **F-1**, **F-2**, **$SF_7$**, **$SF_8$-$SF_{10}$**, **$SF_{26}$-$SF_{30}$**, and **$SF_{31}$-$SF_{37}$**, which have a high amount of sugared components.

Carrageenan-induced rat paw oedema has been used widely for the discovery and evaluation of anti-inflammatory drugs (Morikawa et al., 2003; Tapas et al., 2008). This method has also been the most frequently used in the search of the antiedematous effects of natural products (Tadić et al., 2008; Morikawa et al., 2003; Tapas et al., 2008). It is well known that the process of carrageenan-induced inflammation in the rat paw involves different phases of mediators released (Bremmer & Heinrich, 2005; Yu et al., 2009).

The anti-inflammatory drugs, such as aspirin and indomethacin, have been shown to inhibit prostaglandin production and cause anti-inflammatory action in carrageenan-induced paw oedema (Noguchi et al., 2005). Moreover, it has also been reported that NO produced by i-NOS is involved in the inflammatory response on paw oedema (Tan-no et al., 2006). Therefore, plant extracts have been shown inhibitory effects on NO production in RAW 264.7 cells and the inhibition of COX activity in vitro (Schreckinger et al., 2010b).

**Table 2.** Amounts of phenolics needed to inhibit gastric damage by concomitant administration given by indomethacin alone and with *A. chilensis* extracts, fractions and compounds.

| Treatment/(200 mg/kg p.o.)[a] | LGL[b] | GDC[b] | % AWL[b] |
|---|---|---|---|
| Indomethacin | 49.8 ± 8.5a | 4.0 | 100 |
| Indomethacin + sample: | | | |
| **A** | 40.1 ± 9.0b | 3.0 | 100 |
| **B** | 5.97 ± 0.5c | 0.5 | 33 |
| **C** | 30.9 ± 5.4d | 2.5 | 67 |
| **D** | 36.7 ± 4.4b | 2.5 | 67 |
| **F-1** | 32.0 ± 3.8d | 3.0 | 67 |
| **F-2** | 19.8 ± 2.5e | 2.5 | 33 |
| **F-3** | 4.9 ± 0.6c | 0.5 | 33 |
| **F-4** | 5.0 ± 0.7c | 0.5 | 33 |
| **$SF_4$-$SF_6$** | 39.9 ± 8.9b | 3.0 | 67 |
| **$SF_7$** | 20.1 ± 2.8e | 2.0 | 67 |
| **$SF_8$-$SF_{10}$** | 22.8 ± 3.1e | 2.0 | 67 |
| **$SF_{11}$-$SF_{15}$** | 4.8 ± 0.4c | 0 | 33 |
| **$SF_{16}$-$SF_{20}$** | 4.4 ± 0.3c | 0.5 | 33 |
| **$SF_{21}$-$SF_{25}$** | 5.9 ± 0.8c | 0 | 33 |
| **$SF_{26}$-$SF_{30}$** | 13.9 ± 2.0f | 0.5 | 33 |
| **$SF_{31}$-$SF_{37}$** | 20.1 ± 2.9e | 2.0 | 67 |
| **Myricetin** | 9.8 ± 1.5g | 1.0 | 33 |
| **Quercetin** | 6.2 ± 1.1c | 1.0 | 33 |
| **Diosmetin** | 15.4 ± 2.1f | 2.5 | 33 |
| **Luteolin** | 7.7 ± 0.4g | 1.0 | 33 |
| **Ovatifolin** | 5.9 ± 0.2c | 0.5 | 33 |

[a]Mean Confidence Interval 95%, $n$ = 3. Different letters show significant differences at ($P < 0.05$), using Duncan's multiple-range test. [b]Values are expressed as mg/Kg p.o. Mean ± SD, $n$ = 3. Different letters show significant differences at ($P < 0.05$), using Duncan's multiple-range test. [c]LGL: length of gastric lesions (mm), GDC: Gastric damage score (according to Adami et al., 1964), AWL: Animals with Lesions.

Indirect effects related to carrageenan-induced inflammation involves the release of COX-2 as evidenced by the inflammation of the rat paw, so the extent of the reduction of inflammation by carrageenan is an indirect measure of control of the release COX-2 and NO in inflammation induced by bacterial infection, viruses, and external agents such as beatings, injuries and bruises among others. It has been shown that the most active agents (in addition to aspirin and indomethacin) are non-steroidal agents (NSA), such as flavonoid aglycones. However, glycosylated flavonoids do not show the same effectiveness (Maruyama et al., 2010). Such as the aglycone of anthocyanins, cyanidin, has higher efficacy than its glycosides, suggesting that the antioxidant activity (and its anti-inflammatory activities) of anthocyanins is due to their aglycone moiety (Wang et al., 1999). In our case, the subfractions and aglycone flavonoids from fruits of *A. chilensis* were the most active samples

**Gastroprotective activity.**

The effects of extracts and fractions of *A. chilensis* expressed on the substantial reduction of gastric impairs showed an important effect, Table 2. When the extracts, fractions and compounds at doses from 8

to 200 mg/Kg p.o. were applied together with indomethacin, the number of gastric lesions, their length and area were significantly reduced (Table 2).

The results of the experiments suggest that the investigated extracts, fractions and subfractions of *A. chilensis* exhibited a pronounced anti-inflammatory and gastroprotective activity.

Almost all of these samples exhibited a concentration-dependence manner in their anti-inflammatory and gastroprotective activities, particularly extract **B**, **F-3, F-4, $SF_{11}$-$SF_{15}$, $SF_{16}$-$SF_{20}$, $SF_{21}$-$SF_{25}$** and **$SF_{26}$-$SF_{30}$** which showed the highest activity. This action was greater than that of quercetin, a known flavonoid with gastroprotective effects (Martin et al., 1998), which at a dose used in this investigation showed a GDC of 1.0 in similar form to myricetin and luteolin with the same GDC values. Similar performance was observed with ovatifolin a sesquiterpene lactone isolated from *Podanthus ovatifolius* a very used anti-inflammatory medicinal plant in Chile (Cespedes et al., 2000).

In addition to control samples, the subfractions **$SF_4$-$SF_6$, $SF_7$, $SF_8$-$SF_{10}$** and **$SF_{31}$-$SF_{37}$** showed considerable activity, with GDC values of 3.0, 2.0, 2.0, and 2.0, respectively. Nevertheless, **$SF_4$-$SF_6$, $SF_{26}$-$SF_{30}$** and **$SF_{31}$-$SF_{37}$** showed a moderate activity with good GDC values; interestingly, these subfractions showed to possess a high concentration of anthocyanins.

The lowest GDC values for **$SF_{11}$-$SF_{15}$, $SF_{16}$-$SF_{20}$, $SF_{21}$-$SF_{25}$**, and **$SF_{26}$-$SF_{30}$** (0, 0.5, 0, and 0.5, respectively) than for any of the other subfractions, might be due to a synergistic effect of the components due to extraction procedures, mainly gallic acid, quercetin, myricetin, delphinidin-3-glucoside and cyanidin-3-glucoside, (see scheme 1 in Cespedes et al., 2010a) inside this subfraction, similar to that reported for Maruyama et al., 2010, where the aglycones were more active than glycosides.

Finally, when the relative contribution of each subfraction to the total anti-inflammatory activity was evaluated using the carrageenan-induced rat paw oedema inflammation method, all samples showed some protective effects; the GDC values of all subfractions are shown in Table 2. **$SF_{11}$-$SF_{15}$** and **$SF_{21}$-$SF_{25}$** were the most active, with both GDC values of 0 . It is noteworthy, that the values for **$SF_{11}$-$SF_{15}$** and **$SF_{21}$-$SF_{25}$** are very low compared to those values for flavonoids and anthocyanins in general, as well as for myricetin or quercetin (Zayachkivska et al., 2005; Sannomiya et al., 2005; Tadić et al., 2008; Shih et al., 2005; Morikawa et al., 2003; Petrović et al., 2008).

Many reports shows that many flavonoids possess a wide range of pharmacological activities that include anti-inflammatory (Cespedes et al., 2010a), gastroprotective (Zayachkivska et al., 2005; Sannomiya et al., 2005), hepatoprotective, antitumor, antimicrobial, antidiabetic, cardioprotective among others (Tapas et al., 2008; Cespedes et al., 2008). Thus, our extracts, fractions, subfractions and compounds have shown to be very good sources of nutraceuticals.

## CONCLUSIONS

In general these compounds that occur in this *Aristotelia* species have been considered as the active principles of many anti-inflammatory plants. Thus, many phenolic acids, anthocyanins and flavonoids have shown inhibitory activities on nitric oxide implicated in physiological and pathological process as chronic inflammation (Matsuda *et al*., 2000; Odontuya *et al*., 2005).

These findings show that the anthocyanins, flavonoids and phenolic acids may be responsible of the anti-inflammatory and gastroprotective activities of this fruit. We are working on the kinetics of inhibition of these plant extracts and compounds as anti-inflammatory and we are also dissecting the sites and mechanism of action as iNOS, COX, and TNF, among others.

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