

Anti-inflammatory Activity and Antioxidant Potential of Aqueous Extracts from Stem Bark of *Geoffroea decorticans*

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Abstract: In the present work we investigated, for the first time, the anti-inflammatory activity and the antioxidant properties of aqueous and ethanolic extracts, obtained from stem bark of *Geoffroea decorticans* (Gill. ex Hook. et Arn.) Burk. (Fabaceae). *G. decorticans*, commonly known as “chañar” or “chañarcillo”, is a traditional argentinean plant used as emollient, balsamic, antitussive, expectorant and anti-inflammatory. The stem bark was collected from San Francisco del Monte de Oro, San Luis, Argentina. Anti-inflammatory activity was evaluated by carrageenan-induced paw edema in rats. Antioxidant activity was tested using 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging activity (DPPH), 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity (ABTS) and ferric ion-reducing power (RP-Fe) assays. Aqueous extract 10% p/v showed anti-inflammatory activity (3h, 48% inhibition, 5h 37% inhibition and 7h 17% inhibition) and antioxidant activity (DPPH, IC₅₀ (mg/mL) = 0.098 ± 0.032; ABTS, IC₅₀ (mg/mL) = 0.022 ± 0.343, RP-Fe IC₅₀ (mg/mL) = 1.124 ± 0.146). In the other hand, the ethanolic extract 5% p/v, presented anti-inflammatory activity (3h, 34% inhibition, 5h 38% inhibition and 7h 35% inhibition) and antioxidant activity (DPPH, IC₅₀ (mg/mL) = 0.133 ± 0.027; ABTS, IC₅₀ (mg/mL) = 0.086 ± 0.262, RP-Fe IC₅₀ (mg/mL) = 7.089 ± 0.104). These results suggest that, also fruits, the aqueous and ethanolic extracts from the stem bark of *G. decorticans* present significant anti-inflammatory activity and antioxidant properties.

Key words: Anti-inflammatory bioactivity, antioxidant properties, *Geoffroea decorticans*.

1. Introduction

The therapeutic uses of herbs as well as the actions of plant extracts are either as old as human civilization and have evolved along with it. These plant-based traditional medicine systems continue to play an essential role in health care. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [1].

Geoffroea decorticans (Gill. ex Hook. et Arn.) Burk, popularly known as “chañar” or “chañarcillo”, is a middling-size tree that inhabits most arid forests of southern South America. Chañar's leaves, bark,

flowers and fruits are used with medicinal purposes as emollient, balsamic, antitussive, expectorant and anti-inflammatory. There are many studies of chemical constituent and popular uses of fruits of *G. decorticans*, but there are no studies reported about chemical constituent and bioactivities of chañar's stem bark. Fruits of algarrobo and Chañar have been a food source for humans and domestic goats in rural communities in the Monte desert since ancient times. Traditionally, Chañar fruits are consumed in añapa and arropes with and without sugar. The antinociceptive action of Chañar arrope and aqueous extract has been demonstrated [2, 3]. However, in argentinean popular medicine, the infusion of the stem bark is used to combat respiratory disorders (cough and asthma) and it is recommended in case of whooping cough. Also, the

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chañar's stem bark is used to combat hemorrhoids [4, 5]. In addition, stem bark of *G. decorticans* detaches itself in strips, relevant feature to comply with the rules that make the plant conservation.

On the other hand, inflammation process is an intrinsic defense response of the host which aims to remove an aggressive agent from the organism. This process is initiated primarily by exposure of macrophages, neutrophils, and innate immune cells to pathogen or damage associated molecular patterns [6]. Recent reports have indicated that plant based antioxidants are of great importance as therapeutic agents to combat oxidative stress associated with the inflammatory process [7].

This work report, for the first time, the anti-inflammatory activity and the antioxidant properties of aqueous and ethanolic extracts obtained from stem bark of *G. decorticans*.

2. Experimental

2.1 Plant Material and Preparation of Infusion

The stem bark of *G. decorticans* was collected from San Francisco del Monte de Oro, San Luis, Argentina (August 2013). A voucher specimen is deposited at the Herbarium of the Universidad Nacional de San Luis (UNSL Del Vitto N° 553). From the dried and pulverized stem bark, infusion (10% p/v) and ethanolic extract (5% p/v) were obtained. The infusion at 10% p/v was prepared from the selected plants in the following way: 200 mL of 100 °C water was added to 20 g of dried plant, and let rest for 20 min. It was filtered through paper (Whatman No. 1) and the residue was washed with warm water.

2.2 Test Animals

Female Wistar albino rats weighing between 160 to 180 g were used in the experiments. Animals were purchased, housed and cared for at the Animal Resource Facilities (Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis). Animals were randomly assigned to

different groups ($n = 6$), provided with a standard rodent chow diet and water *ad libitum* and maintained at a constant temperature of 24 ± 1 °C and humidity of $55 \pm 5\%$ with 12 h light/dark cycle.

2.3 Drug Administration

Each group was administered intraperitoneally with 0.5 mL of infusion 10% p/v and ethanolic extract (200 mg/kg). The reference group was administered with ibuprofen (10 mg/kg) suspended in sterile saline. The control group only received sterile saline.

2.4 Carrageenan-induced Paw Edema in Rats

Anti-inflammatory activity was assessed on the paw edema induced by carrageenan [8]. One hour after administration of drugs, animals were injected in the subplantar region of left hind paw with 0.1 mL/rat of carrageenan type IV 2% w/v suspended in saline. The volumes of both hind paws were measured in triplicate using a plethysmograph (Ugo Basile 7140) at intervals of 1, 3, 5, and 7 h after injection of carrageenan. The edema volume is expressed in each animal as the difference between the average volumes of both hind paws. The inhibition percentage of edema was calculated as $(V_{\text{control}} - V_{\text{treated}} / V_{\text{control}}) \times 100$ (V = edema average volumes of groups).

2.5 Antioxidant Activity

2.5.1 DPPH and ABTS Radical Scavenging Assays

The radical scavenging activity was evaluated using the DPPH and ABTS assays. DPPH Experiments were carried out according to the method of Blois with a slight modification briefly [9, 10], a 0.1 mM solution of DPPH radical solution in MeOH/H₂O (8:2) was prepared. Then 1 mL of this solution was mixed with sample solution (3 mL) (1mg/mL final conc.). Finally, after 30 min, the absorbance was measured at 517 nm. Radical cation ABTS⁺ was produced by mixing 2 mM ABTS with 30 μM H₂O₂ and 6 μM horseradish peroxidase (HRP) enzyme in 50 mM phosphate buffered saline (PBS-pH 7.5). Immediately after the

addition of the HRP enzyme, the contents were vigorously mixed, incubated at room temperature in the dark and the reaction was monitored at 730 nm until stable absorbance was obtained. Then, 10 μ L of different extract concentrations were added in the reaction mixture and the decrease in absorbance at 730 nm was measured. Percent inhibition of DPPH and ABTS radicals was calculated by to the formula $IC (\%) = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$ (Abs = absorbance).

2.5.2 Ferric Reducing Power Test

The ferric reducing power test was determined by a slightly modified method of Yen and Duh [11]. Sample of 1.0 mL of various dilutions was mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixtures were incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Upper layer (0.5 mL) of solution is mixed with 2.5 mL of distilled water and 100 mL of 0.1% FeCl₃ and the absorbance was measured at 700 nm. Controlling sample contained 1.0 mL distilled water, 2.5 mL of phosphate buffer, 2.5 mL of 1% potassium ferrocyanide and 2.5 mL of 10% trichloroacetic acid. Blank sample contained 1.0 mL distilled water, 2.5 mL of phosphate buffer, 2.5 mL of 1% potassium ferricyanide and 2.5 mL of 10% trichloroacetic acid. The reducing power of samples was calculated by the following formula: $RP (\%) = [(Abs_{control} - Abs_{sample}) \times 100]$. All Tests were carried out in triplicate. As

positive controls were used ascorbic acid, and quercetin and BHA. The IC₅₀ was determined using GraphPad software.

2.6 Statistical Analysis

The data obtained are presented as mean \pm SEM. The data were analyzed with One-Way ANOVA followed by a Dunnet multiple comparison test. A probability of $p < 0.05$ was considered significant.

3. Results and Discussion

In Table 1 are shown the effects of *G. decorticans* aqueous extracts at 10% (GdIn10), ethanolic extract at 5% p/v (GdEE) and ibuprofen administration on carrageenan-induced paw edema in rats. The GdIn10 showed its greater anti-inflammatory activity at 3 hours of its administration. The GdEE also showed its greatest activity from 3 hs, in a percentage lower than GdIn10, however it remained in the same percentage until 7 hs of its administration. Finally, ibuprofen only exhibited anti-inflammatory activity at 3 h (51% inhibition).

Acute inflammatory response is characterized by an increase in vascular permeability, extravasation of fluid and plasma proteins, and cellular infiltration from blood vessels to the inflamed area, leading to the edema formation. A number of chemical mediators have been identified in the inflammatory response: histamine, serotonin, NO, eicosanoids, cytokines, and products derived from plasma systems (coagulation, complement, and kinins) [12].

Table 1 Effect of *G. decorticans* aqueous and ethanolic extracts on carrageenan induced paw edema in rats

Time (h)	Control	<i>G. decorticans</i> aqueous extract 10% (GdIn10)		<i>G. decorticans</i> ethanolic extract 5% (GdEE)		Ibuprofen (10mg/kg)	
	Paw volume (mL)	Paw volume (mL)	Inhibition (%)	Paw volume (mL)	Inhibition (%)	Paw volume (mL)	Inhibition (%)
1 h	0.34 \pm 0.18	0.25 \pm 0.08	28%	0.27 \pm 0.14	22%	0.25 \pm 0.06	27%
3 h	1.12 \pm 0.33	0.59 \pm 0.41	48%*	0.73 \pm 0.59	34%	0.58 \pm 0.16	51%*
5 h	1.37 \pm 0.07	0.86 \pm 0.38	37%	0.85 \pm 0.56	38%	1.18 \pm 0.38	14%
7 h	1.23 \pm 0.06	1.02 \pm 0.28	17%	0.80 \pm 0.48	35%	1.32 \pm 0.27	0

Tabular values represent the mean \pm SEM of volume differences between both paws for control, different doses of *G. decorticans* aqueous and ethanol extract and ibuprofen groups of six animals and inhibition percentage in paw volume compared with control group. ANOVA with multiple comparison method by Dunnet: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2 Antioxidant activity of extracts of *G. decorticans*

Natural Extracts and controls	DPPH IC ₅₀ (mg/mL)	ABTS IC ₅₀ (mg/mL)	RP-Fe IC ₅₀ (mg/mL)
GdIn10	0.098 ± 0.032	0.022 ± 0.343	1.124 ± 0.146
GdEE	0.133 ± 0.027	0.086 ± 0.262	7.089 ± 0.104
BHA	0.065 ± 0.001	0.048 ± 0.451	1.032 ± 0.093
Quercetin	0.041 ± 0.001	0.022 ± 0.001	ND
Ascorbic acid	ND	0.003 ± 0.174	ND

Tabular values represent the mean ± SEM of volume differences between both paws for control, different extracts of *G. decorticans* and ibuprofen groups of six animals and inhibition percentage in paw volume compared with control group. ANOVA with multiple comparison method by Dunnet: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Inhib: Inhibition. GdIn10: Infusion 10% p/v; GdEE: Ethanolic extract 5% p/v. All the values are mean ± SD; SD: standard deviation; BHA= tert-Butyl-4-hydroxyanisole; ND: not determined.

Carrageenan induced paw edema has been described as a biphasic process. The initial phase of edema formation (0-1 h), which is not inhibited by NSAIDs, is attributed to the release of histamine, serotonin, and bradykinin. The second phase (1-6 h) is associated with a local infiltration and activation of neutrophils and the production of prostaglandins [13]. An increase in NO production is also associated with the increase of paw volume. The results shown that both extract act preferentially on the second rather than the first phase, which could be explained by a better specificity against the release/action of mediators involved in the second phase (neutrophils, prostaglandins, and NO), whose production usually peaks between 3 and 6 h after injection of carrageenan [14].

It is observed that both the ethanol and aqueous extracts possess high anti-inflammatory potency and therefore decrease the side effects. In chronic diseases such as arthritis, rheumatism, it is an advantage.

The results of antioxidant activity are shown in Table 2. GdIn10 possesses better radical scavenging activity as well as reducing capacity. GdEE do not show significantly antioxidant properties. In addition, available literature has revealed the role of natural antioxidant in the treatment of inflammation and associated problems [15].

4. Conclusions

These results suggest that, also fruits, the aqueous extract from the stem bark of *G. decorticans* present significant anti-inflammatory activity and antioxidant

properties that can provide opportunities for application in areas such as pharmacy, alternative medicine, and natural therapy. Taking into account the significant results obtained, phytochemical studies are in progress. At the same time, from the aqueous extract of *G. decorticans*, we are carrying out the design of a semi-solid pharmaceutical form to external use (gel and emulsion).

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