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## Vicenin-2 and vitexin participate in the *in vitro* modulation of the anti-inflammatory and antioxidant activities exerted by two *Urtica circularis* extracts

[Participación de vicenina-2 y vitexina en la modulación de la actividad anti-inflamatoria *in vitro* y en la actividad antioxidante de dos extractos de *Urtica circularis*]

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**Abstract:** *Urtica circularis* is an Argentinean species traditionally used to treat inflammation symptoms and oxidative stress-related diseases. Considering the uses in folk medicine, the purpose of this work was to evaluate and compare the anti-inflammatory and antioxidant activities of two different *U. circularis* extracts. The contribution of vicenin-2 and vitexin, two compounds identified in the phytochemical analysis, in the biological activity of the extracts was evaluated. The anti-inflammatory activity of the extracts and the isolated compounds was tested on lipopolysaccharide (LPS)-stimulated macrophages, while the antioxidant activity was evaluated through the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical and 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) scavenging activities. The popular uses of both extracts were validated, i.e., the use of *U. circularis* ethanol extract for the treatment of inflammation, and the use of the aqueous extract to treat oxidative stress related-diseases. The differences in the biological activities observed between the extracts are probably due to qualitative and/or quantitative differences in the chemical composition and/or the occurrence of synergism between compounds.

**Keywords:** *Urtica circularis*; Vicenin-2; Vitexin; Inflammation; Antioxidant activity.

**Resumen:** *Urtica circularis* es una especie argentina utilizada para tratar los síntomas de la inflamación y enfermedades relacionadas con el estrés oxidativo. El objetivo de este trabajo fue evaluar y comparar las actividades anti-inflamatoria y antioxidante de dos extractos teniendo en cuenta su uso popular. Además, se analizó la participación de vicenina-2 y vitexina, compuestos identificados en el análisis fitoquímico, en la actividad de los extractos. La actividad anti-inflamatoria fue evaluada en macrófagos activados con lipopolisacárido (LPS). Se midió su actividad antioxidante con los métodos del 1,1-difenil-2-picrilhidrazilo (DPPH) y del ácido 2,2'-azinobis-3 etilbenzotiazolin-6-sulfónico (ABTS). Los usos populares de ambos extractos fueron validados: el extracto etanólico para la inflamación y el extracto acuoso para el tratamiento de enfermedades relacionadas con el estrés oxidativo. Las diferencias en las actividades biológicas observadas entre los extractos están probablemente relacionadas con diferencias cualitativas y/o cuantitativas en su composición química y/o a la presencia de sinergismo entre compuestos.

**Palabras clave:** *Urtica circularis*; Vicenin-2; Vitexina; Inflamación; Actividad antioxidante.

## ABBREVIATIONS

ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

ATCC: The American Type Culture Collection

DPPH: 2,2'-diphenyl-1-picrylhydrazyl

iNOS: inducible NO synthase

LPS: lipopolysaccharide

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NO: nitric oxide

Rt: retention time

TNF- $\alpha$ : tumor necrosis factor alpha

## INTRODUCTION

*Urtica circularis* (Hicken) Sorarú is an Argentinean native herb also found in Paraguay, Uruguay, and Brazil. It is commonly known as “ortiga”, “ortiga crespá”, “caá poropí” and “urtiginha miúda”. The alcoholic solution of the aerial parts is used in folk medicine to relieve pain in inflammatory processes. As infusion, *U. circularis* is used as a diuretic and antihypertensive agent, and to treat hepatic affections and cough (Martínez Crovetto, 1981). *U. circularis* is also a highly nutritious herb because of its minerals and vitamins content (Rondina *et al.*, 2003).

The anti-inflammatory activity of the ethanol extract has previously been demonstrated in a model of carrageenan-induced plantar edema in rats. Moreover, its major compound, the flavonoid vicenin-2, has proved to exert a biphasic effect on nitric oxide (NO) release, tumor necrosis factor alpha (TNF- $\alpha$ ) production, and NF- $\kappa$ B translocation in macrophages stimulated with lipopolysaccharide (LPS) (Marrassini *et al.*, 2011). The same extract also presents central nervous system activity (Anzoise *et al.*, 2013) and a hypotensive effect (Rodríguez Basso *et al.*, 2016; Marrassini & Anesini, 2018). The ethanol extract, but not the decoction, displays antinociceptive effects related to the anti-inflammatory effects (Gorzalczany *et al.*, 2011).

Macrophages are known to play a fundamental role during inflammation and in the immune response. These cells are also responsible for the overproduction of pro-inflammatory cytokines and inflammatory mediators such as IL-1 $\beta$ , IL-6, NO, iNOS, COX-2, and TNF- $\alpha$ . Among these, NO plays diverse roles in inflammatory reactions as it induces vasodilation and the generation of edema. NO also modulates the activity of sensory nerve endings and leukocytes and contributes to tissue cytotoxicity (Christopherson & Bredt, 1997). NO is involved in

both acute and chronic inflammation, participating in the development of inflammatory diseases like rheumatoid arthritis, autoimmune disorders, and oxidative stress-related diseases (Yoon *et al.*, 2009). Taking into account the biological activity of NO, any compound presenting inhibitory effects over the excessive production of NO can be considered potential anti-inflammatory drugs (Sarkar *et al.*, 2005). NO is produced as a free radical during the conversion of L-arginine to L-citrulline by the inducible NO synthase (iNOS). In this regard, and unlike L-arginine, which enhances inflammation, iNOS inhibitors reduce acute inflammation or adjuvant-induced arthritis in rats (Bogdan, 2000).

The toxic side effects of synthetic anti-inflammatory medicines are well known (Bennett & Brown, 2003). Many medicinal plants have been used for years to treat inflammation with none or few undesired side effects. Plants are rich sources of structurally diverse secondary metabolites, many of which possess potential anti-inflammatory activity by acting on different molecular targets (Burk *et al.*, 2009). Thus, the screening of natural products displaying anti-inflammatory activity is a good strategy for the development of new drugs with fewer side effects (Suba *et al.*, 2005).

The aim of this work was to evaluate and compare the capacity of the ethanol and aqueous extracts of *Urtica circularis* to modulate the NO production in macrophages stimulated with LPS. This model has proved to be suitable to study the effect of drugs on the production of NO. LPS, which is obtained from cell wall of Gram-negative bacteria, activates multiple signaling pathways in macrophages leading to the transcription of the iNOS gene and enhances the production of inflammatory mediators like NO (Jachak, 2007). The free radical scavenger activity of the extracts was also assayed. Finally, the effects of some of the flavonoids identified in the extracts were studied, analysing their contribution to the overall effect of the extract.

## MATERIALS AND METHODS

### *Plant material*

*Urtica circularis* (Hicken) Sorarú was collected in Estancia “La Merced”, Saladas Department, Corrientes province, Argentina and identified by Dr Martha Gattuso. A voucher specimen (No. 54) was deposited in the Museum of Facultad de Ciencias Químicas, Universidad Nacional de Rosario, Argentina.

**Preparation of plant extracts**

The dried and powdered aerial parts of *U. circularis* parts were used for the preparation of both extracts. The ethanol extract was prepared by macerating the dried plant material (20 g) with 80% ethanol (200 mL) at room temperature (seven times). The extract was then concentrated by evaporation and lyophilized. The yield of this procedure was 11.47% w/w. The decoction or aqueous extract was prepared by boiling 20 g of the dried plant material in 400 mL distilled water. The extract obtained was then concentrated by evaporation and lyophilized. The extraction yield was 16.70% w/w.

**Phytochemical analysis**

The HPLC profiles were obtained by applying an HPLC validated method for polyphenols (Filip *et al.*, 2001). A Varian 9000 HPLC device equipped with a diode array detector and rheodyne injector fitted with a 20  $\mu$ L loop was employed. A C18 column was used (Gemini, 5  $\mu$ m, 150 mm $\times$ 4.6 mm). Two mobile phases were used: A: H<sub>2</sub>O/AcOH (98:2) and B: MeOH/AcOH (98:2) and the gradient was 15 - 40% B, 30 min; 40 - 75% B, 10 min; 75 - 85% B, 5 min. The flow rate was 1.2 mL/min and the detection was carried out at 325 nm.

**Cell and culture conditions**

The RAW264.7 (ATCC) murine macrophage cell line was used. Cells were cultured in Dulbecco's Modified Eagle's medium without phenol red and supplemented with 10% FBS, 2 mM glutamine and antibiotics (100 IU/mL penicillin and 100  $\mu$ g/mL streptomycin). Cells were incubated under a 5% CO<sub>2</sub> atmosphere at 37°C.

**Cell proliferation and determination of the levels of nitric oxide**

RAW264.7 macrophages were incubated with or without treatment with LPS (1  $\mu$ g/mL) alone or in the presence of different concentrations of the extracts or the isolated compounds vicenin-2 and vitexin. After 24 h incubation, the supernatant was collected to determine NO levels and the proliferation rate of cells still bound to the plate microwells.

Nitrite levels, which is an indicator of NO production, were measured by the Griess' reaction (Becherel *et al.*, 1997). As standard curve, serial dilutions of a sodium nitrite solution were employed. Briefly, 100  $\mu$ L of each supernatant or dilution of the standard curve were mixed with 50  $\mu$ L of a 1% sulfanilamide solution in 5% phosphoric acid and 50

$\mu$ L of a 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride solution. After incubating at room temperature for 20 min, the absorbance at 540 nm was read. Results were expressed as mean  $\pm$  SEM of three determinations performed in triplicate. Cell proliferation was determined by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma). Briefly, cells were incubated for 4 h in 100  $\mu$ L RPMI 1640 cultured medium and 10  $\mu$ L of 5 mg/mL MTT (Sigma, St. Louis, MO, USA). After incubation, the formazan formed was dissolved in acidified isopropanol (0.04 N HCl in isopropanol). The absorbance was read at 540 nm.

**2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity**

The DPPH scavenging activity of the extracts was assayed (Blois, 1958). The bleaching rate of the free radical DPPH was monitored in the presence of the sample at 517 nm. Briefly, 100  $\mu$ L of an aqueous dilution of the extract were mixed with 500  $\mu$ L of a 500  $\mu$ M DPPH solution in absolute ethanol and 400  $\mu$ L of 0.1 M Tris-HCl buffer, pH 7.4. After incubating for 20 min in the dark, the absorbance was read. The DPPH inhibition percentage was calculated by the following equation:

$$\% \text{ of inhibition} = [1 - (As/Ab)] \times 100$$

where As is absorbance of sample and Ab is the absorbance of the DPPH solution.

**2,2'-Azino bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) scavenger activity**

The scavenging activity of the extracts on ABTS was measured (Re *et al.*, 1999). A 7 mM ABTS solution in distilled water was prepared. This solution was mixed with 2.45 mM potassium persulfate (final concentration) and incubated 12-16 h in the dark at room temperature to produce the ABTS radical cation. The ABTS radical cation solution was then diluted with ethanol to an absorbance of  $0.70 \pm 0.03$  at 734 nm. A 950  $\mu$ L volume of the ABTS radical cation solution was mixed with 50  $\mu$ L of an aqueous dilution of the extracts, compounds, or distilled water (blank). Three minutes after mixing, the absorbance was read at 734 nm. The percentage inhibition was calculated as:

$$\% \text{ of inhibition} = (Ab - As)/Ab] \times 100$$

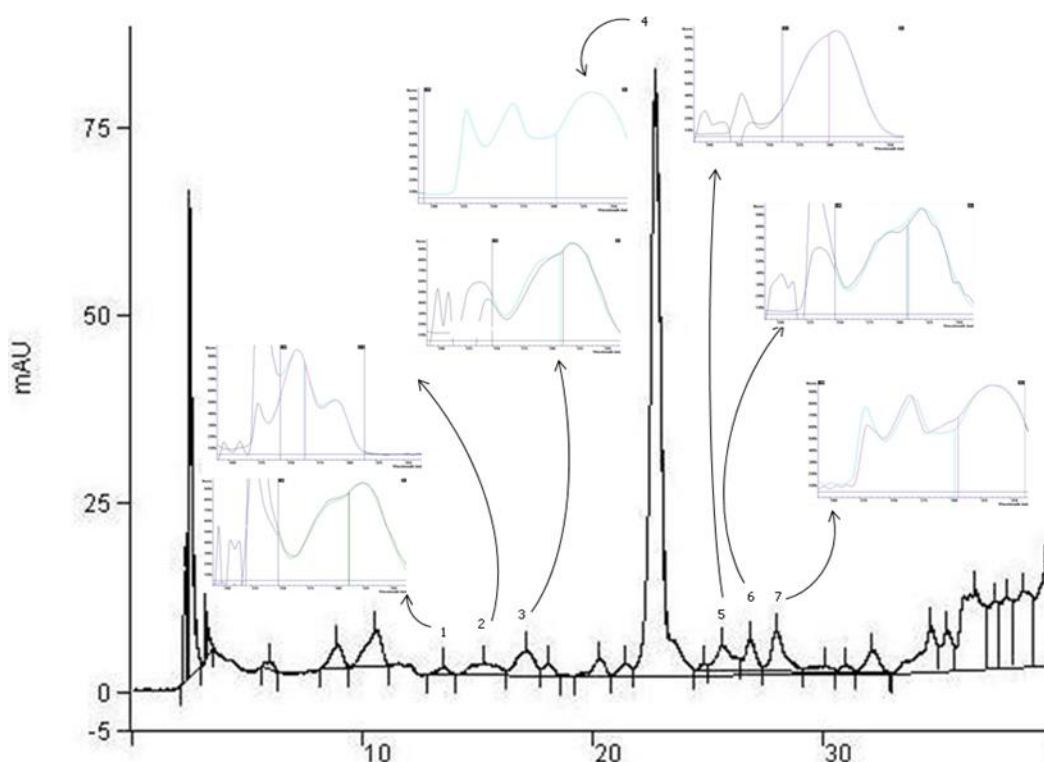
where  $A_s$  is the absorbance of sample and  $A_b$  is the absorbance of the ABTS radical cation solution plus water.

### Statistical analysis

Data were expressed as the average of triplicate values of three independent experiments. Comparisons were performed by analysis of variance (ANOVA) and the Dunnett's test. A  $p < 0.05$  was considered statistically significant.

### RESULTS

In this work two extracts of *Urtica circularis* were studied. The extraction was done considering the most common forms in which the plant is used in popular medicine (decoction and ethanol extract). The HPLC extracts profiles were analyzed to define the extracts composition (Figures No. 1 and No. 2). The major compound in both extracts was found to be vicenin-2. Chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, and ferulic acid were also identified and quantified in both extracts (Table No. 1). Vitexin was found only in the ethanol extract.



**Figure No. 1**

#### Ethanol extract HPLC profile

Peak 1 Rt: 13.30 min chlorogenic acid

Peak 2 Rt: 15.05 min vanillic acid

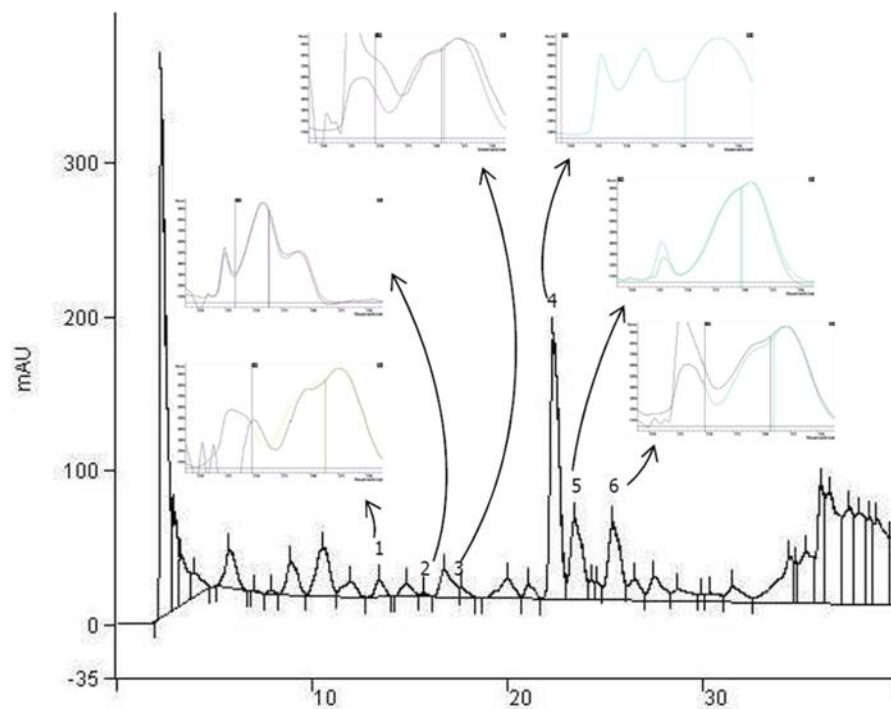
Peak 3 Rt: 17.10 min caffeic acid

Peak 4 Rt: 22.08 min vicenin-2

Peak 5 Rt: 25.29 min *p*-coumaric acid

Peak 6 Rt: 25.62 min ferulic acid

Peak 7 Rt: 27.99 min vitexin



**Figure No. 2**

**Aqueous extract HPLC profile**

Peak 1 Rt: 13.71 min chlorogenic acid

Peak 2 Rt: 15.45 min vanillic acid

Peak 3 Rt: 17.11 min caffeic acid

Peak 4 Rt: 22.39 min vicenin-2

Peak 5 Rt: 23.47 min *p*-coumaric acid

Peak 6 Rt: 25.30 min ferulic acid

**Table No. 1**

**Identification and quantification of polyphenolic compounds in ethanol and aqueous extracts of *Urtica circularis***

	Ethanol extract	Aqueous extract
	mg compound/ mg extract	
<b>Vicenin-2</b>	$4.66 \times 10^{-3}$	$8.74 \times 10^{-3}$
<b>Vitexin</b>	$3.7 \times 10^{-4}$	-
<b>Chlorogenic acid</b>	$3.8 \times 10^{-4}$	$1.41 \times 10^{-3}$
<b>Vanillic acid</b>	$1.07 \times 10^{-4}$	$2.76 \times 10^{-5}$
<b>Caffeic acid</b>	$2.98 \times 10^{-4}$	$2.62 \times 10^{-4}$
<b><i>p</i>-Coumaric acid</b>	$3.59 \times 10^{-5}$	$3.64 \times 10^{-3}$
<b>Ferulic acid</b>	$9.52 \times 10^{-5}$	$3.61 \times 10^{-3}$

The effect of the extracts on the nitrosative stress was studied on LPS-stimulated macrophages. As expected, LPS increased the NO release by macrophages (Figures No. 3A and Figures No. 3A

3B). On LPS-stimulated macrophages, the ethanol extract presented a biphasic effect, increasing the NO production at low concentrations and decreasing it at higher ones. The ethanol extract inhibited the effect

of LPS at 100, 250, 500, and 1000  $\mu\text{g/mL}$  (28.4%, 69.6%, 86.2%, and 72.9% inhibition, respectively). Moreover, in the presence of LPS and the extract at 250, 500, and 1000  $\mu\text{g/mL}$ , NO levels were similar to baseline ones. On the other hand, the aqueous extract did not modify the effect exerted by LPS, in fact, it enhanced it at some concentrations (500 and 1000  $\mu\text{g/mL}$ , 20% and 30% enhancement, respectively).

The effects exerted by the isolated compounds on the nitrosative stress were also studied. Vicenin-2 was chosen because it was the majority compound in both extracts, whereas vitexin was evaluated because it was found to be present only in the ethanol extract. The aim was to analyze and compare the effects exerted by both compounds and assess their contribution to the overall effect of the aqueous and ethanol extracts. Both vicenin-2 and vitexin were able to reduce the high levels of NO produced under stimulation of macrophages with LPS (Figure No. 3C). Vicenin-2 reduced NO levels by 14.8%, 20.3%, and 29.6% at 0.1  $\mu\text{g/mL}$ , 1  $\mu\text{g/mL}$ , and 10  $\mu\text{g/mL}$ , respectively. On the other hand, vitexin reduced NO levels by 25.9%, 44.4%, and 81.5% at 0.1  $\mu\text{g/mL}$ , 1  $\mu\text{g/mL}$ , and 10  $\mu\text{g/mL}$ , respectively, when compared with the treatment with LPS alone. At some doses, the compounds administered together displayed a synergic effect (Figure No. 3D). The most effective combination was 0.1  $\mu\text{g/mL}$  vicenin-2 + 1  $\mu\text{g/mL}$  vitexin with which the effect was more potent than the sum of the individual effects (32.9% vs. 26.3% of inhibition). Although with other combinations the effect was not potentiated, the inhibition was higher than the one achieved by one of the compounds alone; for example, 0.1  $\mu\text{g/mL}$  vicenin-2 + 0.1  $\mu\text{g/mL}$  vitexin or 1  $\mu\text{g/mL}$  vicenin-2 + 10  $\mu\text{g/mL}$  vitexin.

The antioxidant activity of the extracts was assessed by the DPPH and ABTS tests (Figures No. 4 and No. 5). Both extracts presented antioxidant activity, but the activity of the aqueous extract was higher than that exhibited by the ethanol extract. In the DPPH test, the aqueous extract reached a maximum effect of 80% of inhibition at 500  $\mu\text{g/mL}$  ( $\text{EC}_{50} = 52.5 \mu\text{g/mL}$ ). On the other hand, at the same concentration, the ethanol extract exerted a 40% inhibition and its maximum effect was 50% of inhibition at 1000  $\mu\text{g/mL}$  ( $\text{EC}_{50} = 396.3 \mu\text{g/mL}$ ). Antioxidant activity was also detected with the isolated compounds vicenin-2 and vitexin (Figure No. 4B), with which the maximum inhibition was approximately 30%. It is noteworthy that when these compounds were administered together, a synergistic

effect was observed (Figure No. 4C), reaching 40% of inhibition (10  $\mu\text{g/mL}$  vicenin-2 + 10  $\mu\text{g/mL}$  vitexin). In the ABTS test, similar results were obtained. The antioxidant activity displayed by the aqueous extract was higher than that of the ethanol extract ( $\text{EC}_{50} = 5.4 \mu\text{g/mL}$  vs.  $\text{EC}_{50} = 20.9 \mu\text{g/mL}$ , respectively). At 250  $\mu\text{g/mL}$ , the aqueous extract exerted almost 100% inhibition, while the ethanol extract at the same concentration only inhibited the free radical formation by 66%. Vicenin-2 and vitexin also had antioxidant effect (Figure No. 5B) and their synergistic effect was marked in the presence of 10  $\mu\text{g/mL}$  vitexin, reaching 70% inhibition (10  $\mu\text{g/mL}$  vicenin-2, Figure No. 5C). However, the antioxidant effect of the extracts was higher than that obtained with the isolated compounds.

## DISCUSSION

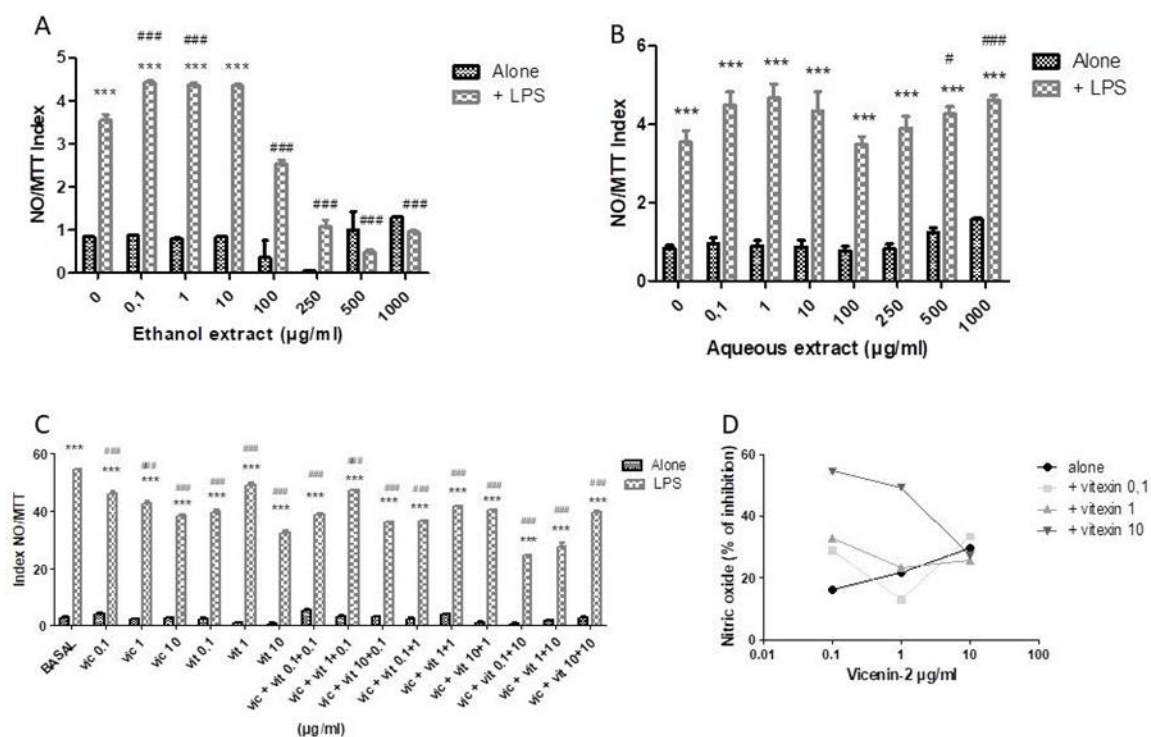
In this work, the activity of two *Urtica circularis* extracts on NO production was analyzed. The ethanol extract exerted a modulatory activity on the production of NO by macrophages, decreasing the levels of this mediator when added at high concentrations. Conversely, the decoction increased NO levels.

The ethanol extract had previously been demonstrated to have anti-inflammatory activity in the carrageenan-induced edema assay in rats (Marrassini *et al.*, 2011). The capacity of the ethanol extract to revert the increase of NO levels induced by LPS in macrophages could explain the anti-inflammatory effect observed *in vivo*.

The aqueous extract did not revert the effects of LPS at any of the concentrations tested; in fact, it increased NO levels, thus showing a pro-inflammatory rather than an anti-inflammatory action. In agreement with these results, it has previously been shown that the aqueous extract does not display any activity in the formalin test, while the ethanol extract proved to have an antinociceptive effect at the same doses (500 mg/kg p.o.) (Gorzalczany *et al.*, 2011). The nociception induced by formalin is associated with tissue injury and inflammation. It is well known that the subcutaneous injection of formalin in the rat paw induces a biphasic response. The early phase is characterized by C-fibre activation by peripheral stimuli and the late phase is characterized by local tissue inflammation and functional changes in the dorsal horn of the spinal cord. Therefore, the late phase is inhibited both by opioids and anti-inflammatory drugs. It could then be hypothesized that the aqueous extract would lack

anti-inflammatory properties, which was verified in the macrophage model *in vitro*. These results

reinforce the use of ethanol preparations for the treatment of inflammatory diseases.



**Figure No. 3**

**Effect of the extracts and isolated compounds on nitrite release by RAW264.7 macrophages**  
**Results are expressed as NO/MTT Index and represent means  $\pm$  SEM of three experiments made in triplicate). Basal: cells without treatment, LPS: cells previously treated with LPS. The statistical differences were determined by ANOVA followed by Dunnet's test. (\* $p < 0.05$ , \*\*\* $p < 0.001$  with respect to basal conditions; # $p < 0.05$ , ### $p < 0.001$  vs. Respect to LPS).**

**A: Effect of the ethanol extract on nitrite release.**

**B: Effect of the aqueous extract on nitrite release.**

**C: Effect of vicenin-2 (vic) and vitexin (vit) on nitrite release.**

**D: Effect of different concentrations of vicenin-2 and vitexin on nitric oxide production together in the presence of LPS.**

In the phytochemical analysis, chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, and ferulic acid were identified in both extracts. Vicenin-2 was found to be the major component in both extracts, but its concentration was 86.7% higher in the decoction than in the ethanol extract. Vitexin was only present in the ethanol extract.

The *in vitro* anti-inflammatory activity (NO release, TNF- $\alpha$  production, and NF-kB translocation) of vicenin-2 has been previously reported (Marrassini *et al.*, 2011). In those experiments vicenin-2 exerted a biphasic effect: at low concentrations, the

compound decreased the levels of NO and the production of TNF- $\alpha$  as well as the NF-kB translocation rate. Conversely, high concentrations resulted in a pro-inflammatory effect, as assessed through TNF- $\alpha$  and NO release, and NF-kB translocation. The biphasic effect of vicenin-2 on NO release could explain the behavior of the aqueous extract, which exerted a pro-inflammatory effect at certain concentrations. It must be noted that in our experiments, the amount of vicenin-2 of the ethanol extract always corresponded to the anti-inflammatory action.

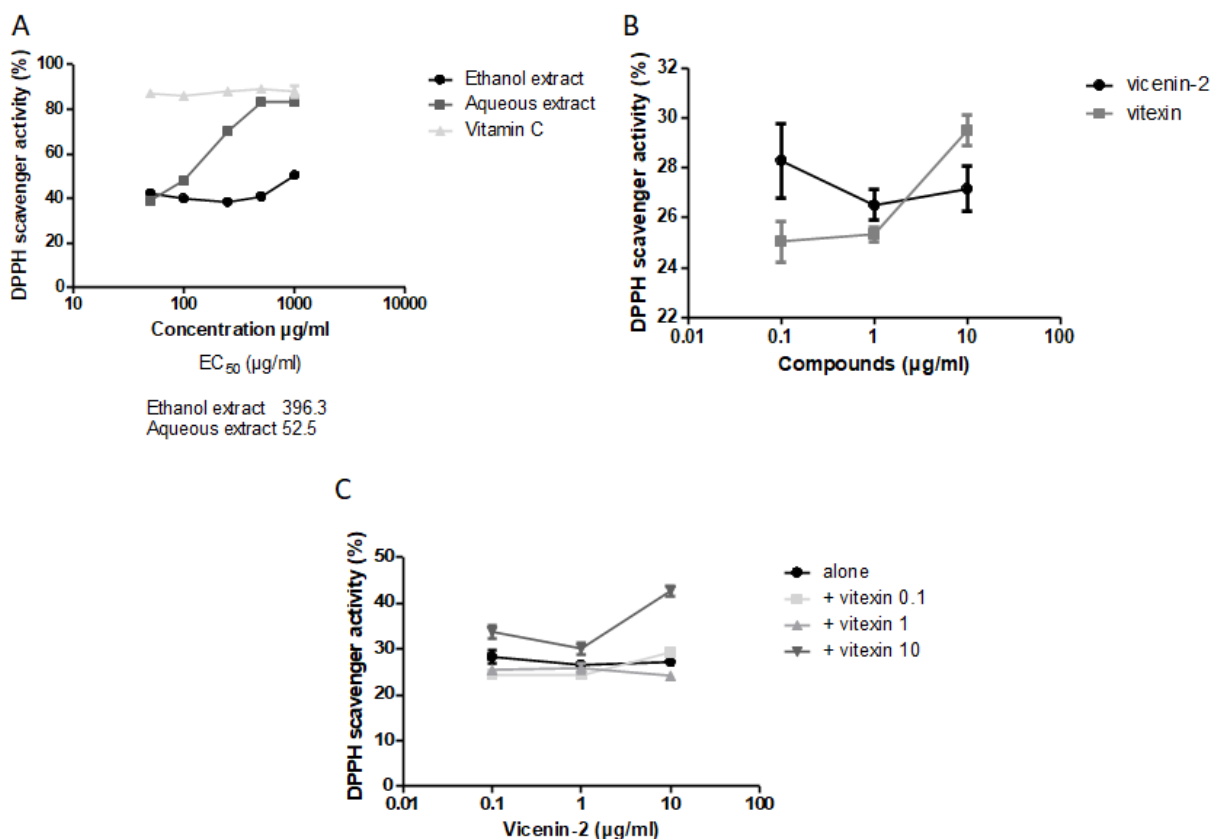


Figure No. 4

## DPPH scavenger activity

**A: DPPH antioxidant activity as a function of the extracts or vitamin C (control) concentration**

**B: DPPH antioxidant activity as a function of the vicenin-2 or vitexin concentration**

**C: DPPH antioxidant activity as a function of the vicenin-2 concentration in the presence of different concentrations of vitexin**

The activity of the ethanol extract could be related to the presence of its major compound, vicenin-2, but also to other compounds that may be not present in the decoction. In this sense, as vitexin was only present in the ethanol extract, its activity and possible participation in the overall effect of the extract was evaluated. The anti-inflammatory effect of vitexin had previously been described. In that work, the authors demonstrated that this compound is capable of decreasing NO and TNF- $\alpha$  levels released by neutrophils stimulated with phorbol 12-myristate 13-acetate (Nikfarjam *et al.*, 2017). In the present study, vitexin was able to diminish the levels of NO by LPS-stimulated macrophages. Moreover, vitexin was more potent than vicenin-2 since vicenin-2 decreased NO levels by 15% at 0.1  $\mu\text{g/mL}$ , by 21% at 1  $\mu\text{g/mL}$ , and by 29% at 10  $\mu\text{g/mL}$ ; while vitexin induced decreases of 26%, 44%, and 80%, respectively. Furthermore, vitexin and vicenin-2 displayed a synergistic effect (98-200%).

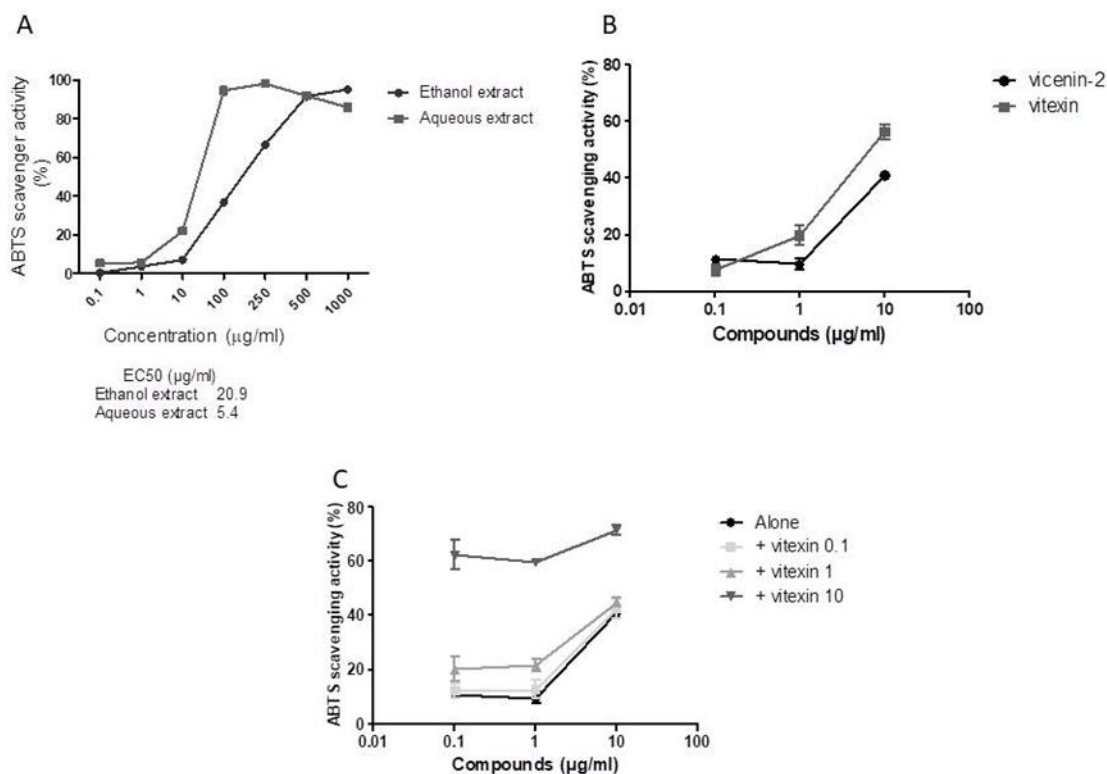
It is interesting to note that under basal conditions, the ethanol extract decreased NO levels while an increase was observed with the aqueous extract. These findings support the hypothesis that compounds that stimulate the production of NO might be present in the decoction and would also explain the differences observed between the ethanol and the aqueous extracts *in vivo*.

Even though the aqueous extract does not exert anti-inflammatory effects, it is used in popular medicine for the treatment of hepatic affections. This use would then be justified by its antioxidant activity, as determined by the DPPH and ABTS free radical scavenging tests. In this regard, the aqueous extract was the most potent since its antioxidant activity was 7.5 times higher for the DPPH test and 3.9 times higher for the ABTS test in comparison with the activity exerted by the ethanol extract. The flavonoids vicenin-2 and vitexin also had antioxidant activity and acted synergistically when tested together. The



high antioxidant activity exerted by the aqueous extract could be attributed to chlorogenic acid, *p*-coumaric acid, and ferulic acid (Kadoma & Fujisawa,

2008), which are present in the aqueous extract at concentrations 3.68 to 100 times higher than those present in the ethanol extract.



**Figure No. 5**

**ABTS scavenger activity**

**A: ABTS antioxidant activity as a function of the extract concentration**

**B: ABTS antioxidant activity as a function of the vicenin-2 or vitexin concentration**

**C: ABTS antioxidant activity as a function of the vicenin-2 concentration in the presence of different concentrations of vitexin**

**CONCLUSION**

*Urtica circularis* has been used in Argentinean folk medicine with different purposes. The preparation of the extract varies according to the purpose. In this sense, for the treatment of inflammation an ethanol extract is recommended, while for oxidative stress-related diseases the aqueous extract is preferred. In this work, the extracts were analyzed phytochemically and the differences in their composition were considered to explain the effects observed. Thus, both popular uses were validated since the presence of vitexin seems to be a determinant factor for the anti-inflammatory activity. Furthermore, this compound was also found to synergize with vicenin-2. The high amount of

vicenin-2 present in the aqueous extract could also explain the lack of anti-inflammatory activity, and the pro-inflammatory effect observed at high doses. On the other hand, the antioxidant activity displayed by the aqueous extract was higher than that of the ethanol extract, which is in line with the popular use. The antioxidant activity of the aqueous extract could be related to either the high content of polyphenols that are also present in the ethanol extract, though in smaller quantities, or to the presence of other antioxidant compounds that were not identified in this work. Taking into account the findings presented herein, it can be concluded the mode of preparation of the extracts strongly influences the biological effect.

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