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Anti-Inflammatory Properties of Extract and Quercetin from *Urtica dioica* L

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Abstract

Introduction: The *Urtica dioica* extract and quercetin have the ability to decrease the inflammatory response, through multiple mechanisms whose consequences are the reduction of pro-inflammatory cytokines, IL-2, IL-1β, IFN γ, TNF-α and TNF-κ.

Objective: The aim of this study was to analyze the anti-inflammatory properties of extract and quercetin from *Urtica dioica* L.

Materials and Methods: The extract was prepared by maceration and dried by spray dryer. The quercetin was isolated by HPLC and it was obtained 263.61 µg/g. *In vitro* anti-inflammatory effect of extract and quercetin was evaluated against denaturation of egg albumin.

Results: The effect of extract (1000 µg/mL) was significantly greater ($p < 0.05$) than quercetin (100 and 200 µg/mL) and diclofenac sodium (100 µg/mL). The effect of extract (100 µg/mL) was significantly greater ($p < 0.05$) than quercetin (100 µg/mL) and 200 µg/mL of extract was more potent than 200 µg/mL of quercetin.

Conclusion: The *Urtica dioica* extract showed a greater anti-inflammatory potential than quercetin, suggesting that the extract may have an anti-inflammatory effect more intense than quercetin.

1. **Keywords**: Anti-Inflammatory Activity; Quercetin; *Urtica Dioica* Extract

2. **Abbreviations**

ELISA	:	Enzyme-Linked Immunosorbent Assay
HPLC	:	High-Performance Liquid Chromatography
IC50	:	Half Maximal Inhibitory Concentration
iNOS	:	Inducible Nitric Oxide Synthase
IFNγ	:	Interferon Gamma
NF-kB	:	Nuclear Factor Kappa B

NO	**:**	Nitric Oxide
TNF-α	**:**	Tumor Necrosis Factor
UV-VIS	**:**	Ultraviolet-Visible
COXs (COX-1 and COX-2)	**:**	Cyclooxygenases
(IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13)	**:**	Interleukins

Introduction

Urtica dioica extracts have the ability to decrease the inflammatory response, through multiple mechanisms whose consequences are the reduction of pro-inflammatory cytokines, IL-2, IL-1β, IFN γ, TNF-α and TNF-κ [1-4] reported that leaves hydroethanolic extract (20, 40, 60 and 80% ethanol) of the *Urtica dioica* inhibits the biosynthesis of arachidonic acid cascade enzymes, particularly, the COX-1 and COX-2, thereby blocking the biosynthesis of prostaglandins and thromboxanes. The IC_{50} values of extract were 160 and 275 µg/mL for COX-1 and COX-2 respectively. This effect was demonstrated on the nuclear factor kappa B (NF-kB) system involved in immune inflammatory response [5,6]. In another study, treatment of different cells with 160 µg/mL of *Urtica dioica* extract potently inhibits transcription factor NF-kB activation. The inhibitory effect was demonstrated in different cell types including T-cells, macrophages and epithelial cells, as well as in response to several stimuli, suggesting that this extract interfered with a common target in the NF-kB pathway [7]. Thus, by decreasing transcription of these various pro-inflammatory genes in concert NF-kB inhibition should modulate several aspects of inflammation. [8] reported that 500 µg/mL of *Urtica dioica* extract in Balb-c mice (female and male) stimulated the proliferation of T-lymphocytes and suppressed nitric oxide production in lipopolysaccharide, even as stimulated macrophages without affecting cell viability.

It is suggested that the anti-inflammatory effect of *Urtica dioica* extracts is related to the presence of flavonoids, such as quercetin, kaempferol and rutin [9-12], because these compounds has been affect the function of T-cell, mast cell and enzyme systems involved in immune response and generation of the inflammatory process, such as inhibition of NF-kB activation, cyclooxygenase enzymes (COX-1 and COX-2) and Inducible Nitric Oxide Synthase (iNOS) [13-15]. In addition, these secondary metabolites inhibited gene expressions, decreased pro-inflammatory mediators (TNF-α, IL-1β, IL-6, and IL-8) in human mast cells [16].

Quercetin has the potential to inhibit inflammatory processes, including eosinophil and neutrophil recruitment, bronchial epithelial cell activation, mucus production and airway hyperactivity [17-19]. These compounds inhibit macrophage-derived cytokines, Nitric Oxide (NO) and Th2 cytokine production, increased IFN-γ and Th1 cytokine production in mice [20-22] reported that quercetin inhibited leukocyte and eosinophil recruitment in the bronchoalveolar lavage fluid, and significantly reduced neutrophil, IL-5 and IL-4 levels. Moreover, it inhibited iNOS expression, COX-2 and NF-kB activation in IL-1β-activated rat hepatocytes [23,24].

Thereby, in this research, we analyzed the anti-inflammatory properties of extract and quercetin from *Urtica dioica* L.

Materials and Methods

Preparation of Extract

Urtica dioica material was collected in Paraíba/Brazil (07º06'54" S, 34º51'47"W), in 2018. The plant material was dehydrated in an oven at 40 ºC and was subsequently milled and pulverized. Two hundred grams of plant powder was subjected to dynamic maceration in 2000 mL hydroethanolic solution (ethanol 70%) for six hours at 1500 rpm. The mixture was filtered using filter paper. The spray-dried filtrate was prepared from a suspension containing 20 % of colloidal silicon dioxide. During the atomization procedure, the mixture was mixed with a magnetic stirring bar. The drying temperature was 160 ºC and the pump flow was 8 mL min^{-1}.

Quantitative Analysis of Total Flavonoids

The content of total flavonoids was measured by UV/VIS spectroscopy using the ELISA Reader. The samples were analyzed in triplicates and the data obtained were expressed as mean ± standard deviation. In 96-well microplate, 100 µL of each test solution and 100 µL $AlCl_3$ (2% w/v) in MeOH were added. After 10 minutes of standing, the absorption of the reaction mixture was measured at 415 nm. The blank was prepared with 100 µL MeOH and 100 µL $AlCl_3$ (2% w/v) in MeOH. The total flavonoids were determined by the calibration curve using standard quercetin at the concentrations of 4, 8, 10, 16, 20, 24 and 28 µg/mL. The results were expressed in mg of quercetin equivalents per gram of extract, determined by the equation of the calibration curve (y=0.0141x+0.12).

Flavonoids Extraction

The extraction was performed in an Erlenmeyer flask with reflux in a water bath for 30 min. The extract was then cooled, filtered, and filled to volume with acetone (100 mL). 25 mL of

this extract were then transferred to a separating funnel, 50 ml, of water was added and extraction with ethylacetate was repeated 3 times with 15 rnL each. The ethylacetate fractions were collected and washed three times with 50 mL of water each, then dried with anhydrous Na_2SO_4 filtered, and evaporated to dryness under low pressure. The residue was dissolved in 10 mL of methanol and this solution was used for identification of flavonoids and quantification of quercetin by HPLC.

HPLC Analysis

It was used HPLC-DAD system prominence series by SHIMADZU, Japan, control system was performed by software LC Solutions; octodecilsilano C18 stationary phase Gemini nx 5 micrometers (µm) 150 x 4.6 millimeters x 0.5 µm; pre-column gemini C18 4 x 3.0 mm; membrane-filtered mobile phase PTFE 0.45 µm and degassed: methanol: phosphoric acid 1% (47: 53%). mobile phase flow: 1.2 ml/min; Oven temperature at 40 °C, monitored wave number at 370 nm, injection volume 20 microliters (µL), chromatographic run time 30 min.

Anti-Inflammatory Activity

Denaturation of tissue proteins is one of the well-documented causes of inflammatory diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo* [25,26]. Agents that can prevent protein denaturation, would be worthwhile for anti-inflammatory drug development [27]. Thus, the *in vitro* anti-inflammatory effect of extract and quercetin from *Urtica dioica* was evaluated against denaturation of egg albumin. The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin, 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of extract and quercetin (100, 200, 400, 600, 800 and 1000 µg/mL for extract, 100 and 200 µg/mL for quercetin). Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37±2 °C in an incubator for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) using vehicle as blank. Diclofenac sodium (100 µg/mL) was used as reference drug and treated similarly for determination of absorbance. The inhibition percentage of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Statistical Analysis

The comparison of the experimental groups was performed by one-way analysis of variance (ANOVA), were considered significant when $p < 0.05$.

Results

Quantification of Total Flavonoids And Quercetin

The concentration of total flavonoids in the dry *Urtica dioica* extract was 53.241±0.003 mg/g. The purity of the quercetin peak was observed by comparing the standard (Figure 1a) and extract sample scanning chromatogram (Figure 1b). The retention time was 10.2 and 9.7 minutes for standard and extract sample, respectively (Figure 1a, Figure 1b). The spectra of both samples showed the purity of the quercetin peaks at 370 nm, and peak purity index of 0.99. It was obtained 263.48 µg/g of quercetin.

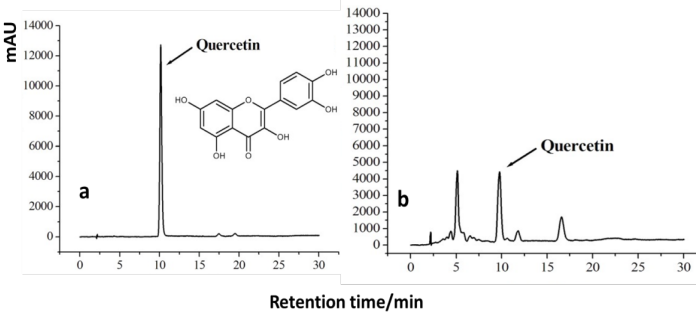

Figure 1: Chromatographic profiles of the quercetin: a) Standard sample and b) Test sample.

Anti-Inflammatory Activity

The *Urtica dioica* extract (100 to 1000 µg/mL) and quercetin (100 and 200 µg/mL) showed concentration dependent inhibition of protein denaturation (Table 1). Diclofenac sodium (100 µg/mL) was used as reference drug which also exhibited inhibition of protein denaturation (Table 1). The effect of extract (1000 µg/mL) was significantly greater ($p < 0.05$) than quercetin (100 and 200 µg/mL) and diclofenac sodium (100 µg/mL). The effect of extract (100 µg/mL) was significantly greater ($p < 0.05$) than quercetin (100 µg/mL) and 200 µg/mL of extract was more potent than 200 µg/mL of quercetin.

Concentration (µg/mL)	% Inhibition		
	Diclofenac	Quercetin	Extract
Control	–	–	–
100	92.45±4.15	26.57±2.63	37.09±1.31
200	–	41.93±1.24	49.71±2.82
400	–	–	63.16±2.73
600	–	–	78.32±1.59
800	–	–	87.87±2.63
1000	–	–	94.16±2.34

Table 1: Effect of quercetin and *Urtica dioica* extract on protein denaturation.

Discussion

Anti-Inflammatory Activity

The present findings exhibited a concentration dependent inhibition of protein denaturation by *Urtica dioica* extract throughout the concentration range of 100 to 1000 µg/mL, and quercetin showed similar results in 100 and 200 µg/mL. Similar results were observed by [26]. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH (pH = 6.2-6.5) [15]. It was observed that the effect of extract was significantly greater than quercetin, suggesting that the anti-inflammatory action of plant extracts may be due to the synergistic effect of several compounds rather than single constituent [28].

Conclusion

The results provide evidence that *Urtica dioica* extract may have a more intense anti-inflammatory effect than quercetin, and may be a suitable raw material for the technological production of anti-inflammatory phytotherapeutic drugs.

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Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. Yilmaz B, Basar Ö, Aktas B, Altinbas A (2014) Effects of *Urtica dioica* extract on experimental acute pancreatitis model in rats. International journal of clinical and experimental medicine 7: 1313-1318.

2. Konrad A, Mahler M, Ari S, Flogerzi B, Klingelhofer S, et al. (2005) Ameliorative effect of IDS 30, a stinging nettle leaf extract, on chronic colitis. International Journal of Colorectal Diseases 20: 9-17.

3. Said AAH, Otmani IS, Derfoufi S, Benmoussa A (2015) Highlights on nutritional and therapeutic value of stinging nettle (*Urtica dioica*). International Journal of Pharmacy and Pharmaceutical Sciences 7: 8-14.

4. Roschek BJr, Fink RC, McMichael M, Alberte RS (2009) Nettle extract (*Urtica dioica*) affects key receptors and enzymes associated with allergic rhinitis. Phytotherapy Research 23: 920-926.

5. Farahpour MR, Khoshgozaran L (2015) Antinociceptive and anti-Inflammatory activities of hydroethanolic extract of *Urtica dioica.* International Journal of Biology, Pharmacy and Allied Sciences 4: 160-170.

6. Safari VZ, Ngugi MP, Orinda G, Njagi EM (2016) Anti-pyretic, Anti-inflammatory and Analgesic Activities of Aqueous Leaf Extract of *Urtica dioica* (L.) in Albino Mice. Medicinal Aromatic Plants 5: 237.

7. Riehemann K, Behnke B, Schulze-Osthoff K (1999) Plant extracts from stinging nettle (*Urtica dioica*) an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-kB. FEBS Letters 442: 89-94.

8. Harput US, Saracoglu I, Ogihara Y (2005) Stimulation of lymphocyte proliferation and inhibition of nitric oxide production by aqueous *Urtica dioica* extract. Phytotherapy Research 19: 346-348.

9. Akbay P, Basaran AA, Undeger U, Basaran N (2003) *In vitro* Immunomodulatory Activity of Flavonoid Glycosides from *Urtica dioica* L. Phytotherapy Research 17: 34-37.

10. Nair MP, Mahajan S, Reynolds JL, Aalinkeel R, Nair H, et al. (2006) The flavonoid quercetin inhibits proinflammatory cytokine (Tumor necrosis factor alpha) gene expression in normal peripheral mononuclear cells via modulation of the NF-κB system. Clinical and Vaccine Immunology 13: 319-328.

11. Joskova M, Franova S, Sadlonova V (2011) Acute bronchodilator effect of quercetin in experimental allergic asthma. Bratisl Lek Listy 112: 9-12.

12. Hajhashemi V, Klooshani V (2013) Antinociceptive and anti-inflammatory effects of *Urtica dioica* leaf extract in animal models. Avicenna Journal of Phytomedicine 3: 193-200.

13. Middleton E, Kandaswami C (1992) Effects of flavonoids on immune and inflammatory cell function. Biochemical Pharmacology 43: 1167-1179.

14. Kwon KH, Murakami A, Tanaka T, Ohigashi H (2005) Dietary rutin, but not its aglycon quercetin, ameliorates dextran sulfate sodium-induced experimental colitis in mice: attenuation of pro-inflammatory gene expression. Biochemical Pharmacology 69: 395-406.

15. García-Mediavilla V, Crespo I, Collado PS, Esteller A, Sánchez-Campos S, et al. (2007) The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2, and reactive C-protein, and down-regulation of the nuclear factor kappa B pathway in Chang Liver cells. European Journal of Pharmacology 557: 221-229.

16. Park HH, Lee S, Son HY, Park SB, Kim EJ, et al. (2008) Flavonoids inhibit histamine release and expression of proinflammatory cytokines in mast cells. Archives of Pharmacal Research 31: 303-1311.

17. Rogerio AP, Kanashiro A, Fontanari C, Da Silva EV, Lucisano-Valim YM, et al. (2007) Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma. Inflammation Research 56: 402-408.

18. Park HJ, Lee CM, Jung ID, Lee JS, Jeong YI, et al. (2009) Quercetin regulates Th1/Th2 balance in a murine model of asthma. International Immunopharmacology 9: 261-267.

19. Fortunato LR, Alves CF, Teixeira MM, Rogerio AP (2012) Quercetin: a flavonoid with the potential to treat asthma. Brazilian Journal of Pharmaceutical Sciences 48: 589-599.

20. Mamani-Matsuda M, Kauss T, Al-Kharrat A, Rambert J, Fawaz F, et al. (2006) Therapeutic and preventive properties of quercetin in experimental arthritis correlate with decreased macrophage inflammatory mediators. Biochemical Pharmacology 72: 1304-1310.

21. Saggini A, Maccauro G, Tripodi D, De Lutiis MA, Conti F, et al. (2011) Allergic inflammation: role of cytokines with special emphasis on IL-4. International Journal of Immunopathology and Pharmacology 24: 305-311.

22. Rogerio AP, Dora CL, Andrade EL, Chaves JS, Silva LFC, et al. (2010) Anti-inflammatory effect of quercetin-loaded microemulsion in the airways allergic inflammatory model in mice. Pharmacological Research 61: 288-297.

23. Raso GM, Meli R, Di Carlo G, Pacilio M, Di Carlo R (2001) Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A.1. Life Sciences 68: 921-931.

24. Martínez-Flórez S, Gutiérrez MB, Sánchez-Campos S, González-Gallego J, Tuñón MJ (2005) Quercetin prevents nitric oxide production and nuclear factor kappa B activation in interleukin-1β-activated rat hepatocytes. Journal of Nutrition 135: 1359-1365.

25. Opie EL (1962) On the relation of necrosis and inflammation to denaturation of proteins. Journal of Experimental Medicine 115: 597-608.

26. Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, et al. (2010) An experimental evaluation of Albuca setosa aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. Journal of Medicinal Plants Research 4: 789-795.

27. Anson ML, Mirsky AE (1932) The effect of denaturation on the viscosity of protein systems. Journal of General Physiology 15: 341-350.

28. Abdulkadir MN, Adedokun A, John E (2015) Phytochemical composition and antimicrobial evaluation of *Kigelia africana* LAM. Asian Journal of Plant Science and Research 5: 14-17.