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Chemical study and anti-inflammatory activity of *Capsicum chacoense* and *C. baccatum*

Paula López,[1] Susana Gorzalczany,[2] Cristina Acevedo,[2] Rosario Alonso,[1] Graciela Ferraro*,[1]

[1]Cátedra de Farmacognosia, IQUIMEFA (UBA-CONICET), Argentina;
[2]Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

Abstract: *Capsicum* species, Solanaceae, have been used for centuries as food additives by populations of different regions of the world. Capsaicin (*trans*-methyl-*N*-vainillyl-nonenamide) is the main pungent compound extracted from the red hot pepper fruit (*Capsicum* spp.). The capsaicin content was determined by means of a HPLC method. The results showed that *Capsicum chacoense* Hunz., contains similar amounts of capsaicin (13.9 mg/100 g of dry fruit) in comparison with *Capsicum baccatum* L. (12.6 mg/100 g) and *Capsicum annum* L. (10.1 mg/100 g). Dichloromethane (CH_2Cl_2) and ethanol (EtOH) extracts of *C. chacoense* elicited a 46% and 38 % of inhibition on the arachidonic acid (AA) pathway in ear edema respectively while the CH_2Cl_2 and EtOH extracts of *C. baccatum* inhibited 52% and 35% the arachidonic acid response respectively.

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Introduction

Capsicum, or red hot chili pepper is a popular culinary spice valued for its sensory attributes of color, pungency and flavor. There are about 23 species of chili peppers (genus *Capsicum*) around the world, but only 4 or 5 of them are cultivated. Due to its chemical composition, nutritional and pharmacological properties *C. annuum* has been thoroughly reviewed (Topuz & Odzemir, 2007; Pino et al., 2007).

In Argentina there are two native *Capsicum* species: *Capsicum chacoense* Hunz and *Capsicum baccatum* L., Solanaceae. *C. chacoense* is a bush of 60 to 90 cm height, very ramified from the base, with alternating leaves, acuminated in their superior part, rounded in the base. The fruits are oval red 8-14 mm long berries. This specie is characteristic of areas of South America ranging from the south of Bolivia and west of Paraguay to the central region of Argentina. Its popular names are "ají del monte", "puta parió". *Capsicum baccatum* is a bush of a little more than half a meter, very laxly pubescent, with oval leaves, acuminated. Their fruits are red oval berries 10 mm long. It grows in the south of Bolivia and Brazil. In Paraguay and in the north of Argentina it is usually found in the lower grounds of the forest. Its popular name is "Ají quitucho". For centuries both species have been used locally as spicy food additives and in traditional medicine (Cabrera, 1983; Loayza, 2001).

Capsaicin (*trans*-methyl-*N*-vainillyl-nonenamide) is the most pungent of the active group of compounds known as capsaicinoids that have been isolated from the genus. It is sparingly soluble in water, very soluble in fats, oils and alcohol. Other minor capsaicinoids are nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin. Capsaicin is extracted together with its homologues from the mature fruits placentas external layers of *Capsicum* species. The capsaicin content depends on the botanical species, the climatic conditions and of the degree of the fruit maturity degree (Howard et al., 2000).

Capsaicin binds to the vanilloid receptor type 1 on sensory neurons to convey the pain sensation. The topical application of capsaicin has a therapeutic value in a variety of neuropathic pain conditions such as rheumatoid arthritis, diabetic neuropathy and postherpetic neuralgia (Bruneton, 2001; McCarthy & McCarthy, 1992).

Topical creams containing 0.025-0.075% pure capsaicin, now available in the form of a prescription drug, are applied to the skin to control the pain associated with *Herpes Zoster* - also known as shingles-, neuralgia and postoperative amputation trauma (Brooks et al., 2002). In addition, scientists have also observed that people whose diet is high in red peppers experienced a much lower incidence of blood clotting diseases (Jaiarj et al., 1998).

Taking into account the information described, it was therefore reasonable to study *C. chacoense* capsaisin content and anti-inflammatory activity, which

have not been previously studied and compare them with known *Capsicum* spp. species (*Capsicum annum* and *C. baccatum*).

Material and Methods

Plant material

Capsicum annum L., Solanaceae, was obtained from a cultivated commercial source. *Capsicum chacoense* Hunz., Solanaceae, was harvested in Tartagal, Salta and was provided and classified by R. Newmann, INTA. Salta, Argentina and *C. baccatum* L. was provided and classified by Prof. Dr. D. Vignale, Jujuy, Argentina. Both voucher herbarium specimens have been deposited at the herbarium of the Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina: *C. chacoense* Hunz. (13520 BAF) and *C. baccatum* L. (BAF 8694).

Plant extracts

Dried fruits (10 g) of each species were extracted three times with 100 mL of dichloromethane followed by extraction with ethanol 80° using the same procedure. The CH_2Cl_2 and EtOH extracts were evaporated to dryness.

Purification of extracts for HPLC analysis

One part of each CH_2Cl_2 extract was mixed with methanol: water (70:30) and was left 24 h in the refrigerator. The solvent was decanted and the solid residue was separated. The methanol fraction was evaporated to dryness (Perucka & Oleszek, 2000).

HPLC method

Preparation of the samples: 150 mg of the purified dichloromethane extracts were dissolved in 0.2 mL of DMSO and were taken to volume up to 10.0 mL with methanol.

Standard solution: capsaicin (1.40 mg, SIGMA) was dissolved in 1.0 mL of DMSO and added methanol to 10.0 mL.

The analytical column used was RP18 (5 mm, 250 x 4.6 mm). The controlled solvent gradient conditions were as follows: 0-50 min, linear gradient from 0% to 100% CH_3CN at 1 mL/min in 0.1% aqueous trifluoroacetic acid; 50-65 min, 100% CH_3CN. The injection volume was 20 mL.

The effluent was monitored by a UV-detector (280 nm) and a photodiode-array detector (Sakamoto et al., 1994; Perucka & Oleszek, 2000; Manizakiza et al., 1999).

Animals

The animals were housed in standard environmental conditions (23±1 °C, humidity 60±5% and a 12 h light/dark cycle), with free access to a standard commercial diet and water *ad libitum* following international recommendations (Olfertet et al., 1993). Ethics approval (EXP-FYB: 0738658/2011) was from The Ethical Committee for the Care and Use of Laboratory Animals of Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

Arachidonic-acid induced ear edema

Female Swiss mice (25-30 g) were used. They were acclimatized in the laboratory for at least one week before the experience with free access to food and water. Mice were randomly assigned into groups consisting of ten animals per group. Arachidonic acid (AA) (Sigma) was dissolved in reagent-grade acetone at a concentration of 100 mg/mL and applied in a volume of 10 µL (2 mg AA/ear) by an automatic pipette to both the inner and the outer surfaces of the right ear.

CH_2Cl2 and EtOH extracts of *C. baccatum* and *C. chacoense* dissolved in acetone were applied topically (1 mg/ear) inmmediately after the application of AA. A reference group was treated with indomethacin (2 mg/ear).

One hour after AA treatment, mice were sacrificed and an ear punch of 6 mm was removed from both right and left ears. The ears were weighed and the edema induced by AA was determined by subtracting the weight of the left punch biopsies from that of the right ear. The anti-inflammatory activity was calculated and was expressed as a percentage of the edema inhibition in treated mice compared to the control mice (Carlson et al., 1985).

Carrageenan-induced edema in rats

Antiinflamatory activity was measured according to Winter et al. (1962) in female Wistar rats (170-200 g). Paw swelling was elicited by subplantar injection of 0.1 mL 1% carrageenan in 0.9% saline (w/v) in to the right hind paw.

Rats were starved for 18 h before the experiment with free access to water. Groups of six animals each were used. CH_2Cl_2 and EtOH extracts (300 mg/kg) were dissolved in ethanol:tween 80:water 5:5:90 and the reference drug indomethacin (3 mg/kg) were administered orally 1 h before carrageenan injection. A control group received vehicle only (5 mL/kg). The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer (Ugo Basile) at time 0 and 1, 3, 4 and 5 h after carrageenan injection. The difference

between the left and the right paw volume was determined and the percent inhibition of edema was calculated in comparison to the control animals.

Results and Discussion

The present work showed that the percentage of capsaicin of the two species of *Capsicum* that grow in North and Central Argentine regions (*C. chacoense* and *C. baccatum*) is similar to the one in *C. annum*, the most commercialized *Capsicum* species in the world. As capsaicin represents the main constituent of the total pungent acid amides present in the *Capsicum* species (Calixto et al., 2005) its content was determined by HPLC. Similar concentrations in the three species were found. *C. chacoense* showed the highest content (Table 1). The recovery of capsaicin was determined as 99.2 %, providing there was no loss of capsaicin in the purification process.

Table 1. Capsaicin content in *Capsicum* species.

	mg capsaicin/g CH_2Cl_2 extract	mg capsaicin/100 g dry fruit
C. annum	0.86±0.03	10.1±0.3
C. chacoense	1.10±0.05	13.9±0.6
C. baccatum	1.16±0.04	12.6±0.4

Results are expressed as means ± SD (n=7)

Capsaicin as single oral dose moderately lowered inflammation in the carrageenan induced paw edema (Manjunatha & Srinivasan, 2006; Reddy & Lokesh, 1994). Moreover *C. baccatum* juice demonstrated an anti-inflammatory activity in a peritonitis animal model induced by carrageenan (Spiller et al., 2008). Polar and non polar extracts obtained from fruits of *C. chacoense* and *C. baccatum* were tested using two classical models of inflammation, AA-induced ear edema in mice and carrageenan induced paw edema in rats.

This study demonstrates for the first time that the CH_2Cl_2 and EtOH extract of *C. chacoense* (1 mg/ear) produced a significant inhibition of the AA-ear edema (46 and 38% respectively) and in a similar way, *C. baccatum* at the same dose induced a 52 and 35% inhibition respectively (Table 2). However, the oral administration (300 mg/kg) of all tested extracts showed no antiinflamatory activity in the carrageenan-induced paw edema (Table 3).

The vanilloid receptor 1, TRPV1, was the first mammalian member of TRPV subfamily to be identified. It is activated by vanilloid compounds such as capsaicin and resiniferatoxin. The intracellular binding domain has recently been located (Pedersen et al., 2005). Traditionally, this receptor has been considered as a pathological one, having a role in pain transduction pathway as well as pro-inflammatory effect. However, in the last years, a paradoxical protective role in certain disease state has been shown and this effect is continually expanding. Interestingly, TPRV1 are involved in the reduction of ear edema and TNF-α levels in an animal model of contact dermatitis (Alawi & Keeble, 2010; Banvolgyi et al., 2005). Due to the effect was observed only by topical administration and capsaicin is one of the active compounds, it is possible that the TPRV1 receptors could be involved in the activity observed in this study. However it can't be discarded the absorption of the extract had not been enough to show systemic activity.

In conclusion, the present data shows that *C. chacoense* and *C. baccatum* could be potential substitutes for *Capsicum annum* in human nutrition, as phytomedicines and sources for drugs pharmaceutical development.

Table 2. Effect of *Capsicum chacoense* and *C. baccatum* extracts on AA- induced mouse ear edema.

Treatment	Edema (mg)	Inhibition (%)
Control	7.36±0.44	-----
C. chacoense EtOH	4.3±0.61*	38.55
C. chacoense CH_2Cl_2	3.97±0.91**	46.06
C. baccatum EtOH	4.73±0.63*	35.73
C. baccatum CH_2Cl_2	3.50±1.09**	52.44
Indomethacine	2.62±0.39**	64.40

Results are expressed as means±SD (n=10). *p<0.05, **p<0.01 vs control. (ANOVA followed Dunnett's test).

Table 3. Effect of *C. chacoense* and *C. baccatum* extracts on carrageenan-induced paw edema in rat.

Treatment	Edema volume (mL)			
	1 h	3 h	4 h	5 h
Control	0.21±0.03	1.06±0.08	1.49±0.07	1.70±0.08
C. chacoense CH_2Cl_2	0.18±0.06	1.42±0.21	2.03±0.04	2.05±0.10
C. chacoense EtOH	0.19±0.03	1.36±0.11	1.86±0.16	2.01±0.17
C. baccatum CH_2Cl_2	0.26±0.07	1.51±0.15	1.85±0.07	1.92±0.13
C. baccatum EtOH	0.20±0.05	1.21±0.14	1.56±0.09	1.54±0.13
Indomethacine	0.19±0.03	0.43±0.07*	0.94±0.11*	1.30±0.13*

Results are expressed as means±SD (n=5). *p<0.01 vs control. (ANOVA followed Dunnett's test).

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***Correspondence**

Graciela Ferraro
Cátedra de Farmacognosia, IQUIMEFA (UBA-CONICET)
Junín 956 (1113), Buenos Aires, Argentina
gferraro@ffyb.uba.ar
Tel: +54 11 4964 8247/8
Fax: +54 11 4508 3642