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Centaurium cachanlahuen (Mol.) Robinson, a Chilean native plant with a vasodilatory effect

[*Centaurium cachanlahuen* (Mol.) Robinson una planta nativa chilena con efecto vasodilatador]

Raúl VINET[a,b,*] , Magdalena CORTÉS[a], Rocío ÁLVAREZ[a], Leda GUZMÁN[c], Eva FLORES[a]

[a]Laboratorio de Farmacología y Bioquímica, Facultad de Farmacia, Universidad de Valparaíso, 2360102 Valparaíso, Chile.
[b]Centro Regional de Estudios en Alimentos Saludables (CREAS), 2362696 Valparaíso, Chile.
[c]Departamento de Bioquímica, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, 2373223 Valparaíso, Chile.
****Contactos | Contacts: Raúl Vinet - E-mail address: rvinet@uv.cl***

Abstract
Centaurium cachanlahuen (Mol.) Robinson is a chilean native plant widely used in traditional medicine for the treatment of many diseases, including cardiovascular disorders. Studies carried out in normal and hypertensive rats suggested that extract of *Centaurium cachanlahuen* has antihypertensive effect. In this work, we aim to evaluate the effect of aqueous and hydroalcoholic extracts of *Centaurium cachanlahuen* on the vascular reactivity using rat aorta rings precontrated with phenylephrine (0.1 µM). Results showed that both aqueous (3 mg/mL) and hydroalcoholic extracts (3 mg/mL) produced rat aorta vasodilatation that was higher ($P < 0.001$) in the hydroalcoholic extract compared to the aqueous extract. This effect had an important endothelium-dependent component that was mediated by nitric oxide (NO), as supported by the inhibition of the response in the presence of N-nitro-L-arginine (L-NNA, 100 µM), a nitric oxide synthase (NOS) inhibitor. We suggest that xanthones present in the plant may play a key role in the vasodilator effect of *Centaurium cachanlahuen* extracts. The present study provides experimental evidence supporting the folkloric use of *Centaurium cachanlahuen* as hypotensive agent.

Keywords: *Centaurium cachanlahuen,* chilean native plant, vasodilator activity, vasorelaxation.

Resumen
Centaurium cachanlahuen (Mol.) Robinson es una planta nativa chilena ampliamente utilizada en medicina tradicional para el tratamiento de varias enfermedades, que incluyen alteraciones cardiovasculares. Estudios llevados a cabo en ratas normales e hipertensas sugieren que el extracto de *Centaurium cachanlahuen* tiene efecto antihipertensivo. El propósito de este trabajo fue evaluar el efecto de extractos acuosos e hidroalcohólicos de *Centaurium cachanlahuen* sobre la reactividad vascular de aorta de rata precontraída con fenilefrina (0.1 µM). Tanto el extracto acuoso (3 mg/mL) como el extracto hidroalcohólico (3 mg/mL) produjeron relajación de aorta de rata, la cual fue de mayor magnitud ($P < 0.001$) con el extracto hidroalcohólico respecto del extracto acuoso. El efecto observado tuvo un importante componente mediado por óxido nítrico (NO), tal como lo demuestra la inhibición de esta respuesta en presencia de N-nitro-L-arginina (L-NNA, 100 µM), un inhibidor de la óxido nítrico sintasa (NOS). Se sugiere que las xantonas presente en la planta pueden jugar un papel clave en el efecto vasodilatador observado por los extractos de *Centaurium cachanlahuen*. Este estudio constituye una evidencia experimental que apoya el uso popular de *Centaurium cachanlahuen* como agente hipotensor.

Palabras Clave: *Centaurium cachanlahuen*, planta nativa chilena, actividad vasodilatadora, relajación vascular.

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INTRODUCCIÓN

Centaurium cachanlahuen (Mol.) Robinson is a chilean native plant belonging to the Magnoliophyta, Gentianiaceae Family (Cronquist, 1988). Annual herb 5-40 cm tall, erect and smooth. Stem quadrangular, leaves opposite, sessile and entire, 1-3 cm long by 0.5-2 cm wide, the upper smaller. Deep-pink flowers, peduncles 1-4 mm length. Fruit in membranous capsule up to 1.2 cm long. Seeds numerous, oval, rough, 0.2-0.3 mm long. It is endemic to Chile and has a distribution between Atacama and Valdivia, from the Andes Mountains to the coast, also present in the Juan Fernández Archipelago (Montes and Wilkomirsky, 1985; Muñoz *et al*., 2004).

Centaurium cachanlahuen infusion is widely used in chilean traditional medicine for its anti-rheumatic (Houghton and Manby, 1985), analgesic/anti-inflammatory (Estomba *et al*., 2006) and hypotensive (Muñoz *et al*., 2004) therapeutic effects. The name *cachanlahuen* is "mapudungun", the language of the Mapuche. It is a compound word which comes from the word "kacán", which means "side ache" and "lawen" which means remedy; literally translated means "side ache remedy" (Gunkel, 1960). Previously isolated compounds in this plant indicate the presence of swertiaperenine, swercherine, decusatine, oleanolic acid and the xanthone 1, 8-dihydroxy-2, 3, 4, 6-tetramethoxy-9H-xanthone (Versluys *et al*., 1982). Other constituents include tannins, resins, mucilage, albumins, fatty acids and eritrocentaurine (Montes and Wilkomirsky, 1985).

Evidence shows that oral administration of aqueous extract of *Centaurium cachanlahuen* in hypertensive rats decreased systolic blood pressure, compared with control group, suggesting the presence of chemical(s) with antihypertensive property (Gómez, 1988). Similar results have been reported in normotensive rats (Schmeda-Hirschmann *et al*., 1992).

According to the hypotensive use of this plant, the aim of the present study was to evaluate the vasodilator activity of aqueous and hydroalcoholic extracts of *Centaurium cachanlahuen* on isolated rat aorta. The role of endothelium, and especially the involvement of NO in this response, was evaluated.

MATERIALS AND METHODS

Plant material and extraction

The plant was collected in Olmué, Chile in November 2008. Plant material and extracts preparation were carried at Laboratorio de Botánica, Facultad de Farmacia, Univerdidad de Valparaíso, Chile. The species and a voucher specimen (register Nº 1589) can be found in the Jardín Botánico Nacional Herbarium, Viña del Mar, Chile.

Aqueous extract of dried and powered plant material (stems, flowers and leaves) was obtained in preboiled hot water (10% w/w) with occasional stirring for 20 min. Aqueous extract was filtered and evaporated *in vacuo*. The dried extract (yield: 21.9% w/w) was stored at 4 °C. Hydroalcoholic extract of dried and powered plant material (stems, flowers and leaves) was obtained at room temperature with ethanol-water (1:1) after 5 days maceration and then filtered and evaporated *in vacuo*. The dried extract (yield: 14.8% w/w) was stored at 4 °C. Appropriate stock solutions were prepared on the day of the experiment. Phytochemical screening (Trease and Evans, 1989) gave positive tests for alkaloids, flavonoids (rutin and quercetin), mucilages, resins, saponins and tannins.

Animals

Male Sprague-Dawley rats weighing 200-300 g were used. They were housed in a standard environmental condition. Food and water were freely available. Animals were used in accordance with the United States animal use and care guidelines (NIH publication #85-23, 1985).

Preparation of rat thoracic aorta rings and recording

Rats were killed by decapitation and the thoracic aorta was carefully removed and mounted on a tissue chamber as previously described (Vinet *et al*., 1991). Briefly, aorta were dissected, clean of connective tissue and divided into 5 mm rings segments. In some rings, endothelium was mechanically removed by gently rubbing the intimal surface of the vessel with a stainless steel rod. The rings were suspended between two L-shaped stainless steel hooks and placed in a 30 mL organ chamber containing a modified Krebs-Henseleit solution, maintained at 37° C and oxygenated continuously with a 95% O_2 - 5% CO_2 gas mixture as described elsewhere (Vinet *et al*., 1991).

Isometric tensions were measured using a FT-03 (Grass Instruments) force displacement transducer connected to a Grass polygraph. The rings were allowed to equilibrate in the tissue bath for 60 min under an optimal resting tension of 1.5 g. Integrity of endothelium was assessed by testing the relaxation produced by the addition of acetylcholine (1 µM) in phenylephrine (0.1 µM)-precontracted rings. Aortic rings were repeatedly washed and allowed to re-equilibrate for an additional 30 min before testing the

extracts. After this period the rings were precontracted with phenylephrine (0.1 µM) and once a stable contraction was achieved, cumulative concentration-response curves were obtained by a stepwise increase in the extract concentration (10^{-4} to 10 mg/mL). Nitro-L-arginine (L-NNA, 100 µM) was incubated with the tissue 10 min before extract addition.

Maximum relaxation and concentration inducing 50% of maximal relaxation (EC_{50}) were determined from each concentration-response curve as described earlier (Fleming *et al*., 1972) and was expressed as pD_2 (-log EC_{50}). The relaxation from the precontracted level to the baseline was considered as 100% relaxation.

Statistical analysis

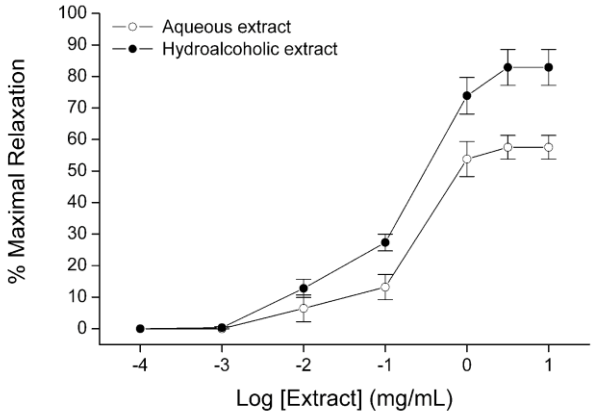
All data are expressed as mean ± standard error (SE). The significance of differences was assessed by Student's *t*-test for unpaired data. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Centaurium cachanlahuen aqueous and hydroalcoholic extracts induced a concentration-dependent vasorelaxation in aortic rings with functional endothelium precontracted with phenylephrine (0.1 µM) (Figure 1). Maximal relaxation induced by the hydroalcoholic extract (82.9 ± 5.7 %) was significantly larger ($P < 0.05$) than the effect produced by the aqueous extract (57.5 ± 3.4 %). pD_2 values calculated for the aqueous (0.598 ± 0.071) and hydroalcoholic extracts (0.723 ± 0.056) showed no significant differences.

To evaluate the participation of endothelium and nitric oxide (NO) in the vasodilatation induced by *Centaurium cachanlahuen* extracts, the subsequent experiments were run with the hydroalcoholic extract. Maximal vasodilatation induced by this extract was significantly larger ($P < 0.001$) in aortic rings with endothelium (84.2 ± 5.1 %) than without endothelium (30.3 ± 3.5%) (Figure 2A). There were no significant differences between pD_2 values, in the presence (0.716 ± 0.063) and in the absence (0.575 ± 0.052) of functional endothelium.

Figure 1



Concentration-response curve for relaxation induced by *Centaurium cachanlahuen* aqueous and hydroalcoholic extracts on phenylephrine (0.1 µM)-precontracted rat aortic rings with functional endothelium. Each data point represent mean ± SE of n = 6 experiments.

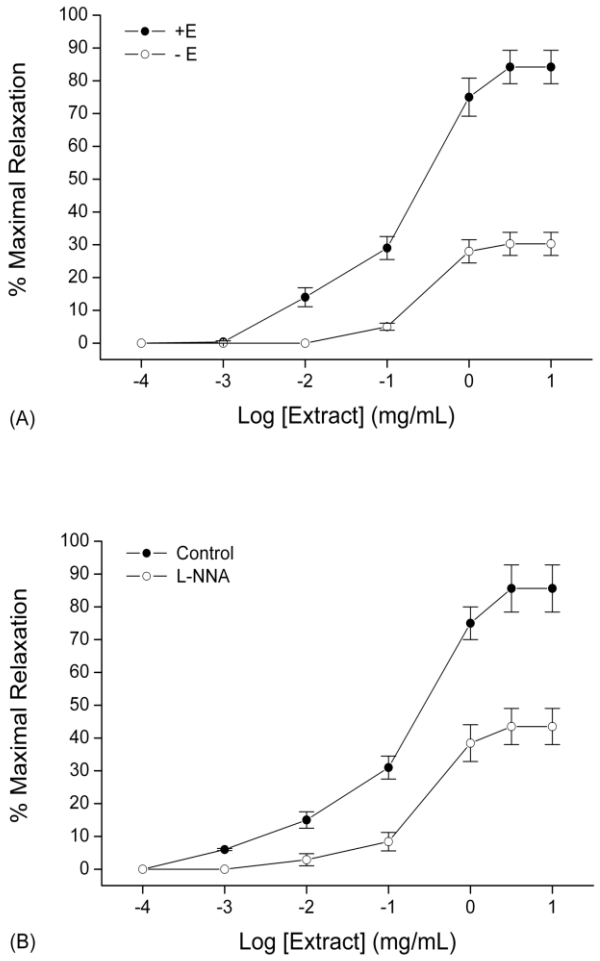
To study the involvement of NO in the vasodilatory effect induced by *Centaurium cachanlahuen* extract we used L-NNA to block NO synthesis. L-NNA significantly reduced ($P < 0.001$) the maximal relaxation elicited by the hydroalcoholic extract (43.5 ± 5.5%) compared to control (82.9 ± 4.7%) (Figure 2B). There were no significant differences between pD_2 values, in the presence of L-NNA (0.564 ± 0.068) compared to the control vessels (0.722 ± 0.074).

The present work is the first report on the vasodilatory effects of *Centaurium cachanlahuen* extract in rat aortic rings. The higher vasodilatory effect (efficacy) of the hydroalcoholic extract compared with the aqueous extract suggest that hydroalcoholic extraction was more efficient in obtain bioactive substances. Consequently, similar pD_2 values indicate that both extracts have the same potency.

The extracts vasodilatory effect was mainly endothelium-dependent, since mechanical removal of the endothelium significantly reduced the maximal relaxation. Participation of NO was revealed by the drastic decrease of maximal relaxation in the presence of L-NNA, a known NO synthase inhibitor (Rees *et al*., 1990). Because a fraction of the aortic vasorelaxation induced by the extracts was observed in the absence of endothelium or in the presence of L-NNA, the study shows evidences of a direct

vasodilatory effect of the extract on aortic smooth muscle cells.

Figure 2



Effect of endothelium removal and nitro-L-arginine (L-NNA) on *Centaurium cachanlahuen* hydroalcoholic extract-induced relaxation in rat aortic rings. (A) Concentration-response curves in the presence (+E) and absence (-E) of functional endothelium. (B) Concentration-response curves in the presence and absence of L-NNA (100 µM). Each data point represent mean ± SE of n = 6 experiments.

The vasodilator effect induced by extracts of *Centaurium cachanlahuen* may be attributed to the xanthones that have been identified in this plant (Versluys *et al*., 1982). The vasodilator action of 1-hydroxy-2, 3, 5-trimethoxyxanthone (HM-1) and 1, 5-dihydroxy-2, 3-dimethoxy-xanthone (HM-5), xanthones isolated from *Halenia elliptica*, a Tibetan medicinal herb, support this hypothesis; as the relaxation induces by extracts of *Centaurium cachanlahuen*, the effect of HM-1 and HM-5 involved both an endothelium-dependent mechanism implicating NO and an endothelium-independent mechanism (Wang *et al*., 2007; Wang *et al*., 2008).

On the other hand, epidemiological studies indicate that consumption of dietary polyphenolic compounds, as xanthones, is beneficial in the prevention of cardiovascular diseases, including ischemic heart disease, atherosclerosis, hypertension and thrombosis; the protective effects of xanthones in the cardiovascular system may be due to their antioxidant, antiinflammatory, platelet aggregation inhibitory, antithrombotic and/or vasorelaxant activities (Jiang *et al*., 2004; Schini-Kerth *et al*., 2011).

The present study provides experimental evidence supporting the folkloric use of *Centaurium cachanlahuen* as hypotensive agent. Further chemical and pharmacological investigations to understand the specific mechanism of action involved in the vasodilatory effect of *Centaurium cachanlahuen* extract, are required.

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