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Endothelium-dependent and independent vasorelaxation induced by an n-butanolic fraction of bark of *Scutia buxifolia* Reiss (Rhamanaceae)

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ABSTRACT

Ethnopharmacological relevance: Scutia buxifolia has been widely used in Brazilian folk medicine as an anti-hypertensive agent.

We evaluated the vascular effects and mechanism involved in the relaxation of aorta induced by an n-butanolic fraction (BuOH) from *Scutia buxifolia*.

Materials and Methods: Rat aortic rings precontracted by phenylephrine (1 μM) were exposed to cumulative concentrations (3–3000 μg/ml) of crude extracts or fractions obtained from bark or leaves of *Scutia buxifolia*. Classical receptor antagonists, channel and enzymatic inhibitors were used to check the mechanisms involved.

Results: The crude extracts of both leaves and bark of *Scutia buxifolia*, as well as several fractions, were able to induce partial or total relaxation of rat aortic rings. The BuOH fraction of bark of *Scutia buxifolia* was the most potent in endothelium-intact (E+) preparations, and also induced a partial, but very significant relaxation in endothelium-denuded (E−) vessels. The non-selective nitric oxide synthase inhibitor L-NAME, as well as the soluble guanylate cyclase inhibitor ODQ, vanished the relaxation in E+. In E− preparations, K^+ channel blockers, such as tetraethylammonium, glibenclamide, 4-aminopyridine, and the large-conductance calcium-activated K^+ channel blocker iberiotoxin, were able to significantly reduce the maximum relaxation elicited by BuOH fraction.

Conclusion: Our results demonstrated that BuOH fraction obtained from barks of *Scutia buxifolia* induced both endothelium-dependent and -independent relaxation in rat aortic rings. The endothelium-dependent relaxation is fully dependent on NO/cGMP system, while direct activation of K^+ channels may explain, at least in part, the endothelium-independent relaxation induced by BuOH fraction of *Scutia buxifolia*.

1. Introduction

Scutia buxifolia Reissek (Rhamnaceae), popularly known in Brazil as "coronilha", is a native tree of Southern Brazil, Uruguay and Northern Argentina. In these regions, an aqueous infusion prepared with stem bark of *Scutia buxifolia* has been described and widely used in folk medicine for diuretic and antihypertensive purposes (Wasicky et al., 1964). Phytochemical screening of fractions of bark of *Scutia buxifolia* revealed the presence of cyclopeptide alkaloids (Maldaner et al., 2011), polyphenols and flavonoids in fractions from leaves and stem bark of *Scutia buxifolia* (Boligon et al., 2012, 2009a, 2011). Among the few studies that were conducted, alkaloids isolated from *Scutia buxifolia* displayed *in vitro* antimicrobial activity (Menezes et al., 1995; Morel et al., 2005; Maldaner

Abbreviations: ACh, acetylcholine; AcOEt, ethyl acetate fraction; BK_{Ca}, large conductance Ca^{2+}-activated K^+ channel; BuOH, butanolic fraction; cGMP, guanosine 3′,5′-cyclic monophosphate; DCM, dichloromethane fraction; GLI, glibenclamide; ibTX, iberiotoxin; L-NAME, N_ω-nitro-L-arginine methyl ester; NO, nitric oxide; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; PE, phenylephrine; PSS, physiological saline solution; TEA, tetraethylammonium.

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et al., 2011), while polyphenols and flavonoids were associated with antioxidant and antymycobacterial activity (Boligon et al., 2011, 2009b).

Despite its traditional usage, there is a lack of data supporting or refuting the anti-hypertensive properties of *Scutia buxifolia*. Hypertension is one of the most prevalent cardiovascular diseases around the world. As a chronic disease, untreated hypertension can lead to several complications, such as stroke, heart failure and kidney dysfunction. The current pharmacological treatment of hypertension includes a range of drugs acting on vessels, heart, central nervous system and kidneys. Independently of the molecular mechanism involved (*e.g.* inhibiting the renin–angiotensin–aldosterone system, blocking adrenergic receptors) the therapeutic aim is reduce systolic and diastolic arterial pressure to more physiological and less dangerous values. Unfortunately, a number of people suffering from hypertension do not have its arterial pressure efficiently reduced by current antihypertensive drugs, even when multidrug therapy is adopted (for review see Mancia et al., 2007). In this way, developing new pharmacological tools may help to improve the clinical management of hypertension.

Since the vascular effects of *Scutia buxifolia* have never been investigated, in this work we studied the effects of its crude extracts of bark and leaves, as well as their fractions, when directly applied on arterial vessels. We highlighted that both the extract and all fractions obtained from *Scutia buxifolia* were able to cause relaxation of rat aortic rings by mechanisms involving both nitric oxide production and direct activation of K^+ channels.

2. Materials and methods

2.1. Plant material, obtainment and fractionation of the crude extract

Stem bark and leaves of *Scutia buxifolia* used in our study were collected in Dom Pedrito, a city in the state of Rio Grande do Sul (Brazil), where this plant is popularly used in the treatment of hypertension. A voucher specimen is deposited in the herbarium of the Department of Biology from Universidade Federal de Santa Maria (Santa Maria, Brazil) cataloged under number SMBD 10919.

The collected leaves and stem bark were completely dried in open air and the material was crushed and milled. The crude extract was obtained by maceration in hydroalcoholic solution (EtOH:H_2O 7:3, v/v), using a container covered with the solvent. The mash was daily subjected to manual agitation for seven days. To remove ethanol, at this time the content was filtered through cotton and concentrated using a rotating evaporator under reduced pressure at a low temperature. Following evaporation of ethanol, the crude extract of leaves or bark were partitioned by sequential extraction using solvents with increasing polarity: dichloromethane (DCM), ethyl acetate (AcOEt), and n-butanol (BuOH). Detailed description of preparation and characterization of extracts and fractions, as well as phytochemical screening of the fractions have been previously published (Boligon et al., 2012, 2009a, 2010).

2.2. Animals

Male Wistar rats (200–280 g) were obtained from the colony of Universidade Federal do Paraná (UFPR, Brazil). The animals were kept under standard laboratory conditions, with a constant temperature ($22 \pm 1\,^{\circ}C$), and a 12 h light/dark cycle with free access to food (Nuvital®, Curitiba/PR, Brazil) and water. The Institutional Ethics Committee from UFPR approved the procedures and protocols adopted in this study (authorization number 454).

2.3. Drugs

Phenylephrine hydrochloride, acetylcholine chloride, Nω-nitro-L-arginine methyl ester (L-NAME), indomethacin, atropine, tetraethylammonium (TEA), glibenclamide, 4-aminopyridine, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), iberiotoxin, pyrilamine, histamine, and HOE-140, were purchased from Sigma (St. Louis, MO, USA). Stock solutions of glibenclamide and ODQ were dissolved in dimethyl sulfoxide (DMSO). Indomethacin was dissolved in sodium bicarbonate (0.5%). All other drugs were prepared in freshly saline solution.

2.4. Preparation of aortic rings and tension measurement

The animals were killed under anesthesia and the thoracic aorta was quickly removed and placed in recipients containing physiological saline solution (PSS; pH 7.4; composition in mM: NaCl 115.3, KCl 4.9, $CaCl_2 \cdot 2H_2O$ 1.46, KH_2PO_4 1.2, $MgSO_4$ 1.2, D-glucose 11.1, $NaHCO_3$ 25), at 37 °C. The aorta was dissected from adhering fat and connective tissues and sliced into rings (3–4 mm in length). The isolated aortic rings, with or without functional endothelium, were kept in organ baths containing 2 ml of PSS under a resting tension of 1 g, maintained at 37 °C and continuously aerated with 95% O_2 and 5% CO_2. An interval of 60 min was respected for stabilization in the beginning of the experimental protocol, as well as between each set of exposition to drugs. The presence of endothelium was confirmed when the relaxation induced by acetylcholine (1 μM) in phenylephrine (1 μM) pre-contracted preparations was greater or equal to 80%, while only rings that did not relax when exposed to ACh were considered without endothelium. Changes in isometric tension were recorded using a MacLab® recording system (MacLab/8) and its application program (Chart, v 4.1 for Mac), both from ADI Instruments (Castle Hill, Australia).

2.5. Experimental protocols

2.5.1. Investigation of vascular effects of crude extracts and fractions of Scutia buxifolia

After the stabilization period, aortic rings with or without functional endothelium were contracted by phenylephrine (PE; 1 μM), and in the tonic phase of contraction were incubated with cumulative concentrations (3–3000 μg/ml) of the crude extracts of bark or leaves of *Scutia buxifolia*, or the fraction obtained by DCM, AcOEt, or BuOH extraction. Each vessel was exposed to a single extract or fraction. In order to investigate a potential deleterious effect promoted by exposition to crude extracts or fractions on vascular functionality, after recording their effects on vascular tone the baths were washed and the rings were allowed for a new resting of 60 min, when the contraction and relaxation induced by PE and ACh were measured again.

2.5.2. Evaluation of the role of membrane receptors and endothelial mediators

Comparing the results obtained in the initial experiments developed in this study we found that BuOH fraction obtained from barks of *Scutia buxifolia* was the most efficient to relax PE-contracted rat aortic rings with functional endothelium, and also induced partial relaxation in endothelium-denuded vessels (see Table 1). For this, the subsequent experiments were conducted using the BuOH fraction.

In this set of experiments, endothelium-intact rat aortic rings were pre-incubated with atropine (1 μM, a muscarinic receptor antagonist), HOE-140 (1 μM, B2 bradykinin receptor antagonist of), or pyrilamine (10 μM, an histamine H1 receptor antagonist), L-NAME (100 μM, a non-selective NO synthase inhibitor), ODQ (10 μM, an inhibitor of the soluble guanylate cyclase), or