Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Ethnopharmacology 104 (2006) 328–335
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Nitric oxide-dependent vasorelaxation induced by extractive solutions and fractions of *Maytenus ilicifolia* Mart ex Reissek (Celastraceae) leaves

Yanna D. Rattmann [a], Thales R. Cipriani [b], Guilherme L. Sassaki [a], Marcello Iacomini [b], Lia Rieck [a], Maria C.A. Marques [a], José E. da Silva-Santos [c,*]

[a] *Department of Pharmacology, Centro Politécnico, Universidade Federal do Parana, Curitiba, PR, Brazil*
[b] *Department of Biochemistry and Molecular Biology, Centro Politécnico, Universidade Federal do Parana, Curitiba, PR, Brazil*
[c] *Department of Pharmacy, Universidade da Região de Joinville, Campus Joinville, Bom Retiro, Caixa Postal: 246, CEP 89201-972, Joinville, SC, Brazil*

Received 23 February 2005; received in revised form 10 September 2005; accepted 16 September 2005
Available online 21 October 2005

Abstract

This study reveals that an ethanolic supernatant obtained from an aqueous extractive solution prepared from residues of methanolic extracts of ground leaves of *Maytenus ilicifolia* is able to cause a concentration- and endothelium-dependent relaxation in pre-contract rat aorta rings, with EC_{50} of 199.7 (190–210) μg/ml. The non-selective nitric oxide synthase inhibitors L-NAME and L-NMMA abolished this effect, while superoxide dismutase and MnTBAP (a non-enzymatic superoxide dismutase mimetic) enhanced it. Further, relaxation induced by this ethanolic supernatant have been strongly inhibited by the guanylate cyclase inhibitors methylene blue and ODQ, as well as by the potassium channel blockers 4-aminopyridine and tetraethylammonium, but was unchanged by the cyclooxigenase inhibitor indomethacin and the membrane receptor antagonists atropine, HOE-140 and pirilamine. Partition of the ethanolic supernatant between H_2O and EtOAc generated a fraction several times more potent, able to fully relax endothelium-intact aorta rings with an EC_{50} of 4.3 (3.9–4.8) μg/ml. ^{13}C NMR spectrum of this fraction showed signals typical of catechin. This study reveals that the leaves of *M. ilicifolia* possess one or more potent substances able to relax endothelium-intact rat aorta rings, an event that appears to involve nitric oxide production, guanylate cyclase activation and potassium channel opening.
© 2005 Elsevier Ireland Ltd. All rights reserved.

Keywords: *Maytenus ilicifolia*; Aorta; Celastraceae; Nitric oxide; Guanylate cyclase; Potassium channels

1. Introduction

Maytenus ilicifolia Mart ex Reissek (Celastraceae), popularly known in Brazil as "espinheira santa" (holy spines), is a native plant from southern Brazil, Paraguay, Uruguay, and northern Argentina (Bruneton, 1995). The leaves infusion of *M. ilicifolia* is used as a contraceptive and an emmenagogue in Paraguay, especially among rural and indigenous populations (Arenas and Azorero, 1977), as an abortive, an emmenagogue and an anti-cancer agent in Argentina (Arenas and Azorero, 1977; Martinez-Crovetto, 1987). In addition, *M. ilicifolia* is largely used against gastric disorders in Brazil (Cruz, 1982; Carlini, 1988).

The antiulcer effectiveness of *M. ilicifolia* extracts have been experimentally proven (Souza-Formigoni et al., 1991; Tabach and Oliveira, 2003; Ferreira et al., 2004; Jorge et al., 2004). Moreover, cytotoxicity (Shirota et al., 1994), antioxidation (Melo et al., 2001), antimutagenicity (Horn and Vargas, 2003), and contraception (Montanari and Bevilacqua, 2002) are among the other biological activities attributed to *M. ilicifolia* preparations in preclinical experiments. Phytochemistry evaluations have revealed the presence of maytenin and pristimerine (Pereira and Borges, 1960; de Lima et al., 1971), flavonoid glycosides (Leite et al., 2001), an arabinogalactan (Cipriani et al., 2004), catechin and epicatechin (Soares et al., 2004), and triterpenes (Shirota et al., 1994), besides the new triterpenoids maytefolins A–C (1–3) and uvaol-3-caffeate (4) (Ohsaki et al., 2004) in both leaves and roots of *M. ilicifolia*.

Despite the wide variety of secondary metabolites found in aerial parts of *Maytenus* species, which could explain much of their popular usage and even effectiveness as a phytomedicine, its cardiovascular effect have not been investigated yet. In this study, the ethanolic supernatant from a methanolic extraction of

* Corresponding author. Tel.: +55 47 461 9091; fax: +55 47 473 0131.
E-mail address: jesilvasantos@ufpr.br (J.E. da Silva-Santos).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.09.026

M. ilicifolia leaves (ES) was tested with the aim of investigating its effects on rat aorta rings.

2. Materials and methods

2.1. Plant material, obtainment and fractionation of the ethanolic supernatant (ES)

M. ilicifolia leaves collected in the metropolitan region of Curitiba (a city located in southern Brazil) were a donation from Central de Produção e Comercialização de Plantas Medicinais, Aromáticas e Condimentares do Paraná Ltda (PR, Brazil). The plant was identified by Dr. O. Guimarães (Department of Botany, Federal University of Paraná, PR, Brazil). Voucher specimens are deposited at the Herbarium of this university under number 30842.

The acquisition of the ES from *M. ilicifolia* leaves was processed according to the schematic representation shown in Fig. 1. Briefly, ground leaves (118 g) were subjected to $CHCl_3$:MeOH (2:1, v/v; 0.5 l) at 60 °C for 2 h (3×). Then, the residue I was subjected to MeOH:H_2O (4:1, v/v; 0.5 l) at 80 °C for 2 h (2×). Finally, residue II was extracted with H_2O (0.5 l) at 100 °C for 3 h (2×) and the aqueous extract treated with 3 V EtOH, which gave rise to a precipitate and an ethanolic supernatant (ES) that was studied in this work.

The ES (1 g) was partitioned between H_2O and EtOAc (60 ml:30 ml), generating the fractions named F_{H_2O} and F_{EtOAc}, respectively. The aqueous layer was then shaker with EtOAc (30 ml, 3×). The combined organic layers were evaporated and the residue suspended in H_2O, which was freeze-dried (yield 170 mg). When this was treated with a small volume of EtOAc (20 ml), a portion (64 mg) remained insoluble (named F_{INS}) while another was soluble (named F_{SOL}).

2.2. ^{13}C NMR analysis

^{13}C NMR spectra of the F_{INS} (the most potent fraction of ES) were obtained using a 400 MHz Bruker DRX Avance spectrometer with a 5 mm inverse probe, at 40 °C in DMSO-d_6. Chemical shifts are expressed in ppm (δ) relative to DMSO-d_6 at δ 39.5.

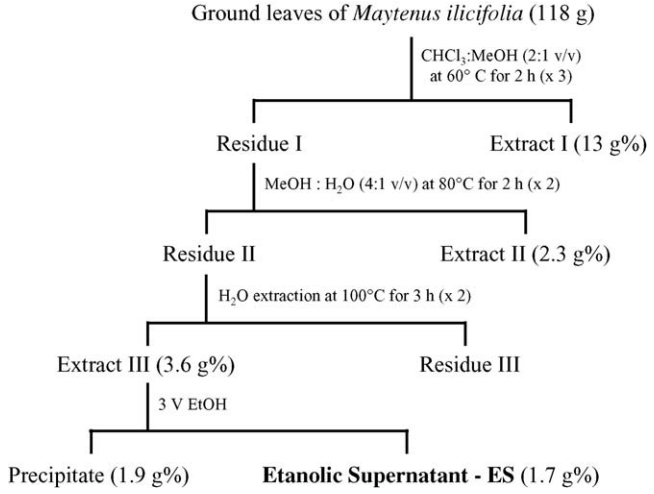

Fig. 1. Fractionation of the crude *M. ilicifolia* leaves extract.

2.3. Animals

Male Wistar rats (3–4 months old) from the colony of the Federal University of Paraná were used. The animals were maintained under standard laboratory conditions, with a constant 12 h light/dark cycle and controlled temperature (22 ± 2 °C). Standard pellet food (Nuvital®, Curitiba/PR, Brazil) and water were available ad libitum. The Institutional Ethics Committee of the Federal University of Paraná approved all the procedures adopted in this study (authorization number 052).

2.4. Preparation of rat aorta rings

Isolated aorta rings, with or without functional endothelium, were prepared according to the standard procedures previously described (Da Silva-Santos et al., 2002), using organ baths containing 3 ml of Krebs–Henseleit (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). The tissues were equilibrated for 60 min at a resting tension of 1 g before the addition of any drug. Tension was recorded via isometric force transducers (Letica Scientific Instruments, Barcelone, Spain) coupled to a MacLab® recording system (MacLab/8) and an application program (Chart, v 3.3), both from ADI Instruments (Castle Hill, Australia), working on an Apple Computer®.

2.5. Experimental protocols

2.5.1. Characterization of the vascular effects of the extractive solutions and fractions obtained from M. ilicifolia leaves

Phenylephrine (1 μM) pre-contracted aorta rings with or without functional endothelium were exposed to 300, 400, 600 and 900 μg/ml of the extract I, extract II or the precipitate obtained during the preparation of the ES (as illustrated in Fig. 1). The baths were then washed and after a further 60 min interval, the contraction and relaxation evoked by phenylephrine and acetylcholine were measured again. This same protocol was followed using smaller concentrations of the ES (150, 200 and 300 μg/ml) and its fractions F_{H_2O}, F_{EtOAc}, F_{INS} and F_{SOL} (3–100 μg/ml).

2.5.2. Evaluation of the role of endothelium derived factors on the ES effects

Endothelium-intact aorta rings were incubated with L-NAME (10 μM), or L-NMMA (1 mM), or D-NMMA (300 μM), or aminoguanidine (1 mM), or methylene blue (100 μM), or ODQ (10 μM), or indomethacin (1 μM), or SOD (300 UI/ml), or MnTBAP (100 μM), 15–30 min prior to the addition of phenylephrine (1 μM) into the bath. Then, while under the tonic phase of the phenylephrine-induced contraction, the effect of the ES (300 μg/ml) was recorded and compared with the data obtained in the absence of any other drug. The relaxation induced by

Download English Version:

https://daneshyari.com/en/article/2547827

Download Persian Version:

https://daneshyari.com/article/2547827

Daneshyari.com