Journal of Ethnopharmacology 133 (2011) 345–352

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

From the vasodilator and hypotensive effects of an extract fraction from *Agelanthus dodoneifolius* (DC) Danser (Loranthaceae) to the active compound dodoneine

M. Ouedraogo[a,c,1], M. Ruiz[a,1], E. Vardelle[b], H. Carreyre[b], J.M. Coustard[b], D. Potreau[a], L.L. Sawadogo[c], C. Cognard[a], F. Becq[a], C. Vandebrouck[a], J. Bescond[a,*]

[a] Institut de Physiologie et Biologie Cellulaires, Université de Poitiers, CNRS/UMR 6187, 40 avenue du Recteur Pineau F-86022 Poitiers Cedex, France
[b] Laboratoire de Synthèse et Réactivité des Substances Naturelles, Université de Poitiers, CNRS/UMR 6514, 40 avenue du Recteur Pineau, F-86022 Poitiers Cedex, France
[c] Université de Ouagadougou, 03 BP 7021, Ouagadougou 01, Burkina Faso

ARTICLE INFO

Article history:
Received 12 May 2010
Received in revised form
23 September 2010
Accepted 2 October 2010
Available online 16 October 2010

Keywords:
Active fraction of *Agelanthus dodoneifolius*
Rat blood pressure
Hypotensive agent
Smooth muscle relaxation
Dodoneine

ABSTRACT

Aim of the study: Effects of the different fractions obtained by partition of ethanolic extract (EE) of *Agelanthus dodoneifolius* through column chromatography were investigated on rat blood pressure and aortic relaxation and compared to those observed in the presence of crude EE.
Materials and methods: The acute hypotensive activity of EE, fractions and dodoneine, administrated intravenously, was evaluated in anaesthetized rats using the invasive method of blood pressure recording. Bioassay-guided fractionation using rat aorta pre-contracted by norepinephrine to monitor the relaxant activity led to the isolation of dodoneine.
Results: In normotensive rats, injection of EE (0.01–10 mg/kg) produced a dose-dependent decrease in both systolic and diastolic blood pressure without any significant change in heart rate. In a similar way, the EE (0.001–3 mg/mL) caused relaxation of rat pre-contracted aorta in a concentration-dependent manner. Fractionation of the EE afforded 14 fractions, F1–F14, that were tested on rat precontracted aortic rings. At the concentration level of 1 mg/mL, a maximum relaxation effect was observed for fractions F2–F5. F4 was the most effective to elicit a concentration-dependent relaxation effect with an $ED_{50} = 160 \pm 1.1$ μg/mL ($n = 5$) and to decreased systolic and diastolic control pressure by 56.9% and 81.6% respectively. F4 contains most of the dihydropyranone dodoneine, with 93% of the sample mass. Dodoneine separated from this fraction was also able to decrease both systolic and diastolic arterial pressure by 32.5% and 38.7% at 100 μg/kg, respectively.
Conclusion: For the first time, this study demonstrates the hypotensive property of the dodoneine present in *Agelanthus dodoneifolius*.

1. Introduction

Hypertension is one of the most common diseases in the world. As it is a multiparametric pathology, its treatment generally involves a large panel of drugs. In developing countries where it also affects a significant part of people (for instance 23% of the population in Ouagadougou (Burkina Faso), Niankara et al., 2002), hypertension is usually treated by plant decoctions or extracts such as infusions of *Agelanthus dodoneifolius*. These natural products recognized from the very beginning as an important source of therapeutically effective medicines are still largely used since approximately 60% of the present world population relies almost entirely on plants for medication (Ajaikumar et al., 2005). Because they contain a number of metabolites, these plants have generally a wide spectrum of applications. From several decades, one of the challenges of pharmaceutics is to isolate the active compounds and to characterize their specific properties.

Agelanthus dodoneifolius (also called "African mistletoe") from the Loranthaceae family is one of these medicinal plants used in African pharmacopeia. *Agelanthus dodoneifolius* (DC) Danser, the most widespread specie of the family (Sallé et al., 1987; Boussim et al., 2004) was shown to contain, tannins, anthracenosides, anthraquinones, alkaloids, saponins, sterols and triterpenes (Deeni and Sadiq, 2002; Traore et al., 2004) using simple group reagent tests. So, it is used in traditional medicine as hypotensive, antispasmodic, antiparasitic, in the treatment of wound infections, cancer,

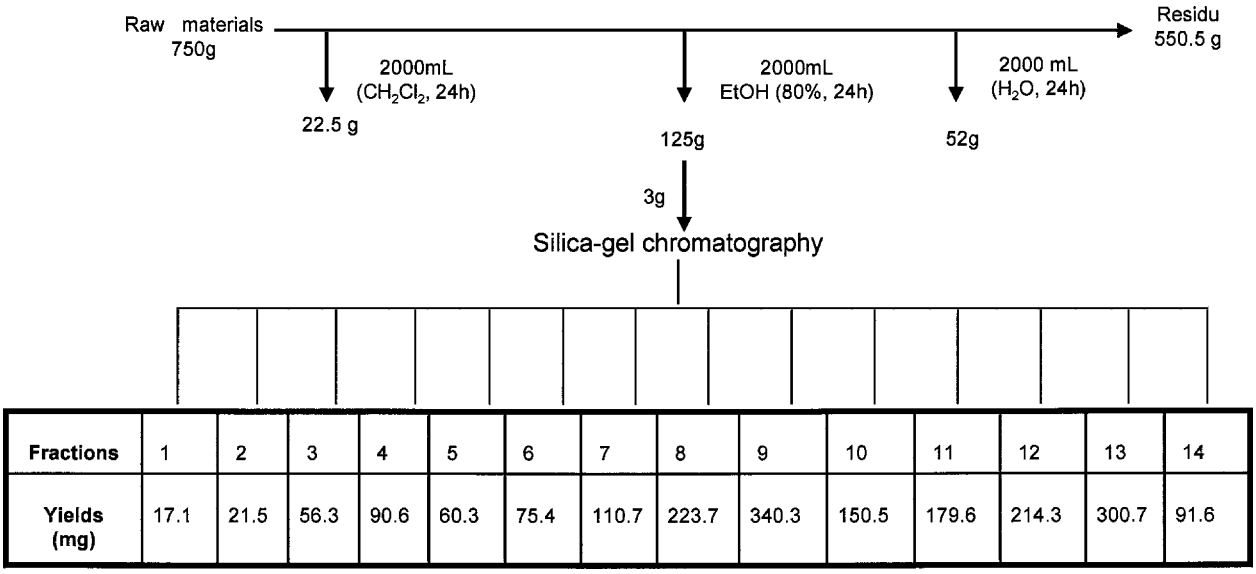
Abbreviations: AE, aqueous extract; DE, dichloromethane extract; EE, ethanolic extract; EtOAc, ethyl acetate; TLC, thin layer chromatography; DMSO, dimethylsulfoxide.

* Corresponding author. Tel.: +33 5 49 45 35 28; fax: +33 5 49 45 40 14.
E-mail address: Jocelyn.Bescond@univ-poitiers.fr (J. Bescond).

[1] These authors contributed equally to this work.

0378-8741/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2010.10.002

Table 1
Scheme of bioassay-guided fractionation of the ethanolic extract (EE) from *Agelanthus dodoneifolius*.



Fractions	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Yields (mg)	17.1	21.5	56.3	90.6	60.3	75.4	110.7	223.7	340.3	150.5	179.6	214.3	300.7	91.6

cholera, asthma, diabetes and gastrointestinal, gynaecologic and nervous disorders (Boussim, 2002, for review). It has also been shown to have larvicidal and molluscidal activities (Cepleanu et al., 1994). Moreover it was extensively used for treatment of cardiovascular diseases (Nacoulma/Ouedraogo, 1996).

Ouedraogo et al. (2005a,b) have shown that the crude aqueous extract AE and the ethanolic extract EE of *Agelanthus dodoneifolius* inhibited acetylcholine-induced bronchoconstriction on rat trachea. It was also reported that the crude aqueous extract AE had a vasorelaxant effect on rat aorta (Ouedraogo et al., 2005c).

The aim of the present study was (i) to fractionate the EE of *Agelanthus dodoneifolius* by column chromatography and identify the fractions inducing vasorelaxant and hypotensive effects, (ii) to determine the level of the active principle identified to be dodoneine and (iii) to estimate its hypotensive property.

2. Materials and methods

2.1. Plant material and extraction

Agelanthus dodoneifolius was collected in the region close to Ouagadougou (Burkina Faso) in June 2005. The plant was identified by Prof. Boussim I.J., Taxonomist, Department of Biological Sciences, University of Ouagadougou. A voucher specimen was deposited in the herbarium of this institute with the reference no. 002. The air-dried and powdered plant materials (750 g) were successively macerated and extracted with CH_2Cl_2 (2 × 2 L), 80% EtOH (2 × 2 L) and H_2O (2 × 2 L). Each extract was evaporated under reduce pressure and/or lyophilized to dryness. The extraction afforded (i) 22.5 g of a dark green gummy material with CH_2Cl_2 (dichloromethane extract or DE), (ii) then 125 g of ethanolic extract (ethanolic extract or EE) and finally, (iii) 52.5 g of water extract (aqueous extract or AE).

A sample of the EE (3 g) was fractionated by column chromatography (Acros Organics, Silicagel for column chromatography 0.035–0.070 mm, 60 Å). Weight of silica: 75 g, column: Φ_i 2.9 cm, length 30 cm. Eluent volume (400 mL each). The following fractions were obtained: fraction F1: CH_2Cl_2; fraction F2: CH_2Cl_2/AcOEt 4/1; fraction F3: CH_2Cl_2/AcOEt 3/2; fraction F4: CH_2Cl_2/AcOEt 2/3; fraction F5: CH_2Cl_2/AcOEt 1/5; fraction F6: AcOEt; fraction F7: AcOEt/EtOH 4/1; fraction F8: AcOEt/EtOH 3/2; fraction F9: AcOEt/EtOH 2/3; fraction F10: AcOEt/EtOH 1/4; fraction F11: EtOH; fraction F12: EtOH/H_2O 4/1; fraction F13: EtOH/H_2O 3/2; fraction F14 (3 × 400 mL): EtOH/H_2O 2/3, EtOH/H_2O 1/3, H_2O. Every fraction was analyzed by TLC (Table 1).

2.2. Apparatus and chromatographic conditions

The chromatographic system (Dionex Ultimate 3000) consisted of a gradient pump with degas option and gradient mixer, a UV detector, and a Chromeleon® chromatography workstation (Dionex Corporation, Sunnyvale, CA, USA). Chromatographic separations were achieved on a C18 column (250 mm × 4.6 mm, 120 Å, Acclaim120, Dionex, USA) associated with a guard column packed with the same phase. 20 μL was injected through a loop injection valve (Rheodyne). The analyses were performed at 30 °C using isocratic elution (water/acetonitrile 7/3) at 1.6 mL/min and UV detection at 275 nm.

2.3. Determination of dodoneine

A set of calibrating standards at 0, 28, 55, 139, 277 and 555 mg/L was prepared from the 1110 mg/L stock solution in acetonitrile. A five-point calibration curve was constructed in the range of 22–555 mg/L with each batch of samples. The calibration curve was linear with a correlation coefficient of 0.999 and an intercept value not statistically different from zero.

The analytical chromatographic conditions were optimized for the determination of dodoneine in the fractions F1–F14 obtained by column chromatography of the EE, as describe in Table 1.

2.4. Animals

Wistar rats (250–300 g) were anaesthetized with intraperitoneal injection of sodium pentobarbital (30–50 mg/kg) for *in vivo* studies, and with chloral hydrate (2 g/kg) for *in vitro* studies. All animal handling and procedures strictly conformed to the European Community Guidelines (EEC Directive of 1986; 86/609/EEC).

2.5. In vivo experiments

Once animals were anaesthetized they were turned to a dorsal decubitus position on a dissecting table under an overhead lamp to maintain constant the body temperature. A longitudinal mid-