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SCIENCE DIRECT
Journal of Ethnopharmacology 102 (2005) 170–176
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Bioassay-guided isolation of iridoid glucosides with antinociceptive and anti-inflammatory activities from *Veronica anagallis-aquatica* L.

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Received 24 September 2004; received in revised form 11 May 2005; accepted 26 May 2005
Available online 12 July 2005

Abstract

Extracts obtained from the herbs of various *Veronica* species are used as folk remedy worldwide for the treatment of various inflammatory ailments including rheumatism. In vivo anti-inflammatory and antinociceptive activities of *Veronica anagallis-aquatica* L. aerial parts were investigated. Methanolic extract of the plant was shown to possess significant inhibitory activity against carrageenan-induced hind paw edema model and of *p*-benzoquinone-induced writhings in mice. Through bioassay-guided fractionation and isolation procedures eight compounds, aquaticoside A (1), aquaticoside B (2), aquaticoside C (3), veronicoside (4), catalposide (5), verproside (6), verminoside (7) and martynoside (8) were isolated and their structures were elucidated by spectral techniques. Catapol derivative iridoid glucosides, verproside (6) and catalposide (5), were found to possess potent antinociceptive and anti-inflammatory activities, per os without inducing any apparent acute toxicity as well as gastric damage. Results of the present study supported the utilization of the plant in Turkish folk medicine.

Keywords: *Veronica anagallis-aquatica*; Scrophulariaceae; Antinociceptive; Anti-inflammatory; Iridoid glucosides; Catalpol derivatives; Carrageenan-induced paw edema; *p*-Benzoquinone-induced writhings

1. Introduction

Several *Veronica* species are reported to possess application in traditional medicines worldwide for the treatment of a wide range of disorders; in respiratory diseases against cough or as expectorant, as antiscorbutic, as diuretics and for wound healing (Baytop, 1984; Harput et al., 2002). In Chinese traditional medicine, *Veronica anagallis-aquatica* L. is used for the treatment of influenza, hemoptysis, laryngopharyngitis and hernia (Su et al., 1999). During our field expeditions on Turkish folk medicine, we have recorded that aerial parts of *Veronica anagallis-aquatica* is boiled in milk to obtain poultice and then is applied to abdomen for abdominal pain or its warm aqueous extract without removing the boiled herbs is used as a bath remedy to alleviate rheumatic pain in northwest Anatolia (Fujita et al., 1995).

Previously, in vitro anti-inflammatory activity of five *Veronica* species (*Veronica cymbalaria*, *Veronica hederifolia*, *Veronica pectinata* var. *glandulosa*, *Veronica persica* and *Veronica polita)* were investigated through evaluation the inhibitory effects on nitric oxide (NO) production in lipopolysaccharide-stimulated mouse peritoneal macrophages as well as cytotoxic activity against KB epidermoid carcinoma and B16 melanoma (Harput et al., 2002). MeOH extracts of five plant materials were found to possess inhibitory effects on NO synthesis in varying degrees. MeOH extracts were further partitioned between water and chloroform, and water fractions showed the activity without inducing any cytotoxicity, while chloroform fractions were cytotoxic dose-dependently. Moreover, water fractions were found to possess remarkable effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH), suggesting that the inhibitory effect on NO production might be due to their radical scavenging activity.

Veronica species have been known to be rich in iridoid glucosides. Mainly aucubin, catalpol, benzoic and

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0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.05.042

cinnamic acid esters of catalpol, mussaenoside and mussaenosidic acid esters were reported in the investigated *Veronica* species (Harput et al., 2003). Various biological activities have been reported previously for the iridoid glucosides including antidiabetic, anti-inflammatory, anticancer and immunostimulant activities (Vijayavitthal et al., 1998; Konoshima et al., 2000; Stevenson et al., 2002; Ahmed et al., 2003).

As related with the above-presented data, this study deals with the anti-inflammatory and antinociceptive effects of *Veronica anagallis-aquatica* herbs, in order to evaluate the folkloric information and isolation and chemical characterization of the active constituent(s) through bioassay-guided fractionation procedures.

2. Material and methods

2.1. Plant material

Aerial part of *Veronica anagallis-aquatica* L. (Scrophulariaceae) was collected from Beytepe Campus of Hacettepe University, Ankara, Turkey in June, 2002. Voucher specimen (HUEF 02021) is deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University.

2.2. Bioassay-guided fractionation and isolation of active ingredients

2.2.1. Preparation of H_2O and MeOH extracts

Two flasks each containing 10 g of dried and powdered aerial parts of *Veronica anagallis-aquatica* were extracted either with MeOH and distilled water, separately, at 40 °C overnight (3 × 250 ml). Each of combined extract was evaporated to dryness under reduced pressure to give 2.3 g MeOH extract and 1.9 g H_2O extract and was administered to mice for activity assessment.

2.2.2. Fractionation of the MeOH extract

The air-dried aerial parts of *Veronica anagallis-aquatica* (453 g) were extracted with MeOH at 40 °C for 12 h (3 × 3 l). The MeOH solution was evaporated under reduced pressure to give "MeOH extract" (75 g). The MeOH extract was redissolved in 90% MeOH (0.2 l) and extracted with hexane (10 × 100 ml) to remove chlorophyl and other lipophylic constituents. The aqueous methanolic layer was concentrated to give a crude extract (68 g). The extract was then subjected to polyamide (Fluka, 50–60 μm) column chromatography eluting with H_2O, followed by increasing concentrations of MeOH to give six main fractions: Frs. A–F (Fr. A, 12.4 g; Fr. B, 29 g; Fr. C, 2.6 g; Fr. D, 2.2 g; Fr. E 3.2 g; Fr. F. 1.54 g).

2.2.3. Chromatographic separation and isolation of the active constituents

Fr. B, the most active fraction, was applied to the medium pressure liquid chromatography (MPLC) was performed on Labormatic (18.5 × 352 mm) and Buchi (25 × 460 mm) glass columns using reversed phase (LiChroprep RP-18; 40–63 μm, Merck) material with increasing concentrations of MeOH in H_2O (0 → 100% MeOH) to yield Frs. B_1–B_7.

Frs. B_1–B_7 were further applied to a series of silica gel (Merck, Kieselgel 60, 60–230 mesh) column chromatography eluting with $CHCl_3$/MeOH (99:1 → 65:35) and compound **6** (200.2 mg) from Fr. B_1 (250 mg), compound **5** (213.1 mg) and compound **2** (10.3 mg) from Fr. B_2 (500 mg), compound **7** (2.2 mg) from Fr. B_3 (120 mg), compound **3** (8.0 mg) from Fr. B_4 (35 mg), compound **1** (15.5 mg) from Fr. B_5 (64 mg), compound **8** (2.5 mg) from Fr. B_6 (31 mg) and compound **4** (59.5 mg) from Fr. B_7 (350 mg) were isolated in pure form.

2.2.4. Structure elucidation of the compounds *1–8*

Structure elucidation of the isolated components **1–8** from Fr. B was carried out by spectral techniques; UV, 1D and 2D NMR (^1H, ^{13}C NMR) and mass spectroscopy (HR-ESI-FTMS) and detailed data were recently published elsewhere (Harput et al., 2004). The structures of compounds **1–8** were as follows (Fig. 1): (**1**) 6′-*O*-benzoyl-8-epiloganic acid, aquaticoside A; (**2**) 6′-*O*-*p*-hydroxybenzoyl-8-epiloganic acid, aquaticoside B; (**3**) 6′-*O*-benzoyl-gardoside, aquaticoside C; (**4**) veronicoside; (**5**) catalposide; (**6**) verproside; (**7**) verminoside; (**8**) martynoside.

2.3. Biological activity tests

2.3.1. Test animals

Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey). The animals left for two days for acclimatization to animal room conditions were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but allowed free access of water. A minimum of six animals was used in each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals.

2.3.2. Preparation of test samples for bioassay

Test samples were given orally to test animals after suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg) or acetyl salicylic acid (ASA) (100 mg/kg and 200 mg/kg) in 0.5% CMC was used as reference drug.

2.3.3. Antinociceptive activity

2.3.3.1. p-Benzoquinone-induced abdominal constriction test in mice (Okun et al., 1963). Sixty minutes after the