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ScienceDirect
Food Chemistry 107 (2008) 990–993
Food Chemistry
www.elsevier.com/locate/foodchem

Anticonvulsant activity of furanocoumarins and the essential oil obtained from the fruits of *Heracleum crenatifolium*

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Received 15 July 2007; accepted 30 August 2007

Abstract

The anticonvulsant activity of furanocoumarins, coumarin mixture and the essential oil obtained from the fruits of *Heracleum crenatifolium* was examined against maximal electroshock (MES)-induced seizures in mice. Bergapten showed significant anticonvulsant activity. The furanocoumarins isolated from the fruits of the plant were identified using thin-layer chromatography, melting points and spectroscopic methods (IR, MS, ^{1}H NMR) as isobergapten (1), pimpinellin (2), bergapten (3), isopimpinellin (4), sphondin (5) and byak-angelicol (6). The essential oil content of the fruits were found as 5.5%. Twenty-two compounds representing 99.3% of the essential oil obtained from the fruits of *H. crenatifolium* were determined and the major components were identified as octanol and octyl acetate (3.1% and 88.4% respectively) by GC and GC–MS.

Keywords: *Heracleum crenatifolium*; Furanocoumarin; Essential oil; Anticonvulsant; MES

1. Introduction

There are almost 125 *Heracleum* species in the world (Pimenov & Leonov, 2004). The genus *Heracleum*, belonging to the family Apiaceae, is represented in the flora of Turkey by 17 species, nine of which are endemic. *Heracleum crenatifolium* is an endemic plant distributed in Gümüşhane, Ağrı and Bitlis (east and north-eastern part of Turkey) (Davis, 1972; Davis, Mill, & Tan, 1988; Duman, 2000).

In traditional medicine, some *Heracleum* species are used as antipyretic, analgesic, diaphoretic (Taniguchi, Yokota, Shibano, Wang, & Baba, 2005), antiseptic, carminative, digestive and also as a flavouring agent and spice for foods (Souri, Farsam, Sarkheil, & Ebadi, 2004; Sonboli, Azizian, Yousefzadi, Kanani, & Mehrabian, 2007) for rheumatic disease, lumbago, gastralgia, and injuries from falls, fractures, contusions and strains (Niu et al., 2004) and in the treatment of hypertension (Eddouks, Maghrani, Lemhadri, Ouahidi, & Jouad, 2002), epilepsy (Eadie, 2004; Sayyah, Moaied, & Kamalinejad, 2005) and against arthritis, paralysis (Chacko, Sethuraman, & George, 2000), dysentery and diarrhoea (Baytop, 1999).

Heracleum persicum (Sayyah et al., 2005) and combined furocoumarins from *H. sibiricum* and *H. verticillatum* (Chauhan, Dobhal, & Joshi, 1988; Rusinov, 1966) were reported to possess anticonvulsant activity.

The essential oils obtained from the fruits of *H. crenatifolium* collected from different localities were reported previously (İşcan, Özek, Özek, Duran, & Baser, 2004; Özek, Özek, Baser, & Duran, 2005).

The current study was performed to evaluate the anticonvulsant activity of the furanocoumarins, coumarin

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doi:10.1016/j.foodchem.2007.08.085

mixture and the essential oil obtained from the fruits of *H. crenatifolium*. This is the first report on the furanocoumarin composition and anticonvulsant activity of *H. crenatifolium*.

2. Materials and methods

2.1. Materials

Bergapten was purchased from the Aldrich Chemical Company (Milwaukee, WI, USA).

2.2. Plant material

Plant material was collected (10.07.2004) in the vicinity of Gumushane-Karamustafa (Turkey). A voucher specimen (AEF 23809) is preserved at the Herbarium of the Faculty of Pharmacy, Ankara University, Ankara, Turkey.

2.3. Isolation of furanocoumarins

Dried and powdered fruits of *H. crenatifolium* (500 g) were extracted with petroleum ether at room temperature. The petroleum ether extract was concentrated under vacuum. By keeping in the refrigerator, the coumarin mixture (4.2 g) was separated. The coumarin mixture (1.5 g) was subjected to column chromatography on silica gel and eluted successively with a *n*-hexane-ethyl acetate solvent system, with increasing polarity (99:1 → 80:20) to afford 28 fractions. The collected fractions were applied to preparative-TLC and 6 compounds were obtained (Fig. 1). After chromatography on silica gel plates using *n*-hexane-ethyl acetate (3:1), fractions 5–8 yielded compound **1** (45.6 mg) and compound **2** (10 mg), fractions 9–12 yielded compound **2** (6 mg). Fractions 13–15 were chromatographed on silica gel plates with *n*-hexane-dichloromethane-ethyl acetate (4:4:2) to give compound **3** (58 mg). After chromatography on silica gel plates using toluene-ethyl acetate (9:1), fractions 16–18 yielded compound **4** (30 mg), fractions 19–23 yielded compound **4** (24.3 mg) and compound **5** (14 mg), fractions 24–28 yielded compound **6** (64 mg).

2.4. Identification of furanocoumarins

The furanocoumarins isolated from the fruits of *H. crenatifolium* were identified using thin-layer chromatography, melting points and spectroscopic methods (IR, MS and ^{1}H NMR) as isobergapten, pimpinellin, bergapten, isopimpinellin, sphondin, and byak-angelicol. Melting points were determined on a Electrothermal 9300 Digital Melting Point Apparatus. IR spectra were recorded on a Bruker Vector 22 IR Spectrophotometer. MS was measured on a HP model 6890 Gas Chromatograph combined with a HP model 5972 A MS detector. ^{1}H NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. Thin-layer chromatography was performed on silica gel 60 F_{254} plates (Merck No.5554). Column chromatography was performed on silica gel 60 (0.040–0.063 mm, Merck No. 9385) column. Preparative thin-layer chromatography was carried out on silica gel 60 F_{254} plates (Merck No. 5744).

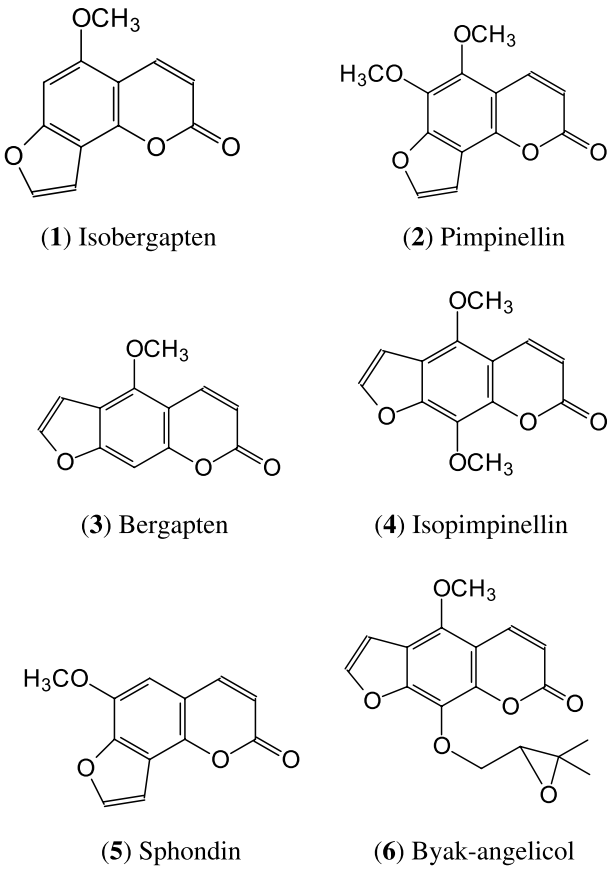

Fig. 1. Structures of compounds **1**–**6** isolated from the fruits of *H. crenatifolium*.

2.5. Distillation of the essential oil

The air-dried and crushed seeds were submitted to hydro-distillation for 3 h using a Clevenger apparatus. Oil was dried over anhydrous sodium sulfate and stored at +4 °C.

2.6. Analysis of essential oil

2.6.1. GC condition

The GC analysis was carried out using a Hewlett–Packard 6890 GC system. An HP-Innowax FSC column (60 m × 0.25 mm ∅, with 0.25 μm film thickness) was used with nitrogen as the carrier gas (1 mL/min). The oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, then kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min. The split ratio was adjusted at 50:1. The injector temperature was set at 250 °C. The percentage composi-

tions were obtained from electronic integration measurements using flame ionisation detection (FID, 250 °C).

2.6.2. GC/MS condition

For GC/MS analysis, a Hewlett–Packard GCD system was used. The same column and operational conditions as in GC were applied. The carrier gas was helium. MS were taken at 70 eV. The mass range was between m/z 35 and 425. Alkanes were used as reference points in the calculation of relative retention indices (RRI). The components were identified by comparing their relative retention times and mass spectra with the data from the Baser library of essential oil constituents, Wiley, Mass-Finder and Adams GC/MS libraries.

2.7. Anticonvulsant activities of the furanocoumarins, coumarin mixture and the essential oil

The Animal Use and Care Committee approved all experiments for animal testing. Male albino mice weighing 30–40 g were used. The laboratory temperature was maintained at 20 ± 1 °C under conditions of a 12 h light and dark schedule. Before the experiments mice were allowed 1wk of adaptation. They were used only once. The experiments were performed between 9 and 12 am in the morning. Furanocoumarins, coumarin mixture and the essential oil were suspended in 0.5% methylcellulose. Furanocoumarins and coumarin mixture (60 mg/kg), and essential oil (0.84 ml/kg) were injected intraperitoneally. Methylcellulose (0.1 ml) was given intraperitoneally to control animals. The rotarod test was carried out to determine minimal neurotoxicity before the experiments. Maximal electroshock seizures (MES) were induced 1 h after administration of the test materials, by application of a 60 Hz current of 60 mA and 0.4 pulse width for 0.2 s via ear electrodes by using a Ugo Basile electroshock device. The anticonvulsive activity of the test materials was evaluated by defining the abolition of the hind-leg tonic maximal extension component of the seizure (Uçar et al., 1998). Fisher's Exact X2 test was used for statistical analysis.

3. Results and discussion

3.1. Identification of compounds 1–6

Coumarin mixture was obtained from the petroleum ether extract of the air-dried fruits of *H. crenatifolium*. Isolation of the compounds from the coumarin mixture was carried out with silica gel column chromatography and preparative-TLC. This led to the isolation of six furanocoumarins. The furanocoumarins (compounds **1**–**6**) isolated were identified using thin-layer chromatography, melting points and spectroscopic methods (IR, MS and ^{1}H NMR) as isobergapten, pimpinellin, bergapten, isopimpinellin, sphondin and byak-angelicol, respectively. Identification of compound **3** was performed by comparing R_f values, melting point and IR spectra with an authentic sample. Compounds **1**, **2**, **4**, **5** were identified by comparing melting point and MS data with those reported in the literature (Ibadullaeva & Serkerov, 2000; Niu, Li, Jiang, Zhao, & Sun, 2002). Identification of compound 6 was performed by comparing melting point, MS and ^{1}H NMR data with those reported in the literature (Saiki et al., 1971; Setzer et al., 2003; Sun, Lin, & Niu, 1978).

All the identified furanocoumarins are known compounds and have been reported for the first time from *H. crenatifolium*.

3.2. Yield and chemical composition of essential oil

The oil of the *H. crenatifolium* obtained by hydro-distillation (yield 5.5%) was analysed using GC and GC/MS techniques. In the oil of *H. crenatifolium* 22 compounds, representing 99.3% of the total oil, were characterised. The compounds identified are given in Table 1 with their relative percentage amounts and relative retention indices (RRI). Octylacetate (88.4%) and octanol (3.1%) were found as main constituents.

The essential oils obtained from the crushed fruits (yield 3.7%) of *H. crenatifolium* collected from Konya: Hadım-Kızılkaya road (20.06.03) were reported previously to contain octyl acetate (93.7%) as the major constituent (İşcan et al., 2004; Özek et al., 2005).

3.3. Anticonvulsant activities of the furanocoumarins, coumarin mixture and the essential oil

The anticonvulsant activity of the test materials was evaluated against maximal electroshock seizures induced in mice. The Rotarod test was used to demonstrate the possible neurotoxicity. The anticonvulsant and neurotoxicity

Table 1
Compounds identified in the essential oil of *H. crenatifolium*

RRI	Compound	%
1032	α-Pinene	0.7
1174	Myrcene	tr
1203	Limonen	0.3
1296	Octanal	0.6
1438	Hexyl 2-methyl butyrate	0.7
1483	Octyl acetate	88.4
1506	Decanal	0.3
1516	(*Z*)-4-Octenyl acetate	1.0
1547	Octyl isobutyrate	0.3
1553	Linalool	0.2
1562	Octanol	3.1
1584	Nonyl acetate	0.1
1590	Bornyl acetate	tr
1623	Octyl butyrate	0.2
1634	Octyl 2-methyl butyrate	0.9
1654	Octyl isovalerate	0.1
1687	Decyl acetate	0.5
1766	1-Decanol	0.2
1829	Octyl hexanoate	0.7
1988	2-Phenylethyl 2-methyl butyrate	0.2
2020	Octyl octanoate	0.4
1296	Myristicin	0.4

Table 2
Anticonvulsant and neurotoxicity screening data of the test materials

Test materials	MES[a]	Toxicity[b]
0.5% methylcellulose	6/8	0/8
Essential oil	4/8	0/8
Coumarin mixture	6/8	0/8
Isobergapten	3/8	0/8
Bergapten	1/8*	0/8
Isopimpinellin	4/8	0/8
Byak-angelicol	5/8	0/8

[a] Protected animals to tested animals.
[b] Animals exhibited neurotoxicity to tested animals.
* $p < 0.05$.

screening data are presented in Table 2. According to the rotarod test none of the test materials showed neurotoxicity at the applied dose. Bergapten showed significant anticonvulsant activity.

Acknowledgement

This study was financially supported by the Research Foundation of the Gazi University (research grant no. SBE-11/2002-11), Ankara, Turkey.

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