



# The analysis of phenolic compounds from the aerial parts of *Eupatorium cannabinum* L. subsp. *cannabinum*

Jakub P. Piwowarski<sup>a</sup>, Birgit Waltenberger<sup>b</sup>, Hermann Stuppner<sup>b</sup>, Anna K. Kiss<sup>a</sup>, Sebastian Granica<sup>a,\*</sup>

<sup>a</sup> Department of Pharmacognosy and Molecular Basis of Phytotherapy, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097, Warsaw, Poland

<sup>b</sup> Institute of Pharmacy/Pharmacognosy and Centre for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innrain 80-82, A-6020 Innsbruck, Austria

## ARTICLE INFO

### Keywords:

*Eupatorium cannabinum*  
Polyphenols  
Asteraceae  
Flavonoids  
Phenolic acids

## ABSTRACT

Thirteen compounds were isolated and identified from the aerial parts of *Eupatorium cannabinum* subsp. *cannabinum* comprising six caffeic acid derivatives, five flavonol glycosides and two flavone aglycones. Additionally, the first HPLC-DAD-MS method for the phytochemical screening of phenolics in the analyzed plant material was developed allowing unequivocal identification of further six constituents occurring in the raw extract including four caffeic acid derivatives and two malonylated flavonol glycosides. This report is the first comprehensive study focused on phenolic compounds in the aerial parts of *E. cannabinum*. The chemotaxonomic significance in respect to the genus *Eupatorium* is discussed.

## 1. Introduction

*Eupatorium cannabinum* L. subsp. *cannabinum* (Asteraceae), commonly known as hemp-agrimony, St John's herb or holy rope is a perennial herb with erect puberulent stems (30–175 cm height) and palmately 3- to 5-fid, opposite leaves (Hansen, 1976). It is distributed all over Europe, Asia, and Northwest Africa (Witkowska-Żuk, 2013). It has been shown that phenolics including flavonoids are useful chemotaxonomic markers for taxa of the Asteraceae family (Emerenciano et al., 2001).

Several studies focusing on the phytochemistry of *E. cannabinum* have been carried out. It has been shown that aerial and subaerial parts of hemp-agrimony contain monoterpenoids, sesquiterpenoids, diterpenoids, triterpenes, benzofuranoids, steroids, pyrolizidine alkaloids, and coumarins (Pedersen, 1975; Hendriks et al., 1983, 1985, 1987; Siebertz et al., 1989; Bachelier et al., 2006; Chen et al., 2011, 2014). Several studies focusing on polyphenolic compounds present in the aerial parts of *E. cannabinum* have also been performed. First Grzybowska and Jerzmanowska (1954) isolated and identified rutin (21) as a major flavonoid occurring in the aerial parts of hemp-agrimony. Next Hendriks et al. (1983) reported that *E. cannabinum* contains flavonoid glycosides in low quantities and that rutin (21) can be suspected as a typical compound for the genus. Later Aquino et al. (1988) also investigated the herb of *E. cannabinum* and isolated and identified three major compounds, namely kaempferol 3-O-rutinoside, quercetin 3-O-rutinoside (rutin, 21), and quercetin 3-O-glucoside (isoquercitrin, 22).

The major study focusing on flavonoids present in *E. cannabinum* was performed by Elma and co-workers (Elema et al., 1989). The group confirmed the presence of kaempferol and quercetin 3-O-rutinosides (21) and quercetin 3-O-glucoside (22) in the analyzed material. Additionally, two 6-methoxyflavones – eupafolin (34) and hispidulin (35) – as well as kaempferol 3-O-glucoside (astragalin, 29) and quercetin 3-O-galactoside (hyperoside) were identified for the first time from the aerial parts of *E. cannabinum*. A further study on flavonoid aglycones present in the herb of *E. cannabinum* led to the detection of hispidulin (35), eupafolin (34), and four additional compounds, namely pectolinarigenin, jaceosidin, santin, and centaureidin. The latest report on polyphenols present in hemp-agrimony based on TLC screening of the biologically active extract revealed that apart from flavonoids like rutin (21) and hyperoside, aerial parts of *E. cannabinum* also contain some phenolic acids including chlorogenic (8) and caffeic acids (Ionita et al., 2013).

In the literature there are no reports on hyphenated HPLC methods that could be used for the routine screening of constituents present in *Eupatorium* species. The HPLC method presented by Maas (Maas et al., 2009, 2011) was developed for major constituents of *Eupatorium perforliatum* but no DAD or MS data were provided. Thus, the first aim of the present study was to develop a fast and sensitive method for the screening of polyphenols in an extract from the aerial parts of *Eupatorium cannabinum* L. subsp. *cannabinum* that could be also used for phytochemical screening of phenolics of other species in the *Eupatoriae*. The second aim was to isolate and identify major polyphenols

\* Corresponding author.

E-mail address: [sgranica@wum.edu.pl](mailto:sgranica@wum.edu.pl) (S. Granica).

(flavonoids and phenolic acids) occurring in the analyzed plant material.

## 2. Materials and methods

### 2.1. Plant material

The aerial parts of *Eupatorium cannabinum* L. subsp. *cannabinum* were collected in the wild at banks of Średnie Lake, near Piduń, warmińsko-mazurskie, Poland (N 53°30'20", E 20°47'40", 148 m a.m.s.l.). The plant material was identified by Anna K. Kiss according to Rutkowski (2014). The voucher specimen (voucher no. EC20150802\_A) was deposited in the herbarium of the Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland. A copy of the voucher specimen is available as supplementary material.

### 2.2. HPLC-DAD-MS<sup>3</sup> analysis

One hundred mg of dried and ground aerial parts of *E. cannabinum* were extracted with 1 ml of a methanol:water mixture (1:1, v/v) for 15 min using an ultrasonic bath. The extract was filtered through a 0.45 µm syringe filter and subjected to HPLC-DAD-MS analysis using a Dionex Ultimate 3000RS system (Dionex, Germany) coupled with an Amazon SL ion trap mass spectrometer or a micrOTOF-QII time-of-flight mass spectrometer (Bruker Daltonics, Germany). The separation was carried out with a Kinetex XB-C18 column (150 mm × 3.0 mm × 2.6 µm, Phenomenex, USA) maintained at 25 °C. The flow rate was set at 0.4 ml/min. The mobile phase A was 0.1% HCOOH in water and B was 0.1% HCOOH in acetonitrile. The following gradient elution was used: 0 min–1%B, 60 min–26%B, 70 min–40%B. Five µl extract solution was injected to the HPLC column. UV–Vis spectra of detected compounds were recorded over the range from 190 to 450 nm. The chromatogram was acquired at 325 nm. Mass spectra were recorded in the negative ion mode. Compounds were identified basing on their UV–Vis and MS spectra. Comparisons with the available chemical standards and suitable literature were performed (Clifford et al., 2003, 2005; Granica et al., 2013, 2015).

### 2.3. Isolation of phenolics

Dried and finely powdered plant material (570 g) was extracted with 15 l of a methanol:water mixture (75:25, v/v) under reflux for 3 h. The extraction procedure was repeated twice. The obtained extracts were combined and the methanol was evaporated under reduced pressure at 40 °C. The water residue was freeze-dried to yield 111.5 g of crude extract. Then the extract was suspended in methanol:water (5:2, v/v, 700 ml) and subjected to liquid-liquid fractionation with petroleum ether (PE, 5 × 500 ml), ethyl acetate (AcOEt, 6 × 500 ml), and finally *n*-butanol (*n*-BuOH, 5 × 500 ml) to obtain 13.0 g of PE, 15.0 g of AcOEt, 20.2 g of *n*-BuOH, and 74.3 g of water residue. The AcOEt residue (13.0 g) was dissolved in methanol (500 ml), adsorbed on silica gel (20.0 g) and subjected to chromatography on a silica gel column (100 mm × 150 mm) eluted with a mixture of dichloromethane:methanol (from 100:0 to 0:100 in 11 steps – 0%, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 75%, and 100% of methanol – 1000 ml per step) to obtain 47 fractions, which were combined based on TLC analysis (silica gel, 100:11:11:26 – ethyl acetate:formic acid:acetic acid:water, v/v/v/v, sprayed with 1% Naturstoffreagenz A in methanol) into twelve main fractions Z<sub>1</sub>–Z<sub>12</sub>. Fraction Z<sub>4</sub> (440 mg) was chromatographed on a Sephadex® LH-20 column (2.5 cm × 145 cm) eluted with methanol to obtain 180 fractions combined into seven main fractions ZS<sub>1</sub>–ZS<sub>7</sub>. Fraction ZS<sub>7</sub> yielded pure compound **35** (10.9 mg). Fraction Z<sub>6</sub> (1.2 g) was subjected to a Sephadex® LH-20 column (2.5 cm × 145 cm) eluted with methanol to obtain 160 fractions combined into ten main fractions ZU<sub>1</sub>–ZU<sub>10</sub>. Fraction ZU<sub>2</sub> (113 mg) was

dissolved in DMSO and separated using preparative HPLC (Dionex Summit P850 instrument) and a Zorbax SB-C<sub>18</sub> (21.2 × 150 mm × 5 µm) column, eluted with A: 0.1% HCOOH in H<sub>2</sub>O, B: 0.1% HCOOH in MeCN at 5 ml/min, and using the following gradient: 0–35 min, 20–60% B, 25 °C, detection at 254 and 350 nm) to give pure compound **34** (t<sub>r</sub> = 22.3–24.0 min, 6.2 mg). Fraction Z<sub>8</sub> (2.3 g) was separated on a Sephadex® LH-20 column (2.5 cm × 145 cm) eluted with methanol to obtain 180 fractions combined into ten main fractions ZX<sub>1</sub>–ZX<sub>10</sub>. Fraction ZX<sub>5</sub> (92 mg) was dissolved in MeOH and separated using preparative HPLC (the same conditions except the gradient: 0–35 min, 10–40% B) to give pure compound **29** (t<sub>r</sub> = 30.3–33.4 min, 25.3 mg). Fraction ZX<sub>6</sub> (223 mg) was dissolved in DMSO and subjected to preparative HPLC (the same conditions as for ZX<sub>5</sub>) to give pure compounds **14** (t<sub>r</sub> = 19.3–20.1 min, 8.2 mg) and **22** (t<sub>r</sub> = 22.4–24.2 min, 92.9 mg). Fraction Z<sub>9</sub> (1.5 g) was subjected to Sephadex® LH-20 column (2.5 cm × 145 cm) eluted with methanol to obtain 190 fractions combined into twelve main fractions ZZ<sub>1</sub>–ZZ<sub>12</sub>. Fraction ZZ<sub>6</sub> (400 mg) was dissolved in DMSO and separated using preparative HPLC (the same conditions as for ZX<sub>5</sub>) to give pure compounds **21** (t<sub>r</sub> = 20.5–22.4 min, 137.3 mg) and **26** (t<sub>r</sub> = 23.7–24.7 min, 207.4 mg). Fraction ZZ<sub>9</sub> (400 mg) was dissolved in MeOH and subjected to HPLC (the same conditions except the gradient: 0–50 min, 10–40% B) to give compound **20** (t<sub>r</sub> = 25.6–26.8 min, 3.2 mg). Fraction Z<sub>12</sub> (850 mg) was chromatographed on a Sephadex® LH-20 column (2.5 cm × 145 cm) eluted with methanol to obtain 180 fractions combined into nine main fractions ZI<sub>1</sub>–ZI<sub>9</sub>. The combined fractions ZI<sub>6</sub>–ZI<sub>8</sub> (240 mg) were dissolved in DMSO and separated using preparative HPLC (the same conditions as for ZZ<sub>6</sub>) to give pure compounds **8** (t<sub>r</sub> = 17.2–18.5 min, 5.5 mg), **30** (t<sub>r</sub> = 35.5–36.8 min, 40.1 mg) and **32** (t<sub>r</sub> = 37.7–39.5 min, 58.0 mg). The *n*-BuOH residue (15.0 g) was dissolved in water (400 ml) and subjected to a Diaion HP-40 column (5.0 cm × 90 cm) eluted with a mixture of water:methanol (from 100:0 to 0:100 in 5 steps – 0%, 20%, 50%, 70%, and 100% of methanol – 1000 ml per step) to obtain 60 fractions combined based on HPLC analysis into 15 main fractions B<sub>1</sub>–B<sub>15</sub>. Fraction B<sub>2</sub> (3.2 g) was dissolved in 7 ml of DMSO and was directly separated using a preparative HPLC system (Shimadzu LC-20AP instrument) and a Kinetex XB-C<sub>18</sub> (21.2 × 150 mm × 5 µm) column, eluted with A: 0.1% HCOOH in H<sub>2</sub>O, B: 0.1% HCOOH in MeCN at 7 ml/min, and using the following gradient: 0–30 min, 0–20% B, 25 °C, detection at 254 and 325 nm, 30 injections, 200 µl per run) to give pure compounds **2** (t<sub>r</sub> = 16.0–16.7 min, 3.2 mg) and **4** (t<sub>r</sub> = 23.1–23.6 min, 2.5 mg).

## 3. Results and discussion

### 3.1. HPLC analysis

The HPLC analysis of the *E. cannabinum* subsp. *cannabinum* raw extract led to the detection of 35 major polyphenols (Fig. 1 and Table 1). Names of caffeoylquinic acid derivatives are given according to IUPAC as suggested by Clifford et al. (2003).

Compounds **1–19**, **24**, **28**, and **30** showed maxima in UV–Vis spectra at approximately 240, 300, and 310–325 nm. Thus they were classified as phenolic acids, most probably caffeic acid derivatives. Further identification was performed based on MS spectra. Compound **1** to **7** had major pseudomolecular ion at *m/z* = 371 in the negative ion mode. The HR-MS analysis (Table 1) suggested that obtained mass corresponds to the molecular formula of C<sub>15</sub>H<sub>15</sub>O<sub>11</sub>. The fragmentation of the primary ion at 371 revealed the neutral loss of 162 amu resulting in the production of a major fragmentation ion at *m/z* = 209. The neutral loss was assigned to the cleavage of a caffeic acid moiety in each of the investigated compounds (**1–7**). The ion observed in the MS<sup>2</sup> fragmentation spectrum suggested that the detected compounds are hexaric acid esters (molecular weight of altaric and glucaric acid is 210 amu). A similar set of compounds was recently detected in our study devoted to the phytochemical analysis of *Syringa vulgaris* L. (Dudek

Download English Version:

<https://daneshyari.com/en/article/7767563>

Download Persian Version:

<https://daneshyari.com/article/7767563>

[Daneshyari.com](https://daneshyari.com)