Three novel constituents from the roots of *Rhaponticum carthamoides*

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Aims: The purpose of our study was to conduct a phytochemical analysis of *Rhaponticum carthamoides* in order to gain a better understanding of its chemical profile and the active constituents responsible for its bioactivities.

Methods: The methanolic extract of the root was fractionated using multiple chromatographic methods, including normal, medium and high-pressure chromatography, rotation planar chromatography with a variety of stationary phases and eluents. The structures of the isolated compounds were identified by comparing their NMR spectra with those reported in the literature.

Results: As a result of our experiments, the mixture of *cis* and *trans* isomers of tiophene derivatives, vanillic acid, chloro-genic acid methyl ester, ajugasterone C, makisterone C, tachioside, 20-hydroxyecdysone, 24-epi-makisterone A and evofo-lin B were isolated and identified in the root of the plant.

Conclusion: Tachioside, evofolin B and chlorogenic acid methyl ester have been reported for the first time from this species.

Keywords: *Rhaponticum carthamoides, Leuzea carthamoides*, tachioside, evofolin B, chlorogenic acid methyl ester

1 Introduction

Rhaponticum carthamoides (Willd) Iljin, also known as *Leuzea carthamoides* (Willd) DC. or Maral root is a perennial plant belonging to the Asteraceae family. It is indigenous in the subalpine zone of Altay and Sayan Mountains, Siberia, Kazakhstan and Eastern Europe. The plant has been used in the folk medicine for its beneficial properties and thus widely cultivated, especially in Russia. Its main therapeutic indication is based on the adaptogenic effects of the roots and it is used as a general tonic and anabolic to enhance physical and mental performance in physical workers and athletes, and to promote recovery after illnesses and surgical interventions (1–6).

The main constituents of *R. carthamoides* belong to phytoecdysteroids, which are playing a defensive role against insects, acting as inhibitors of insect growth and moulting. In the last decades more than 50 ecdysteroids and their derivatives have been isolated from the plant, the majority of these compounds being identified in the roots, but the seeds and leaves also contain this type of substances (2,7–17). The most abundant constituent is 20-hydroxyecdysone, also known as β-ecdysone or polypodine A. The adaptogenic effect of the plant can be explained by the presence of ecdysteroids. Besides phytoecdysteroids, flavonoids, phenolic acids, lignans, polyacetylenes, sesquiterpene lactones and triterpenes have also been detected from this species (18).

Because of the adaptogenic properties of the *R. carthamoides*, numerous studies were carried out with the plant, aiming to understand its pharmacology. Various animal studies have been performed with the extracts of the plant and its main ecdysteroid, 20-hydroxyecdysone. Increase in protein synthesis and body mass were observed in different experiments, moreover, increased work capacity was also reported (19–23). The adaptogenic effect was studied in human trials as well. In clinical studies with athletes, significant muscle mass increase was observed in the groups treated with *R. carthamoides* or 20-hydroxyecdysone compared with the control (19,24).

However, the pharmacological profile of *R. carthamoides* goes beyond its anabolic effects. The plant extract exerts immunomodulatory activities, which may be explained by the presence of other compounds than ecdysteroids (25,26). The same applies to its central nervous system (27–29) and hemorheological effects (30).

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Considering the perspective use of *R. carthamoides* in the medicine and its undiscovered phytochemical profile, our aim was to identify further compounds from this plant.

2 Materials and methods

2.1 General experimental procedures

The HPLC system comprised of Waters 600 pump, Waters In-line degasser AF, Waters 2487 dual channel UV detector modules connected with Waters 600 control module. Rotation planar chromatography (RPC) was performed with a Chromatotron instrument (model 8924; Harrison Research) on manually coated SiO_2 plates.

The isolated compounds were analysed via NMR. NMR spectra (Supplementary material) were recorded mostly in MeOH-d_4 for ^{1}H spectra, but DMSO-d_6 and $CDCl_3$ were also used for 2D on a Bruker Avance 600 III spectrometer (^{1}H: 600.13 MHz; ^{13}C: 150.9 MHz) equipped with a 5 mm cryo-TXI probe. The peaks of the residual solvents were taken as reference points. Chemical shifts are expressed in parts per million and coupling constants (*J*) values are reported in Hz. Data were acquired and processed with the MestReNova v6.0.2-5475 software and were compared to literature data to identify the compounds.

2.2 Plant material

Rhaponticum carthamoides roots ("Roots of *Leuzea carthamoides* [Lujza]" 2,0 kg) were purchased from Herbosus (Finland, Espoo). The plant material was identified by dr. Zsuzsanna Hajdú (University of Szeged, Institute of Pharmacognosy).

2.3 Extraction and isolation

The dry roots (1.75 kg) were ground, then extracted with MeOH, using an ultrasonic bath at room temperature. The extract then was filtered and was evaporated to dryness, yielding 112.52 grams of dry extract. To the dry residue 1000 mL of MeOH – H_2O (1:1) was added, then extracted with *n*-hexane, CH_2Cl_2 and EtOAc, resulting dry fractions 9.91 g, 28.79 g and 103.37 g, respectively (Figure 1). The EtOAc fraction was fractionated by column chromatography on SiO_2 stationary phase with a gradient elution using CH_2Cl_2 – MeOH (95:5–0:100). After TLC analysis the fractions were merged to subfractions **A-T**. Purification of subfraction **E** by rotation planar chroma-

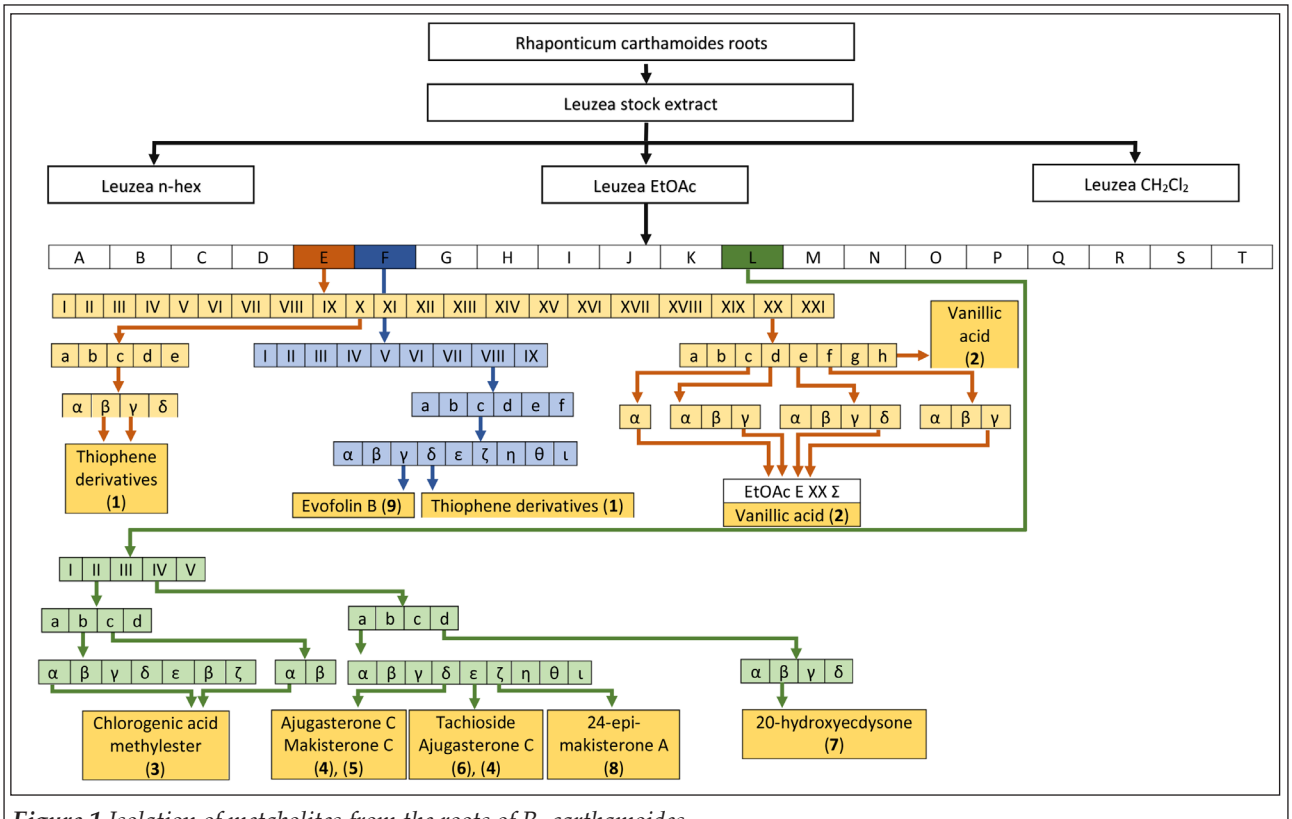

Figure 1 *Isolation of metabolites from the roots of R. carthamoides*

tography (RPC) on 4 mm SiO_2 plates using an *n*-hexane – EtOAc gradient (100:0–0:100) led to subfractions **EI-EXXI**. With the help of HPLC fraction **EX** was further purified on a Phenomenex Kinetex C18 column (250×21.1 mm, 100 Å, 5 μm) using H_2O – MeOH (0 min: 40:60, 15 min: 0:100, 16 min: 0:100, 20 min: 40:60) as eluent. The resulting fractions were further refined with atmospheric pressure column using SiO_2 as stationary and CH_2Cl_2 – EtOAc [100:0–0:100, l0% (20ml each) increments] as mobile phase. As a result, the pure compound **1** (21 mg) were obtained. The subfraction **EXX** was further purified using RPC [1 mm SiO_2 CH_2Cl_2 – EtOH (100:0–0:100, 3.0 mL/min)] and preparative TLC (SiO_2, elution with EtOAc – EtOH – H_2O (4:0.5:0.25) to obtain the pure compound **2** (13 mg).

Subfraction **L** was fractionated with help of medium pressure liquid chromatography (MPLC) using SiO_2 (0.043-0.060 mm) as stationary phase eluted with CH_2Cl_2 – EtOAc gradient (100:0–0:100). After TLC analysis the gained fractions were combined to five subfractions, namely **LI-LV**. The **LII** was further processed on an atmospheric column filled with SiO_2 (0.043–0.060 mm). EtOAc – MeOH (100:0–0:100) was used as eluent. With a subsequent purification using HPLC [LiChrospher RP-C18e column (250×4.6mm, 5 μm), gradient elution with H_2O – MeOH (90:10–50:50) in fifteen minutes], one pure compound (**3**, 10 mg) was obtained. The subfraction **LIV** was further purified by MPLC. As stationary phase SiO_2 (0.043–0.060 mm) was chosen. The gradient elution consisted of EtOAc – MeOH (0 min: 100:0, 60 min: 90:10, 70

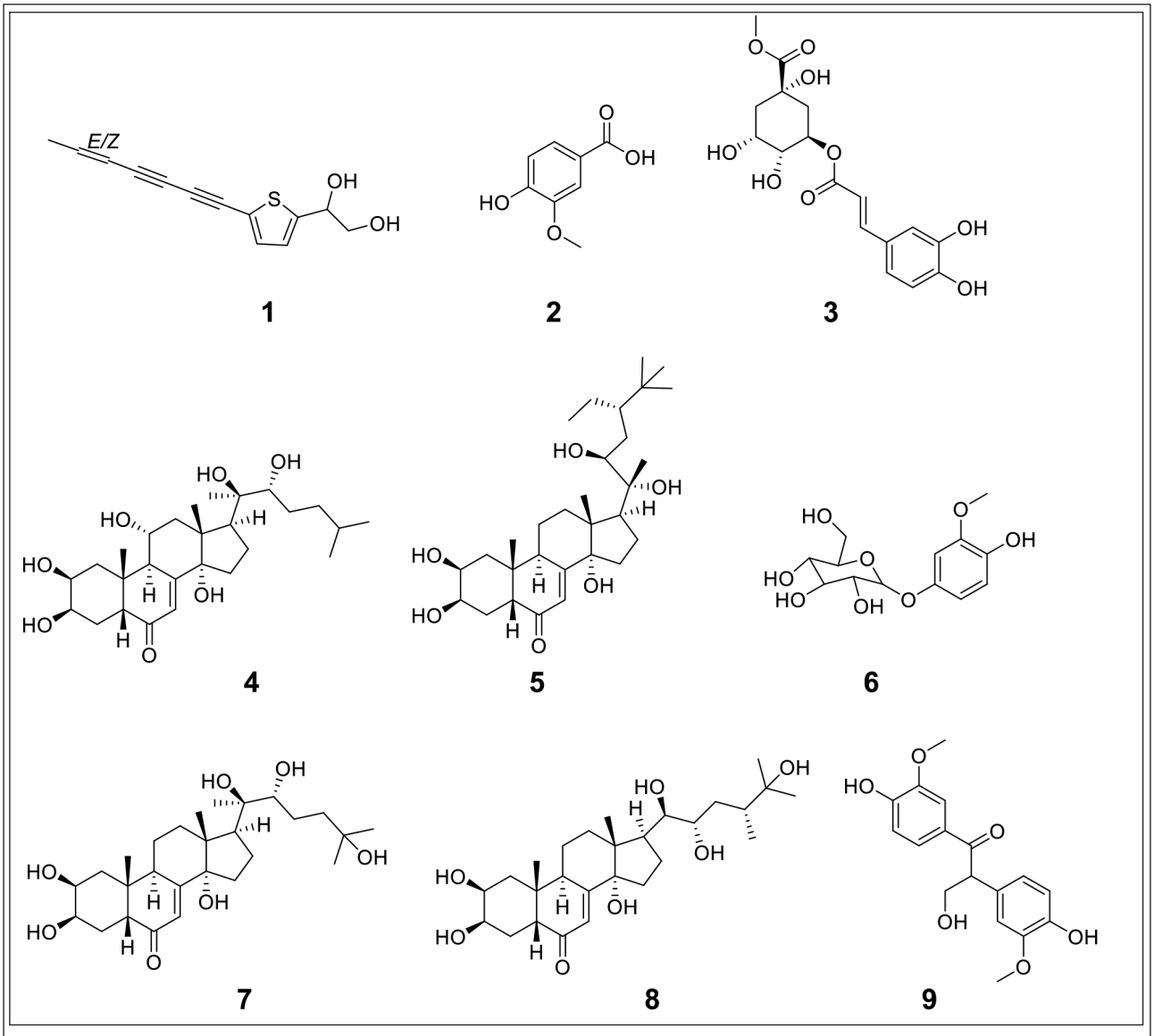

Figure 2 *Compounds 1–9 Isolated from R. carthamoides*

min: 90:10). A further fractionation by HPLC (LiChrospher RP-C18e, 250×4.6 mm, 5 μm column, H_2O – MeOH gradient) resulted in the isolation of the pure compounds **4** (8 mg)**, 5** (2 mg), **6** (2 mg), **7** (43 mg) and **8** (2 mg).

The fraction **F** was further processed by MPLC. To this SiO_2 (0.043–0.060 mm) as stationary phase was used. As eluent hexane – CH_2Cl_2– MeOH (100:0:0–0:100:0–0:50:50), with a flow rate of 15 mL/min was utilised. After TLC analysis and combination of fractions the resulting subfractions were **FI-IX**. The fraction **FVIII** was further purified on MPLC on SiO_2 stationary phase (0.043–0.060 mm) using a gradient elution of CH_2Cl_2 – MeOH – H_2O (100:0:0–100:0:0 to 0:50:50), with a flow rate of 25 mL/min with 5% increments. A final purification with HPLC (Kinetex Phenomenex C18, 150×4.6 mm, 5 μm; H_2O – MeCN gradient) resulted in the isolation of the pure compound **9** (5 mg).

3 Results

As result of a series of various chromatographic purifications, such as normal, medium and high-pressure chromatography, rotation planar chromatography with a variety of stationary phases and eluents, combined with modern structure elucidation methods (NMR, spectra see in Supplementary material) in total nine compounds were isolated from the plant. These compounds were identified by comparing their NMR spectra with those reported in the literature as the mixture of *cis* and *trans* isomers of tiophene derivatives (compound **1**) (31) vanillic acid (**2**) (32), chlorogenic acid methyl ester (**3**) (33), ajugasterone C (**4**) (34), makisterone C (**5**) (35) tachioside (**6**) (36), 20-hydroxyecdysone (**7**) (37) 24-epi-makisterone A (**8**) (35), evofolin B (**9**) (38) (Figure 2).

4 Discussion

Thiophene derivatives (31), vanillic acid (39), ajugasterone C (40), makisterone C (41), 20-hydroxyecdysone (41), 24-epi-makisterone A (35) have been previously reported from this plant, however, the presence of tachioside, evofolin B and chlorogenic acid methyl ester have been reported from *R. carthamoides* for the first time by our research group.

The ecdysteroids ajugasterone C, makisterone C, 20-hydroxyecdysone and 24-epi-makisterone contribute to the adaptogenic effect of the plant. The contribution of other compounds than ecdysteroids in the clinical effects of the plant is slightly discovered. The specific role of vanillic acid and chlorogenic acid methyl ester is not likely, since these compounds are ubiquitous in the plant kingdom and were isolated in small amounts from the plant. Evofolin B, a benzenoid isolated for the first time in 1995 (42), has received little pharmacological attention. The weak *in vitro* quinone reductase inducing (43), the *in vitro* superoxide anion generation inhibitory (44) the moderate *in vitro* lipolytic (45) activities are not directly related to the therapeutic use of *R. carthamoides*. Tachioside, an aromatic glycoside that was first isolated from *Berchemia racemosa* (46) is reported to have *in vitro* tyrosinase (47), moderate alpha-glucosidase (48) and 15-lipoxygenase inhibitiory activities (49) and possesses antioxidant effect (50). However, some of the reported activities of these compounds make them interesting for future investigations and could also open up new opportunites for *R. carthamoides* research.

5 Conclusions

As the result our experiments we isolated and characterised three, previously undescribed compounds (tachioside, evofolin B and chlorogenic acid methyl ester) from this *R. carthamoides* along with 6 already reported constituents.

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