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Phenolic compounds in the flowering herbs of Cirsium esculentum (Siev.) C. A. Mey

Związki fenolowe w kwitnącym zielu *Cirsium esculentum* (Siev.) C. A. Mey

INTRODUCTION

According to literature, plants from the *Cirsium* genus are rich in phenolic compounds [8–11,16–20].

The plants showed biological activity like larval growth inhibition, antibacterial activity and as insect attractant.

Phenolic acids are known to occur widely in plants, they are a prominent group of substances that play an important role in plant physiology, as stimulators of plant growth. Phenolic acids are known to have various biological activities, especially fungistatic, bacteriostatic, choleretic, potential sedative-hypnotic, antianxiety and anticolvulsant ones. Flavonoids, have been shown to possess vasoprotective, hepatoprotective, anti-inflammatory, anticarcinogenic, and free radical-scavenging properties [12,16–19,21,26].

In the investigated plant *Cirsium esculentum (*Siev.) C. A. Mey., flavonoids and the phenolic acids have not been studied yet. For the first time a qualitative and quantitative analysis of flavonoids and phenolic acids in the flowering herb was carried out.

The aim of this study was to carry out the separation of the active components of flowering herb of *Cirsium esculentum* (Siev.) C. A. Mey. using Accelerated Solvent Extraction. The obtained extracts were combined and purified by SPE. The SPE-eluates were analyzed by RP-HPLC. A quantitative analysis of flavonoids and phenolic acids was made.

EXPERIMENTAL DESIGN

Flowering herbs were collected in Mongolia in 2008. They were identified by a specialist from the Herbarium of the Botanical Institute of the Mongolian Academy of Science, Ulaanbaatar, Mongolia, where specimens of the plants were deposited. The aerial parts were dried in the air at room temperature and immediately powdered according to the accepted normal procedures.

EXTRACTION

The plant material (1g) was placed in a stainless-steel cell of Dionex (Sunnyvale, CA, USA) ASE 100 accelerated solvent extractor using methanol as a solvent. The extraction conditions were optimalized, giving the best parameters of extraction for: methanol concentration 70%, temperature: 85 °C, number of cycles: 3. Extraction was performed at 100 bar. All the methanolic extracts were concentrated under reduced pressure, dissolved in a small portion of methanol, and transferred to a 10 mL graduated flask.

Solid Phase Extraction is a popular procedure used for the isolation, purification and preconcentration of organic compounds present in biological material. It is often considered an alternative to other methods. In this study, SPE has been used for the isolation of flavonoids and phenolic acids in the *Cirsium esculentum* (Siev.) C. A. Mey.

Samples containing phenolic compounds were purified from chlorophylls with SPE. Samples were evaporated to dryness, dissolved in 30% aqueous methanol and applied to Octadecyl BakerBond SPE microcolums (500 mg, 3 mL, J.T. Baker Phillipsburg, NJ,USA) previously activated with 10mL methanol and then 10 mL water. The isolated compounds were obtained by the elution of the columns with 7 mL methanol – water 80:20, under reduced pressure (SPE-12G chamber, J.T. Baker USA, Groß-Gerau, Germany).

The eluates obtained were free from ballast compounds and contained flavonoids and phenolic acids.

RP-LC ANALYSIS

The isolation and separation of natural compounds, including phenolic compounds, from plants is a very important analytical problem in phytochemistry [22, 24, 25, 27]. Standard procedures based on TLC still play a major role in the isolation, purification and identification of phenolic compounds. Thin-layer chromatography is the technique most frequently applied for the qualitative analysis of plant extract. [6, 22].

In the present paper, flavonoids and phenolic acids from the flowering herbs of *C. esculentum* were analyzed by 1D TLC and 2D TLC. For the optimization of the separation, several mobile phases were used. Derivatization (after both 1D and 2D TLC) was performed by spraying with a 3% methanolic solution of iron (III) chloride and diazotized sulfanilic acid (dSa) in 20% sodium carbonate solution 1:1 v/v for phenolic acids, and with a 1% methanolic solution of Naturstoffreagenz A and polietylenoglikol 4000 for flavonoids.

The results were confirmed by RP HPLC analysis. RP LC was performed with an Agilent 1100 system coupled with an auto-sampler, a column thermostat; and diode – array detector (DAD). Compounds were separated on 250 X 4.6 mm stainless-steel column packed with 5 μm Hypersil XDB-C18 (ZORBAX Eclipse), using a stepwise mobile phase gradient prepared from 1% aqueous acetic acid (component A) and methanol (component B) (v/v). The gradient was: 0 min, 2%B in A; 8 min 5% B in A; 12 min 10% B in A; 20 min 25% B in A; 35 min 45% B in A; 40min 60% B in A, 45 min 75% B in A. The mobile phase flow rate was 1 mL min^{-1}, the sample injection volume was 10

μL, and elution was performed at 25 °C. The LC pumps, auto sampler, column oven, and DAD were monitored and controlled using HP Chem. Station rev.10.0 software (Agilent)

Identity of the compounds examined was performed by comparing retention times (t_R) and UV spectra with the compounds examined and standard substances analyzed under the same conditions. They were purchased from Sigma. The qualitative and quantitative analysis was performed. Retention times were compared with those of standards, (rutosid, kaempferol 3-rhamnoglucoside and chlorogenic acid) using UV spectra as a comparative parameter. (Fig. 1–3).

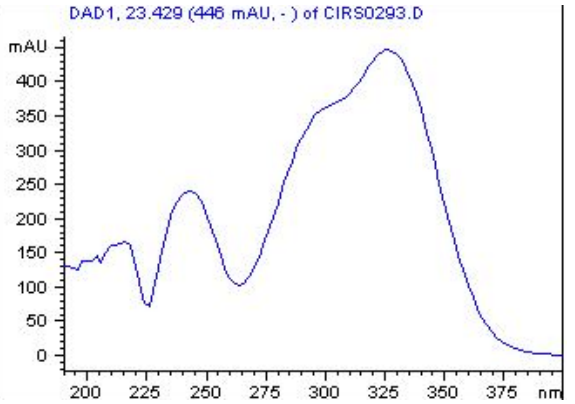

Fig 1. UV spectra (λ=200-400 nm) of compound 1 and chlorogenic acid standard present in the methanolic extract from flowering herbs scanned with DAD

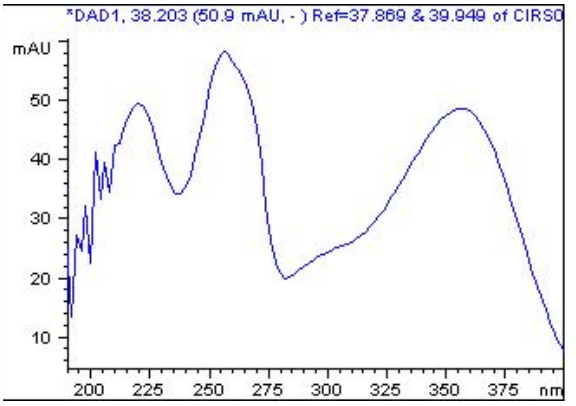

Fig 2. UV spectra (λ=200-400 nm) of compound 2 and rutosid standard present in the methanolic extract from flowering herbs scanned with DAD

Quantitative determination was performed at the wavelength of maximum absorption of flavonoids and chlorogenic acid – 320 nm.

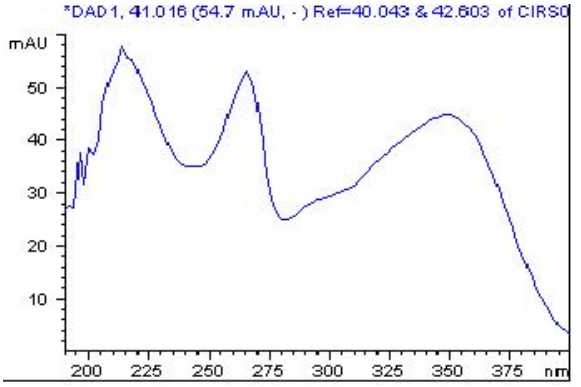

Fig 3. UV spectra (λ=200-400 nm) of compound 3 and kaemferol 3-rhamnoglucoside standard, present in the methanolic extract from flowering herbs scanned with DAD

Each extract was injected in triplicate on the same day. The RSD (relative standard deviation, %) of retention times and peak arras was used as a measure of precision. Method precision was evaluated using intra-day and inter-day tests. Intra – day experiments were performed by replicate analysis of three aliquots of the same sample on the same day, inter-day tests were performed on three consecutive working days in the same way as intraassay experiments [21]. The peak area of each of the extract components was measured three times.

Each calibration plot was prepared three times after chromatography of five different concentrations (1, 0.75, 0.5, 0.25, 0.1 mg per 10mL for rutosid and chlorogenic acid except kaempferol 3-rhamnoglucoside, for which the additional concentration of 0.05 mg per 10mL. was also used). Quantification was performed by comparing the chromatographic peak areas for the external standard.

RESULTS AND DISCUSSION

According to the literature plants from *Cirsium* genus are rich in phenolic compounds [17–20].

Nazaruk et al. [20] performed simultaneous identification of eight phenolic acids (gallic, protocatechuic, chlorogenic, p-hydroxybenzoic, vanillic, caffeic, p-coumaric and ferulic) and free flavonoid aglycones (luteolin, kaempferol and apigenin) in Et_2O-fractions of inflorescences and leaves of five species of *Cirsium* genus – *C. arvense, C.palustre, C.rivulare and C.vulgare* by HPLC method. Qualitative analyses were prepared by extraction of each source (50g) with methanol and with 80% methanol under reflux. The content of phenolic acids, determined by spectroscopic method with Arnov's reagent, were higher in leaves than in flowers. The flavonoid content was examined by Christ-Müller's method.

In contrast in our work 1g plant material was extracted using Accelerated Solvent Extractor and combining method Solid Phase Extraction and Reversed Phase High-Performance Liquid Chromatography for isolation and qualitative determination of flavonoids and phenolic acids. We

found our extract from *C. esculentum* to be poorer then the respective phenolic compounds content compared to that presented by Nazaruk et al. [20].

In the investigated flowering herbs of *Cirsium esculentum* (Siev.) C. A. Mey. qualitative HPLC analysis was performed for some of the identified compounds. The following flavonoids were identified: kaempferol 3- rhamnoglucoside, rutosid and chlorogenic acid.

Standard deviation was calculated for all the results leading to the conclusion that the results are statistically significant. Calibration plots for the phenolic acids were highly linear (R^2>0.991) in the concentration range 0.05–1.00 mg per 10 mL (n=3).

HPLC analysis of the extracts enabled identification of two flavonoids and one phenolic acid. Typical chromatograms obtained from the extracts from aerial parts are shown in Fig 4.

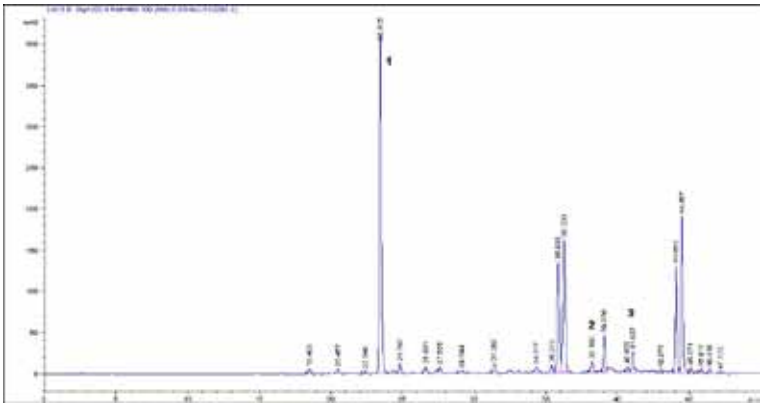

Fig. 4. LC chromatogram of an extract of the flowering herb of *C. esculentum* obtained by ASE at 85°C, repeated three times; peaks: 1) chlorogenic acid, 2) rutosid, 3) kaempferol 3-rhamnoglucoside

The analysis of methanol extracts of flowering herbs of the *Cirsium esculentum* (Siev.) C. A. Mey as well as their quantitative analysis were carried out for the first time.

Amounts of all flavonoids and phenolic acid were estimated by HPLC. Chlorogenic acid was found in the studied fraction; it was also the predominant acid in the flowering herbs (46122.13) [μg/g] Table1.

Table 1. Flavonoids and phenolic acid content of extracts obtained from the investigated plant

	c	**±S.D.**	**RSD**	**Calibration curve**	***R²***
Kaempferol 3- rhamnoglucoside	6437.51	9.66	0.15	y = 157.51x – 144.64	0.9917
rutosid	1184.85	5.45	0.46	y = 173.78x – 113.76	0.9908
chlorogenic acid	46122.13	30.18	0.06	y = 646.75x – 629.77	0.9919

Each value is the mean (μg per 1 g dry sample) from three replicate analyses, SD – standard deviation, RSD – relative standard deviation

Results of the study suggest that chlorogenic acid may by responsible for the activity of plants of the *Cirsium* genus. This is important because this acid has a widely known antioxidant activity [1, 15, 18, 26]. Flavonoids are of particular interest because of their various pharmacological activities (including antianginal, antihepatotoxic, antimicrobial, antiulcer, spasmolytic, antiallergic, antiinflammatory, antiviral, anticarcinogenic and antioxidant) [2, 7, 19]. *Cirsium esculentum* (Siev.) C. A. Mey. are also rich in kaempferol 3- rhamnoglucoside (6437.51 μg/g) and rutosid 1184.85 μg/g Table1.

These compounds may by responsible for the activity of the *Cirsium* genus. Rutoside, which is more commonly known as Rutin but also called quercetin-3-rutinoside a well-known natural antioxidant, is one of the medicinally important flavonoids. Rutin can reduce capillary fragility, swelling and bruising and has been used in the treatment of venous insufficiency (varicose veins, hemorrhoids, diabetic vascular disease, and diabetic retinopathy), and for improving micro-vascular blood flow (pain, tired legs, night cramps, and restless legs) [3, 5].

The obtained methanol extracts proved to be very rich in flavonoids, some of which have not been identified. Therefore, more research is required.

The flowering herbs of *C. esculentum* (Siev.) C. A. Mey. have not previously been studied. Flavonoids and phenolic acids have been quantitatively analyzed for the first time.

CONCLUSIONS

This is the first report of simultaneous quantification of two flavonoids and one phenolic acid in flowering herbs of *C. esculentum* (Siev.) C. A. Mey. TLC is not only inexpensive but also a very suitable method for rapid separation and identification of flavonoids and phenolic acids present in the *Cirsium* species extracts. ASE and SPE proved to be an inexpensive but very efficient method for rapid isolation, separation and identification of the flavonoids and phenolic acids present in the extract examined.

The result of our investigation enabled us to establish a simple RP-HPLC method. This method for simultaneous analysis is very simple, economical and suitable for rapid screening of phenolic compounds in plants.

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SUMMARY

In the investigated aerial parts of flowering herbs of *Cirsium esculentum* (Siev.) C. A. Mey. the following have been identified: kaempferol 3- rhamnoglucoside, rutoside and chlorogenic acid. The analysis of methanol extracts as well as their quantitative analysis were carried out for the first time. The methanol extract is very rich in flavonoids but some of the flavonoids were not identified. Therefore, more research is required. Calibration plots for the phenolic acids were highly linear (R^2>0.991) in the concentration range 0.05–1.00 mg per 10 mL (n=3).

Standard deviation was calculated for all of the results leading to the conclusion that the results are statistically significant. ASE and SPE proved to be an inexpensive but very efficient method for rapid isolation, separation and identification of the flavonoids and phenolic acids present in the extract examined.

Keywords: Accelerated Solvent Extraction, flavonoids, chlorogenic acid, *Cirsium esculentum* (Siev.) C. A. Mey.

STRESZCZENIE

W pracy omówiono analizę jakościową i ilościową zidentyfikowanych substancji aktywnych uzyskanych z nadziemnych kwitnących części *Cirsium esculentum* (Siev.) C. A. Mey. W celu identyfikacji kwasów fenolowych i flawonoidów w kwitnącym zielu *Cirsium esculentum* (Siev.) C. A. przeprowadzono ekstrakcję przy użyciu ASE oraz jakościową i ilościową analizę TLC oraz SPE-HPLC badanych związków. Zebrano i porównano widma z dostępnymi wzorcami. Przeprowadzona kalibracja dla kwasów fenolowych i flawonoidów jest wysoce linearna (R^2 > 0.991) w stężeniu 0.05–1.00 mg na 10 mL (n = 3). W wyniku przeprowadzonych badań w zielu stwierdzono obecność kwasu chlorogenowego oraz flawonoidów: 3 ramnoglukozyd kemferolu (6437.51 μg/g) i rutozydu (1184.85 μg/g). Otrzymany wyciąg jest bogaty w związki flawonoidowe, które zostały niezidentyfikowane, dlatego też będą prowadzone dalsze badania. Badania składu jakościowego i ilościowego nadziemnych części *Cirsium esculentum* (Siev.) C. A. Mey. były przeprowadzone po raz pierwszy.

Słowa kluczowe: ASE, flawonoidy, kwas chlorogenowy, *Cirsium esculentum* (Siev.) C. A. Mey.