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Pharmacochemistry

HPLC Analysis of an Anticonvulsant Fraction from the Roots of *Cephalaria Gigantea*

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ABSTRACT. The chemical composition and biological effectiveness of a Caucasian endemic plant - *Cephalaria gigantea* were studied. The presence of alkaloids, flavonoids and phenol carboxylic acids was revealed in the roots of this plant. The main components, however, are triterpene glycosides, derivatives of hederagenin and oleanolic acid. They are represented by 15 compounds, named giganteosides. Giganteosides D, G, L, M and N turned out to be new organic compounds. Saponins containing glucuronic acid in the sugar chain were revealed in *Cephalaria* species. Antifungal and antiprotozoal activities of several giganteosides and enriched fractions were evaluated. The alkaloid fraction showed an antimalarial effect. The aqueous extract of *C. gigantea* roots has high anticonvulsive effect and minimal toxicity in pentylenetetrazol and audiogenic seizure models. The present paper describes isolation of an enriched fraction of phenolic compounds from the anticonvulsive substance, named "Epicef". The high performance liquid chromatographic (HPLC) analysis method of this fraction was proposed. Several dominant compounds were identified, on the basis on the retention time, using correspoding standards and UV spectra. Three main compounds were identified: chlorogenic acid, caffeic acid and a flavonoid glycoside quercitrin. This method will be used for the standartization of the expected anticonvulsive drug form "Epicef". © *2017 Bull. Georg. Natl. Acad. Sci.*

Key words: Triterpene saponins, giganteoside, HPLC, antifungal, anticonvulsive activity

Out of 60 species of the genus *Cephalaria*, 12 are found in Georgia. Among those *Cephalaria gigantea (Ledeb*.) Bobr. (fam. Dipsacaceae) is a Caucasian endemic plant, widespread throughout the Caucasus [1].

The presence of alkaloids, flavonoids, phenolcarboxylic acids and triterpene compounds was demonstrated in different species of the genus *Cephalaria* [2].

In the 1970s the Director of the Institute of Experimental and Clinical Neurology, Member of the USSR and Georgian Academy of Sciences, Peter Sarajishvili, became interested by the fact that V. and G. Japaridze, residents of Chiatura, were successfully using aque-

ous extracts of the *Cephalaria* roots in the treatment of epilepsy and he offered us to study this plant. We gladly accepted academician P. Sarajishvili's offer and began the phytochemical study of the plant.

Preliminary phytochemical analysis showed the presence of 0.2 % alkaloids in the plant's roots. These include 3 known alkaloids: gentianine, gentianidine, gentianaine [3]. The main constituents of the roots are triterpene saponins, represented by 15 compounds, named giganteosides. With respect to the increase of polarity they are represented as: A, B, C, D, E, F, G, H, I, J, J', K, L, M, N.

Chemical structures of individual compounds isolated from the crude extract of *C. gigantea* were established by physical-chemical methods, by 1D and 2D NMR experiments (^{1}H, ^{13}C, gs-COSY, gs-HMBC, gs-HMQC and gs-HSQC-TOCSY) and mass spectrometry (MALDI-TOF, ESI-HR-MS [4-10].

Giganteosides are the derivative of two aglycones: oleanolic acid and hederagenin. Saponins containing glucuronic acid in the sugar chain were reported in *Cephalaria* species. Giganteosides D, G, L, M and N represent new organic compounds: Giganteoside D – α-L-Rhap(1→2)-β-D-Xylp(1→3)- oleanolic acid; Giganteoside G - α-L-Rhap(1→2)-β-D-Xylp(1→3)-oleanolic acid 28-O-β-D-Glcp; Giganteoside L - α-L-Rhap(1→2)-β-D-GlcAp(1→3)- hederagenin -28-O-β-D-Glcp(1→6)-β-D-Glcp; Giganteoside M -β-D-Galp(1→2)-β-D-GlcAp(1→3)- oleanolic acid 28-O-β-D-Glcp(1→6)-β-D-Glcp; Giganteoside N -β-D-Galp(1→2)-β-D-GlcAp(1→3)- hederagenin - 28-O-β-D-Glcp(1→6)-β-D-Glcp [4,8].

The fungicidal and antiprotozoal activities of individual substances and enriched fractions from the roots of *Cephalaria* were studied in the Laboratory of Botany and Parasitology of the faculty of Pharmacy at the University of Mediterranean (Marseille, France). Giganteoside E exhibited a strong effect on HL-60 leukemia cells [9, 10]. The alkaloid containing fractions of the roots of *C. gigantea* were active against *Plazmodium falciparum*, parasite that causes malaria [2].

The anticonvulsive effects of the aqueous extracts of *C. gigantea* were studied on pentylenetetrazol and audiogenic seizure models (Krushinsky-Molodkina and Wistar rat strains). It was shown that the aqueous extract is effective in both types (single and repeated) of oral administration and injection. At the same time, significant increase in the duration of a barbiturate induced sleep in mice shows that the extract possesses sedative properties.

We have developed a method for obtaining an active fraction from *C. gigantea* [11] named "Epicef", which has a strong anticonvulsive effect and a very low toxicity. This gives an opportunity for creating of a new medicine of plant origin for prevention and treatment of psychosomatic forms of epilepsy [12].

The present paper describes the qualitative HPLC study of the phenolic fraction of "Epicef". In order to achieve our goal we have obtained enriched fraction of phenolic compounds from the active extract of *C. gignatea*, by multiple extractions of the aqueous extract with ethylacetate. Namely, 10 g of dry extract were dissolved in 80 mL of distilled water with the use of an ultrasonic bath. The obtained solution was placed in a 200 mL separation funnel and extracted with 200 mL ethylacetate 5 times with careful stirring. The united ethylacetate layers were placed in a vacuum rotating evaporator to remove the solvent. The final stage of evaporation was performed in a vacuum dryer at 50-60°C with the presence of calcium chloride.

The obtained aqueous and ethylacetate (phenolic) fractions were assessed using thin layer chromatography. Nine phenolic components were identified in both fractions. The mixture of water (0.1%HCOOH) - acetonitrile (0.1%HCOOH) was used as a mobile phase with gradient condition (acetonitrile 5% → 40%). All solutions were of HPLC purity, the concentrations of the sample and standards were 10 mg/mL and 1 mg/mL in MeOH, respectively. Solid phase - C18 reversed phase column Kinetex XB-Rp18, 250X4.6mm (Phenomenex), run time 25 min. UV detection was performed at 254, 280, 325 and 365 nm.

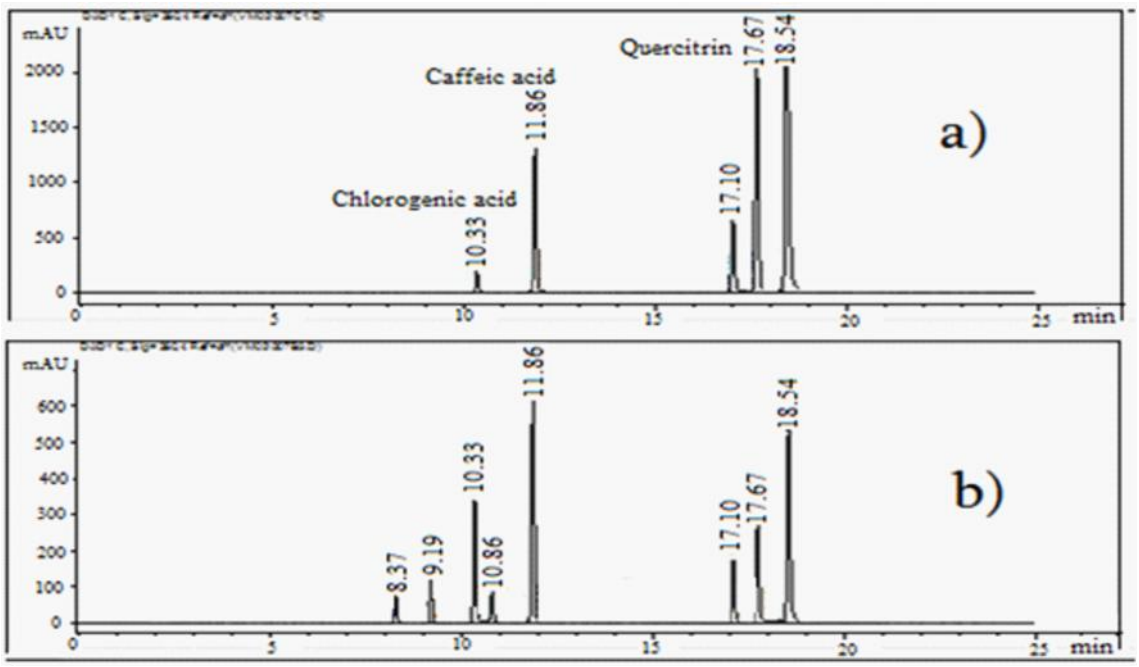

Fig. 1. Comparative HPLC analyses of ethylacetate (a) and aqueous fractions (b) of *C. gigantea* extract (280 nm). Chlorogenic acid (r. t. 10.33 min), caffeic acid (r. t. 11.86 min) and quercitrin (r. t. 17.67 min).

The following compounds were used as standards: rutin, caffeic acid, chlorogenic acid, cumaric acid, ferulic acid, hyperoside, luteolin and quercitrin.

A much higher concentration of phenolic compounds (both flavonoids and phenolic acids) was found in the ethyl acetate fraction compared to the aqueous fraction. Several dominant compounds were identified in both fractions with the more polar phenolic acids prevailing in the aqueous fraction, while the less polar flavonoids prevailing in the ethyl acetate fraction.

On the basis of the retention time, using correspoding standards and UV spectra, three main compounds were identified: chlorogenic acid, caffeic acid and flavonoid glycoside quercitrin in both fractions (Fig. 1).

Thus, an HPLC analysis method was developed for the qualitative detection of phenolcarboxylic acids (caffeic and chlorogenic acids) and a flavonoid glycoside quercitrin in the roots of *Cephalaria gigantea*. The obtained results allow us to perform standardization of biological and chemical markers of the anticonvulsive drug form “Epicef”.

Thus, the *Cephalaria gigantea* is a very perspective medicinal source due to its large botanical resources in Georgia having rich chemical composition and wide range of biological activities.

ფარმაკოქიმია

Cephalaria gigantea-ს ფესვების კრუნჩხვების საწინააღმდეგო ფრაქციის HPLC ანალიზი

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**თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, იოველ ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი, ტერპენული შენაერთების ლაბორატორია, თბილისი, საქართველო*
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გამოკვლეულია კავკასიის ენდემური სახეობის - ***Cephalaria gigantea***-ს ფესვების ქიმიური შედგენილობა და ბიოლოგიური ეფექტურობა. მათში აღმოჩენილია ალკალოიდები, ფლავონოიდები, ფენოლკარბონმჟავები, ძირითადი კომპონენტები კი ჰედერაგენინის და ოლეანოლის მჟავას წარმოებული ტრიტერპენული გლიკოზიდებია, რომლებიც 15 ნივთიერებითაა წარმოდგენილი. მათ გიგანტეოზიდები ეწოდათ. გამოყოფილი და დახასიათებულია ყველა მათგანი. გიგანტეოზიდები **D, G, L, M** და **N** ახალი ორგანული ნივთიერებები აღმოჩნდნენ.

ნაჩვენებია ზოგიერთი გიგანტეოზიდის და გამდიდრებული ფრაქციის ფუნგიციდური და ანტიპროტოზოული აქტივობები. ალკალოიდების ჯამმა მალარიის პლაზმოდიების საწინააღმდეგო მოქმედება გამოავლინა. განსაკუთრებით აღსანიშნავია ***C. gigantea***-ს ფესვების წყლიანი ექსტრაქტის კრუნჩხვების საწინააღმდეგო ეფექტურობა, რომელიც შიგნით მიღების ან ინექციის პირობებში მოქმედებს პენტილენტეტრაზოლური და აუდიოგენური კრუნჩხვების მოდელებზე ძლიერ დაბალი ტოქსიკურობის პირობებში.

სტატიაში აღწერილია კრუნჩხვების საწინააღმდეგო მოქმედების აქტიური სუბსტანციიდან („ეპიცეფი“) ფენოლებით გამდიდრებული ფრაქციის მიღება და შემუშავებულია მათი იდენტიფიცირების **HPLC** მეთოდი. ფენოლურ ფრაქციაში იდენტიფიცირებულ იქნა ხუთამდე დომინანტი ნივთიერება, მათ შორის: ქლოროგენის მჟავა, ყავის მჟავა და ფლავანოიდური გლიკოზიდი ქვერცეტრინი. მიღებული შედეგები საშუალებას გვაძლევს, შემდგომ ეტაპზე მოვახდინოთ კრუნჩხვების საწინააღმდეგო პრეპარატ „ეპიცეფის“ სტანდარტიზაცია.

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