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# CHOICE OF THE OPTIMAL METHOD OF EXTRACTION OF FLAVONOIDS AND POLYPHENOLS OF *CENTAURIUM ERYTHRAEA* RAFN. HERB

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**Introduction.** Using knowledge of traditional medicine and recent scientific advancements, a significant number of medicines based on biological active substances from medicinal plants have been developed. Important biological active substances with pronounced pharmacological effects are flavonoids and polyphenols. Therefore, the aim of the study was to investigate the effect of the method of extracting total flavonoids and total polyphenols from *Centaurium erythraea* Rafn. herb.

**Materials and methods.** The object of the study was the *Centaurium erythraea* Rafn. herb. 69% ethanol was used as the extractant, with a raw material to extractant ratio of 1:5. Extracts from common centaury herb were obtained using the following methods: maceration, remaceration, maceration with stirring, and ultrasonic extraction. The evaluation criterion was the yield of total polyphenols and total flavonoids. The HPLC method was used to determine the qualitative composition and quantify the individual flavonoids.

**Results.** The research results showed that the maceration with stirring method extracted the highest total amounts of polyphenols ((18.2±0.19) mg/ml) and flavonoids ((16.57±0.19) mg/ml) from the investigated raw material. The qualitative composition and quantitative content of individual flavonoids were determined in the extract obtained via this method. It was found that the predominant flavonoid was neohesperidin (641.44 µg/ml). Rutin (217.07 µg/ml), kaempferol (42.36 µg/ml), quercetin (24.58 µg/ml), quercetin 3-O-beta-D-glucoside (22.16 µg/ml), and kaempferol 3-O-beta-D-glucoside (10.83 µg/ml) were also detected in slightly smaller amounts.

**Conclusions.** The highest amount of total flavonoids and total polyphenols was extracted from extracts obtained by the maceration with stirring method. Among the individual flavonoids determined by the HPLC method, neohesperidin prevailed.

**Key words:** *Centaurium erythraea* Rafn., herb, flavonoids, polyphenols, HPLC.

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**ВИБІР ОПТИМАЛЬНОГО МЕТОДУ ЕКСТРАГУВАННЯ ДЛЯ ВИЛУЧЕННЯ ФЛАВОНОЇДІВ І ПОЛІФЕНОЛІВ ІЗ ТРАВИ *CENTAURIUM ERYTHRAEA* RAFN.**

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Метою дослідження було вивчення впливу методу екстрагування на вилучення суми флавоноїдів та суми поліфенолів із трави золототисячника звичайного.

Об'єктом для досліджень була трава золототисячника звичайного; екстрагент – 69% етанол, співвідношення сировина : екстрагент – 1:5. Екстракти одержували методами мацерації, ремацерації, мацерації з перемішуванням, ультразвукової екстракції. Критерієм оцінювання був вихід суми поліфенолів та суми флавоноїдів. Методом ВЕРХ встановлювали якісний склад та визначали кількісний вміст індивідуальних флавоноїдів.

Методом мацерації з перемішуванням вилучено з досліджуваної сировини найбільшу кількість поліфенолів ((18,2±0,19) мг/мл) і флавоноїдів ((16,57±0,19) мг/мл). Домінуючим флавоноїдом був неогесперидин (641,44 мкг/мл); дещо менше виявлено рутину (217,07 мкг/мл).

**Ключові слова:** *Centaurium erythraea* Rafn., трава, флавоноїди, поліфеноли, ВЕРХ.

**Introduction.** Each year, the range of medications in the pharmaceutical market continues to expand. Drawing on historical experience and recent scientific advancements, a significant number of drugs have been developed based on biologically active substances obtained from medicinal plant raw materials. It is important to note the advantages of herbal medicines over synthetic ones, as they rarely cause side effects and are well tolerated by patients of all ages [1]. To ensure effective prevention and comprehensive treatment of diseases, the development and implementation of medications based on medicinal plant raw materials remain a pertinent issue. Despite the wide range of phytopreparations presented on the pharmaceutical market of Ukraine, most of them are medications of foreign origin. Therefore, an important task of modern pharmaceutical science is the search for plants with a sufficient raw material base, which can lay the groundwork for medicinal products. *Centaurium erythraea* Rafn is among such plants.

The herb of common centaury is included in twenty-three Pharmacopoeias worldwide, involving the State Pharmacopoeia of Ukraine.

The plant is used for metabolic disorders. It stimulates the secretion of gastric juice without changing its acidity and increases bile secretion. It is also used for treating liver and kidney diseases, diabetes, and hemorrhoids, and exhibits anti-inflammatory, analgesic, and antioxidant activity [2]. The properties of common centaury are determined by the presence of many biologically active substances, including flavonoids and polyphenols.

**The aim of the article** – to study the effect of the method of extracting flavonoids and polyphenols from *Centaurium erythraea* Rafn. herb.

**Materials and methods.** The object of the study was the herb of *Centaurium erythraea* Rafn., harvested at the beginning of plant flowering in 2023 in the outskirts of the city of Zboriv, Ternopil region. The herb was dried in a warm-air convection dryer at a temperature of 40°C and stored in paper bags in a dry place [3].

As an extractant, 69% ethanol was used, with a raw material to extractant ratio of 1:5 [4]. For the extraction of biologically active substances from the *Centaurium erythraea* Rafn. herb, the following methods were used: maceration, remaceration, maceration with stirring and ultrasonic extraction.

When establishing the optimal extraction method, the evaluation criterion was the yield of the total amount of polyphenols and total flavonoids, whose quantitative content was determined by the spectrophotometric method using the LabAnalyt SP-V1000 spectrophotometer (China).

The quantitative content of total flavonoids was measured using rutin as a standard at a wavelength of 408 nm in a 10 mm path length cuvette. The total polyphenols were measured using pyrogallol as a standard at a wavelength of 760 nm [5].

High-performance liquid chromatography (HPLC) was used to determine the qualitative composition and quantitative content of individual flavonoids in the extract with the highest total flavonoid content. Analysis was performed on an Agilent Technologies 1200 liquid chromatograph.

To do this, the obtained extract from the *Centaurium erythraea* Rafn. herb was diluted 8 times with 80% ethanol. The resulting solution was centrifuged at 3000 rpm. Then, the centrifugate was filtered through disposable membrane filters with pores of 0.22 μm [6].

Acetonitrile (A) and 0.1% formic acid solution in water (B) were used as the mobile phase. Elution was performed in gradient mode (Table 1).

Table 1

**HPLC-DAD gradient solvent system for flavonoids separation**

| Time, min | Solvent A (%) | Solvent B (%) |
|---|---|---|
| 0 | 5 | 95 |
| 20 | 30 | 70 |
| 50 | 100 | 0 |
| 60 | 100 | 0 |

The separation was carried out on a Zorbax SB-C18 chromatographic column (5 μm, 150 mm x 4.6 mm) (Agilent Technologies, USA). The flow rate through the column was 0.25 mL/min, the thermostat temperature was set to 30°C, and the injection volume was 4 μL.

Detection was performed using a diode array detector with signal registration at 280 nm and recording of absorption spectra in the range of 210–700 nm.

Identification and quantitative analysis were conducted using standard solutions of flavonoids, namely: rutin, kaempferol, kaempferol 3-O-beta-D-glucoside, rhamnetin, naringin, neohesperidin, naringenin, apigenin, fisetin, silibinin, baicalein, quercetin 3-O-beta-D-glucoside, quercetin, naringin, luteolin, kasticin. Calibration was performed using the external standards method [7]. The content of flavonoids was calculated in μg/mL of the extract [8].

The results obtained from the research were expressed as the mean ± SEM. The analysis was conducted using the Statistica v 10.0 (StatSoft Inc.) software. The statistical significance of differences between the mean values was evaluated using the Student's t-test. The significance level was set at $*p<0.05$ [9].

**Results and Discussion.** The impact of different extraction methods on the extraction of total polyphenols from *Centaurium erythraea* Rafn. herb demonstrates the order of advantages as: maceration with stirring > maceration > ultrasound extraction > remaceration.

Analyzing the obtained results (Fig. 1), it was found that the highest amount of polyphenols was extracted when using the maceration with stirring method – (18.2±0.19) mg/ml. Using maceration extracted slightly less of these compounds from *Centaurium erythraea* Rafn. herb – (18±0.22) mg/ml. The ultrasound extraction method extracted (17.35±0.17) mg/ml of polyphenols. The lowest amount of these compounds was extracted from the investigated raw material using remaceration – (16.21±0.11) mg/ml.

The influence of the extraction method on the extraction of total flavonoids from *Centaurium erythraea* Rafn. herb shows the following advantages: maceration with stirring > maceration > ultrasound extraction = remaceration, as shown in Figure 2.

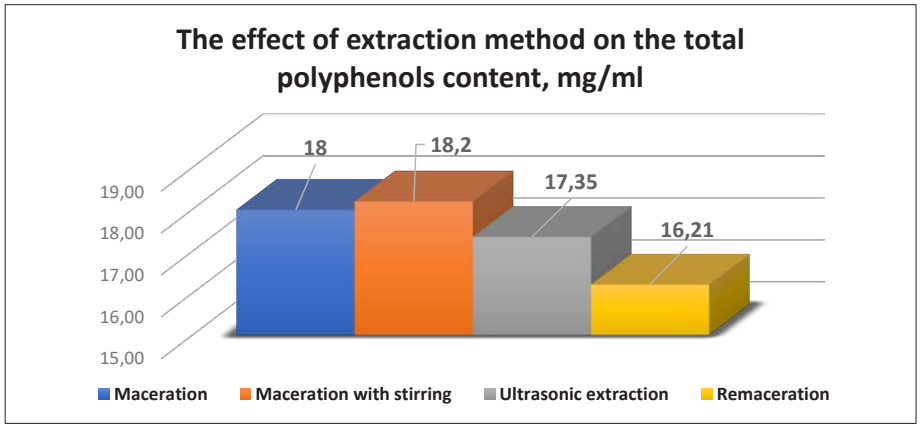


**Fig. 1. The influence of the extraction method on the extraction of total polyphenols from *Centaurium erythraea* Rafn. herb**

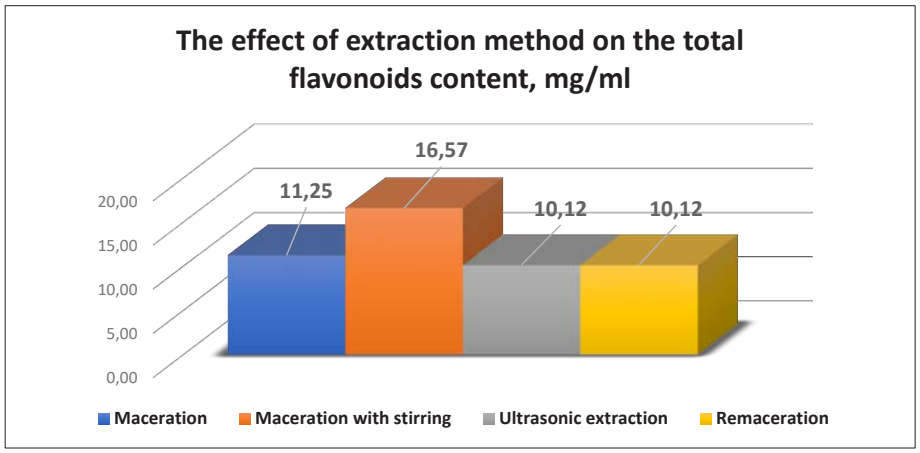


**Fig. 2. The influence of the extraction method on the extraction of total flavonoids from *Centaurium erythraea* Rafn. herb**

Research results indicate that the highest amount of total flavonoids was extracted using the maceration with stirring method, with an amount of (16.57±0.19) mg/ml. A lower amount, approximately 1.5 times less, was extracted from the common centaury herb using the maceration method, with a quantitative content of (11.25±0.37) mg/ml. Ultrasound extraction and remaceration extracted the same amount of flavonoids – (10.12±0.26) mg/ml and (10.12±0.16) mg/ml, respectively.

Flavonoids represent a crucial group of secondary metabolites produced by plants [10]. Given the current trends in the study of plant metabolites, flavonoids have gained prominence due to their significant bioactive properties, including antioxidant, antimicrobial, anti-inflammatory, and anti-cancer activities [11].

The HPLC analysis of the extract from the herb of *Centaurium erythraea* Rafn. shows the presence of some flavonoids. The HPLC chromatogram of flavonoids of the common centaury herb is presented in Figure 3.

Six flavonoids were detected in the extract from the studied raw material, including rutin, kaempferol, kaempferol 3-O-beta-D-glucoside, quercetin, quercetin 3-O-beta-D-glucoside, and neohesperidin (Table 2).

Neohesperidin was found in the largest amounts among the flavonoids in the extract from the herb of *Centaurium erythraea* Rafn. Its content was 641.44 µg/ml. Neohesperidin (hesperetin 7-O-neohesperidoside) is a known flavanone glycoside that has demonstrated a variety of biological activities, with potential applications ranging from food ingredients to therapeutics. It is an emerging therapeutic agent for the management of different etiologically complex diseases. Neohesperidin exhibits a wide range of biological and therapeutic activities in the treatment of different complex illnesses, including diabetes, obesity, hepato-cardiac conditions, infectious diseases, allergies, inflammatory, neurodegenerative, and cancer diseases [12; 13].

Additionally, a high amount of rutin (217.07 µg/ml) was determined among the flavonoids in the extract from the herb of *Centaurium erythraea* Rafn. Rutin is a glycoside that combines the flavonol quercetin with the disaccharide rutinose (glucose and rhamnose). Rutin exhibits various effects, such as antioxidant, cardioprotective, antidiabetic, hepatoprotective, antibacterial, antifungal, anti-inflammatory, neuroprotective, nephroprotective, haematoprotective, and anticancer properties [14; 15].

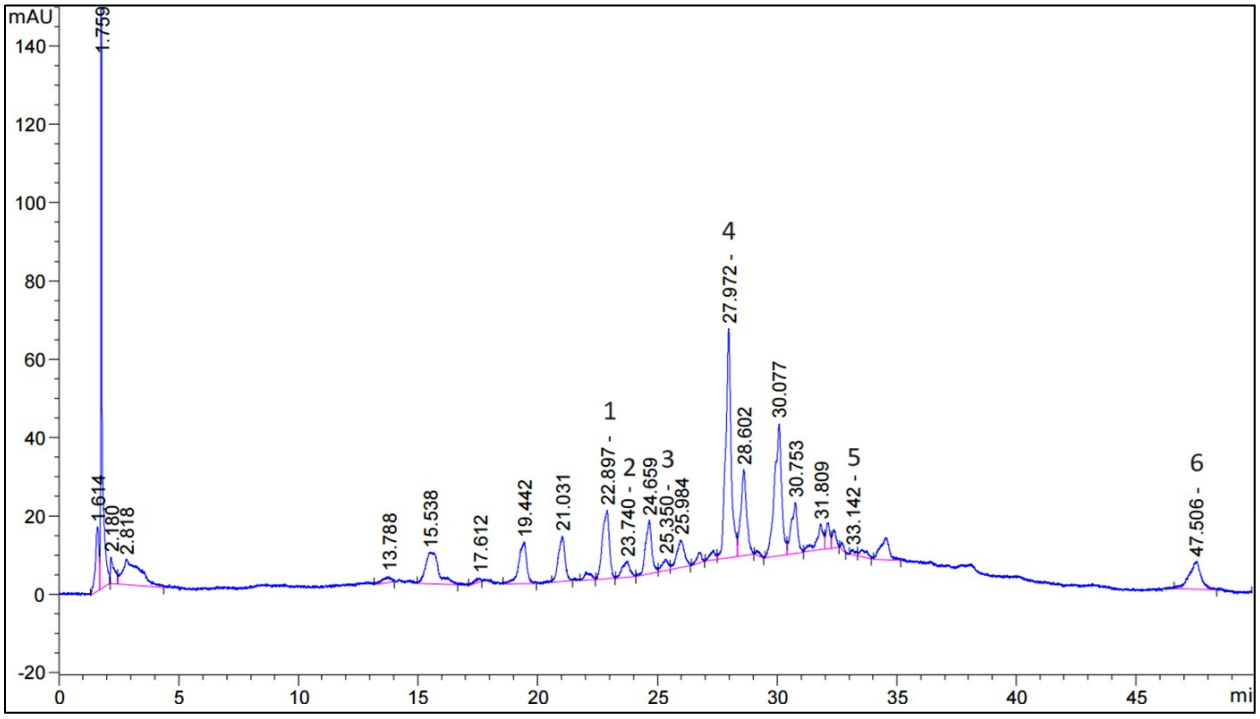


**Fig. 3. HPLC chromatogram of flavonoids identified in the extract from *Centaurium erythraea* Rafn. herb**

Table 2

**HPLC analysis of flavonoids in the extract from *Centaurium erythraea* Rafn. herb**

| No. | Retention time | Common name of identified compound | Quantitative content, µg/ml |
|---|---|---|---|
| 1 | 22.90 | rutin | 217.07 |
| 2 | 23.74 | kaempferol | 42.36 |
| 3 | 25.35 | kaempferol 3-O-beta-D-glucoside | 10.83 |
| 4 | 27.97 | quercetin | 24.58 |
| 5 | 33.14 | quercetin 3-O-beta-D-glucoside | 22.16 |
| 6 | 47.51 | neohesperidin | 641.44 |

**Conclusions.** The quantitative content of total flavonoids and total polyphenols was determined in the extracts obtained by different methods. The highest amount of flavonoids was extracted using the maceration with stirring method, with a content of (16.57±0.19) mg/ml. Similarly, the highest amount of polyphenols was also extracted using the maceration with stirring method, with a content of (18.2±0.19) mg/ml.

Since the maceration with stirring method resulted in the highest amount of bioactive compounds, the content of individual flavonoids was further investigated using the HPLC method.

The qualitative composition and quantitative content of flavonoids in the extract from *Centaurium erythraea* Rafn. herb was determined, with neohesperidin being the predominant compound (641.44 µg/ml).

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