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TOTAL PHENOLIC AND FLAVONOID CONTENT, ANTIOXIDANT ACTIVITY OF *THALICTRUM FOETIDUM*

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The objective of the present study was to determine the total content of phenolics and flavonoids in extracts of *Thalictrum foetidum*. The total phenolic content was estimated spectrophotometrically using Folin Ciocalteu method. The total content of flavonoids was determined by spectrophotometric analysis, based on the measurement of the absorption of the complex of aluminum chloride with flavonoids. The antioxidant effects of the extracts *Thalictrum foetidum* were investigated.

Key words: extracts; bioactive compounds; biological activity; flavonoids; antioxidant effect; *Thalictrum foetidum*.

Introduction

Nowadays, medicinal preparations of plant origin, due to their useful and healing properties, have found wide application in traditional phytotherapy of many countries. After all, plants are a source for obtaining various medicinal substances.

More than 30 % of all medicines are obtained from plant raw materials. Plants are used to obtain alkaloids, cardiac glycosides, vitamins, etc. Medicinal plants are most widely used in folk medicine. The low toxicity of most medicinal plants allows their use in the treatment of chronic diseases, for anti-relapse or rehabilitation treatment. The price of medicinal products from plants is in most cases significantly lower than synthetic ones, and therefore their use is economically more profitable. Every third drug on the world market is a drug of herbal origin. Because of this, in recent times, modern medicine has paid attention to phytotherapeutic methods of treatment with the possibility of independent preparation of medicinal raw materials.

One of the promising sources of biologically active compounds used in traditional medicine are representatives of the *Ranunculaceae* family, namely the *Thalictrum genus*, which in the world flora includes from 120 to 200 species of perennial plants of the *Ranunculaceae* family.

Thalictrum foetidum is widespread in the territory of on Podilla (Tovtrovy kryaz), Opilli, Roztochchi and Prykarpattia. Administrative regions – Chernivtsi, Ivano-Frankivsk, Lviv, Ternopil and Khmelnytskyi. The plant grows in deciduous forests, forest edges, meadows, steppes, open meadow gravel and stony cliffs and rocks. It is found even in mountains at an altitude of 4 km above sea level [1, 2].

Thalictrum foetidum is rich in biologically active substances of primary and secondary origin. The grass of the plant contains alkaloids – more than 6 % (berberine, thalictrinin, isotetrandrin, berbamine, talfin, fetidine, magnoflorin, corunine, talmetin, thalikmidin, glaucin, isoboldin), coumarins, triterpene saponins, flavonoids – more than 1 % (rutin, kaempferol, quercitin, flavesuetin, ranunculetin, glucorhamnin), tannins – 5.4 %, organic acids, cardiac glycosides, vitamin C. The fresh plant contains traces of essential oil, which includes camphor. The leaves and roots of the stinking beetroot are rich in alkaloids – up to 2.2 %. Two flavonoid glycosides, rutin and glucorhamnin with aglione, which is very rare in nature, can be detected in *Thalictrum foetidum* grass using paper chromatography. Fetidine from *Thalictrum foetidum* showed a high hypotensive effect in the experiment [1–4].

Thalictrum foetidum is a non-official plant that has analgesic, diuretic, expectorant, sedative, hemostatic, anti-inflammatory, hypotensive, and antiseptic properties.

The purpose of our study is to investigate the chemical composition of the ethanol extracts of *Thalictrum foetidum*, in particular phenolic compounds and flavonoids and to study their antioxidant effects.

Material and research methods

Plant material. Medicinal plant raw materials should be collected when the maximum amount of active substances accumulates in them. In this regard, the grass *Thalictrum foetidum* was collected during the flowering period (June–July) in the territory of the Lviv region (Pluhiv village, Zolochiv city).

Drying and standardization were carried out according to the requirements of the State Pharmacopoeia of Ukraine.

Preparation of extracts. The extracts were obtained by maceration. Aqueous ethanol solutions in concentrations of 20 % (RS-1 extract), 40 % (RS-2 extract), 70 % (RS-3 extract) and 90 % (RS-4 extract) were used as extractants. The ratio of raw material and extractant was 1:20.

Determination of total phenolic content. The determination was performed using a spectrophotometric analysis using a modified Folin-Ciocalteu method.

1 ml of Folin reagent, 20 ml of distilled water and 3 ml of 20 % Na2CO3 solution were added to 1 ml of the analyzed solution, diluted in a ratio of 1:20. The prepared mixture was shaken for 10 min, then kept in a water bath at 40 for 20 min. The solution was cooled and the optical density of the resulting solution was measured at 760 nm. The conversion was performed per gallic acid according to a calibration curve that was constructed under similar conditions, replacing the analyte with the gallic acid solution used as standard. A 3-fold measurement was performed for data validity [5, 6; 12].

Determination of total flavonoid content. Quantitative determination of flavonoids was carried out according to the method of K. Muller using a spectrometric method [50]. For this, a control solution was prepared, which consisted of 0.8 ml of methyl alcohol, 0.2 ml of 96 % ethyl alcohol and 60 μL of sodium nitrite solution (5 % concentration). The resulting solution was kept for 5 minutes, and then 60 μL of AlCl3 solution (concentration 10 %) was added to it. It was kept for another 5 minutes, and 0.4 ml of 1M NaOH solution and 0.48 ml of ethyl alcohol were added, and it was kept again for 5 minutes in a dark place.

The studied solution was prepared by taking 0.8 ml of methyl alcohol, to which was added 0.2 ml of a solution consisting of 500 μL of the extract and 1500 μL of ethyl alcohol, 60 μL of sodium nitrite solution (5 % concentration). Then, similarly to the standard solution, it was kept for 5 minutes and 0.4 ml of 1M NaOH solution and 0.48 ml of ethyl alcohol were added. They lasted 5 minutes.

The measurements were performed on a Hitachi U-2810 spectrophotometer at a wavelength of 510 nm. For calibration, a standard curve was constructed using the solution of quercetin as standard, and the content of flavonoids was determined in terms of quercetin. A 3-fold measurement was performed for the accuracy of the data [7, 8; 13, 14].

Determination of the antioxidant effect. DPPH radical scavenging effect

We have conducted studies to study its biological activity of the BAR complex, which are contained in the obtained extract of St. John's wort in order to know the ways of application. To do this, the antioxidant activity of the extract was evaluated using a method based on the reaction with the relatively stable radical 2.2–diphenyl-1-picrylhydrazyl (DPPH) dissolved in methanol. The solution of 2.2-diphenyl-1-picrylhydrazyl has a purple color.

As a result of the reduction of DPPH free radicals, antioxidant molecules turn them into a colorless or slightly yellow product, which changed the optical density at a wavelength of 517 nm on a spectrophotometer.

The study was conducted according to the following method: 4.5 ml of 2.2-diphenyl-1-picrylhydrazyl with a concentration of 0.1 mol was added to 500 μL of the studied extract in a test tube. The resulting mixture was kept for 90 minutes in a dark place. Then a sample was taken and the optical density was measured on a spectrophotometer at a wavelength of 517 nm.

The results of the determination were calculated according to the following formula:

$$\% \text{ inhibition} = ((A_{control} - A_{sample}) / A_{control}) 100 \%, \quad (1)$$

where Acontrol is the optical density of the control solution of the free radical 2.2-diphenyl-1-picrylhydrazyl, Asample is the optical density of a solution of 2.2-diphenyl-1-picrylhydrazyl with the substance under investigation.

Quercetin and ascorbic acid were used as reference standards for all researchers with antioxidant activity determinations [7, 8].

The obtained results were statistically processed using the STATISTICA 8 program and the package of statistical functions of the Microsoft Excel program. The average arithmetic deviation m, average arithmetic value M, Student's t-test, number of repetitions n were determined.

ABTS radical scavenging effect. The extract's free radical scavenging effect was also determined by ABTS radical cation decolorization assay. Concentration of ABTS was dissolved to a 0.014 mM in water. Reacting the original ABTS solution with a solution of potassium persulfate (prepared by dissolving 0.0135 g $K_2S_2O_8$ in 10 ml of water) gave a possibility to obtain a radical cation ABTS (ABTS*) and then the mixture was stirred in the dark at room temperature for 20 h. After that 1 ml of the resulting solution was diluted to 100 ml. There was taken reagent blank reading (Acontrol). The absorbance reading was taken after addition of 1.0 ml of diluted ABTS*+ solution to 100 μL of the extract exactly 6 min after initial mixing at 30 (Asample). Blanks of appropriate solvent were run in each assay. A 3-fold measurement was performed for the accuracy of the data [9–11].

Results and discussion

The results of the study of the quantitative content of biologically active substances indicate the presence of biologically active compounds in the studied raw material and their significant content, which allows the use of the plant as a raw material in the development of phytoremedies.

Total phenolic and flavonoid contents. The total content of phenolic compounds in the investigated extracts was determined, the result is expressed in mg of gallic acid per g of plant material. The total content of flavonoids was determined, the result is expressed in mg of quercetin per g of plant material. The results are presented in Table 1.

The content of phenolic compounds in the tested extracts ranged from 2.1685 to 4.8924 mg/g. The highest value was observed for the RS-4 extract, the extractant being 90 % aqueous ethanol solution. The content of flavonoids in the tested extracts ranged from 1.6817 to 3.2095 mg/g. The highest value was observed for the RS-3 extract, the extractant being 70 % aqueous-ethanol solution.

Antioxidant activity. There were used two assays: DPPH radical and ABTS radical cation assays for the sake of appreciation of free radical scavenging properties of *Thalictrum foetidum* extracts. Table 2 shows the results of the analysis.

For the evaluation of antioxidant activity of single compounds has been widely used relatively stable organic radical DPPH as well as the different plant extracts. A rapid decrease in the optical density at 517 nm was induced by the addition of extracts to the DPPH solution. The effect of *Thalictrum foetidum* extracts of different concentrations in comparison with quercetin and vitamin C on the inhibition of DPPH radical is shown in Table 2.

Table 1

Total phenolic and flavonoid content of *Thalictrum foetidum* extracts

Sample	Total phenolic content (mg gallic acid/g) $\bar{x} \pm \Delta\bar{x}$, $n = 4$	Total flavonoid content (mg quercetin/g) $x \pm \Delta x$, $n = 4$
RS-1	2.1685	1.6817
RS-2	3.4695	2.9289
RS-3	4.0137	3.2095
RS-4	4.8924	3.1716

Table 2

DPPH and ABTS radical scavenging activity of *Thalictrum foetidum* extracts

Sample	$\%_{\text{inhibition}}$ of DPPH*	$\%_{\text{inhibition}}$ of ABTS radical cation
RS-1	85.1542	85.8613
RS-2	87.6548	88.5693
RS-3	89.5874	90.8406
RS-4	90.2548	90.6089
Vitamin C	77.6241	87.8119
Quercetin	87.3479	88.4940

Our investigation shows that the free radical scavenging ability of PP3 and PP4 extracts was better than quercetin. Under the test conditions, *Thalictrum foetidum* extracts with vitamin C were better DPPH radical scavengers. The ability to release free radicals of *Thalictrum foetidum* extracts was also determined through the ABTS radical cation.

It is often used to evaluate the antioxidant activity of individual bioactive compounds as well as mixtures of various origins. In this investigation, a stable form of the ABTS radical cation was formed directly by using potassium persulfate. The interference of compounds that affect the formation of radicals before the addition of antioxidants is prevented by a generation of the radicals.

This option makes the investigation less susceptible to artifacts and averts reassesment of antioxidant ability. A sample of antioxidants is added after stable absorption is received to the reaction medium and the antioxidant activity is measured by discoloration.

The obtained results prove that RS-2, RS-3 and RS-4 extracts improve the absorption of radical ABTS cations more than vitamin C or quercetin.

Conclusions

Based on the results of research and analysis of the literature on the content of biologically active substances in *Thalictrum foetidum*, the main aspects of its use in medicine and pharmacy, it can be concluded that *Thalictrum foetidum* is a rather promising medicinal plant from the *Ranunculaceae* family. The results of the work indicate a sufficient content of phenolic compounds and flavonoids, and the revealed antioxidant effect of the studied extracts allows us to consider *Thalictrum foetidum* as an even more promising medicinal raw material for further thorough research.

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ЗАГАЛЬНИЙ ВМІСТ ФЕНОЛІВ ТА ФЛАВОНОЇДІВ, АНТИОКСИДАНТНА АКТИВНІСТЬ РУТВИЦІ СМЕРДЮЧОЇ

Визначено вміст фенольних сполук і флавоноїдів у екстрактах Рутвиці смердючої. Загальний вміст фенолів оцінено спектрофотометрично за методом Фоліна Чокальтеу. Загальний вміст флавоноїдів визначено за допомогою спектрофотометричного аналізу, базуючись на вимірюванні абсорбції комплексу алюміній хлориду з флавоноїдами. Досліджено антиоксидантні ефекти екстрактів Рутвиці смердючої.

Ключові слова: екстракти; біоактивні сполуки; біологічна активність; флавоноїди; антиоксидантна дія; Рутвиця смердюча.