TOTAL PHENOLIC AND FLAVONOID CONTENT, ANTIOXIDANT ACTIVITY OF FICARIA VERNA

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Summary

The objective of the present study was to determine the total content of phenolics and flavonoids in ethanol extracts of a plant from the family of *Ranunculaceae – Ficaria verna.* Harvesting of medicinal plant was carried out in ecologically clean regions of west Ukraine in spring 2020. The extracts were obtained by maceration of grass, leaves,and flowers to compare the content of active substances in different types of raw materials.

The total phenolic content was estimated spectrophotometrically using Folin Ciocalteu method. The total flavonoid content was measured by aluminium chloride colorimetric assay. The maximum content of phenolic and flavonoids compounds was observed in the grass Ficaria verna. The antioxidant effects of the extracts were investigated. The Ficaria verna can be regarded as a promising natural plant source of antioxidant effects with a high potential for phytopreparations.

Keywords: Ranunculaceae, extracts, bioactive compounds, biological activity.

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1. Introduction

Despite significant advances in the modeling and creation of synthetic drugs, the popularity of herbal therapy is increasing and its competence is expanding. Today, the world pharmaceutical industry is making extensive use of herbal raw materials, which are the basis for the creation of medicines. Medicinal plants contain evolutionarily formed complexes of native substances engaged in complex interactions.

A large number of drugs that are manufactured worldwide have natural ingredients of plant origin. Natural drugs are known to have a milder effect than synthetic agents. Undesirable side effects of drugs, including those of synthetic origin, are observed in 10-40% of patients and are one of the obstacles in the development of new drugs. The percentage of side effects significantly increases during self-medication. Thus in more than 60% of cases of self-medication, there was observed irrational and unjustified use of drugs. So it is promising to expand the range of herbal medical products with new effective plant-based preparations, in particular those based on the herbs widely used in ethnomedicine.

Therefore, the search for new species of plants that could be a source of biologically active compounds, such as flavonoids, coumarins, hydroxycoric acids, alkaloids, saponins, amino acids,and so on. One of the most relevant and promising representatives of the Ukrainian

flora to be used in modern medicine and pharmacy is a plant from the family of *Ranunculaceae – Ficaria verna*. This plant is typical for the ethnopharmacology of East Slavic peoples. It has been used to treat bronchitis, tracheitis, hemorrhoids, skin rashes, acne, diathesis, gingivitis, polyarthritis, stomatitis, and wounds.*(Liakh and Konechna, 2021)*

The complex of bioactive compounds of plant *Ficaria verna* has diuretic, expectorant, anti-inflammatory and blood purifying properties**.**

The *Ficaria verna* growth range extends from Europe and North Africa to West Asia. Plant populations were found in Belarus, Croatia, Germany, Lithuania, Spain, Algeria, Libya, Tunisia, Israel, Turkey, and Georgia. It was introduced into North America*. (Karpiuk, et al., 2020)*

In Ukraine, it is widespread throughout the territory. it occurs in wet forests, mostly deciduous, often along watercourses, in thickets of shrubs. *Ficaria verna* contains biologically active substances in both primary and secondary synthesis. It consists of saponins, γ-lactones: protoanemonin, anemonin, ascorbic acid (190 mg %),carotene (5,2 mg %). Starch (13.5%), sugars (10%) have been found in underground organs. It also contains triterpenoid saponins**.** *(Hrodzins'kyy, 1992)*

Ficaria verna flowers contain flavonoid compounds (kaempferol 3-O-β-d-(6"-α-1-rhamnopyranosyl)-glucopyranoside (nicotiflorin), apigenin 8-C-β-d-glucopyranoside (vitexin), luteolin 8- C-β-d-glucopyranoside (orientin) and apigenin 8-C-β-d-(2"-O-β-d-gluco-pyranosyl)-glucopyranoside (flavosativazide)), flavonol triglycosides (3-O-[alpha-L-rhamnopyranosyl-(1-6)-beta-D-glucopyranosyl]-7-O-(beta-D-glucopyranosyl)-quercetin (1) and 3-O-[alpha-L-rhamnopyranosyl-(1-6))-beta-D-glucopyranosyl]-7-O-(beta-D-glucopyranosyl (kaempferol)), triterpenes and stearins**.** *(Gudej and Tomczyk,1999)*

Lactone protoanemonin, the main component of the plant, is toxic,but after drying the toxic properties are lost because protoanemonin is converted into anemonin. All parts of the plant contain protoanemonin, but the highest content is found in stems and flowers. *Ficaria verna* leaves contain fewer flavonoids than flowers. The main components in the leaves are derivatives of the C-glycoside apigenin and luteolin. Ranulinculin and its breakdown products are observed in a raw. *(Tomczyk and Gudej,2003; Tomczyk and Gudej,2002)*

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

2. Material and method

2.1 Plant material

Harvesting of medicinal plant material (*Ficaria verna.* herb, leaves, and flowers) was carried out in ecologically clean regions of west Ukraine in spring 2020. Drying and standardization were carried out according to the requirements of the State Pharmacopoeia of Ukraine. *(Derzhavna Farmakopeya Ukrayiny. Dopovnennya 2).*

2.2 Preparation of extracts

The extracts were obtained by maceration from each type of raw material separately. To compare the content of active substances prepared extracts of grass (FH), leaves(FL), and flowers (FF). Aqueous ethanol solutions in concentrations of 20% (FH1, FV1, FF1 extracts), 40% (FH2, FL2, FF2 extracts), 70% (FH3, FL3, FF3 extracts) and 90% (FH4, FL4, FF4 extracts) were used as extractants. The ratio of raw material and extractant was 1:10.

2.3 Determination of total phenolic content

The determination was performed using a spectrophotometric analysis using a modified Folin-Ciocalteu method. 0,1 ml of Folin reagent, 1,5 ml of distilled water, and 0,3 ml of 20% Na_2CO_3 solution were added to 0,1 ml of the analyzed solution, diluted in a ratio of 1:10. Kept for 150 min in the dark place

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 *(Skotti et al., 2014; Krvavich et al., 2019).*

2.4 Determination of total flavonoid

The number of flavonoids was determined by a modified spectrophotometric method by the complexation reaction of flavonoids with $AlCl_3$. For this purpose, a 5% solution of $NaNO_2$, a 0.1M solution of sodium hydroxide NaOH, and a 10% solution of AlCl3 were prepared. 0.2 ml of the obtained *Ficaria verna*. herb extract was taken into a test tube and dissolved in 0.8 ml of ethyl alcohol. 0.06 ml of 5% sodium nitrite solution was added and mixed. After that, the tube was kept for 5 min. 0.06 ml of a 10% solution of aluminum chloride was added and kept for 5 min until the reaction was complete. Then 0.4 ml of 0.1 M sodium hydroxide solution and 0.480 ml of ethyl alcohol were added. After that, the tube was kept for 5 min in a dark place.

The measurements were performed 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 *(Do et al., 2014).*

2.5 Determination of the antioxidant effect

2.5.1 DPPH radical scavenging effect

The DPPH method of measuring the antioxidant effect of the extract was used with some modifications. Freshly prepared solution of DPPH was about 0.1 mM (0.2 g DPPH in 500 mL of ethanol). 4.5 mL of solution of DPPH and 500 µL of the extract were mixed in a test tube, which was incubated for 30 minutes in the dark at room temperature. A UV-VIS spectrophotometer was used for measuring the decrease in absorbance (at 517 nm). *(Konechna, R.et al.,2017)*

The following formula was used for calculating percentage of inhibition of the radicals:

$\%inhibition = (Acontrol - Asample) / Acontrol \times 100\%$

where Acontrol is the absorbance of DPPHsolution without extract and Asample is the absorbance of the sample with the added DPPH solution. A 3-fold measurement was performed for the accuracy of the data *(Do et al., 2014).*

3. Results

3.1 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, Table 2, Table 3.

Table 1

Total phenolic and flavonoid content of Ficaria verna herb extracts

Sample	Total phenolic content (mg gallic acid/g), n=3	Total flavonoid content (mg quercetin/g) n=3
FH1	15,8±0,01	7,41±0,01
FH2	16.75±0,01	9,16±0,01
FH3	20,35±0,01	18,37±0,01
FH4	18,65±0,01	12,975±0,01

It was found that among the extracts from the herb Ficaria verna the maximum content of both phenolic compounds and flavonoids was observed in 70% of water-ethanol extracts.

The content of flavonoids in the tested extracts ranged from 7,41 to 18,37 mg quercetin/g.The highest value was observed for the FH3 extract, the extractant being 70% aqueous-ethanol solution.

Table 2

Total phenolic and flavonoid content of Ficaria verna leaves extracts

Sample	Total phenolic content (mg gallic acid/g) n=3	Total flavonoid content (mg quercetin/g) n=3
FL1	8.3±0,01	3.38±0,01
FL2	8.97±0,01	4.15±0,01
FL3	11.58±0,01	10.37±0,01
FL4	8.97±0,01	0.675±0,01

It was found that among the extracts from leaves of Ficaria verna the maximum content of both phenolic compounds and flavonoids was observed in 70% of water-ethanol extracts.

The content of flavonoids in the tested extracts ranged from 0.675 to 10,37 mg quercetin/g.The highest value was observed for the FL3 extract, the extractant being 70% aqueous-ethanol solution.

Table 3

Total phenolic and flavonoid content of Ficaria verna flowers extracts

Sample	Total phenolic content (mg gallic acid/g) n=3	Total flavonoid content (mg quercetin/g) n=3
FF1	5.87±0,01	2.15±0,01
FF2	6.23±0,01	2.89±0,01
FF3	8.51±0,01	6.32±0,01
FF4	7.35±0,01	0.386±0,01

It was found that among the extracts from flowers of Ficaria verna the maximum content of both phenolic compounds and flavonoids was observed in 70% of water-ethanol extracts.

The content of flavonoids in the tested extracts ranged from 0.386 to 6.32 mg/g. The highest value was observed for the FF3 extract, the extractant being 70% aqueous-ethanol solution.

The maximum content of phenolic compounds and flavonoids was observed in extracts with the herb Ficaria verna, the lowest content in extracts from the flowers of Ficaria verna.

3.2 Antioxidant activity

For the evaluation of the 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 *Ficaria verna* extracts of different concentrations in comparison with quercetin and vitamin C on the inhibition of DPPH radical is shown in Table 4, Table 5, and Table 6.

Table 4

DPPH radical scavenging activity of Ficaria verna herb extracts

Sample	% inhibition of DPPH*
FH1	80,71±0,01
FH2	78,94±0,01
FH3	77,19±0,01
FH4	75,43±0,01
Vitamin C	76,23±0,01
Quercetin	78,54±0,01

Table 5

DPPH radical scavenging activity of Ficaria verna leaves extracts

Sample	% inhibition of DPPH*
FL1	67,33±0,01
FL2	63,02±0,01
FL3	62,33±0,01
FL4	61,86±0,01
Vitamin C	76,23±0,01
Quercetin	78,54±0,01

Table 6

DPPH radical scavenging activity of Ficaria verna flowers extracts

Sample	% inhibition of DPPH*
FF1	8,71±0,01
FF2	7,94±0,01
FF3	77,19±0,01
FF4	75,43±0,01
Vitamin C	76,23±0,01
Quercetin	78,54±0,01

Our investigation shows that the free radical scavenging ability of FH1**,** FH2 –extracts was better than quercetin. The free radical scavenging ability of FH1**,** FH2**,** and FH3 extracts were better than Vitamin C.

The results prove that FH1**,** FH2 extracts improve the scavengers of radical DPPH cations more than vitamin C or quercetin.

4. Conclusions

The research done into the chemical composition of *Ficaria verna* ethanol extracts discovered the quantitative content (strength) of phenol compounds and flavonoids as well as examined their antioxidant effects

Sufficient content phenolic compounds and flavonoids as well as the detected antioxidant effects allow us to consider *Ficaria verna* a promising medicinal plant for the development of herbal preparations and further research of the plant.

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