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DETERMINATION OF FLAVONOIDS AND HYDROXYCINNAMIC ACIDS IN TABLETS WITH THICK EXTRACT OF *PRIMULA DENTICULATA* SMITH

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Abstract

Primula denticulata Smith is a perennial herbaceous plant that has a thick spherical inflorescence of purple, purple-red, white, or blue, commonly known as primrose denticulate. The plant has widely been used in folk medicine. The medicinal properties of primrose denticulate have been known for many years. These properties are the result of the availability of different groups of biologically active compounds. The tablets with a thick extract of primrose denticulate were studied to present flavonoids and hydroxycinnamic acids. The method of paper chromatography and thin-layer chromatography method was identified five hydroxycinnamic acids, namely, rosmarinic, ferulic, *p*-coumaric, and caffeic acids. Rutin, isoquercitrin, luteolin, kaempferol, and apigenin were identified in the study tablets among flavonoids by the thin-layer chromatography method. The quantitative content of flavonoids and hydroxycinnamic acids was determined by absorption spectrophotometry. The results of the determination of biologically active substances in tablets show that the flavonoids content in terms of rutin should be from 0.018 g to 0.021 g in a tablet, the content of hydroxycinnamic acids in terms of rosemary acid should be from 0.020 g to 0.022 g in a tablet.

Keywords: *Primula denticulata* Smith, Primrose denticulate, *tablets, hydroxycinnamic acids, flavonoids*

Introduction

Getting along years, the searching for plants with a long history of use, low side effects, and high tolerability, regardless of the age of patients, is of interest to our society [1-5]. The use of plant raw materials is one of the areas of modern pharmaceutical science in the production of herbal drugs [6]. The purpose of using plants is the control metabolic disorders, as plant metabolites are close to the metabolites of the people's body [7-9]. The manufacture of synthetic drugs, which mainly simulate the biologically active substances of plants, has not lowered the role of herbal medicines [10-13]. Combinations of various medicinal plants need special attention as such herbal mixtures have a variety of biologically active substances [14-18]. Phytotherapy has a number of edges, such as it is low-toxic, has a mild pharmacological effect, and can be used for a long period without great side effects [19-22].

Members of the primrose family (*Primulaceae*), namely the genus *Primula* (*Primula* L.) are widely used in many countries of the world. They unite about 400 species of annual and perennial plants that have a great diversity in morphological features and have their anatomical features [23-27]. These plants contain a variety of valuable BAS (saponins, flavonoids, hydroxycinnamic acids, amino acids, tannins, polyphenols, organic acids, carbohydrates, ascorbic and fatty acids, volatile components, chlorophylls, carotenoids, macro-, and micro-elements, etc.) and have found their application in scientific and folk medicine [28-30].

In medicine, all parts of the plant are valuable: rhizomes with roots are used in diseases of the lungs and respiratory tract; leaves – for the treatment of hypo- and avitaminosis; from primrose flowers prepare an infusion, which is used for fever, headaches, migraines, palpitations, rarely used as a blood cleanser for boils, skin rashes.

9 species of the genus *Primula* grow on the territory of Ukraine. Cowslip primrose (*Primula veris* L.) is the most studied of the genus *Primula* L. Its raw material is the underground and aboveground organs of the plant. Different drugs, such as Herbion, Paralen, Bronchipret, Sinupret, Sinupret Forte, Sinupret Extract, Bronchosol are made based on the medicinal raw material of cowslip primrose.

Since the natural resources of cowslip primrose are declining every year, it is advisable to study other species of the genus Primula [31].

Primrose denticulate (*Primula denticulata* Smith) is the closest and most similar in action. It is cultivated and is undemanding to growing conditions. Primrose denticulate is a perennial herbaceous plant that has a thick spherical inflorescence of purple, blue, purple-red, pink, or white. This plant began to be cultivated in the XIX century. Plants have long been used in folk medicine. Infusion of the leaves is used as an analgesic for rheumatism, as a diuretic for diseases of the kidneys and bladder. Infusion of primrose denticulate flowers is used as a diaphoretic and expectorant, for fever, bronchitis; as an anti-inflammatory – for gingivitis and tonic – for migraine, nervousness, insomnia, tachycardia, etc. [32-36]. During pharmacological studies of primrose denticulate, the expectorant, anti-inflammatory, and antimicrobial activities were found.

Taking into account the above proof points, further studies should be carried out to clarify the relationship between impact on the human body and availability in plant biologically active substances. Therefore, the purpose of the study was to determine the qualitative composition and quantitative content of flavonoids and hydroxycinnamic acids.

Methods

The object of the researches was tablets with thick extract of primrose denticulate which containing thick extract of *Primula denticulata* Smith (0.3 g), Avicel PH – 105 (0.174 g), Prosolv 90 (0.006 g), Croscarmellose sodium (0.09 g), Neusilin® US 2 (0.03 g).

The tablets were developed at the Department of Pharmacy Management, Economics and Technology (I. Horbachevsky Ternopil National Medical University) [37, 38].

Chemicals and reagents

Acetic acid, *n*-butanol, aluminium chloride, hydrochloric acid, sodium molybdate, sodium nitrite,

sodium molybdate, ammonia solution, methanol, sodium hydroxide, potassium hydroxide, were of the highest purity. Chemicals and reagents were purchased from the Ltd. Sfera Sim (Lviv, Ukraine) 39-42].

Identification of flavonoids

Determination of the individual flavonoids of tablets with thick extract of primrose denticulate by was used solvent systems:

- n-butanol – acetic acid – water (4 : 1 : 2) – direction I;
- 15% acetic acid – direction II.

Chromatogram analysis was performed in UV light at a wavelength of 366 nm using a 6% potassium hydroxide solution [43].

Total flavonoids content

Stock solution.

To 0.6 g (exact weight) of the powdered tablets with thick extract of primrose denticulate was added 90 ml of methanol R, heat in a water bath under a reflux condenser until the extraction liquid is colourless, cooled. Transfer the methanolic solution to a 100 ml volumetric flask. Wash out the extraction flask with little milliliters of methanol R. Union the methanolic solutions in a 100 ml volumetric flask and dilute methanol R to the mark. Dilute 10 ml the obtained solution to 100 ml with water R and stir.

Test solution.

Place 10 ml of the stock solution in a 100 ml volumetric flask, added a 20 g/l solution of aluminium chloride R in methanol R to the mark.

Compensation solution.

Place 10 ml of the stock solution in a 100 ml volumetric flask and added methanol R to the mark.

The optical density of the test solution and the compensation solution is measured 15 min after preparation to the compensatory solutions for each one respectively.

The quantitative content of flavonoids in the tablets with thick extract of *Primula denticulata* Smith was determined on a spectrophotometer Lambda 25 UV (Perkin Elmer, USA) at a wavelength of 425 nm [44, 45]. The results were expressed as g.

Used a specific absorption of rutin equal to 370.

Identification of hydroxycinnamic acids

For detection of hydroxycinnamic acids by the method of paper chromatography was used Filtrak FN No. 4 paper. For this purpose, the test tablets solution with thick extract of primrose denticulate was applied to paper, standard samples were applied, and chromatograms were placed in a solvent – 2% acetic acid solution. Hydroxycinnamic acids were also detected by thin-layer chromatography in a solvent system of n-butanol - acetic acid – water (4 : 1 : 2). Chromatographic plates Sorbfil were used for chromatography (Sorbfil plates 10x15, Russia). The chromatogram was dried in a fume hood and examined in daylight and UV light before and after treatment with ammonia vapor.

Total hydroxycinnamic acids content

In a flask introduce 0.6 g (exact weight) of the powdered tablets with thick extract of primrose denticulate and added 80 ml of ethanol 50% R, heat in a water bath under reflux for 30 min, cool, filter. The filter is washing out with 10 ml of ethanol 50% R. The filtrate and wash are united in a 100 ml volumetric flask and added ethanol 50% R to the mark, stirred. (Stock solution).

To 1 ml of the original solution was placed sequentially added, stirring after each addition, 2 ml of 0.5 M hydrochloric acid solution, 2 ml of a solution prepared by dissolving 10 of sodium nitrite R and 10 of sodium molybdate R, in 100 ml of water R, 2 ml of sodium hydroxide solution of diluted R, and was brought the volume of the solution with water R to 10 ml and mixed.

Compensatory solution.

1 ml of the stock solution was placed in a 10 ml volumetric flask, 2 ml of 0.5 M hydrochloric acid solution and 2 ml of dilute sodium hydroxide solution R were successively added, the volume of the solution was brought to mark with water R and mixed.

The content of hydroxycinnamic acids in the tablets with thick extract of *Primula*

denticulata Smith was the determination of spectrophotometry method at a wavelength of 505 nm [46, 47]. The results were expressed as mg.

Used a specific absorption of rosmarinic acid, that is equal to 400 [48, 49].

Statistical analysis

All the assays were carried out five times. Values were determined using Statistica v 10.0 (StatSoft I nc.) program [50, 51]. Results were represented as mean ± SEM [52]. Statistical significance of differences between mean values was assessed by the Student's t-test [53]. The level of significance was set at *p<0.05 [54, 55].

Results and Discussion

The presence of flavonoids and hydroxycinnamic acids, which provide some pharmacological activity was determined in the tablets with thick extract of *Primula denticulata* Smith, using phytochemical methods of analysis.

The thin-layer chromatography method revealed the presence of individual flavonoids, namely:

- rutin (quercetin 3-O-rutinoside) – flavonols;
- isoquercitrin (quercetin 3-O-glucoside) – flavonols;
- luteolin - flavones;
- kaempferol – flavonols;
- apigenin – flavones [56, 57].

The results of thin layer chromatography testify to the presence of flavonoids in tablets with thick extract of primrose denticulate.

Flavonoids are the greatest group of natural phenolic compounds [58]. It is known that flavonoid compounds due to high biological activity, which is due to the free hydroxyl and carbonyl groups in the molecule, undergo various biochemical changes and participate in a number of physiological processes [59. Their relatively low toxicity together with the selective pharmacological action on the human body allows the use of this group of compounds in the development of new drugs. Flavonoid compounds have an antioxidant effect, as a result of which they delay the processes of aging and carcinogenesis, reduce the harmful effects of radiation damage. They have pronounced hepatoprotective and antitoxic properties, show capillary-strengthening (P-vitamin), anti-inflammatory, reparative, diuretic, antihypertensive, cardiotonic, antispasmodic, antiradiation, choleretic, antiallergic, hypoglycemic and hypolipidemic activity. A number of flavonoids can inhibit the release of histamine and Ca2 + - ATPase, which regulates the entry of Ca2 + ions into mast cells after exposure to the antigen [28, 60].

The results of determining the quantitative content of flavonoids in three series of tablets with thick extract of *Primula denticulata* Smith are shown in Table 1.

The method of paper chromatography and thin-layer chromatography method revealed the attendance of hydroxycinnamic acids, such as:

- rosmarinic (3,4-dihydroxycinnamic acid (R)-1-carboxy-2-(3,4-dihydroxyphenyl) ethyl ester);
- ferulic (4-hydroxy-3-methoxycinnamic acid);
- *p*-coumaric (4-hydroxycinnamic acid);
- chlorogenic (3- (3,4-dihydroxycinnamoyl)quinic acid);
- caffeic (3,4-dihydroxycinnamic acid).

Hydroxycinnamic acids are present in plants, mostly in the free state. Almost every higher plant contains one or more acids in different combinations. Caffeic, chlorogenic, p-coumaric, ferulic acids are the typical representatives of this class of compounds. Studies show that caffeic acid inhibits carcinogenesis, has immunomodulatory and anti-inflammatory activity. Chlorogenic acid has a strong antioxidant effect. In terms of antioxidant activity, the flavonoid naringenin is 27 times superior, but inferior to ferulic and caffeic acids. Chlorogenic acid inhibits the biosynthesis of leukotrienes by blocking lipoxygenase that oxidizes arachidonic acid, reduces the level of malonic dialdehyde in blood plasma, and the composition of low-density lipoproteins. By reducing the sensitivity of low-density lipoproteins to oxidation, chlorogenic acid may reduce the risk of cardiovascular disease. Its enzymatically oxidized forms show antiviral activity against herpes pathogens. Extracts rich in

chlorogenic acid inhibit the expression of HIV reverse transcriptase. It is active against *Escherichia coli* and *Staphylococcus aureus* strains. Hypoglycemic, hypocholesterolemic, hepatoprotective, antitumor action of chlorogenic acid has also been noted [61, 62]. Rosmarinic acid loosens allergic diseases, slows the development of Alzheimer's disease, attenuates T-cell receptor-mediated signaling, and defends from neurotoxicity [49, 63]. *p*-Coumaric acid also has antioxidant properties that reduce the risk of gastric cancer by reducing the production of carcinogenic nitrosamines. In addition, in *vitro* experiments, showed pronounced anti-inflammatory activity. Ferulic acid exhibits a wide range of pharmacological properties, including anti-inflammatory, anti-allergic, antiplatelet, antitumor, antitoxic, hepatoprotective, cardioprotective, antibacterial, antiviral, and other activities, mainly due to the antioxidant effect – inhibition of lipid peroxidation and inhibition of free radical stages of prostaglandin synthesis [64-66].

The results of determining the content of hydroxycinnamic acids in study series tablets with thick extract of primrose denticulate are shown in Table 2.

Since flavonoids and hydroxycinnamic acids show many pharmacological activities, it is important to expand the range of drugs with these biologically active substances.

Conclusions

Investigation of the tablets with thick extract of *Primula denticulata* Smith indicated the presence of five flavonoids and five hydroxycinnamic acids. Rutin, isoquercitrin, luteolin, kaempferol, and apigenin were identified in the study tablets among flavonoids by the thin-layer chromatography method. The method of paper chromatography and thin-layer chromatography method was identified the following hydroxycinnamic acids in tablets: rosmarinic, ferulic, p-coumaric, and caffeic acids. The quantitative content of biologically active substances was determined by the absorption spectrophotometry at a wavelength of 425 nm (for flavonoids) and 505 nm (for hydroxycinnamic acids). The total content of flavonoids in recalculation to rutin was in sample 1 - 0.019 g, sample 2 - 0.018 g, sample 3 - 0.021 g. The content of hydroxycinnamic acids in recalculation to rosmarinic acid was in sample 1 - 0.022 g, sample 2 - 0.020 g, sample 3 - 0.021 g.

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Table 1. The results of determination of flavonoids in tablets with thick extract of *Primula denticulata* Smith (M ± SEM, n = 5)

Sample No.	The content of total flavonoids in the recalculation of rutin, g
1	0,019 ± 0,0008
2	0,018 ± 0,0006
3	0,021 ± 0,001

Table 2. The results of determination of hydroxycinnamic acids in tablets with thick extract of *Primula denticulata* Smith (M ± SEM, n = 5)

Sample No.	The content of total hydroxycinnamic acids in the recalculation of rosmarinic acid, g
1	0,022 ± 0,001
2	0,020 ± 0,001
3	0,021 ± 0,001