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DETERMINATION OF POLYSACCHARIDES IN *GENTIANA CRUCIATA* L. HERB

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Abstract

Gentiana cruciata L. herb is widespread in central Europe, Central and Western Asia, Kazakhstan and the Caucasus. *Gentiana cruciata* L. is known in most of the world. It is contains xanthones, polyphenols, iridoids, flavonoids, tannins, hydroxycinnamic acids, volatile components, organic and fatty acids, ascorbic acid, amino acids, chlorophylls and carotenoids. However, there has been no scientific information on the content of *Gentiana cruciata* L. polysaccharides. Thus the aim of this study was to determine the content of polysaccharides in the study raw materials. To identify polysaccharides in *Gentiana cruciata* L. herb, generally accepted qualitative reactions were carried out, which confirm the presence of these biologically active substances. Polysaccharides content in the herb of *Gentiana cruciata* L. was determined by the gravimetric method. The fractions of pectin substances and water-soluble polysaccharides were distinguished in the raw material of *Gentiana cruciata* L., the quantitative content of which was the following: pectin substances (5.77%), water-soluble polysaccharides (3.26%).

The character of processes in which polysaccharides participate are associated with the therapeutic properties of *Gentiana cruciata* L.

Keywords: *Gentiana cruciata* L., herb, polysaccharides, water-soluble polysaccharides, pectin substances

Introduction

Recently, there is a tendency to prescribe herbal medicines in modern medicine, despite significant advances in the development of synthetic drugs for the treatment of various diseases [1-4]. The phytotherapeutic method of treatment and prevention of diseases is recognized by scientific medicine and has a well-founded basis – the biological activity of chemical components of medicinal plant raw materials. The plant medicines are well tolerable, often used in the fight against many diseases, and have minor side effects [5-9]. Like synthetic drugs, herbal medicines accomplish their pharmacological effects through certain biologically active substances in their composition, which are able to affect physiological processes in the body. The importance of medicinal plants did not reduce the increase in the number of synthetic medicines, which often model their biologically active substances [10-12]. However, this effect of natural compounds is milder compared to xenobiotic drugs of synthetic origin. Despite scientific progress in the field of organic synthesis and pharmaceutical chemistry, the development of drugs based on plant raw materials remains relevant given their effectiveness, safety, without significant side effects, as well as the lack of difficulties to get them [13, 14]. The pharmaceutical development of new herbal medicines is preceded by the search for new medicinal plant raw materials. To do this, the experience of folk medicine and traditional medical systems is studied, phytochemical screening of many plants is carried out to find valuable biologically active substances with predicted pharmacological activity in their raw materials, and plants are studied on the principle of phylogenetic relationship with known plants with proven pharmacological activity [15]. An urgent task of modern pharmacy is the search for plants with a sufficient raw material base that can supplement the range of official species, rational and comprehensive use of raw materials, as well as the creation of new drugs based on them [1]. These plants include a member of the family *Gentianaceae* genus *Gentiana L. – Gentiana cruciata L.*

Gentiana cruciata L. is widespread in central Europe, Western and Central Asia (northwest), the Caucasus and Kazakhstan [16]. On the territory of Ukraine it grows in the southern part of forest areas, forest-steppe (including Donetsk forest-steppe), in the Crimea and the Carpathians to the middle mountain and mountain belts. It grows on dry meadows, meadows, slopes, among shrubs and on the outskirts; mainly in places with shallow carbonate rocks.

Remedies based on *Gentiana cruciata* L. improve the function of the digestive organs, liver, gallbladder, stimulate the secretion of gastric glands, stimulate appetite, increase the motility of the digestive tract, and have anticholinesterase, anti-inflammatory antiseptic, antigenotoxic, antimicrobial, antioxidant and anthelmintic properties. It is used for heartburn, jaundice, dysentery, as well as an antifebrile for colds [17, 18]. As a lotion, the infusion of *Gentiana cruciata* L. is used to heal purulent wounds and burns.

Gentiana cruciata L. contains a complex of the following biologically active substances: hydroxycinnamic acids, xanthones, secoiridoid glycosides (sweroside, swertiamarine, gentiopicroside), tannins, flavonoids, organic and fatty acids, volatile components, amino acids, chlorophylls, carotenoids, polyphenols and ascorbic acid [19-24].

However, there has been no scientific information on the content of *Gentiana cruciata* L. polysaccharides. In this regard, this work is carried out to determine the content of these compounds in the research of raw materials.

Methods

Plant materials

The object of the study was *Gentiana cruciata* L. herb, which was collected at the territory of Volove, Ternopil region (Western Ukraine), in 2017. The raw material of the study object was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine) [25]. A voucher specimen of *Gentiana cruciata* L. no. 133 is kept at the Department of Pharmacognosy and Medical Botany (TNMU) [26]. The herb of *Gentiana cruciata* L. was dried using the conventional method and stored in a dry place, protected from direct sunlight [27-29].

Chemicals and reagents

Hydrochloric acid and Fehling's solution were of analytical grade. Reagents were purchased from the Ltd. Sfera Sim (Lviv, Ukraine) [30, 31].

Determination of polysaccharides

To carry out qualitative reactions to polysaccharides, aqueous extracts of *Gentiana cruciata* L. were prepared. To do this, 30 g of crushed dry raw material (particle size 2–5 cm) was poured into 250 ml of purified hot water P and infused during the day. The aqueous extracts were filtered, and the raw material was poured into 100 ml of purified hot water. The operation was repeated 3–5 times. The aqueous extracts were combined and evaporated to 15 ml. The obtained extract was used to detect polysaccharides.

The presence of polysaccharides in the studied extracts was confirmed by the following reactions:

- with 96% ethanol: 2–3 drops of 96% ethanol was added to 2–3 ml of extract, infused. The precipitate was filtered off and reacted to detect reducing (neutral) sugars.

- with Fehling's solution: the precipitate was transferred into a test tube, 5 ml of dilute hydrochloric acid was added and boiled for 30 min, 10 ml of Fehling's solution was added to the cooled hydrolyzate and boiled again [1].

Polysaccharides content in the herb of *Gentiana cruciata* L. was determined by the gravimetric method according to the State Pharmacopoeia of Ukraine [32].

5.00 g (exact weight) of the powdered herb of *Gentiana cruciata* L. was placed in a 250 ml volumetric flask, 100 ml of water R was added, heat in a water bath under reflux for 30 min, cooled, decanted through a glass funnel.

Extraction continued in two servings, the first – 100 ml of water R, the second – 50 ml of water R, each time heat in a water bath under reflux for 30 min. The obtained extracts were cooled and decanted into the same flask. The filter was washed with water R and diluted the volume of solution with water R to the mark.

To 5 ml of the obtained solution added 15 ml of 96% alcohol R stirred was placed in a centrifuge test tube, heated in a water bath under reflux for 5 min at 30 °C. Then the solution is left to stand for 1 hour and centrifuged for 30 min at a speed of 5000 revs/min.

The supernatant was filtered through a glass filter under a vacuum. The precipitate is quantitatively transferred to the filter and washed with 10 ml of 96% alcohol R. The filter with precipitate is air-dried, then dried to constant weight at a temperature of 100 °C to 105 °C.

The content of polysaccharides in the herb of *Gentiana cruciata* L., in terms of dry raw materials, was a percentage [33].

Statistical analysis

The tests were carried out five times. Results were determined using Statistica v 10.0 (StatSoft I nc.) program. The level of significance was mounted at *p<0.05 [34, 35].

Results and Discussion

When interacted with 96 % ethanol P, lamellar clots appeared, which precipitated during infusion. This indicates the presence of polysaccharides.

As a result of the reaction with Fehling's reagent, a brick-red precipitate appeared, which indicates the presence of reducing sugars.

As a result of the research, water-soluble polysaccharides and pectin substances of *Gentiana cruciata* L. were isolated.

The results of determining the quantitative content of polysaccharides in the herb of *Gentiana cruciata* L. are shown in Figure 1.

Water-soluble polysaccharides are an amorphous powder of light brown color, which is easily soluble in water, soluble in aqueous solutions of alkalis and acids and insoluble in organic solvents.

Pectin substances are amorphous white-cream colored powder, which forms a gel-like colloidal solution when heated and dissolves rather slowly in water.

Carbohydrates play an important role in plant life, being on the one hand structural, supporting

substances (fiber, hemicellulose, pectin), and on the other hand, carbohydrates are directly involved in metabolism (starch, inulin, sugars), and they are one of the main energy sources. The bulk of carbohydrates found in nature exist in the form of polysaccharides that have a wide range of pharmacological activity: expectorant, analgesic, antitumor, laxative, hypoglycemic, immunomodulatory, hypocholesterolemic, anti-inflammatory, antiviral, antimicrobial, tonic, anabolic, antiulcer, wound healing [36, 37].

They also increase the body's resistance, reduce the side effects of glucocorticoids, antibiotics and cytostatics. All polysaccharides are adsorbents, the most active of which are pectins. In addition to the important functional significance and specific pharmacological activity, polysaccharides affect the development of the total pharmacological effect of drugs derived from herbal raw materials [1].

Conclusions

To identify polysaccharides in *Gentiana cruciata* L. herb, generally accepted qualitative reactions were carried out, which confirm the presence of these biologically active substances in the study object. The content of polysaccharides, present in the herb of *Gentiana cruciata* L., was studied by the gravimetric method. The fractions of pectin substances and water-soluble polysaccharides were distinguished in the raw material of *Gentiana cruciata* L., the quantitative content of which was the following: pectin substances (5.77%), water-soluble polysaccharides (3.26%).

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Figure 1. The content of polysaccharides in the herb of *Gentiana cruciata* L.