

## THE STUDY OF BIOLOGICALLY ACTIVE FORMATIONS OF THE MAIN LICORICE

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### Annotation

Glycyrrhizic acid, water-soluble polysaccharides and pectin substances have been isolated from licorice root. Physical and chemical parameters, monosaccharide composition of water-soluble polysaccharides and pectin substances were studied. In the composition of carbohydrates, the predominant monosaccharides are uronic acids, galactose, glucose and arbinose, xylose and rhamnose are in trace amounts.

**Keywords:** licorice root, glycyrrhizic acid, pectin substances, water-soluble polysaccharides, quantitative and qualitative composition of carbohydrates.

### Introduction

Among the representatives of the flora used by humans as therapeutic agents, it is perhaps difficult to find a plant with such an ancient, documented history as licorice has. The term "licorice" or licorice refers to the roots and rhizomes of the sweet licorice species *Glycyrrhiza glabra* L. and *Glycyrrhiza uralensis* Fisch. It is these most common types of licorice in Eurasia that were used in ancient medical recipes and medical recommendations, as well as in cosmetics and cooking /1/.

The raw material of licorice root has three pharmacopoeial species: licorice naked (*Glycyrrhiza glabra* L.), Ural licorice (*Glycyrrhiza uralensis* Fisch) and Korzhinsky licorice (*G. Korshinskyi* Grig.). These are the most valuable medicinal plants, underground organs of which are harvested in large quantities for subsequent use in many areas of the national economy. The main active substance of the roots and rhizomes of these species is glycyrrhizic acid, which determines their sweet taste and biological activity /2/.

Licorice is a valuable plant that is classified as a medicinal raw material not only in Russia, but also in many countries of the world. The main value of licorice is its root, since it has a high content (up to 25%) of triterpene glycoside - glycyrrhizic acid. This

compound is the main active component of licorice and exhibits anti-inflammatory, antiviral, immunomodulatory, antiallergic, hepatoprotective and other activities /3/. A wide spectrum of therapeutic action of glycyrrhizonic acid determined the high scientific and practical interest in this compound. The aim of the study is to study biologically active substances in licorice root is of interest. In this regard, this work is devoted to the isolation of biologically active substances from licorice roots.

The object of the study is the dry roots of licorice, the "Boyan" variety, grown in the territory of the city of Khodjeyli in Karakalpakstan.

### Research Methods

To extract glycyrrhizonic acids, aqueous solutions of alkalis are used as an extractant /4/. However, the resulting extracts contain a significant amount of ballast substances in the form of carbohydrates and proteins. Therefore, another approach was proposed for the extraction of glycyrrhizic acid - extraction of licorice root with acetone acidified with mineral acid ( $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ ). In this case, the resulting extracts contain various phenolic compounds and simple carbohydrates as concomitants.

The qualitative composition of the extracts, as well as the quantitative content of glycyrrhizic acid in them, was determined by spectrophotometry on the METLERTOLEDO laboratory complex. Fast Track UV-Visible technology combines reliability and compactness. Thanks to FastTrack, a compact, reliable and accurate instrument has been developed. High measurement speed and ease of use are achieved through the combination of Fast Track technology and the One Click concept, which are at the heart of the UV Vis spectrophotometers of the Excellence series.

### Research Results

To obtain glycyrrhizic acid, air-dry raw materials of licorice root are crushed and sifted, a sieve (opening 0.2 mm) is used /5/. 2 g of the prepared sample is placed in a flask (150 ml) and a 3% solution of nitric acid in an amount of 20 ml is added. The mixture is shaken frequently and vigorously for 1 hour. Next, the mixture is filtered into a cylinder (100 ml). The raw material powder is washed in a flask with 10 ml of acetone, filtering through the same filter into a cylinder. The powder remaining on the filter is washed back into the flask using 20 ml of acetone. The resulting solution is boiled on a water bath for 5 minutes using a reflux condenser. The mixture is then filtered into the same cylinder through the same filter. Thus, the extraction with hot acetone is repeated twice more. Washing with acetone of the raw material powder continues until the liquid in the cylinder reaches 100 ml, which is then poured from the cylinder into a glass (200 ml). In the amount of 40 ml of alcohol, the cylinder is rinsed and alcohol is poured, then into the same glass. Further, a solution of concentrated ammonia is added in drops to the glass, while stirring vigorously, until an abundant cheesy light

yellow precipitate is formed, they are determined using a pH potentiometer in the range of 8.3–8.6. The precipitate with the mother liquid is transferred to the filter placed in the Buchner funnel, the liquid is sucked off. The filter with sediment and the beaker are washed 3-4 times with 50 ml of acetone. A filter with a precipitate is placed in a beaker and the precipitate is dissolved in 50 ml of water. The resulting solution is poured into a flask (250 ml). The filter is repeatedly washed with small portions of water, which are then added to the main solution, bringing its volume in a volumetric flask with water to the mark of 250 ml. On the spectrophotometer, setting the wavelength at 258 nm, the optical density of solution 2 is determined. Water is used as a control solution. The calculation of the quantitative content (X, in percent) of glycyrrhizic acid is performed using the formula:

$$x = \frac{(D - 822 \times 250 \times 500 \times 100)}{(m \times v \times 1100)}$$

D-optical density - 0.62698 822 - molecular weight of glycyrrhizic acid, m, v - weight of raw materials, grams; 250 - solution volume 1100 is the molar absorption index.

#### **Thus the Content of Glycyrrhizic Acid is 11%**

To obtain water-soluble polysaccharides, 10 g (accurately weighed) of crushed raw materials are placed in a flask with a capacity of 500 ml, 200 ml of purified water, heated to the boiling point, are added, and extracted for 20 minutes. The extraction is repeated 2 times and water-soluble polysaccharides are precipitated with 95% ethyl alcohol. The precipitate on the filter is successively washed with 15 ml of a solution of 95% ethyl alcohol and dried /6/.

#### **Yield -2%.**

Water-soluble polysaccharides are amorphous powders of light cream color, highly soluble in water. Aqueous solutions give a negative reaction to starch and they do not belong to starch-like glucans.

To isolate pectin 65 g of pre-peeled licorice roots, add 250 ml of distilled water. In the hydrolysis of pectin, mineral acids are used. Add hydrochloric acid HCl, pH in the range of 3.5-4.0. Boil for 3 hours at 90 °C. Then gradually 1:2 volume of 96% ethyl alcohol is added to the solution until a yellow precipitate is completely precipitated. The precipitate formed is separated through a paper filter, then washed twice with ethyl alcohol and dried /7.8/.

#### **The Output of Pectin -3.5%.**

The resulting pectin is amorphous yellow powders, readily soluble in water. To determine the monosaccharide composition of carbohydrates, acid hydrolysis is performed. For this, polysaccharides and pectin substances. 100 mg of pectin substances were hydrolyzed with 3 ml of 2N sulfuric acid for 20 hours at a temperature

of 100 ° 50 mg, and polysaccharides were hydrolyzed with 3 ml of 1N sulfuric acid at 100 ° C for 10 hours. The hydrolysates were neutralized with BaCO<sub>3</sub>, deionized with a KU-2CH-form cation exchanger, evaporated, and chromatographed on Filtrak-FN-12.16 paper using the descending method. System-butanol-1-pyridine-water (6:4:3) developer aniline phthalate acidic, uronic acids and pentose (arabinose, xylose) are found in the form of pink spots. Hexoses (glucose, galactose, rhamnose) in the form of brown spots.

The isolated pectin was analyzed by IR spectroscopy /9/. In the IR spectrum of pectin there is an intense asymmetric band with a maximum of 3412 cm<sup>-1</sup>, indicating the presence of hydroxyl groups-OH. The absorption band at 2952 cm<sup>-1</sup> is weak, but shows that hydrogen bonds are involved in the formation of hydroxyl groups.

Pectic substances, as acidic polysaccharides - carboxy polysaccharides, give new absorption bands in contrast to neutral polysaccharides. Thus, the absorption bands in the region of 1743 cm<sup>-1</sup> correspond to vibrations of carbonyls (C=O) of carboxyl groups.

Pectins in nature are usually in the form of Na<sup>+</sup>, K<sup>+</sup> or CA<sup>++</sup> salts, as evidenced by the absorption bands in the region of 1638 cm<sup>-1</sup> and 1444 cm<sup>-1</sup>, corresponding to vibrations of the ionized carboxyl.

The carboxyl group can contain not only metals, it can be esterified with methyl alcohol, the presence of methoxyl groups of pectin substances. It is also reflected in the IR spectrum as an absorption band at 1370 cm<sup>-1</sup>. Absorption bands in the region 1327,1146,1103,1018 (cm<sup>-1</sup>) show vibrations of fragments of pyranose rings: O-CH, C-OH, -C-C-H, -C-C-. The absorption band at 953 cm<sup>-1</sup> reflects the vibrations of the methyl and methylene groups. The triplet 920.886.832 cm<sup>-1</sup> of the pyranose rings indicates the presence of a 1-4 glycosidic bond.

Thus, the analysis of the IR spectrum of pectin substances showed that the studied pectin substances are indeed an esterified carboxy polysaccharide with - 1→4 glycosidic bonds between the residues of uronic acid galactopyranosyl.

Thus obtained diterpinoid and carbohydrate compounds from licorice root can be appropriately used as biologically active additives for food enrichment /10/.

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