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Isolation of the Individual Substance Liquiritin from the Plant Glycyrrhiza Glabra L

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Annotation. The individual substance liquiritin was isolated from the root of the plant Glycyrrhiza glabra L. The extraction was carried out in a water bath equipped with a reflux condenser for 2 hours at a temperature of 40-50 oC. To extract the residue of lipophilic substances and chlorophyll, the aqueous extract was treated with chloroform in a ratio of 1:4. The purified aqueous extract was fractionated with ethyl acetate, the fractions were separated on a separating funnel. All of the above fractions were concentrated on a rotary evaporator to a residue of 1/10. Ethyl acetate fractions were precipitated with hexane in ratios from 1 : 1 to 1 : 5.

Keywords: Glycyrrhiza glabra, liquiritin,plant, substances.

Introduction

Licorice naked - Glycyrrhiza glabra, belongs to the legume family (Fabaceae) [1]. Other names: licorice root, smooth licorice, liquorice, liquorice, liquorice, glycyrrhiza. Licorice is a Mediterranean species. In Uzbekistan, this plant grows in the floodplains of the Amudarya, Syrdarya and Zarafshan rivers, on the banks of their tributaries. Rising to the middle belt of mountains to a height of 2000 m above sea level, licorice in some places forms almost pure licorice thickets [2].

The chemical composition of licorice is up to 23% saponin-glycyrrhizin (potassium and calcium salt of glycyrrhizic acid), which gives them a sweet taste, and 27 flavonoids (quercetin, kaempferol, apigenin, etc.), the total content of which reaches 4%, glabric (glycyrrhetic) acid , steroids, essential oil, asparagine, ascorbic acid (up to 30 mg%), tannins (8.3-14.2%), bitterness, pigments, gum resins, asparagine, higher aliphatic hydrocarbons and alcohols, higher fatty acids, alkaloids and others [3,4]. Licorice roots and rhizomes - in addition to traces of essential oils, vitamins, proteins, bitter (up to 4%) and resinous (3-4%) substances, lipids (about 4%), polysaccharides (pectic substances 4-6% and

starch up to 34%), monosaccharides and disaccharides (up to 20% in total), contain more interesting from a pharmacological point of view flavonoids (3-4%) and triterpene saponins (about 20%). Among the 27 different flavonoids, the most important are flavonol and chalcone, as well as their isoforms - licurazide, kaempferol, liquiritoside, liquiritin, isoliquiritin, neoliquiritin, rhamnoliquiritin, uraloside, rhamnoisoliquiritin, etc. It is flavonoids, derivatives of flavonol and chalcone, that make it possible apply appropriate preparations of licorice. Glycyrrhizin is the main triterpene saponin. In addition, an aglycone of uralenoglucuronic acid, oxyglycyrrhetinic (uralenic) acid, was found in the roots and rhizomes of Ural licorice [5,6]. Licorice, as glycyrrhizin acid, has anti-inflammatory, antiviral, antibacterial, anticancer, immunostimulating, antiulcer,

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hepatoprotective and antioxidant properties. In addition, licorice preparations naked can be used in the treatment of heart disease, cancer, asthma and diabetes [7].

In this regard, the purpose of our work is to study the chemical composition of phenolic compounds and the isolation of the individual substance liquiritin from the plant Glycyrrhiza glabra L.

Material and Methods

Object of study. The object of the study was the root of Licorice collected at the end of October 2018. The place of selection and sampling was the Khorezm region, the Republic of Uzbekistan.

Isolation of polyphenols. Dried licorice root was extracted with 40% acetone. The extraction was carried out on a water bath equipped with a reflux condenser for 2 hours at a temperature of 40-50°C. The next day, the extract is filtered off and the experiment is repeated 4-5 times. The combined acetone extracts are evaporated on a rotary evaporator (t=35-400C, P=30 mmHg) to the remainder of the water part. To extract the residue of lipophilic substances and chlorophyll, the aqueous extract was treated with chloroform in a ratio of 1:4. The resulting layered parts of the solvents were separated on a separating funnel. The purified aqueous extract was fractionated with ethyl acetate, the fractions were separated on a separating funnel. All of the above fractions were concentrated on a rotary evaporator to a residue of 1/10. The ethyl acetate fractions were thickened and precipitated with hexane in ratios from 1 : 1 to 1 : 5 (concentrate : precipitant), and a flocculent precipitate formed. Qualitative reactions of phenolic compounds were carried out according to the generally accepted method [8, 9].

Column chromatography on silica gel grade 100/160. KSK from Tianjin Sinomed Pharmaceutical (China), the second adsorbent Sephadex LH-20 sorbent from GE Healthcare Bio-Sciences AB (Sweden). For both adsorbents, the column size is 60.0/2.0 cm. Gravity flow rate. The following solvent systems were used for TLC: I chloroform-methanol (10:1); to II - chloroform-methanol (4:1) and finally washed with methanol.

The individual liquiritin substances were identified by high performance liquid chromatography-mass spectrometry (HPLC-MS/MS).

Chromatography conditions:

- HPLC Ultimate 3000 chromatograph, Thermo Fisher Scientific (USA), equipped with an automatic sampler,
- column Hypersil GOLDaQ 100 mm x 2.1 mm, particle size 1.9 μm;
- mobile phase - bidistilled water acidified with 0.1% formic acid (A) and acetonitrile (B), flow rate 0.1 ml/min. 15:85 isocratic mode.

Names of the device and conditions of gas chromatography-mass spectrometry:

Electron Impact Ionization Mass Spectrometry (HESI-II)

Spray chamber voltage - 4000 V; evaporator temperature - 250 °C;
shell gas pressure - 20 psi; auxiliary gas pressure - 10 psi;
capillary temperature – 100 °C; collision pressure - 1.5 mTorr;
ionization energy – 20 eV. The discussion of the results

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Results

The dry residue from the ethyl acetate fraction is placed on a chromatographic column filled with silica gel (LH 250 40/100). Separation is carried out by eluting with a column of chloroform:ethanol 96% in a ratio of 3:1. Identification is carried out using thin layer chromatography (TLC, chloroform:ethanol (96%) 3:1). Ferric chloride (III) is used as a developer. Collect fractions with the same Rf and combine, evaporate to dryness and get the target product, flavanone - liquiritin. The yield of the target product is 0.15-0.45% by weight of air-dry roots (depending on the concentration of the extractant).

The physicochemical properties of the obtained product have been established. Identification was carried out by the HPLC-MS method by the mass of the liquiritin molecular ion. The obtained data are illustrated in Figure 1.

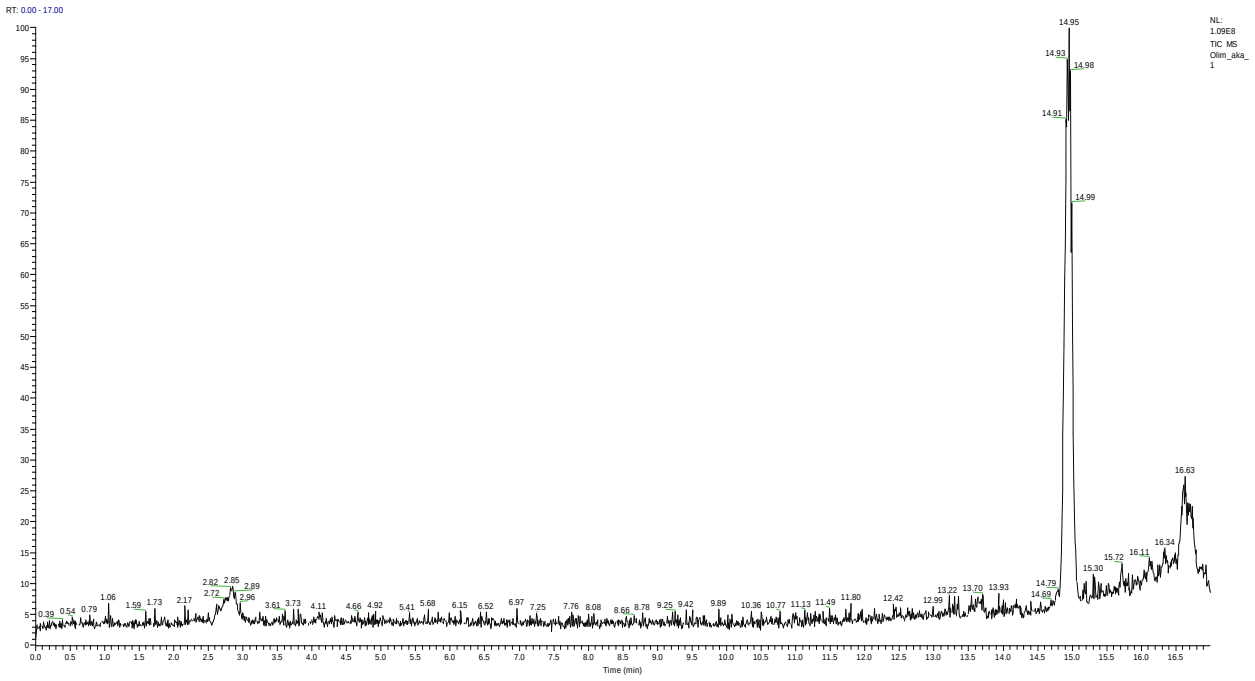

Fig-1. HPLC-MS chromatogram of liquiritin

Liquiritin-C21H22O9, Mm 418, Rf 0.6, m.p. 209-2110C, UV (EtOH, λmax, nm): 289, 325(pl)

Figure 1 shows that a peak with a retention time of 14.9 min is observed in the chromatogram of the isolated substance. The mass spectrum contains the main molecular ion with mass m/z 419 corresponding to liquiritin.

The UV spectrum of the obtained compound was also studied and it was shown that it has 2 characteristic absorptions in the near ultraviolet region of the spectrum. Figure 2 shows the UV spectrum of liquiritin.

Figure 2 shows that in the UV spectrum, in the near ultraviolet region, two main characteristic peaks (275 nm and 315 nm) are observed, corresponding to the absorption of liquiritine.

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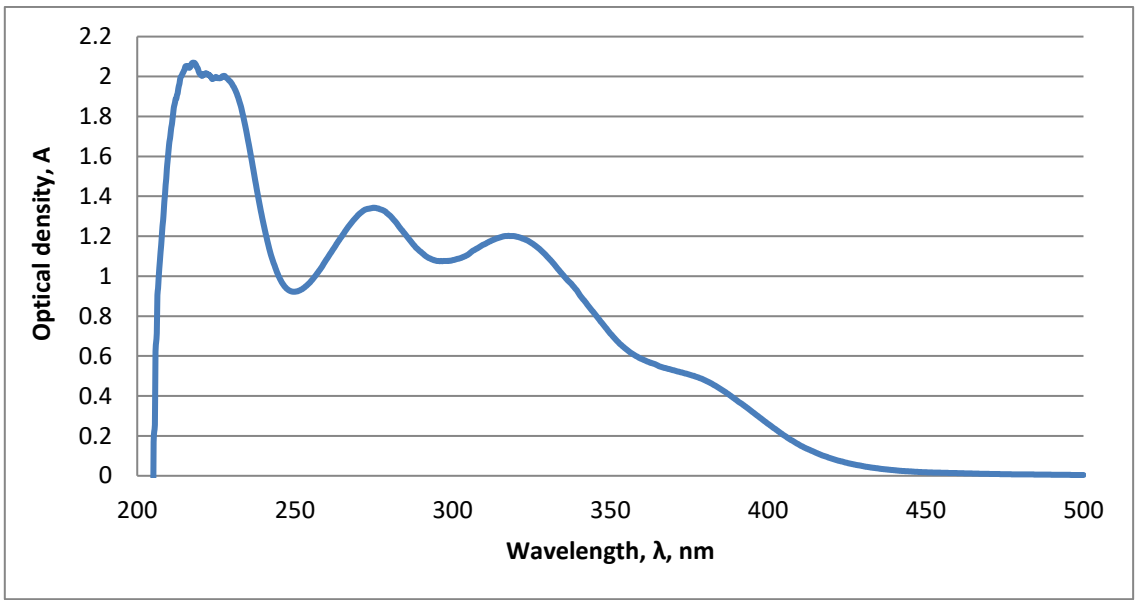
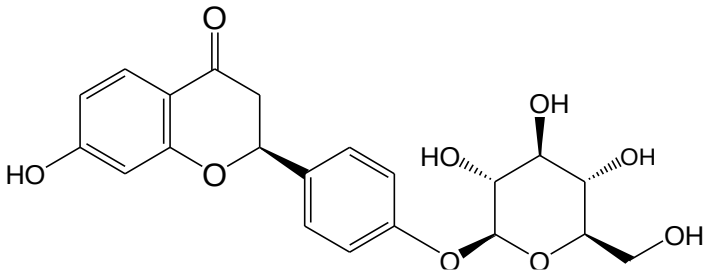


Fig-2. UV spectrum of liquiritin

These data show that the isolated substance is liquiritin.



Conclusion.

1.The number of polyphenols has been isolated from licorice roots.
2.The individual substance liquiritin was isolated and proved by spectra.

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