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Extraction of Biologically Active Substances for Food from the Root of Glycyrrhiza Glabra L.

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Abstract: This article studies the issue of effective extraction of biologically active substances, in particular flavonoids, which are important for the food industry, from the root of Glycyrrhiza glabra (licorice). Flavonoids have strong antioxidant properties and are of great importance in strengthening human health. The study used an ultrasonic extraction method and systematically analyzed the main factors affecting the efficiency of the process - ethanol concentration in the solvent, extraction time, temperature and liquid-solid ratio. The experiments were carried out in 45%, 75% and 96% ethanol solutions, in the temperature range of 40–60 °C, for 30–60 minutes and in different ratios. The obtained extracts were concentrated using a rotary evaporator, and the flavonoid content was determined by HPLC. The results showed that the optimal conditions of 75% ethanol, 50 °C, 45 minutes and a ratio of 1:15 provided the highest (30 mg/g) flavonoid yield. The results of the study are important for the development of effective, environmentally friendly and energy-efficient technologies for the extraction of biologically active substances from licorice root.

Keywords: Glycyrrhiza Glabra, Licorice Root, Flavonoids, Ultrasonic Extraction, Ethanol Solvent, Antioxidants, Biologically Active Substances, HPLC Analysis, Extraction Optimization, Food Industry.

1. Introduction

Licorice (*Glycyrrhiza glabra* L., Fabaceae family) is one of the most abundant and well-studied plants in the world. It is a perennial herb with erect and sparsely branched stems, reaching a height of 50–100 cm (in exceptional cases up to 200 cm). The underground organs are well developed, the roots penetrate the soil to a depth of 6–8 m, forming a dense network that helps to support the licorice population. Despite being a Mediterranean species, the plant is widespread in many regions. Licorice forms thickets in the valleys of large rivers of Central Asia, Uzbekistan and Kazakhstan. The roots of this plant are widely used in medicinal medicine. Many combination drugs (MP) have been developed based on biologically active substances (BAS) isolated from its roots [1,2]. Licorice roots are valuable pharmacopoeial raw materials and are widely used in the production of drugs with expectorant and anti-inflammatory properties [3,4]. In recent decades, scientists have focused on various species belonging to the genus *Glycyrrhiza* L. as promising sources of biologically active substances for therapeutic purposes. [5, 6].

Glabridin is a powerful antioxidant for the food and pharmaceutical industries with anti-inflammatory, neuroprotective, anti-osteoporotic, central nervous system and cardiovascular diseases [14].

Glycyrrhizin and isoliquiritin content of *Glycyrrhiza glabra* have shown anti-inflammatory, antimicrobial, anti-ulcer, antitussive, antioxidant and antidiabetic properties. *Glycyrrhiza glabra* also has potential in homeopathy and is increasingly recognized in complementary medicine [15].

Licorice root extract (*Glycyrrhiza glabra* L.) has long been used as a natural medicine and sweetener in many countries around the world. Therefore, much attention has been paid to developing efficient and environmentally friendly methods for the extraction of bioactive components from licorice root [8]. The recovery efficiency of these bioactive compounds is highly dependent on the extraction technique and the polarity of the solvent used. Traditional methods such as maceration and Soxhlet extraction often require long processing times and high temperatures, which can lead to the degradation of thermolabile flavonoids. To overcome these limitations, ultrasonically assisted extraction (UAE) has emerged as a highly “green” alternative [9]. The choice of solvent is important for the selective extraction of flavonoids. Due to the synergistic effect of water and ethanol in dissolving polar and moderately polar compounds, the use of hydroethanolic mixtures is often more effective than pure solvents [10]. An ethanol concentration of 75% was found to be the optimal equilibrium for extracting high-density flavonoid concentrates by penetrating the lignocellulose matrix of licorice roots. Solvent optimization is essential for the high-performance liquid chromatography (HPLC) analysis of flavonoids from licorice roots [11]. Standardization of the resulting extracts requires advanced analytical protocols. Al-Dhubiab et al. demonstrated that a validated HPLC-DAD method is essential for the simultaneous quantitative determination of key bioactive markers, ensuring the quality and safety of functional beverages and pharmaceutical formulations. Despite extensive research on *Glycyrrhiza glabra*, there is a need to optimize extraction parameters for specific regional varieties to achieve maximum bioactivity [12].

The main known properties of licorice root include its efficacy in the treatment of ulcers, bronchitis, sore throats, and liver diseases, as well as anti-inflammatory, antibacterial, and antiviral effects (especially in the treatment of hepatitis C and HIV). Glabridin (GB), an isoflavone, is known for its cytotoxic, antimicrobial, estrogenic, and antiproliferative effects in breast cancer. It also has antioxidant effects on melanogenesis, low-density lipoprotein (LDL) oxidation, and mitochondrial function. Quantification of active components in licorice root extracts is of great importance. The developed simple, rapid, and cost-effective high-performance liquid chromatography method will help in future studies of bioavailability and quantification of these compounds in extracts [13].

Molecular docking and dynamics simulations revealed that the binding affinities of solasonine, solamargine, rutin, and glycyrrhizin ranged from -9.40 to -8.50 kcal/mol, which is higher than that of the standard NSAID diclofenac (-5.68 kcal/mol). While these docking results provide valuable information, further in vitro validation is needed. Stability analysis of the ligand-receptor complexes showed minimal structural fluctuations. Furthermore, cardiotoxicity predictions indicated that solamargine, rutin, and glycyrrhizin pose a lower risk compared to diclofenac. ADMET profiling highlighted favorable pharmacokinetic properties for rutin, indicating its potential as a promising COX-2 inhibitor with a favorable safety profile. Further MM-GBSA calculations revealed free bond energy values of -11.316 kcal/mol for rutin and -35.190 kcal/mol for diclofenac, indicating strong binding interactions. Overall, this study highlights the potential of these natural compounds as safer alternatives in anti-inflammatory therapy and paves the way for future experimental validation and clinical application [16].

This study focused on optimizing flavonoid extraction by ultrasonically extracting locally grown licorice roots from 75% ethanol mixtures. The resulting high-density

concentrates were characterized by HPLC analysis in order to provide a solid basis for the development of enriched functional beverages.

2. Materials and Methods

2.1. Plant raw materials

Licorice (*Glycyrrhiza glabra* L.) roots were selected as the object of research. The roots were collected from the territory of the Khorezm region in December 2025 by "Shovot Boyon Tomir" LLC and dried at 55-60 °C. The dried roots were ground to a size of 0.5-1 mm, and the particles were sieved to ensure uniform size.

2.2. Reagents and chemicals

All chemicals used during the experiment were of analytical purity. Standard samples: rutin and quercetin standards (purity $\geq 95.0\%$) were used as controls in the analysis process. Ethyl alcohol solutions of different concentrations (45%, 75%, 96%) were used for extraction

Reagents for HPLC: acetonitrile and phosphoric acid for chromatographic analysis

Water: Distilled (deionized) water was used to prepare all solutions and extracts.

2.3. Extraction in an ultrasonic bath.

The extraction process was carried out in a digital ultrasonic bath (GT SONIC VGT-series). The device was equipped with a 40 kG frequency, temperature and power control system.

The extraction of flavonoids was carried out using ethanol solutions of different concentrations. For each experiment, 100 g of *G. glabra* powder was weighed using an analytical balance and mixed with ethanol of different concentrations at predetermined solid-liquid ratios (1:10, 1:15, 1:20 g/ml). The extraction process was also carried out in different temperatures and for 3 different times:

- Solid-liquid ratios (1:15, 1:10, 1:20 g/ml);
- Ethanol concentrations: 96%, 75% and 45% (v/v);
- Extraction temperatures: 40°C, 50°C and 60°C;
- Extraction times: 30, 45, and 60 minutes.

Table 1. Extraction of flavonoids from red mulberry root in an ultrasonic water bath.

Nº	Dry mass, g	Ethanol concentration, %	Temperature °C	Extraction time, min	Solid-liquid ratio, m
1	100	45	40	30	1:10
2	100	45	50	45	1:15
3	100	45	60	60	1:20
4	100	75	40	30	1:10
5	100	75	50	45	1:15
6	100	75	60	60	1:20
7	100	96	40	30	1:10
8	100	96	50	45	1:15
9	100	96	60	60	1:20



Figure 1. Extraction of licorice root.

2.4. Concentration

The obtained extract was filtered through laboratory filter paper. The prepared extract was concentrated in a rotary evaporator for 2 hours 45 minutes, at 50 °C, 240 mBar pressure, rotation speed 120 r/min, cooling water temperature -6 °C, until the density was 1.26.



Figure 2. Separation of alcohol from the extract in a rotary evaporator.

2.5 HPLC analysis

The obtained concentrates were analyzed by high-performance liquid chromatography. The flavonoids in the sample were determined using the high-performance liquid chromatography method. The concentrated solution was centrifuged at 8000 rpm for 20 minutes. The resulting solution was taken from the supernatant for analysis.

Phosphate buffer system and acetonitrile were used as eluents for the determination of flavonoids by HPLC.

Chromatography conditions:

- Agilent-1200 chromatograph (equipped with an autodispenser)
- Exlipse XDB C 18 column (reversed-phase), 5 μ m, 4.6 x 250 mm
- Diode array detector (DAD), 254 nm, 276 nm were identified.
- Flow rate 0.8 ml/min
- Eluent phosphate buffer: acetonitrile:
 - 0-5 min 95:5,
 - 6-12 min 70:30,
 - 12-13 min 50:50,
 - 13-15 min 95:5,
- thermostat temperature 300°C, -10 μ l injected amount (vkol)

First, working standard solutions were introduced into the chromatograph, and then the prepared working solutions were introduced.

Table 2. Determination of flavonoids in samples prepared from red mulberry root.

№	Dry mass, g	Ethanol concentration, %	Temperature, °C	Extraction time, min	Solid-liquid ratio, m	Total flavonoid content, %
1	100	45	40	30	1:10	0,4
2	100	45	50	45	1:15	0,41
3	100	45	60	60	1:20	0,41
4	100	75	40	30	1:10	1,46
5	100	75	50	45	1:15	1,47
6	100	75	60	60	1:20	1,46
7	100	96	40	30	1:10	1,41
8	100	96	50	45	1:15	1,4

9	100	96	60	60	1:20	1,42
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Table 3. Flavonoid content in licorice root determined by HPLC analysis.

Samples	Flavonoid content, %		
	Rutin	Quercetin	Total flavonoids
96 % ethanol solution	0,3±0,03	0,26±0,07	1,42±0,07
75 % ethanol solution	0,56±0,03	0,3±0,03	1,47±0,03
45 % ethanol solution	0,09±0,07	0,78±0,03	0,42±0,07

3. Results and Discussion

Analysis of results. The results of the study showed that the main technological factors have a significant effect on the extraction of flavonoids from *Glycyrrhiza glabra* roots using ultrasonic extraction. According to Table 3, the content of flavonoids varies significantly depending on the extraction conditions.

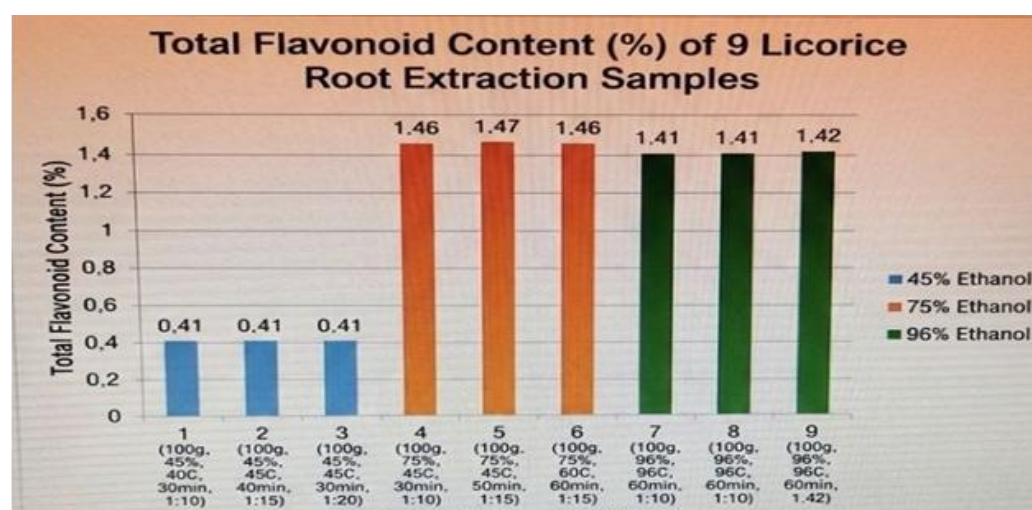


Figure 3. Flavonoid content in samples.

The lowest flavonoid content was observed in a 45% ethanol solution (0.40–0.41%), which indicates that the low concentration of the solvent cannot completely dissolve the bioactive substances. On the contrary, the content of flavonoids reached a maximum in a 75% ethanol solution (1.46–1.47%), which indicates that the hydroethanolic medium has an optimal degree of polarization and ensures effective separation of flavonoids from the cell matrix. The slightly lower content of flavonoids in a 96% ethanol solution (1.40–1.42%) is explained by the inability of high-concentration alcohol to penetrate deeply into the cell structure.

The extraction temperature and time also appeared to be important factors. While 50°C and 45 min gave the optimal results, increasing the temperature and time did not lead to a significant increase in the amount of flavonoids, but in some cases showed the possibility of thermal degradation. Also, the value of the liquid-solid ratio of 1:15 was found to be the most optimal, since under these conditions the maximum mass transfer between the solvent and the raw material was ensured. HPLC analyses clearly confirmed the presence of flavonoids in the extract and showed that their content was high in the concentrate obtained under optimal conditions. Overall, the obtained results prove the effectiveness of the ultrasonic extraction method and its potential as a promising technology for the extraction of biologically active substances in the food industry.

4. Conclusion

During the study, the main factors affecting flavonoid extraction, including ethanol concentration in the solvent, extraction time, extraction temperature, liquid-solid ratio, were studied. Licorice root was extracted using 96%, 75% and 45% ethanol solutions at 40, 50, 60 °C for 30, 45, 60 minutes, solid-liquid ratios (1:15, 1:10, 1:20 g/ml). The extract was concentrated in a rotary evaporator for 2 hours and 45 minutes at a bath temperature of 50 °C, a vacuum pressure of 240 mbar, a rotation speed of 120 r/min, and a cooling water temperature of -6 °C until its density was 1.26. The maximum flavonoid content (30 mg/g) in the concentrate was obtained under optimal conditions, with 75% ethanol as the extraction solvent, at 50 °C for 45 minutes, with two extraction cycles at a material-solvent ratio of 1:15.

In conclusion, based on the experiments conducted, it can be concluded that the most important factors affecting flavonoid yield are the ethanol concentration in the solvent, extraction time, extraction temperature, and liquid-solid ratio. It was found that these parameters have a significant and positive effect on the amount of flavonoid obtained during the extraction process.

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