Original research article

Development of a technology for obtaining a substance from Glycyrrhiza glabra L. roots by sequential extraction

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Abstract

Background. Differences in the physicochemical properties of biologically active compounds in Glycyrrhiza glabra L. roots, including triterpene saponins, flavonoids and other natural compounds, make their extraction with a single extraction system difficult. A sequential technological approach using extractants of different composition is therefore relevant.

Objective. To develop a technology and laboratory procedure for obtaining a dry substance from Glycyrrhiza glabra L. roots by sequential extraction.

Materials and methods. Prepared Glycyrrhiza glabra L. roots were extracted three times with 1% aqueous ammonia at a raw material-to-extractant ratio of 1:10, 75-85 °C and 1 h per cycle. Pooled extracts were cooled to 5 °C, centrifuged at 8000 rpm for 20 min, decanted and lyophilized. The residual plant material was subsequently extracted three times with 50% and 90% ethanol at 50-60 °C. Ethanolic extracts were cooled, centrifuged and decanted; ethanol was removed under vacuum at no more than 45 °C and the concentrates were lyophilized.

Results. A multistage technological scheme and the principal process parameters were established. The resulting substance was a hygroscopic light- to dark-brown powder readily soluble in water and hydrophilic media. The dry-substance yield was 16.73 ± 0.42%; glycyrrhizic acid content was 20.81 ± 0.17%, and total flavonoids expressed as liquiritin were 8.36 ± 0.21%.

Conclusion. A technology and laboratory procedure for obtaining a dry substance from Glycyrrhiza glabra L. roots by sequential extraction were developed. The approach provides a basis for subsequent assessment of reproducibility and process scale-up.

Keywords: Glycyrrhiza glabra L.; licorice; glycyrrhizic acid; liquiritin; flavonoids; sequential extraction; laboratory procedure; ethanolic extraction; lyophilization

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Introduction

Licorice (Glycyrrhiza glabra L.) is a valuable medicinal plant material and a source of biologically active compounds from several chemical classes. Its roots contain triterpene saponins, flavonoids and other phenolic compounds, as well as polysaccharides, coumarins, phytosterols and other natural constituents [1-3]. Glycyrrhizic acid is one of the principal marker compounds, while flavonoids including liquiritin, isoliquiritin, liquiritigenin, isoliquiritigenin and glabridin are also of significant interest [1-5]. The multicomponent nature of the raw material requires a justified selection of processing conditions.

Extraction is a key stage in production of plant-derived substances. Its efficiency depends on the nature and polarity of the extractant, the raw material-to-extractant ratio, temperature, extraction time and number of cycles, particle size, and subsequent separation and concentration operations [6-8]. For multicomponent plant material, one extractant may limit recovery of compounds with substantially different physicochemical properties and solubility.

This problem is particularly relevant to G. glabra roots. Recovery of glycyrrhizic acid depends on extractant composition and pH; water, aqueous-alcoholic systems and ammonia-containing solutions have been used [4,8,9]. Flavonoid components of licorice also differ in polarity, and extractant composition therefore affects the profile of recovered compounds [6,10]. Studies using water and aqueous ethanol of different concentrations likewise demonstrate differences in the amount and composition of extracted substances [11].

Contemporary studies seek to intensify extraction using ultrasound, enzymes and other forms of treatment and alternative solvent systems such as deep eutectic solvents [7,10,12-14]. However, the efficiency of obtaining a plant substance depends not only on intensified recovery of individual compounds but also on the sequence of processing operations and on separation, concentration and drying of the resulting extracts [8].

Because licorice constituents differ in solubility, sequential processing of the same plant material with extractants of different composition is a promising technological approach. This principle enables stepwise processing with extraction systems of different physicochemical properties and may broaden recovery of different groups of biologically active compounds.

The aim of the study was to develop a technology and laboratory procedure for obtaining a dry substance from Glycyrrhiza glabra L. roots by sequential extraction.

Materials and methods

The study material consisted of Glycyrrhiza glabra L. roots harvested in September 2024 in Almaty Region, Republic of Kazakhstan. One kilogram of roots was used for the technological process. The raw material was washed with running water to remove mechanical impurities and cut into 2-5 cm fragments [15].

The prepared raw material was dried at 45-55 °C to a residual moisture content of no more than 10%. The dried roots were milled on a roller crusher to a particle size of 0.5-2.0 mm. The process scheme was organized as a 17-stage sequential workflow.

Stage 1 - raw-material preparation. Dried, milled Glycyrrhiza glabra L. roots (0.5-2.0 mm), 96% ethanol, ammonia and purified water were used. All raw materials and auxiliary materials were required to comply with applicable specifications [16-18]; regulated quantities were weighed.

Stage 2 - preparation of extractant 1. Ammonia was mixed with purified water to obtain a 1% aqueous NH_4OH solution. Mixing was performed at 20-25 rpm for 10-20 min and concentration was controlled.

Stage 3 - extraction 1. The plant material was loaded into a reactor and 1% aqueous ammonia was added at a raw material-to-extractant ratio of 1:10, taking absorption into account. Extraction was conducted at 75-85 °C for 1 h and repeated three times with fresh extractant. After each cycle the liquid phase was separated.

Stage 4 - clarification of extract 1. The marc was pressed after each extraction. Extracts were pooled and cooled to 5 °C, centrifuged at 8000 rpm for 20 min and the supernatant was separated by decantation. The marc was retained for subsequent 50% ethanol extraction.

Stage 5 - drying of clarified extract 1. The clarified aqueous-ammonia extract was lyophilized at -40 °C and pressure not exceeding 0.1 mmHg for 24 h to obtain dry extract 1.

Stage 6 - preparation of extractant 2. Ninety-six percent ethanol was mixed with purified water to obtain 50% aqueous ethanol. Mixing was carried out at 20-25 rpm for 10-20 min and ethanol concentration was controlled.

Stage 7 - extraction 2. The marc from the aqueous-ammonia stage was extracted three times with 50% ethanol at a marc-to-extractant ratio of 1:10, 50-60 °C and 72 h per cycle. After each cycle the liquid phase was separated and the marc was treated with a fresh portion of extractant.

Stage 8 - clarification of extract 2. After each cycle the marc was pressed; ethanolic extracts were pooled, cooled to 5 °C, centrifuged at 8000 rpm for 20 min and decanted. The

marc was then transferred to the next sequential extraction stage.

Stage 9 - concentration of extract 2. The clarified ethanolic extract was concentrated on a rotary vacuum evaporator with a chiller at no more than 45 °C. Pressure was gradually reduced from 200 to 60 mbar until a concentrated extract was obtained.

Stage 10 - drying of concentrated extract 2. The concentrate was lyophilized at -40 °C and pressure not exceeding 0.1 mmHg for 24 h to obtain dry extract 2.

Stage 11 - preparation of extractant 3. Ninety-six percent ethanol and purified water were mixed to obtain 90% aqueous ethanol at 20-25 rpm for 10-20 min; ethanol concentration was controlled.

Stage 12 - extraction 3. The marc remaining after 50% ethanol extraction was extracted three times with 90% ethanol at a marc-to-extractant ratio of 1:5, 50-60 °C and 72 h per cycle. The liquid phase was separated after each cycle.

Stage 13 - clarification of extract 3. The marc was pressed after each cycle; extracts were pooled, cooled to 5 °C, centrifuged at 8000 rpm for 20 min and decanted. The exhausted plant material was then directed to disposal.

Stage 14 - concentration of extract 3. The clarified extract was concentrated under vacuum with a chiller at no more than 45 °C while pressure was reduced from 200 to 60 mbar until a concentrate was obtained.

Stage 15 - drying of concentrated extract 3. The concentrate was lyophilized at -40 °C and pressure not exceeding 0.1 mmHg for 24 h to obtain dry extract 3.

Stage 16 - mixing and milling. Dry extracts 1, 2 and 3 were combined and mixed at 20-25 rpm until homogeneous. If necessary, the mixture was additionally milled, resulting in the final dry substance from Glycyrrhiza glabra L. roots.

Stage 17 - filling and labelling. The dry substance was filled into bags for vacuum sealing, sealed and labelled. The finished substance was submitted to quality control in accordance with the approved specification.

For characterization of the dry substance, glycyrrhizic acid and total flavonoids were quantified. Glycyrrhizic acid was determined by high-performance liquid chromatography with UV detection [19], and total flavonoids were determined spectrophotometrically as liquiritin equivalents [20].

Dry-substance yield was determined gravimetrically as the ratio of the final dry product mass (m_1) to the mass of the initial air-dry plant raw material (m_0): $Y\ (\%) = (m_1 / m_0) \times 100$. Quantitative results are presented as mean ± standard deviation (M ± SD, n = 3).

Results

A sequential technological scheme for obtaining a substance from Glycyrrhiza glabra L. roots was developed. It is based on stepwise processing of the same plant material with extractants of different composition. The process comprises triple aqueous-ammonia extraction followed by triple extraction of the resulting marc with 50% and 90% ethanol.

The process parameters established for the three extraction stages included extractant composition and concentration, raw material/marc-to-extractant ratio, extraction temperature and duration, number of cycles, cooling and clarification conditions, vacuum concentration and lyophilization.

Three dry extracts were obtained through the sequential process and, after pooling and mixing, formed the final dry substance.

The resulting dry substance was a hygroscopic powder ranging from light brown to dark brown and readily soluble in water and hydrophilic media.

The dry-substance yield was 16.73 ± 0.42% of the initial air-dry raw material. Glycyrrhizic acid content was 20.81 ± 0.17%, and total flavonoids expressed as liquiritin were 8.36 ± 0.21%.

Discussion

The use of 1% aqueous ammonia in the first stage is consistent with reports describing diluted ammonia solutions for extraction of glycyrrhizic acid from licorice roots. Carboxyl groups in glycyrrhizic acid make its solubility pH-dependent; an alkaline aqueous medium promotes transition to a more soluble ionized form. Pan et al. used 1-2% ammonia solutions at an extractant-to-raw-material ratio of 10:1, Liao et al. used a system containing 1% ammonia, and Stolyarova et al. used 0.5% NH_4OH during primary extraction [22-24]. The selected 1% concentration therefore lies within the range previously applied to glycyrrhizic-acid recovery.

Temperature is also an important factor in aqueous-ammonia extraction. Increased temperature promotes mass transfer and extraction of licorice constituents [13,23,25]. The developed process used 75-85 °C for 1 h, combined with three cycles using fresh extractant.

The composition of aqueous ethanol affects the profile of compounds recovered from licorice roots. Changing ethanol concentration changes solvent polarity and therefore the relative recovery of biologically active constituents. Boyko et al. showed that extraction of individual Glycyrrhizae radix compound groups depended on ethanol concentration: maximum extraction of the

studied flavonoids occurred with 50% ethanol, whereas chalcones showed maximal values at a higher ethanol concentration of about 71% [26].

Tian et al. likewise demonstrated different extraction behavior of licorice components. As ethanol concentration increased from 10% to 90%, recovery of glycyrrhizic acid decreased, whereas recovery of the less polar glabridin remained relatively high over a broad ethanol range [27]. These results support sequential use of solvents of different polarity.

Zhurinov et al. compared water, 50%, 70% and 90% ethanol and reported extractive-matter contents of 46%, 45%, 38% and 43%, respectively [11]. Both 50% and 90% ethanol therefore extracted substantial amounts of material. Together with data on differential extraction behavior [26,27], this supports sequential use of 50% and 90% ethanol after the aqueous-ammonia stage.

Cooling plant extracts before subsequent processing can reduce the solubility of accompanying constituents and promote precipitation, which can then be removed by centrifugation. Low-temperature holding followed by separation of precipitate has also been described in licorice processing [28]. In the developed technology, cooling to 5 °C followed by centrifugation and decantation served as a preliminary clarification step.

For ethanolic extracts, solvent removal under reduced pressure is important. Vacuum concentration reduces the temperature required for ethanol removal and therefore limits thermal exposure of plant constituents. Rotary vacuum evaporation at reduced temperature has previously been used for Glycyrrhiza glabra L. extracts [29]. In the present process ethanol was removed at no more than 45 °C while pressure was gradually reduced from 200 to 60 mbar; recovered ethanol was directed to rectification for potential reuse.

Lyophilization provides low-temperature removal of water and has been used for dry Glycyrrhiza glabra L. extracts [29,30]. Here it was conducted at -40 °C and pressure not exceeding 0.1 mmHg for 24 h. The three dry extracts were then pooled and mixed to form the final substance.

The final yield was 16.73 ± 0.42%; glycyrrhizic acid content was 20.81 ± 0.17% and total flavonoids expressed as liquiritin were 8.36 ± 0.21%. These values characterize the combined substance formed from the three sequentially produced dry extracts.

The distinguishing feature of the proposed approach is sequential processing of the same plant raw material with extractants of different polarity followed by pooling of the resulting dry extracts. Unlike approaches focused mainly on intensification of a single extraction stage, the proposed scheme uses the remaining marc successively and produces one dry substance containing glycyrrhizic acid and flavonoids.

The scientific novelty lies in the sequential use of 1% aqueous ammonia, 50% ethanol and 90% ethanol for the same plant raw material, with subsequent combination of the dry extracts. This changes the extraction environment at each stage and provides a structured technological basis for subsequent reproducibility and scale-up studies.

Conclusion

A technology and laboratory procedure for obtaining a dry substance from Glycyrrhiza glabra L. roots by sequential extraction were developed. The approach is based on stepwise processing of the same plant material with 1% aqueous ammonia, 50% ethanol and 90% ethanol, followed by pooling of the dry extracts.

Key process parameters were established, including extractant composition and concentration, raw material/marc-to-extractant ratios, temperature and duration, number of extraction cycles, low-temperature clarification, vacuum concentration of ethanolic extracts and lyophilization. The scheme also includes regeneration of distilled ethanol by rectification and potential reuse.

Implementation of the laboratory procedure yielded a hygroscopic light- to dark-brown powder readily soluble in water and hydrophilic media. Yield was 16.73 ± 0.42% of the initial air-dry raw material; glycyrrhizic acid content was 20.81 ± 0.17%, and total flavonoids as liquiritin were 8.36 ± 0.21%.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Ibragimova L.N. - study concept and design, methodology development, scientific supervision, analysis and interpretation of results, manuscript preparation and editing.

Yensebayeva G.B. - experimental work, participation in development of the technological scheme, data processing and analysis.

Ibragimova R.Z. - participation in experimental work, systematization of data and preparation of manuscript materials.

Sarsenbekova Zh.A. - participation in experimental work, analysis and interpretation of results.

Sharipova O.F. - participation in research, analytical control and processing of experimental data.

Kurmanaliyeva A.R. - methodological support, analysis and interpretation of results, manuscript editing.

Baigaipova G.K. - experimental work, preparation of plant raw material and performance of technological operations.

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Figure 1. Process scheme for obtaining a dry substance from Glycyrrhiza glabra L. roots by sequential extraction.



Table 1. Principal technological parameters of sequential extraction of Glycyrrhiza glabra L. roots

Parameter	Stage I	Stage II	Stage III
Extractant	Aqueous NH_4OH	Ethanol	Ethanol
Extractant concentration, %	1	50	90
Extraction temperature, °C	80	55	55
Raw material/marc:extractant ratio	1:10	1:10	1:5
Duration of one cycle, h	1	72	72
Number of extraction cycles	3	3	3
Post-extraction treatment	Pressing, pooling of extracts	Pressing, pooling of extracts	Pressing, pooling of extracts
Cooling temperature, °C	5	5	5
Centrifugation speed, rpm	8000	8000	8000
Centrifugation time, min	20	20	20
Vacuum bath temperature for ethanol removal, °C	-	≤45	≤45
Pressure, mbar	-	200→60	200→60
Extractant regeneration	-	Rectification of recovered ethanol with reuse	Rectification of recovered ethanol with reuse
Freezing temperature, °C	−40	−40	−40
Pressure during lyophilization, mmHg	≤0.1	≤0.1	≤0.1
Lyophilization duration, h	24	24	24

Table 2. Characteristics of the substance obtained from Glycyrrhiza glabra L. roots by sequential extraction

Parameter	Obtained value / characteristic
Appearance	Hygroscopic powder
Colour	Light brown to dark brown
Solubility	Readily soluble in water and hydrophilic media
Substance yield	16.73 ± 0.42%
Glycyrrhizic acid content	20.81 ± 0.17%
Total flavonoids as liquiritin	8.36 ± 0.21%