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ON THE ISSUE OF STANDARDIZATION OF DRY EXTRACT "GLYZYRRHIZA 7"

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ABSTRACT

A promising direction for the search and development of new adaptogenic drugs of natural origin is the study of nonspecific drugs from the arsenal of Tibetan medicine, recommended for the weakened, as well as for elderly people, as a general tonic, "giving longevity and health." Based on the prescription recipe "Glyzyrhiza-7", in the indications for use: "with pain in the kidneys, lower back, limb stiffness, sudden flushes of heat in different parts of the body" developed a dry plant extract from the roots *GlyzyrrhizauralensisFisch*, wood *Caraganajubata (Pall.) Poir*, roots *Polygonatumodoratum (Mill) Druse*, roots *PolygonatumhumileFisch ex Maxim*, bulbs *Orchismascula*L., roots *Rheum rhabarbarum* L., roots *Rubiatinctorum* L. HPLC in the dry extract revealed the presence of ten marker components whose raw material source is *Rheum* (deoxy-raponticin, raponticin, rapontigenin, caffeic acid),*Glyzyrrhiza* (likuritin, glycyrrhizinic acid), *Rubia* (alizarin, purpurine, ruberitrinic acid, lucidin-primverozid) The dominant compounds of the licorice 7 extract are anthraquinones, the content of which was 7.65 mg/g; stelben content 6.13 mg/g; terpenes (glycyrrhizic acid) 1.67 mg/g, flavonoids and phenol carboxylic acids account for 2.07 and 1.12 mg/g, respectively. A technique has been developed for the quantitative determination of the content of glycyrrhizic acid in the dry extract (16.5%), which can be used to standardize this object.

KEYWORDS: adaptogens, biologically active substances.

INTRODUCTION

Adaptogenic drugs are widely used in modern preventive medicine. The mechanism of action of adaptogens is associated with their activating effect on metabolic processes, and the main effects of adaptogens are expressed in increasing the body's resistance to adverse factors through adaptive metabolism restructuring. This is due to the fact that adaptogenic drugs more pronouncedly stimulate oxidative phosphorylation during hypoxia and extreme situations, under adverse environmental conditions normalize energy and nucleic acid metabolism, have antioxidant effects, increase the activity of antioxidant defense factors and inhibit lipid peroxidation.

Their influence is distinguished by the wide spectrum of pharmacological action due to the content of various classes of biologically active substances in them, their availability, and interchangeability. The predominant pharmacotherapeutic effectiveness of multicomponent herbal preparations is due not only to their effect on the pathological process, but also to their regulatory effect on various functional systems of the body with an increase in the resistance of the organism as a whole.

A promising direction in the search and development of new adaptogenic drugs of natural origin is the study of nonspecific drugs from the arsenal of Tibetan medicine, recommended for the weakened, as well as people of advanced age, as tonic, “giving longevity and health”.

Based on the prescription “Glyzyrrhiza 7”, in indications for use: “for pain in the region of the kidneys, lower back, stiffness of the extremities, sudden flushes of heat in different parts of the body”,[8] a dry plant extract from the roots and rhizomes of (Glyzyrrhiza uralensisFisch), wood of Caragana jubata (Pall.) Poir, roots Polygonatum odoratum (Mill) Druse, roots Polygonatum humile Fischex Maxim, tubers of Orchis mascula (L.), roots Rheum rhabarbarum L., and rhizomes of Rubia tinctorum (L.).

The components of this tool contain a wide range of biologically active substances belonging to different classes of chemical compounds: terpenes (glycyrrhizic

acid, diosgenin, saponin PO-2, PO-3), flavonoids (liquiquirithin, quercetin, rutin, vitexin, myricitin, chrysoriol, apigenin and their glycosides), phenolcarboxylic acids (caffeic, chlorogenic, neochlorogenic, gallic, coumaric, cinnamon), anthocyanins (chresatemin, seranin, cyanine), anthraquinones (chrysofanol, emodin, alizarin, purpurine, lucidin pririmoroside.[4-7]

The pharmacological activity of these species is known, so glyzyrrhiza extracts exhibit anti-inflammatory and immunomodulating activity,[5] Caragana - hypoglycemic, anti-inflammatory,[5] Polygonatum odoratum – immunomodulatory,[7] Polygonatum humile – coagulant,[7] Orchis mascula,[7] Rheum rhabarbarum L., - anti-inflammatory,[4] Rubia tinctorum - choleretic and antibacterial.[4]

Thus, according to folk and scientific medicine, preparations from from the roots and rhizomes of (Glyzyrrhiza uralensisFisch), wood of Caragana jubata (Pall.) Poir, roots Polygonatum odoratum (Mill) Druse, roots Polygonatum humile Fischex Maxim, tubers of Orchis mascula (L.), roots Rheum rhabarbarum L., and rhizomes of Rubia tinctorum (L.).have a pronounced antioxidant and immunomodulating effect , which indicates the advisability of using this composition as an adaptogenic agent.

The purpose of the work is the determination of the main biologically active substances and the development of a standardization procedure for a complex extract obtained from the roots and rhizomes of (Glyzyrrhiza uralensis Fisch), wood of Caragana jubata (Pall.) Poir, roots Polygonatum odoratum (Mill) Druse, roots Polygonatum humile Fischex Maxim, tubers of Orchis mascula (L.), roots Rheum rhabarbarum L., and rhizomes of Rubia tinctorum (L.).

Experimetal part.

Materials and methods. Plant material - roots of Glyzyrrhiza, woods of Caragana, roots Polygonatum odoratum, roots of Rheum collected at the experimental hospital "Goryachinsk", Buryatia in 2018, roots of Rubia, roots Polygonatum humile, tubers of male Orchis purchased in LLC Dar Altai, Chelyabinsk in 2019. Using the remaceration method, taking into account the optimal extraction parameters, a dry plant extract (DPE) was obtained.

The extraction process was optimized taking into account the yield of the sum of saponins, in terms of glycyrrhizic acid. Determination of the mass loss upon drying was carried out on a Netzsch STA 449 C derivatograph (Germany). Absorption spectra were recorded on an Agilent-8453E spectrophotometer (USA) in quartz cells with an absorbing layer thickness of 10 mm.

The quantitative content of marker components was determined by HPLC. The studies were performed on a Milichrom A-02 microcolumn liquid chromatograph (Ekonova, Russia) equipped with an autosampler, a UV detector, and a ProntoSIL-120-5-C18 AQ reversed-phase sorbent column (2 × 75 mm (5 μm; Metrohm AG, Switzerland). Mobile phase: eluent A - 0.2 M LiClO4 at 0.006 M HClO4, eluent B - acetonitrile; elution mode - gradient; gradient program (% B): 0-20 min 5-10%; mobile phase velocity 200 μl / min; column temperature 35 ° C; detector wavelength 270 nm. Standard samples of glycyram, gallic acid, quercetin, apigeninin were used in the work, routine manufactured by Extrasynthese (France). The compounds were identified on the basis of data on chromatographic mobility, addition method, UV and MS characteristics of individual compounds.[9,10]

Mathematical data processing was performed using Microsoft Excel 2003. After checking the distribution for normality, the statistical significance of differences in the compared values was determined using Student's t-test.[1]

RESULTS AND ITS DISCUSSION

The extract is a fine brown powder with a pleasant odor and a sweet astringent taste, with a moisture content of not more than 5%. The loss in mass during drying does not exceed 5%. Using differential scanning calorimetry, it was found that the main moisture removal begins at a temperature of 50 ° C, and at 150 ° C the decomposition of the extract occurs. The presence of ten marker components was found by HPLC in the dry extract, the raw source of which is Rheum (deoxyraponticin, raponticin, rapontigenin, caffeic acid), Glyzyrrhiza (liquiquitin, glycyrrhizic acid), Rubia (alizarin, purpurin, ruberitric acid , lucidin primrovoside) fig. 1, tab. one.

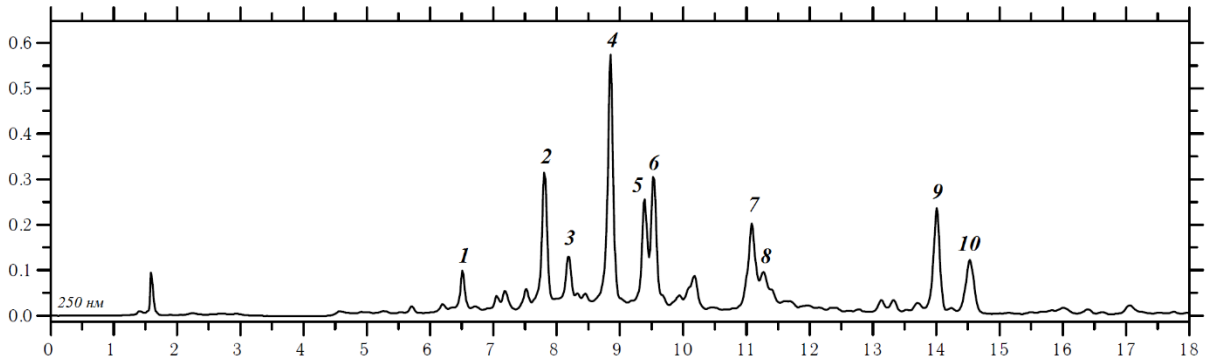

Рис. 1. Chromatogram (HPLC) of alcohol extraction from the «Glyzyrrhiza -7» extract. The numbers indicate the compounds: 1 - caffeic acid; 2 - deoxyraponticin; 3 - glycirithin; 4 - raponticin; 5 - lucidin primrovezide; 6 - ruberitric acid; 7 - rapontigenin; 8 - alizarin; 9 - glycyrrhizic acid; 10 - purpurine.

Table 1: The quantitative content of biologically active substances in the dry extract «Glyzyrrhiza -7».

Compound	Content, mg / g
Coffee acid	1.12 ± 0.01
Deoxyraponticin	1.86 ± 0.05
Likviritin	2.07 ± 0.06
Raponticin	3.35 ± 0.06
Lucidin primveroside	2.83 ± 0.02
Ruberitric Acid	2.96± 0.04
Rapontigenin	0.92± 0.05
Alizarin	0.84± 0.06
Glyirrhizic acid	1.67± 0.03
Purpurin	1.02± 0.01

The dominant compounds of the «Glyzyrrhiza -7» extract are anthraquinones, the content of which was 7.65 mg / g; stelbene content 6.13 mg / g; terpenes (glycyrrhizic acid) 1.67 mg / g, flavonoids and phenolcarboxylic acids account for 2.07 and 1.12 mg / g, respectively.

To develop standardization techniques, the glycyrrhizic acid content was used.[2,3] State standard samples of glycyram (monoammonium salt of glycyrrhizic acid) have been proposed since glycyrrhizic acid is an unstable substance, which prevents the use of this compound as a state standard sample.

In the quantitative determination of the glycyrrhizic acid content, the electronic spectrum of the extract contains one intense absorption maximum at a wavelength of 258 ± 2 nm (Fig. 2), which correlates with that of glycyrrhizic acid and glycyram.[2]

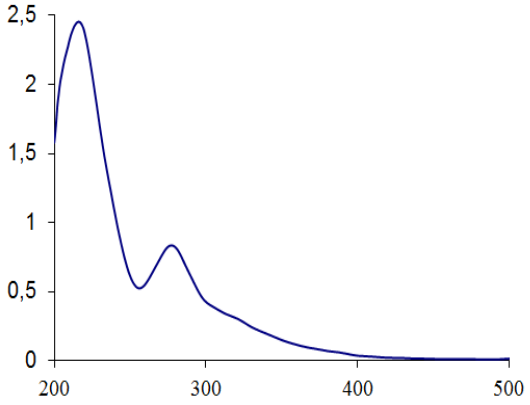

Рис. 2. Electronic spectrum of the extract. Abscissa.

Method for the quantitative determination of glycyrrhizic acid in dry extract. 0.5 g (accurately weighed) of the extract is placed in a conical flask with a thin section with a capacity of 250 ml, 20 ml of alcohol 95% are added and mixed. Then 50 ml of a 3% acetone solution of trichloroacetic acid are added and heated for 10 minutes. After cooling, the resulting solution was filtered through a paper filter, the flask was washed with two portions of a 10% trichloroacetic acid solution of 10 ml each, filtered through the same filter. Concentrated ammonia solution is added dropwise to the obtained filtrate until an abundant precipitate appears (pH from 8.3 to 8.6 according to the universal indicator). The solution with the precipitate is transferred to an ashless filter placed in a Buchner funnel. The flask and filter are washed with 50 ml of acetone in three steps. The filter cake was transferred to a flask in which precipitation was carried out, dissolved in 50 ml of water, quantitatively transferred to a 250 ml volumetric flask and the volume of the solution was adjusted to the mark with water (solution A). 1 ml of solution A is placed in a 25 ml volumetric flask and the volume of the solution is adjusted to the mark with water (solution B). The optical density of solution B was measured on a spectrophotometer at a wavelength of 258 nm in a cuvette with a layer thickness of 10 mm, using water as a comparison solution.

In parallel, the optical density of solution B of glycyram-stadart is measured. The content of glycyrrhizic acid in the polyextract in terms of glycyr in percent (X) is calculated by the formula:

$$x = \frac{D * mo * 250 * 25 * 5 * 100}{m * Do * 1 * 50 * 25}$$

Where D is the optical density of the test solution (B) at a wavelength of 258 nm;

DO - the optical density of the GSO solution of glycyram (B) at a wavelength of 258 nm;

m– mass of dry extract in g; mo is the mass of GSO glycyram in grams.

Tab. 2: Metrological characteristics of quantitative methods determination of glycyrrhizic acid in the extract.

f	*S*	*P*	%	*t*(*P*, *f*)	Δ*x*	*E*,%
10	16.5	0.1746	95	2.23	±0.39	±4.78

The error of a single determination with a confidence probability of 4.78% (Table 1).

It was determined that the total content of saponins, in terms of glycyrrhizic acid in the dry extract with a confidence level of 95%, was 16.5%. The error of a single determination does not exceed 5%; the experimental results can be considered satisfactory.

Findings

The dominant compounds of the «Glyzyrrhiza -7» extract are anthraquinones, the content of which was 7.65 mg / g; stelbene content 6.13 mg / g; terpenes (glycyrrhizic acid) 1.67 mg / g, flavonoids and phenolcarboxylic acids account for 2.07 and 1.12 mg / g, respectively. A method has been developed for the quantitative determination of glycyrrhizic acid in dry extract (16.5%), which can be used to standardize this object.

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