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Abstract

The article presents the results of examination of the pharmaco-technological properties of burnet roots as required for optimization of the technological process of obtaining the dry extract. Dependence of the amount of extractive substances on the degree of grinding of the medicinal herbal raw materials has been examined, the weight loss upon drying and the absorption coefficient of the extraction solvent have been specified, the parameters of specific, volumetric and bulk weights have been calculated, such technological parameters as porosity, porousness have been calculated. Upon examination of the thermal conduct of burnet roots, absence of signs of the herbal constituents' degradation, under the temperature conditions provided by the process of the dry extract production, has been found.

Keywords: medical herbal raw material, burnet roots, dry extract, pharmaco-technological researches, thermogravimetric analysis.

Development of the technology for obtaining extracts from medicinal herbal raw materials requires a series of experimental researches focused on intensifying the process of extraction of a set of biologically active substances that determine the range of the extract's pharmacological effect [7]. With this aim, the optimum degree for grinding the raw material is determined, i.e. the preliminary processing; the pharmaco-technological parameters are examined, which will be further taken into account for preparation of the technological equipment as appropriate to the extract production method and provision of the necessary conditions for optimizing the extraction phase conduct [3].

Substantiation of the temperature conditions at the stages of the liquid extract condensation and dehydration of the extract down to the dry condition provides for the necessity to be convinced that there is no decomposition or destruction of the set of biologically active substances when heated. This can be researched through examination of the thermal conduct of the herbal raw material using the thermogravimetric method, as it is specific to each type of herbal item [4].

Thermogravimetry is used by scientists to survey both particular substances and multicomponent formulations while developing the drug products in various dosage forms, the technological process of which provides for the heat treatment of active and/or auxiliary substances and, consequently, creates a risk of transformation thereof, enables changes in the physicochemical and pharmaco-chemical properties through interaction or destruction [1, 5, 6].

No less acute is the expediency of creation of drug products of herbal origin for gastroenterology, in particular on the basis of herbal raw material: burnet, which has long been used in this area by the traditional medicine [8, 9].

Purpose of the paper is to study the pharmaco-technological properties and thermal conduct of burnet roots to substantiate the process of obtaining the dry extract based on it.

Materials and methods. The object under research is the medicinal herbal raw material: medicinal burnet (Sanguisorba officinalis L., species: Rosaceae) – burnet roots (thickness of roots up to 1.5 cm, the outer colour is dark brown, yellow on fracture, no odour, styptic to smell), which meets the requirements of the specifications.

Analytical sieving has been carried out in accordance with the generally accepted methods of the State Pharmacopoeia of Ukraine [2]. The sieving has been performed by mechanical shaking (dry sieving method).

Specification of content of the extractive substances**.** 1.0 g of crushed burnet roots, sifted through a 1 mm screen, has been placed in a 250 ml conical flask, 50 ml of solvent has been added. The flask has been covered with a lid, weighed and left for 1 hour. Thereafter the flask has been combined with the reflux condenser, heated, with the low boiling maintained for 2 hours. After cooling, the filled flask has been closed once again with the same lid, weighted and supplemented the loss in weight with solvent. The contents of the flask have been shaken and filtered through a dry

paper filter into a 200 ml dry flask. 25 ml of the filtrate has been pipetted into the precisely weighted porcelain bowl 7-9 cm in diameter pre-dried at 100-105 °C to constant weight and evaporated to dryness in the water bath. The bowl with the residue has been dried at 100-105 °C to constant weight, then cooled for 30 min in a desiccator, at the bottom of which there has been anhydrous calcium chloride, and immediately weighed.

Composition of the extractive substances has been calculated under the formula:

$$X = \frac{m \times 200 \times 100}{m_1 \times (100 - W)},$$

where: m – is the dry residue weight, g;

m_1 – is the sample weight of burnet roots, g;

W – means the weight loss on drying, %.

Specification of loss on drying of the herbal raw materials [2]. Moisture content in burnet roots has been determined through the gravimetric method using the ground burnet roots (1-3 mm) with the relevant weight at the given drying time and under the given temperature condition. A sample weight of the raw material (3.0-5.0 g) has been placed in the pre-weighed sample bottle. Burnet roots have been dried to the constant weight at 100-105 °C. The first weighing is carried out after 2 hours.

Specification of the specific weight of the raw material (d_y). We have determined the ratio between the weight of the absolutely dry ground burnet roots and the full volume thereof. The parameter is calculated under the formula:

$$d_y = \frac{P \times d_w}{P + G - F}, \text{ g/cm}^3,$$

where: P – means the weight of the absolutely dry ground burnet roots, g;

G – means the weight of pycnometer with water, g;

F – means the weight of pycnometer with water and the medical herbal raw material, g;

d_w – means the specific weight of water, g/cm3 (d_w = 0.9982 g/cm^3).

Specification of the bulk weight (d_b). We have filled the measuring cylinder with the ground burnet roots, slightly shaken and determined the volume taken by them. The bulk weight has been calculated under the formula:

$$d_b = \frac{P_b}{V_b}, \text{ g/cm}^3,$$

where: P_b – means the weight of the ground burnet roots under the natural or prescribed humidity, g;

V_b – means the volume taken by medical herbal raw material, cm^3.

Specification of the volumetric weight (d_0) has been carried out under the formula as the ratio of the non-ground burnet roots to their full volume (the volume that includes the pores filled with air, cracks and capillaries):

$$d_0 = \frac{P_0}{V_0}, \text{ g/cm}^3,$$

where: P_0 – means the weight of the non-ground medical herbal raw material under the natural humidity, g;

V_0 – means the volume taken by the medical herbal raw material, cm^3.

Specification of the raw material's porosity (P_m). We have calculated this parameter as the ratio of difference between the specific and volumetric weights to the specific weight:

$$P_m = \frac{d_y - d_0}{d_y},$$

where: d_y – means the burnet roots specific weight, g/cm^3;

d_0 – means the burnet roots volumetric weight, g/cm^3.

Specification of the layer's porousness (P_l). We have determined the ratio of the difference between the weight of the volumetric and bulk weight to the volumetric weight:

$$P_l = \frac{d_0 - d_н}{d_0},$$

where: d_0 – means the burnet roots volumetric weight, g/cm^3;

$d_н$ – means the burnet roots bulk weight, g/cm^3.

Calculation of the layer's free volume (V_v). This parameter has been determined as the ratio of the difference between the specific and bulk weight to the specific weight:

$$V_v = \frac{d_y - d_н}{d_н},$$

where: d_y – means the burnet roots specific weight, g/cm^3;

$d_н$ – means the burnet roots bulk weight, g/cm^3.

Extraction solvent's absorption ratio has been calculated as the difference between the volumes of the filled extraction solvent and drained off extraction solvent following absorption of burnet roots to the weight of the ground burnet roots (X, ml/g):

$$X = \frac{V - V_1}{P},$$

where: V – means the volume of the filled extraction solvent, ml;

V_1 – means the volume of the drained off extraction solvent following absorption of burnet roots, ml;

P – means the weight of the ground burnet roots, g.

Thermogravimetric analysis [2] has been conducted on derivatograph Q-1500 D of Paulic, Paulic-Erday system with the platinum-iridium thermocouple. The sample has been heated in silica crucible; the heating rate is 5 °C/min. The thermochemical transformation of burnet roots have been studied in the temperature range from 24 °C to 500 °C in the air, using Al_2O_3 calcined powder as a standard. The broach speed is 2 mm/min. We have recorded the temperature and weight change curves, differentiated heat effects and weight change curves.

Statistical processing of the research results. The results have been statistically processed subject to the pharmacopoeial requirements [2].

Results and discussion. The primary researches, while development of the technology for obtaining burnet roots extract dry, include surveying the derivative of the herbal raw materials: burnet roots. One of the predominating factors affecting the process of extraction of the biologically active substances is the degree of grinding of the raw material, which has been examined through the analytical sieving, determining the weighted average particle size. The results of survey of the fractional composition of burnet roots are shown in the Table 1.

Table 1

Fractional content of burnet roots

Fraction, (mm)	Amount, g	Content, %
>5	60.40	12.08
3-4	137.39	27.48
1-3	240.49	48.10
<1	61.72	12.34

The major aim of grinding the medicinal herbal raw materials is to damage the structures of the particles of the herbal material in order to increase the total contact area with the extraction solvent in the course of extraction. Following the destruction of the structure, a part of the cells opens, the extraction solvent fills the intercellular pores, vacuoles, air cavities, washing out the cells' contents, in particular a set of groups of the biologically active substances.

The optimum degree of grinding of raw materials, which has been determined through examination of dependence of content of the extractives' amount on the degree of the burnet roots' grinding, is no less significant. The obtained data are shown in the Table 2.

According to the Table 2, the highest content of extractive substances is seen in the fractions with the particle size of 1-3 mm, thereby correlates with the data of scientists as for grinding to the specified size of the underground parts of the medicinal herbs and use to obtain the extractive substances pursuant to the selected method (remaceration, percolation, re-percolation, etc.) of the aforesaid faction. Therefore, for further researches we have used burnet roots ground to a specified size.

Table 2

Effect of the burnet roots' grinding degree to the output of extractive substances

Size of the burnet roots' particles, mm	Content of extractive substances, %
>5	49.05±0.04
3-5	51.50±0.03
1-3	71.49±0.04
<1	54.58±0.05

In order to intensify the technological process of obtaining the extracts, the pharmacological parameters of the medical herbal raw material have to be taken into account, which thing has been surveyed. The results of the survey are shown in the Table 3.

Table 3

Characteristics of the pharmaco-technological parameters of burnet roots

Parameter under survey	Measuring unit	Value
Weight loss on drying	%	6.5083 ± 0.5281
Specific weight	g/cm^3	1.4119 ± 0.0875
Bulk weight	g/cm^3	0.3811 ± 0.0055
Volumetric weight	g/cm^3	0.7795 ± 0.0387
Porosity	–	0.4479
Porousness	–	0.5111
Free volume of the layer	–	0.7301

Note. n = 5, P = 95 %

Following the specification of the weight loss on drying of burnet roots, we have found that the humidity figures for the medical herbal raw material under examination are 6.5083 ± 0.5281%. The obtained figures of the technological parameters of burnet roots: specific weight (1.4119 ± 0.0875 g/cm^3), bulk weight (0.3811 ± 0.0055 g/cm^3) and volumetric weight (0.7795 ± 0.0387 g/cm^3). Based on the aforesaid findings, we have calculated other technological parameters of the herbal item: porosity (0.4479), porousness (0.5111) and free volume of the layer (0.7301).

The obtained values of the technological parameters of the raw material will be taken into account when substantiating the technology of extraction from the burnet roots in order to optimize the process of extraction of the biologically active substances from the medicinal herbal raw materials at the stage of extraction.

While developing the technology to obtain the extract, another factor that requires experimental determination is the absorption ratio of the solvent extraction. The above parameter promotes adjustment of the total volume of the extraction solvent, since it is mandatory to take into account the losses of the extraction solvent

remaining in the herbal raw material after the extraction has been drained off.

The Table 4 shows the survey results in respect of the absorption ratio 50% of ethanol by burnet roots, as the above-mentioned extraction solvent has been chosen as the optimal one, based on our researches on specification of major groups of biologically active substances and subject to the microbiological screening.

It is established that the average value of the absorption ratio 50% of ethanol by burnet roots after 2 hours is 1.5; after 4 hours it is 1.9; after 6 hours it is 2.3; after 8 and 10 hours it makes 2.8 and 2.9, accordingly. It is emphasized that 8 and 10 hours after the values of the extraction solvent's absorption ratio are not significantly different.

Substantiation of all the stages of the technological process promotes optimization of the final product output, in particular the dry extract, and among the stages of the production process thereof: thickening of the extract and drying thereof. At the above stages, the intermediate product (liquid and thick extract) is subjected to the temperature effects that can adversely affect the quality, invoke changes in the pharmacological properties of the final product, which fact justifies surveying the thermal conduct of the herbal item under examination.

Table 4

Results of specification of the absorption ratio of the extraction solvent by burnet roots

Test No.	Infusion time, hours	Volume of extraction solvent, ml		Extraction solvent absorption ration
		filled	drained off	
1	2	25.0	23.7	1.3
	4		23.1	1.9
	6		22.8	2.2
	8		22.3	2.7
	10		22.1	2.9
2	2	25.0	23.4	1.6
	4		23.1	1.9
	6		22.6	2.4
	8		22.1	2.9
	10		22.0	3.0
3	2	25.0	23.5	1.5
	4		23.2	1.8
	6		22.7	2.3
	8		22.2	2.8
	10		22.1	2.9

Note. sample weight of burnet roots is 1.0 g

Subject to the analysis of findings on the recorded changes of weight of the ground roots' powder (Fig. 1), depending on the temperature conditions, one can single out the following three stages of destruction: at the first stage with the temperature up to 140 °C the loss of weight is 8% from the sample weight with the maximum velocity at t=95 °C; at the second stage with the temperature range between 140 °C and 220 °C the loss of weight is 7.5% with the maximum velocity at t=205 °C; at the third stage with the temperature range between 220 and 380 °C the weight loss of 31% has been observed with the maximum velocity at t=295 °C.

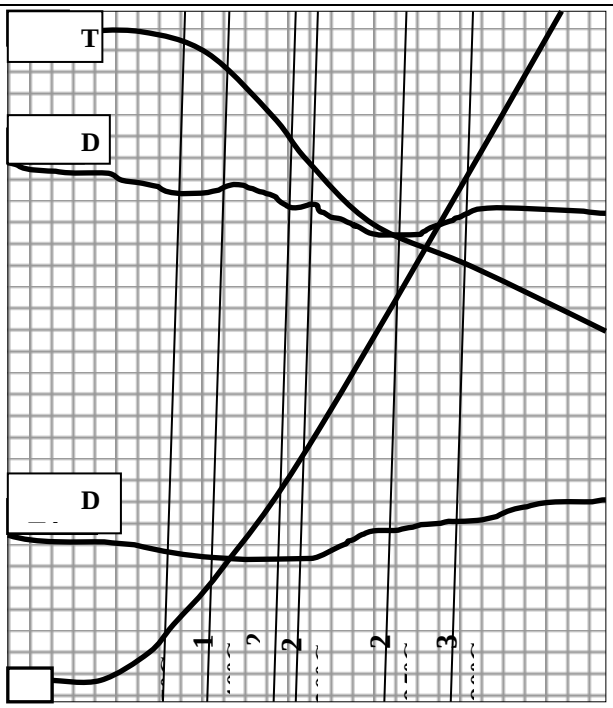

Fig. 1. Derivative chart of burnet roots

All the three stages of the weight change in the burnet roots sample under examination have been accompanied by a low grade endothermic reaction, which fact is related with evaporation as evidenced by the endothermic maxima on the heat effects change curve.

Examination of the thermal conduct of burnet roots through use of the thermogravimetric method and confirmed absence of signs of degradation of the surveyed herbal raw materials in the temperature range from 24 °C to 95 °C make it possible to obtain a dry extract from burnet roots. The critical temperature, exceeding which there occurs a commencement of destruction of the substances in the raw material, is higher than the temperature conditions at the stages of condensation of the liquid extract and drying of the extract, which causes elimination of the adverse effect of the temperature factor onto quality of the burnet roots extract dry.

Conclusions. Expediency of use of the burnet roots fraction, which is ground down to 1-3 mm, as the initial medicinal herbal raw material is determined. The pharmaco-technological parameters of burnet roots are characterized in order to optimize the process of obtaining the liquid extract as an intermediate product at the stage of the raw material extraction.

Practicality of obtaining the burnet roots extract dry subject to the results of thermogravimetric analysis is confirmed, whereupon the absence of signs of destruction of herbal components in the temperature range as provided by the technological process is found.

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DETERMINATION OF OPTIMUM CONCENTRATION OF CEFAZOLIN IN THE OINTMENT FOR THE TREATMENT OF WOUNDS

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Abstract

The development of new drugs for the treatment of purulent wounds in the face of increasing resistance of microorganisms is an urgent problem of medicine and pharmacy.

Keywords: wound, ointment, cefazolin, decamethoxin**,** antimicrobial activity.

According to the authors, in topical medicines, antibiotics and antiseptics are used to treat phase I of the wound process [2, 6, 8, 9]. The combination of several antiseptics in one wound is not used [5, 7, 9]. Their use is stopped with the first signs of wound healing.

We have developed a composition in the form of an ointment for the treatment of phase I of the wound process with cefazolin and decamethoxin. As a basis, we have chosen an emulsion base of the first kind using surfactants. To obtain a stable emulsion, we used a mixture of emulsifiers of the first (25 - 30%) and second kind (70 - 75%). The amount of dispersion medium (purified water) in the composition of the emulsion system of the oil / water type (1st genus) is 55 - 65%, of the dispersed (oil) phase is 20 - 30%.

The purpose of this study was to study the optimal concentration of cefazolin by the in vitro method in the composition of the developed ointment.

Research methods. The study of the optimal concentration of cefazolin was carried out by the method of diffusion into agar on a thick nutrient medium by comparing the size of the growth inhibition zones of the test microorganisms [1].

The following were used as nutrient media: liquid soya-casein medium ("MERCK", Germany); soybean casein agar ("Viomerieux", France); Saburo agar - 4% with glucose ("MERCK", Germany); Antibiotic Agar No. 1 ("MERCK", Germany); nutrient agar (Experimental Medicinal Plant, Ukraine); buffer sodium chloride-peptone pH 7.0 (Experimental Medicinal Plant, Ukraine).

Nutrient media were tested for sterility and growth properties. The work used a sterile nutrient medium that had corresponding growth properties. The nutrient medium was poured into Petri dishes. Thick nutrient medium was prepared according to the manufacturer's instructions. An important point in determining the antimicrobial activity of the drug is the thickness of the agar layer in the Cup. It must be 4.0 mm ± 0.5, which is achieved by introducing strictly 20 ml of agar into a Petri dish with a diameter of 90 mm, 25 ml of agar with a diameter of 100 mm, and 60 ml of agar with a diameter of 150 mm. Petri dishes were filled with nutrient medium by means of a rotating table, through which the nutrient medium was evenly distributed in the Petri dish, and mounted on a strictly horizontal surface (exposed in level, without hollows and bulges). Compliance with these requirements is necessary because the size and shape of the growth inhibition zone depend on the depth and uniformity of the agar layer.

After filling the cup was left at room temperature to solidify the agar.

As test cultures used museum strains of fungi: Candida utilis LIA 01; Candida albicans ATSS 885-653, Candida albicans ATSS 10231 and bacteria: Staphylococus aureus ATCC 6538 (Table 1).

Table 1

Testing the properties of test cultures

Name	Collection	Properties			
		morphological	cultural	tinctorial	Biochemical
Staphylococcus aureus	ATCC 6538	respond	respond	respond	respond
Candida albicans	ATCC 885-653	respond	respond	respond	respond
Candida albicans	ATCC 10231	respond	respond	respond	respond

For testing, working cultures of test microorganisms were prepared according to the requirements of the State Pharmacopoeia of Ukraine [4]. The bacterial test strains were grown on a thick nutrient medium No. 1 at 35 ° C for 18 to 20 hours on soybean casein agar (Staphylococus). Test strains of mushrooms (Candida) were grown at a temperature of 20 - 25 0C for 24 - 48 hours on Saburo agar - 4% with glucose.

Preparation of inoculum. Suspensions of microorganisms were prepared and their optical density was determined at 550 nm using a Densimat