

## BURNET ROOTS EXTRACT DRY: STUDY OF PHARMACO-TECHNOLOGICAL AND ANTIBACTERIAL PROPERTIES

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### Introduction.

Currently, the reference is made to an increase in number of gastroenterological patients; the list of the gastrointestinal tract common diseases includes gastritis and duodenitis, stomach and duodenum ulcers, non-infectious enteritis and colitis, which mean a prerequisite for expediency of pharmaceutical development of new drug products, in particular the herbal drugs for therapy of the aforesaid pathologies [1, 2].

The herbal drug products, where the biologically active substances provide a polyvalent pharmacological effect, hold a specific place in the set of therapeutic and preventive measures in gastroenterology: they are efficient and safe, can be used for a long time, have a mild effect on the underlying disease and a positive effect on the comorbidity [3, 4, 5].

Based on our own researches, we emphasize that despite the offering list of the herbal drug products in sundry dosage forms at the domestic pharmaceutical market, both by domestic and foreign manufacturers, which drug products can be used in treatment of the digestive system various diseases, there is a limited range of the drugs, which are based on the substances extracted from the medicinal herbal raw materials [6]. At the same time, a limited number of extracts and drugs, where the extracts are included, is proposed for use in the gastroenterological practice; and the spectrum of pharmacological activity of the current extracts prevents the pharmaceutical development of the drugs on their basis from been seen as expedient [1].

In previous works on search and selection of promising herbal sources for further studying, development of such medicinal herb as Greater Burnet has been proven as justified. It should also be emphasized that the most traditional medicine prescriptions that include Burnet rhizomes and roots are recommended for treatment of the gastrointestinal tract diseases: stomach and duodenum ulcers, enteritis, acute and chronic colitis [7].

We have obtained the dry extract from Burnet roots under the technological scheme, which covers the stages of obtainment of the primary extract, its purification, thickening, drying and grinding. As the extract obtained is intended to be used for creation of solid drug products for gastroenterological practice, examination of its technological parameters is justified. The results of such examination are taken into account in determining the auxiliary substances while creating the dosage forms, substantiation of the technology for obtainment of the drug product [8, 9].

The scientific sources portray that the subterranean organs of Greater Burnet exert the

bactericidal effect against intestinal microorganisms (*E. coli*), dysentery and paratyphoid group [1]. Microbiological examinations of the tincture from Burnet rhizomes and roots as conducted while developing the composition of the dental herbal drug with the aim to study the effect of its individual components on the spectrum of antimicrobial action have proven the antifungal activity of the tincture from Burnet rhizomes and roots against 10 archival and clinical strains of *Candida* fungi [10]. It is the basis for microbiological screening as to determine the antibacterial effect of the obtained Burnet extract, which is desirable in the spectrum of pharmacological effect of herbal drugs for treatment of gastroenterological diseases, being the purpose of this paper.

### Materials and methods

The study object is Burnet roots extract dry (BRED), being the dark brown powder with a specific odour and a bitter taste.

The shape, size of the particles of the dry extract, description of their surface have been determined by optical microscopy using the fluorescent microscope "Lumam R-1" under the methods of the State Pharmacopoeia of Ukraine [11]. The photos have been taken and processed through use of the software: Scope Photo (version 3.0.12.498).

Loss on drying of Burnet extract has been calculated under the methods as set out in the State Pharmacopoeia of Ukraine [11]. Moisture absorption of the extract has been determined under the weight method by tracking the change in the batch kept in the desiccator at the relative humidity of 75% and 100%. Examinations to determine the pharmaco-technological properties (fluidity, bulk and tapped density, natural slope angle, compressibility parameter and Gausner coefficient) have been carried out under the methods of the State Pharmacopoeia of Ukraine [11].

The antibacterial properties of BRED have been examined through the method of diffusion in agar in the "wells" modification at the facilities of the Biochemistry and Biotechnology Laboratory of the State Institution "Mechnikov Institute of Microbiology and Immunology of NAMS of Ukraine". The following reference testing cultures of gram-positive and gram-negative bacteria belonging to different taxonomic groups have been used for the study: *P. vulgaris* ATCC 4636, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922. The antifungal effect of the extract has been determined on the reference strain *C. albicans* ATCC 885-653. The optical density of the microbial suspension of the said microorganisms has been in line with the scale McFarland 0.5 units according to the Order No. 167 of the Ministry of Health Care of Ukraine dated 05.04.2007.

The drug product "Chlorophyllipt", alcohol solution 10 mg/ml ("Galychfarm" Joint Stock Company, Lviv, Ukraine; "Kyivmedpreparat" Joint Stock Company, Kyiv, Ukraine) has been used as the comparative drug.

The statistical processing of the obtained results has been carried out in Excel program.

### Results and discussion

According to the microscopic analysis, it has been observed that the BRED particles are the plates of anisodiametric shape, having the size in the range of 1-75 microns and that their surface is rough and glossy. The shape of the particles and the size thereof, along with dispersion and moisture content, affect one of the basic

technological characteristics: fluidity [12]. The anisodiametric shape of the particles as specified makes it possible to predict the reduced fluidity and the necessity for its correction while selecting auxiliary substances for powder masses.

The results of crystallographic examination of BRED are given in Fig. 1.

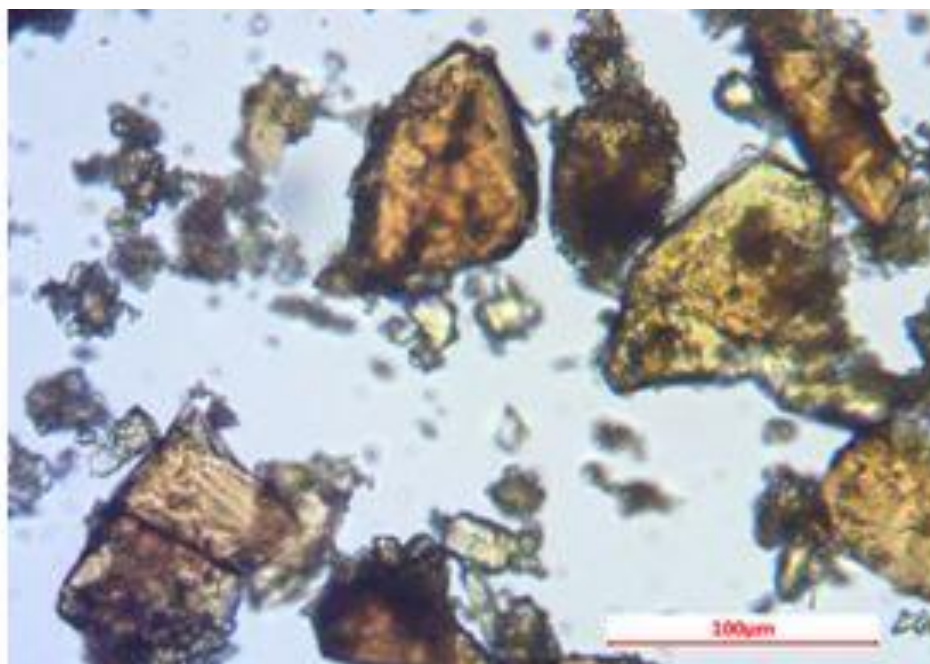


Fig. 1. Microphotography of Burnet roots extract dry

The requirements of the general article of the State Pharmacopoeia of Ukraine "Extracts" for dry extracts (except certain monographs) regulate that the loss on drying or the water content in the dry extracts shall not exceed 5% (*m/m*) [11]. The obtained figures of the loss on drying for Burnet extract have been in line with the requirements and made  $3.77 \pm 0.10$  %.

While developing the solid dosage forms based on dry extracts a special attention is paid, among the

parameters affecting the technological characteristics in the process of obtainment of the drug product and its quality, to the moisture absorbing properties of herbal extracts, because most of them are hygroscopic objects [12]. The results of examination to specify the moisture absorption of BRED subject to the relative air humidity 75 % and 100 % are shown in Fig. 2.

Moisture absorption, %

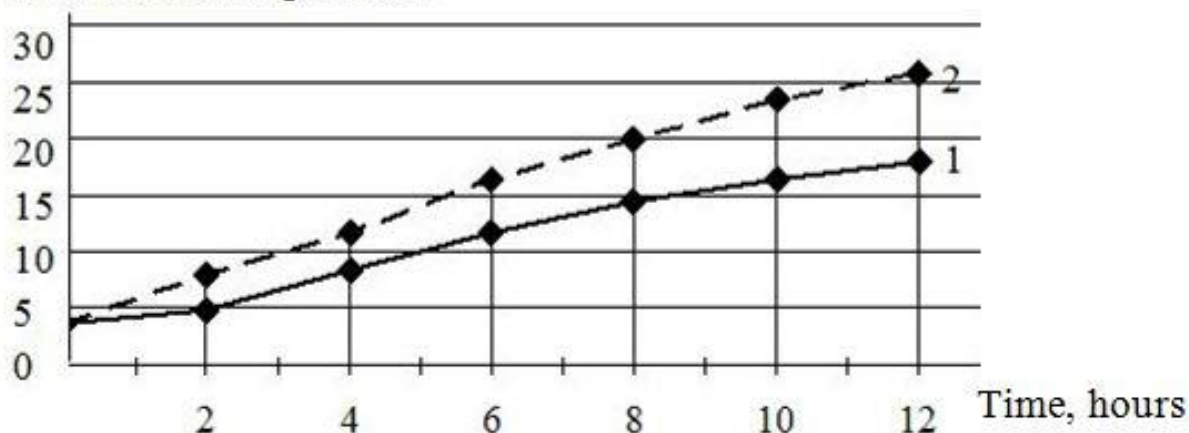


Fig. 2. Moisture absorption of Burnet roots extract dry subject to the relative air humidity: 1 – 75%, 2 – 100%

It is emphasized that subject to the relative air humidity of 75 % the mass of the Burnet extract samples

increases for 1.04% after 2 hours, for 14.15% after 12 hours, for 20.98 % after 24 hours. Subject to keeping the samples under the relative air humidity of 100%, the increase of the samples' mass after 2 hours after commencement of the experiment is 4.17%, after 12 hours: 21.89%, after a day: 29.98%.

Therefore, the results of the study confirm hygroscopicity of the above extract. It can adversely affect fluidity of the powder masses while production of solid drug products, their stability during storage and it speaks for the reason of selection, as constituent masses for tabletizing, of the auxiliary substances with the moisture-absorbing properties.

The experimental data on study of the technological characteristics of BRED are included into Table 1.

Based on the results of examination (according to Table 1), we emphasize that determination of such technological parameter of Burnet extract as fluidity is possible, provided that only the funnel method with the shaker is used, which fact is relevant to the anticipated data of the substance crystallographic examination. The necessity to improve the above technological parameter upon development of the dosage form is pointed out.

**Table 1. Pharmaceutical and technological properties of Burnet roots extract dry**

Parameter under examination	Measuring unit	Value
Fluidity (fixed funnel method)	c/100 g	eternally
Fluidity (funnel method with the shaker)	c/100 g	9.56±0.28
Bulk density	g/ml	0.760±0.006
Tapped density	g/ml	0.834±0.005
Bulk volume before shrinkage ( $V_0$ )	g/ml	92.20±1.16
Bulk volume after shrinkage ( $V_{10}$ )	g/ml	85.60±1.33
Bulk volume after shrinkage ( $V_{500}$ )	g/ml	84.20±1.50
Bulk volume after shrinkage ( $V_{1250}$ )	g/ml	83.70±1.50
Natural slope angle	degree	34.20±0.58
Compressibility degree	%	9.24±0.53
Gausner coefficient	-	1.10±0.01

Note. n = 5, P = 95 %

The powder natural slope angle is another characterizing parameter of its fluidity as it is connected with the interparticle friction or movement resistance between particles. The natural slope angle of Burnet is 34.20±0.58 degrees and is within the satisfactory range acceptable for production.

The volumetric properties of Burnet extract are characterized by such parameters as bulk density (0.760±0.006 g/ml) and tapped density (0.834±0.005 g/ml), the values of which are close.

The powder's tendency to be pressed can be estimated by the compressibility degree and Gausner coefficient, the calculation of which is based on the obtained values of the bulk volume before shrinkage and final bulk volume after shrinkage (table 2). The values found under the generally accepted fluidity scale testify to a good fluidity of the extract, however they are not peculiar to its properties.

The results of microbiological study as to determine the antimicrobial effect of the Burnet extract are given in Table 2.

**Table 2. Antibacterial effect of Burnet roots extract dry**

Items under examination	Growth retardation area diameters, mm					
	<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922	<i>P. vulgaris</i> ATCC 4636	<i>B. subtilis</i> ATCC 6633	<i>P. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 653- 885
Burnet extract	24.17± 0.31	21.33± 0.33	17.33± 0.22	24.33± 0.33	16.50± 0.22	15.50± 0.22
Chlorophyllipt	22.17± 0.31	16.83± 0.17	–	16.50± 0.22	–	12.83± 0.31

Note. "–" means absence of growth retardation areas of the testing strains of microorganisms

According to the microbiological screening data, sensitivity of all the testing strains to the Burnet extract under examination has been noted. The largest diameters of growth retardation areas have been observed for *S. aureus* (24.17 ± 0.31) and *B. subtilis* (24.33±0.33). The obtained extract shows antimicrobial properties against *P.*

*vulgaris* (17.33±0.22) and *P. aeruginosa* (16.50±0.22) in contrast to the comparative drug product "Chlorophyllipt", the antimicrobial effect of which to other testing strains is slightly inferior to the examined extract.

## Conclusions

The comprehensive research on study of pharmaceutical and technological properties of BRED, whereupon creation of the solid dosage forms is anticipated, is performed.

The expediency of introduction, as constituents into masses for tabletizing, of auxiliary substances with the moisture absorbing properties due to the hygroscopicity of the extract and the necessity to improve such technological parameter as fluidity by virtue of processing the modern auxiliary substances are determined.

The antibacterial properties of Burnet extract in respect to the testing strains of microorganisms are determined. Further microbiological studies as to determine the possible anti-helicobacter effect of BRED and to develop the composition of tablets based on BRED for gastroenterology are scheduled.

## Abstract

### BURNET ROOTS EXTRACT DRY: STUDY OF PHARMACO-TECHNOLOGICAL AND ANTIBACTERIAL PROPERTIES

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**Introduction.** The basis for timely development of new drug products, in particular the herbal ones, for therapy of the digestive system diseases is an increase in the total number of gastroenterological patients. The surveys of the domestic pharmaceutical market as for the offer of the herbal drug products used for treatment of various diseases of the digestive system have found a limited range of the drugs based on the substances obtained from medicinal herbs and expediency of search for promising herbal sources. Based on the experience of the traditional medicine, Greater Burnet, the herbal raw material of which is used for production of the extract dry, has been selected for further examination. Whereas Burnet roots extract dry is anticipated to underlie the production of the solid dosage forms, examination of its technological properties is justified. The scientific sources portray that the subterranean organs of Greater Burnet exert the bactericidal effect against microorganisms of intestinal, dysentery and paratyphoid groups, as well as antifungal activity of the tincture of Burnet against 10 archival and clinical strains of *Candida* fungi, which is a reason for microbiological screening of the obtained extract. **The object of this paper** is to study the technological parameters of Burnet roots extract dry and to specify its antibacterial effect. **Materials and methods.** The study object is Burnet roots extract dry. The following methods are used: optical microscopy method for specification of crystallographic characteristics, weight method upon surveying of the extract's moisture absorption. The pharmaco-technological properties (fluidity, bulk density, tapped density, natural slope angle) have been studied; compressibility parameters and Gausner coefficient have been calculated under the methods of the State Pharmacopoeia of Ukraine. The antibacterial properties have been examined at the facilities of the Biochemistry and Biotechnology Laboratory of the State Institution "Mechnikov Institute of Microbiology and Immunology

of NAMS of Ukraine" through the method of diffusion in agar in the "wells" modification on the reference testing cultures of microorganisms: *P. vulgaris* ATCC 4636, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *C. albicans* ATCC 885-653 with the use of the drug product "Chlorophyllipt", alcohol solution 10 mg/ml ("Galychfarm" JSC, "Kyivmedpreparat" JSC, Ukraine) as the comparative drug. The optical density of the microbial suspension of the said microorganisms has been in line with the scale McFarland 0.5 units. The obtained data have been processed statistically in Excel program.

**Results & discussion.** The found anisodiametric shape of the particles of Burnet extract can anticipate a reduction of fluidity. The values of the extract's loss on drying are  $3.77 \pm 0.10$  %. The results of determining the moisture absorption of the extract samples under the conditions of relative air humidity of 75% and 100% prove its hygroscopicity, which, upon creation of a solid dosage form, requires adjustment through processing of the modern auxiliary substances with the moisture-absorbing properties. The technological parameters of Burnet roots extract dry are characterized; reasonableness of selection of the modern auxiliary substances with the aim to improve such technological parameter as fluidity is proven in the paper. The natural slope angle makes  $34.20 \pm 0.58$  degrees, being within the satisfactory range acceptance for the production. The values of Gausner coefficient and compressibility degree show a good fluidity of the extract, however they are not peculiar to its properties. Sensitivity of all the examined testing strains of microorganisms to Burnet extract, including *P. vulgaris* and *P. aeruginosa*, is proven; at the same time the comparative drug: "Chlorophyllipt" has shown no antimicrobial effect against them. **Conclusions.** The pharmaceutical and technological properties of Burnet roots extract dry have been examined; subject to the results our emphasis is given to a thorough approach for selection of auxiliary substances in development of the tablet composition based on it. The antibacterial effect of Burnet extract in respect to the testing strains of microorganisms is determined and microbiological researches as for specification of a potential antihelicobacter effect are scheduled.

**Key words:** Greater Burnet, extract dry, pharmaco-technological properties, antibacterial properties, gastroenterology

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