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DEVELOPMENT OF THE COMPOSITION OF TABLETS WITH THE EXTRACT OF BIRD CHERRY AND THEIR STANDARDISATION

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Key words: tablets; bird cherry fruit extract; technological parameters; anthocyanins; tannins; phenolic compounds; standardisation

The composition of tablets with the bird cherry dense extract under the conditional name "Fructopad" has been developed, and the technological properties of the granulate of the tabletting mass, resistance of tablets to crushing and their friability have been determined. Based on modern approaches to standardisation of herbal medicines the methods for identification and quantitative determination of BAS in tablets with the bird cherry dense extract have been developed. The presence of anthocyanins has been chosen as an identification criterion of quality of the tablets developed when detecting them by TLC. It has been confirmed that the content of tannins calculated with reference to pyrogallol and phenolic compounds not less than (%) 1 and 3.5, respectively, should be chosen as a quantitative criterion.

Periodontal diseases, which have inflammation in their basis, can be treated by different local medications such as antiseptics, anti-inflammatory agents, and among them herbal products occupy an important place. They are frequently used to treat gingivitis, symptomatic gingivitis and localized periodontitis. The examples of such herbal products are kalanchoe juice and ointment independently and in combination with St. John's wort or calendula infusion; green tea (one of its components is catechin that reduces capillary fragility of gums and increases absorption of ascorbic acid) and others. Herbal extracts containing arnica, calendula and eucalyptus with peach oil are also recommended.

Tablets are the most common among dosage forms. The ease of use, transportation and storage, production efficiency, the possibility of accurate dosing are inherent to them; therefore, it is expedient to create a new medicine for the treatment and prevention of oral diseases in the form of tablets.

Bird cherry (*Padus avium* Mill) of the *Rosaceae* family is widely distributed in Ukraine and grows almost throughout the territory, mainly in the forest zone, in the valleys of rivers, the steppe zone. It also is cultivated due to the pleasant sweet-tart fruits, which are used for confectionery [1, 8, 10]. Bird cherry fruits are used in scientific medicine as astringent, anti-inflammatory, phytoncid medicine [9]. The monograph on bird cherry fruits was included in the Pharmacopoeia of the USSR of the 11-th edition, as well as a project of the monograph for this raw material was prepared for the State Pharmacopoeia of Ukraine [2, 11].

Phenolic compounds such as anthocyanins (cyanidine glycosides), flavonoids (rutin, quercetin) hydroxycinnamic acids, and polysaccharides are among biologically active substances of the bird cherry extract. These substances have different effects: they promote regeneration of the epithelium, strengthen of the capillary walls, as well as they have anti-inflammatory action [8, 10, 11].

The dense extract from bird cherry fruits has been prepared, and it has been found that it has the anti-inflammatory, membrane-stabilizing, antimicrobial activity and low toxicity. Therefore, development of a new drug from the bird cherry extract is timely to expand the range of oromucosal medicated products with the anti-inflammatory and vitamin action.

The aim of our research was to develop tablets with the bird cherry dense extract, and to study the technological properties of the granulate of the tabletting mass and parameters of standardisation of the tablets created.

Materials and Methods

The subjects of our study were the granulate of the tabletting mass and tablets with the bird cherry dense extract. Fluidity was determined on an automatic apparatus. To measure fluidity 30-100 g of the granulate was weighed with the accuracy of 0.5% and placed without compaction in a container with the closed opening. The outflow opening was opened, and the time of the sample full outflow was recorded. The result was calculated as the time required for passing 100 g of granules through the container nozzle or the quantity of granules passing through the opening per 1 sec.

The bulk volume (volume before tapping) is the amount of 100.0 g of granules introduced without compacting. It was determined according to the ShPU 2.0 [7].

The volume and density of granules before and after tapping during mechanical tapping were identified on a Pharma Test apparatus. The difference between the bulk volume of the bulk material and the volume after tapping shows the ability of the sample to tapping. In a dry cylinder 100.0 g of the test sample was placed without compacting. Measurements were carried out on the apparatus at 10, 500, 1250 taps of the cylinder determining

Table 1

The tabletting mass composition

Components	Per one tablet, g
Bird cherry dense extract	0.050
Lactose	0.295
Microcrystalline cellulose	0.130
Polyvinyl pyrrolidone	0.035
Aerosil	0.015
Magnesium stearate	0.005
Total	0.53

Table 2

The results of determining the technological properties of the powdered granulate (tabletting mass)

Parameter	Value
Fluidity	14.41 s/100 g of granules (6.9 g/s)
Angle of repose	25°
Bulk volume	254 ml
Settled volume	V_{10}=240 ml V_{500}=223 ml V_{1250}=218 ml
Bulk density	0.39 g/ml
Compressibility index (Carr index)	14.29
Hausner ratio	1.17

the volumes (V_{10}, V_{500}, V_{1250}) with the accuracy to the nearest point. The ability of granules to tapping was determined as the difference between V_{10} and V_{500}.

Identification of the dense extract in tablets was performed by TLC; the content of phenolic compounds was studied by spectrophotometry according to the ShPU method [4, 5].

Results and Discussion

When developing tablets with bird cherry extract it was necessary to choose such excipients, which use could improve the properties of the tabletting mass, its fluidity, compressability, permit to use the wet granulation method, and provide obtaining the high-quality tablets by all indicators. At the stage of developing a solid dosage form one of the most important elements was the choice of excipients, namely disintegrants, diluents, binders, etc. The appropriate granulometric composition allowed providing accuracy of dosing and uniformity of the weight at the stage of tabletting.

Several tablet formulations with the dense bird cherry extract were created with the list of identical excipients, but with different ratios. The optimal tabletting mass composition is given in Tab. 1.

The basic technological properties of the tabletting mass were studied according to the SPhU as scientific and practical base for developing the technology of tablets with the bird cherry extract.

These properties are interrelated and affect the process of compressing and obtaining of quality tablets in some way. Fluidity, the angle of repose, the bulk volume, the bulk density, settling qualities, the tapped density, the compressibility index or the Carr index and the Hausner ratio were identified [7]. The research results of fluidity are given in Tab. 2.

The speed control of the material flow through the nozzle is considered one of the best methods for measuring the powder and granule flowability. Another indicator of fluidity is the angle of repose between a cone generator of a bulk sample and a horizontal plane. The lower this indicator is, the better is fluidity. Its value is in the range of 25 to 30°C for powders with a good looseness. The result of determination of the angle of repose for our tabletting mass was 25°C, which allowed referring it preliminary to the products with a good looseness. Fluidity can be assessed by the powder compressibility index or the Carr index and the Hausner ratio characterizing the interaction between particles that affect the bulk properties of the powder, and also affect the flowability of the material. It was determined that the bulk volume of the granulate was 254 ml. Dosing of tabletting masses in tablet machines are made by volume. Therefore, it is important to determine the bulk density, which predetermines the choice of a press tool. The bulk density was 0.39 g/ml (Tab. 2).

According to our data the Carr index (C) and the Hausner ratio (HR) were calculated:

$$C = \frac{V_0 - V_{1250}}{V_0} \cdot 100; \quad HR = \frac{V_0}{V_{1250}} .$$

It was determined by the scale of fluidity that the Carr index of the tabletting mass studied was in the range of 11-15, and the Hausner ratio was within 1.12-1.18 characterizing fluidity as good.

An important indicator for quality of a tabletting mass is moisture. Prior to compression tabletting granulates should have the optimal residual moisture that is individual for each tabletting material. Such tablet characteristics as mechanical strength, disintegration and uniformity of dosing depend on the granulate moisture [7]. The moisture of the powdered granulate for tablets was 1.81±0.05%.

To obtain tablets the weighed quantity of the powdered granulate was measured on hand scales, granules were compressed with antifriction excipients using a NTM-01EF table tablet machine. The tabletting mass studied was plastic and easily compressed. Tablets had good edges and the right shape, a slightly shiny surface, and a light pink colour. The tablet obtained was weighed on electronic scales; the height was measured by a micrometer. The average tablet weight was 0.53 g.

Tablets with the dense bird cherry extract were called "Fructopad". They refer to tablets for use in the buccal cavity "Oromucosal preparations", ShPU 2.0, p. 1104, Praeparationes buccales. Their physical hardness – resistance of tablets to crushing and friability was measured.

The resistance to crushing was determined according to the SPhU, the 2nd ed., (2.9.8.) [7] by the device for determination the geometric dimensions and hardness

of tablets manufactured by Pharma test of the PTF E/ER type, and it was 118±3 N. The tablet diameter was 12±0.01 mm, and the height was 3.5±0.08 mm.

The friability of tablets or damage under mechanical shock was determined according to the ShPU 2.0 (2.9.7) [7] by the device of the PTF E/ER type. According to the requirements of the ShPU the friability of uncoated tablets should be not more than 1%. The tablets friability was 0.1% (n = 5) according to our study. Analyzing the results obtained it should be noted that the "Fructopad" tablets under study meet the requirements of the SPhU by all criteria, except for resistance of tablets to crushing, which is more than 100 N. However, taking into account that slowly releasing the active substances of the bird cherry dense extract these tablets must act in the buccal cavity, and it should not consider as a disadvantage, but on the contrary, allows to longer maintain a contact of the extract active substances, such as flavonoids, anthocyanins, hydroxycinnamic acids, polysaccharides, providing the anti-inflammatory, reparative, hemostatic, antioxidant membrane-stabilizing local effect on the surface of the gums.

The bird cherry dense extract, which is part of "Fructopad" tablets, contains a variety of biologically active substances (BAS). Among them there are tannins, flavonoids, anthocyanins, hydroxycinnamic acids, polysaccharides, amino acids and vitamins.

It was proposed to conduct identification of BAS in tablets with the extract by the TLC method. This method is generally accepted in the world. It is effective and easy to conduct, the necessary equipment is relatively inexpensive; therefore, the method is used for identification of the medicinal plant raw material, as well as substances obtained from it and drugs [11].

Anthocyanins were selected for identification of the bird cherry fruit dense extract in "Fructopad" tablets by TLC. They are present in the extract in a great amount and, together with other phenolic compounds have a strong and anti-inflammatory, hemostatic and membrane stabilizing action proven in experiments with animals.

Among the mobile phase proven the optimal one was the mixture of solvents of ethyl acetate – anhydrous acetic acid – formic acid – water in the ratio of 100:10:10:25. The plates with silica gel F_{254} Merck, the size of 20×10 cm, on a glass substrate were used for chromatography.

Two tablets were crushed and 25 mL of 1% solution of hydrochloric acid in 95% ethanol was added and treated with ultrasound for 60 min at 50ºC, then filtered. The filtrate was evaporated to of the half volume.

To prepare the reference solution 1 mg of chrysanthemin (Cyanidin-3-glucoside chloride, > 98%, Chengdu Dioputify Phytochemicals Ltd, batch 15041502) was dissolved in 10 mL of 1% solution of hydrochloric acid in 95% ethanol. The test solutions and the reference solution were placed on the chromatographic plate by 5 mcL each. After the plates were dried in air for 5-10 min, they were placed into the chamber. In 15-20 min the plates were taken out when the chromatogram developed, and allowed the solvent to evaporate at room temperature, then the spots were observed in daylight. Two pink-red colour zones were observed on the chromatograms obtained, one of which coincided for Rf and colour with chrysanthemin, the other was slightly lower.

Top of the plate	
A pink-red zone A pink-red zone	A pink-red zone (chrysanthemin)
The test solution	The reference solution

Fig. The scheme of the chromatogram for identification of BAS in tablets with the bird cherry fruit extract when examining in the daylight.

The results of chromatographic analysis of tablets with the bird cherry fruit extract are presented in Fig.

When studying the absorption spectra of the bird cherry extract alcohol solution it has been found that they meet pyrogallol absorption curves by the course of the curves of light absorption and the position of the maximum of absorption. Therefore, determination of the content of tannins in "Fructopad" tablets was carried out by spectrophotometry according to the SPhU method *(2.8.14)* calculated with reference to pyrogallol, and the content of phenolic compounds was calculated with reference to the absolutely dried raw material [5]. The procedures were as follows.

Tannins. Crush 2 tablets (accurate weight), add 15 ml of water *R* to the powder and extract for 30 min, filter in a 25.0 ml volumetric flask. Wash the filter twice with 2 ml of water *R*, dilute with water to the volume and mix. Place 2 ml of the solution obtained in a volumetric flask, add 1.0 ml of the phosphotungstic reagent *R*, 10.0 ml of water *R* and dilute with 290 g/L sodium carbonate solution to the volume of 25.0 ml. Measure the optical density of the test solution in 30 min according to the ShPU method (2.8.14) at the wavelength of 760 nm (A_1) against water blank on a Hewlett Packard 8453 spectrophotometer. The solution of the standard sample of pyrogallol is used as a reference solution.

Polyphenols. To determine polyphenols that are not adsorbed by the leather powder take 10 ml of the filtrate, add 0.10 g of the leather powder *RS* and shake vigorously for 60 min. Filter the mixture, dilute 5.0 ml of the filtrate with water to the volume of 25.0 ml. Mix 2 ml of this solution with 1.0 ml of the phosphotungstic reagent *R*, 10.0 ml of water *R* and dilute to the volume of 25.0 ml with 290 g/L sodium carbonate solution *R*. Measure the optical density of the solution in 30 min according to the SPhU method (*2.8.14*) at the wavelength of 760 nm (A_2) against water blank.

Pyrogallol standard solution. Immediately before the test dissolve 50.0 mg of pyrogallol *R* in water *R*, dilute with water to the volume of 100 ml. Place 5.0 ml of the solution obtained in a volumetric flask and dilute with water *R* to the volume of 100 ml. Mix 2 ml of this solution with 1.0 ml of the phosphotungstic reagent *R*, 10.0 ml of water *R* and dilute to the volume of 25.0 ml with 290 g/L sodium carbonate solution *R*. Measure the optical density of the solution in 30 min according to the SPhU method (2.8.14) at the wavelength of 760 nm (A3) against water blank.

The content of tannins and polyphenols were calculated by the following formulas.

The formula for calculation of tannins is:

$$X = \frac{62.5 \cdot (A_1 - A_2) \cdot m_2}{A_3 \cdot m_1}.$$

The formula for calculation of polyphenols is:

$$X = \frac{62.5 \cdot (A_1) \cdot m_2}{A_3 \cdot m_1},$$

where: A_1 – is the optical density of the test solution; A_2 – is the optical density of the solution with each powder; A_3 – is the optical density of pyrogallol; m_1 – is the weight of the test solution, g; m_2 – is the weight of pyrogallol, g.

The product satisfies the test if the amount of tannins calculated with reference to pyrogallol is at least 1.0%, and the content of polyphenols that is not adsorbed by the leather powder is at least 3.5%.

CONCLUSIONS

The composition of tablets with the bird cherry dense extract under the conditional name "Fructopad" has been developed, and the technological properties of the granulate of the tabletting mass have been determined.

Based on modern approaches to standardisation of herbal medicines the methods for identification and quantitative determination of BAS in tablets with the bird cherry dense extract have been developed. The presence of anthocyanins has been chosen as an identification criterion of quality of the tablets developed when detecting them by TLC. It has been confirmed that the content of tannins with reference to pyrogallol and phenolic compounds not less than (%) 1 and 3.5, respectively, should be chosen as a quantitative criterion calculated.

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РОЗРОБКА СКЛАДУ ТАБЛЕТОК З ЕКСТРАКТОМ ЧЕРЕМХИ ТА ЇХ СТАНДАРТИЗАЦІЯ

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Ключові слова: *таблетки; екстракт плодів черемхи; технологічні показники; антоціани; таніни; фенольні сполуки; стандартизація*

Розроблено склад таблеток з екстрактом черемхи густим під умовною назвою «Фруктопад», визначені технологічні властивості грануляту таблеткової маси та стійкість до роздавлювання таблеток і їх стираність. На підставі сучасних підходів щодо стандартизації лікарських засобів рослинного походження опрацьовані методики ідентифікації та кількісного визначення БАР у таблетках з екстрактом черемхи густим. Ідентифікаційним критерієм якості розроблених таблеток обрано наявність антоціанів при їх виявленні методом ТШХ. Підтверджено, що кількісним критерієм якості розроблених таблеток слід обрати вміст танінів у перерахунку на пірогалол та фенольних сполук не менше (%) 1 та 3,5 відповідно.

РАЗРАБОТКА СОСТАВА ТАБЛЕТОК С ЭКСТРАКТОМ ЧЕРЕМУХИ И ИХ СТАНДАРТИЗАЦИЯ

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Ключевые слова: *таблетки; экстракт плодов черемухи; технологические показатели; антоцианы; танины; фенольные соединения; стандартизация*

Разработан состав таблеток с экстрактом черемухи густым под условным названием «Фруктопад», определены технологические свойства гранулята таблеточной массы, устойчивость к раздавливанию таблеток и их истираемость. На основании современных подходов к стандартизации лекарственных средств растительного происхождения отработаны методики идентификации и количественного определения БАВ в таблетках с экстрактом черемухи густым. Идентификационным критерием качества разработанных таблеток избрано наличие антоцианов при их выявлении методом ТСХ. Подтверждено, что количественным критерием качества разработанных таблеток следует выбрать содержание танинов в пересчете на пирогаллол и фенольных соединений не менее (%) 1 и 3,5 соответственно.