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THEME OF MASTER WORK:
THE PHARMACOGNOSTIC STUDY OF BRYONIA ALBA ROOTS

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INTRODUCTION

Herbalism ("herbology "or" herbal medicine") is use of plants for medicinal purposes, and the study of such use. Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today. Modern medicine recognizes herbalism as a form of alternative medicine, as the practice of herbalism is not strictly based on evidence gathered using the scientific method [18]. Modern medicine, does, however, make use of many plant-derived compounds as the basis for evidence-tested pharmaceutical drugs, and phytotherapy works to apply modern standards of effectiveness testing to herbs and medicines that are derived from natural sources. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts."

"Archaeological evidence indicates that the use of medicinal plants dates at least to the Paleolithic, approximately 60,000 years ago. Written evidence of herbal remedies dates back over 5,000 years, to the Sumerians, who created lists of plants. A number of ancient cultures wrote on plants and their medical uses. In ancient Egypt, herbs are mentioned in Egyptian medical papyri, depicted in tomb illustrations, or on rare occasions found in medical jars containing trace amounts of herbs [27]. The earliest known Greek herbals were those of Diocles of Carystus, written during the 3rd century B.C, and one by Krateuas from the 1st century B.C. Only a few fragments of these works have survived intact, but from what remains scholars have noted that there is a large amount of overlap with the Egyptian herbals. Seeds likely used for herbalism have been found in the archaeological sites of Bronze Age China dating from the Shang Dynasty [28]. Over a hundred of the 224 drugs mentioned in the Huangdi Neijing, an early Chinese medical text, are herbs. Herbs were also common in the medicine of ancient India, where the principal treatment for diseases was diet [29]. De Materia Medica by Pedanius Dioscorides, a Roman physician, is a particularly important example of such writings. The documentation of herbs and their uses was a central part of both

Western and Eastern medical scholarship through to the 1600s, and these works played an important role in the development of the science of botany.

Since some plants used from the ancient times are still believed to be potent medicines, the aim of the present work is to carry out phytochemical study of bryony – a plant widely used in homeopathy.

To achieve the aim of the work we have set the following Tasks:

- ✓ analyze the literature data about the botanical description, chemical constituents, uses in medicine of Bryony plant material;
- ✓ determine the main technological parameters of the plant material;
- ✓ identify the main groups of biologically active compounds present in the Bryony root;
- ✓ determine the content of the biologically active compounds identified.

CHAPTER 1. *BRYONIA ALBA* L. – A PERSPECTIVE MEDICINAL PLANT OF THE *CUCURBITACEAE* FAMILY

Review articles help us to realize the importance and potential of studies of medicinal plants. The world scientific literature shows us the various views and researches of the same medicinal plant and medicinal plant material in different years and induce to find the areae of future researches. Literature investigation is the first stage before pharmacognostic and pharmacological studies and medicines creation as a consequence.

Bryonia alba L. or a white bryony is well-known plant of *Cucurbitaceae* family. The aim of our review was to analyze the world scientific literature about the study of *Bryonia alba* L.

The analysis of world literature about the study of *Bryonia alba* L. have been made. Searched parers included phytochemical, phylogenetic and pharmacological studies, in vitro and in vivo surveys, review reports, etc. The selected papers were puplished in English, Russian, French and German languages. The selected papers were considered and analyzed. The most important studies were made between 1970 to 2019 years. The present review describes phylogenetic studies, botanical characteristics, chemical composition, medicinal use, side effects of *Bryonia alba* L.

1.1 Botanical characteristic *Bryonia alba* L.

Bryonia alba L. belongs to family *Cucurbitaceae*, subfamily *Cucurbitoideae,* tribe *Bryonieae*, genus *Bryonia*. Recent molecular phylogenetic studies (Schaefer & Renner 2011) of the *Cucurbitaceae* family show that genus *Bryonia* includes 10 species: *B. acuta, B. alba, B. Aspera, B. cretica, B. dioica, B. marmorata, B. monoica, B. multiflora, B. syriaca,* and *B. verrucosa* [1-3]. Studies on major chloroplast haplotypes of the *Bryonia* genus indicate that this genusis the *Cucurbitaceae* clade centered in the Mediterranean, Irano-Turanian, and (in part) Holarctic floral kingdoms (Schaefer et. al) [1, 2, 4]. All *Bryonia* species are well adapted to drained soils, such as sand dunes, dry channels, or rocky slopes in

mountainous areas, thanks to the presence of underground tubers for water storage [3,4].

Bryonia alba L.is one of the more common species of the genus. Its life-from is liana and it is a perennial herbaceous monoecious plant. *B. alba* applies to both wild plants and cultivators [1,2,5].

Botanical characteristics (Fig.1 and 2).

Figure 1. *B. alba* stem, leaves, flowers.
Robert Vidéki, Doronicum Kft., Bugwood.org

Figure 2. *B. alba* fruits.
Jan Samanek, Phytosanitary Administration, Bugwood.org

B. alba stems are numerous, 2.0-4.0 m long. They are rigidly pubescent, furrowed, and creeping, clinging by means unbranched spiral tendrils.

The *leaves* are alternate, with long petioles, ovate, five-lobed, are toothed on the edge, with a heart-shaped base, with a rough surface, up to 10.0 cm long.

Flowers are separate, regular, in axillary inflorescences, yellowish-white. Female flowers are collected in corymb-like or umbrella-like inflorescences. The male flowers are collected in whisks located at the tops of the stems.

Fruits are black, spherical, juicy berries 8.0-10.0 mm in diameter with 10 ovate flattened seeds. Unripe fruits are green.

B. alba has a rod-root system. *Roots* are thick and fleshy. The root length can reach 50.0-70.0 cm with width up to 10.0 cm. The surface of the root is transversely wrinkled and hasintermittently ringed grooves. The color of the root surface is yellowish-gray. The cut color is white with milky juice [1,5,6].

All parts of the plant are poisonous!

MPM (Fig.1.3 and 1.4).

The medicinal plant material (MPM) of *B. alba* isthe root - *Radix Bryoniae.* Harvesting is carried out before vegetation or at the end of vegetation. 2-3-year-old plants are preferred. Soil is removed from the roots, which are used fresh or finely sliced and dried [3,5]. Figures 1.3 and 1.4 show the sliced dry roots and the rootpowder. MPM must be kept separately from others, in accordance with the rules for the storage of poisonous plants.

Figure 1.3. *B. alba* sliced dry roots.

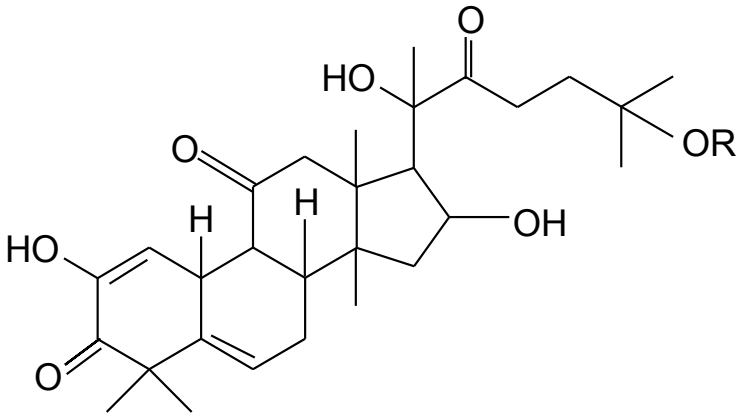
Figure 1.4. *B. alba* root powder.

1.2. Chemical composition

The most studied part of the *B. alba*arethe roots. The works of Pohlmann J., Panossian A., and Wagner H. et al. show that *B. alba*roots contain cucurbitacins. They are tetracyclic triterpenesfound in plants in the form of aglycones and glycosides. The sugar part of the glycosides is most commonly glucose and rhamnose. Glycosides of cucurbitacins are known to be unstable and will split into aglycones when raw materials are dried [7,8].Cucurbitacin E, B, D, I, J, K, L, R, dihydrocucurbitacin B, D, E, tetrahydrocucurbitacin I,2– and 25–glucosylhydrocucurbitacinD,and 2,25-diglucosyldihydrocucurbitacin D have all been found in *B. alba*roots [7,9-16]. Structural formulas of some of these cucurbitacins are given in Figure 1.5.

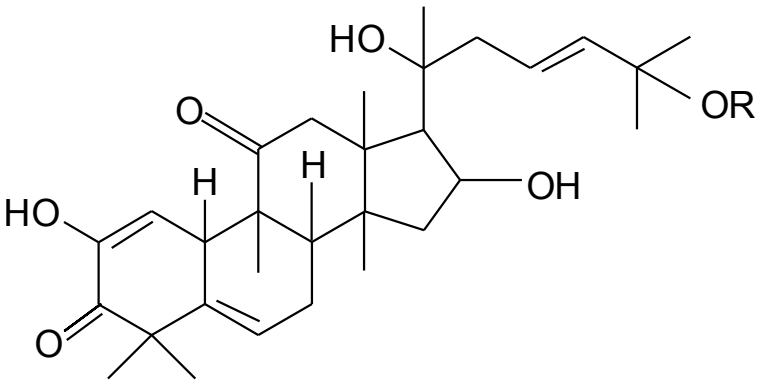
R= H
Cucurbitacin L

R= Ac
23,24-dihydrocucurbitacin E



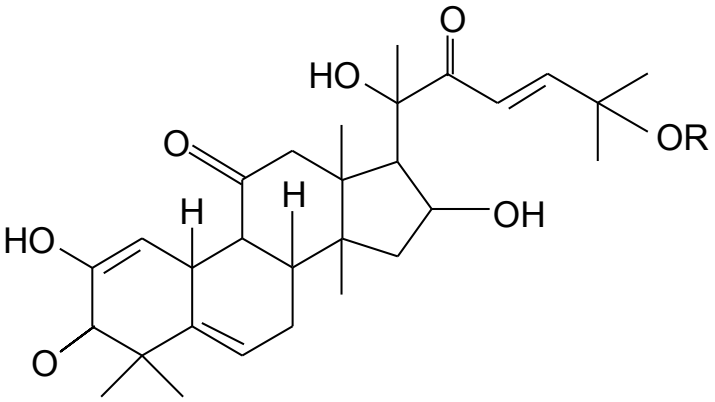
R= H
Cucurbitacin I

R= Ac
Cucurbitacin E



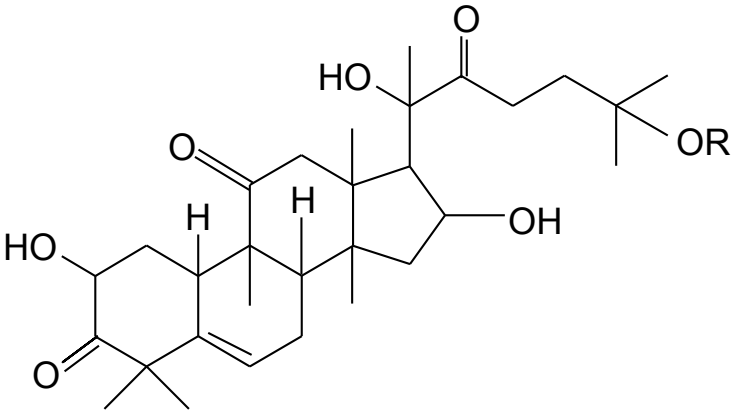
R= H
CucurbitacinD

R = Ac
CucurbitacinB



R= H
CucurbitacinR

R= Ac
23,24-dihydrocucurbitacin B



Brionolic acid

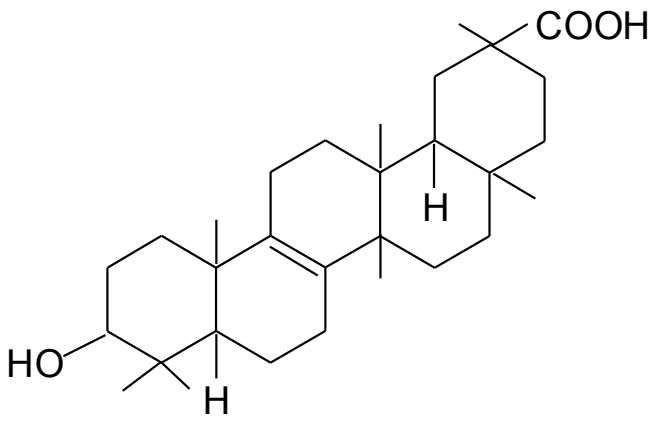


Figure. 1.5. Structural formulas of some triterpenes from *B. alba* roots

HPLC and spectrophotometric methods are used to determinethe quantitative content of cucurbitacins in MPM and extracts from *B. alba*roots [10,17].

The other triterpene of *B. alba*roots is brionolic acid. This pentacyclic triterpene has anti-allergic, antioxidant, and anti-inflammatory activity [18-20].

Information about the compounds responsible for the poisonous activity of *B. alba* is controversial. Many articles say that the cucurbitacins are the most poisonous substances in *B. alba* [3,16,21,22]. Other sources identify not only cucurbitacins but a complex of toxic compounds, including the glycosides brionin, brionidine, and brionicin, which are found in all parts of *B. alba*, especially in the roots and fruits[23].Manvi et al. proved the presence of alkaloids (usually toxic) in *B. alba*in various extracts [24].

Panosyan A.G. et al. confirmed the presence of phytosterols and their glicosides [14] and also studied the lipids [25] in *B. alba* roots. They also confirmed the presence of fatty acids (especially trihydroxyoctadecadienoic acids) and phospholipids [25-27].

Lectins have also been found in the roots of *B. alba* [28].

Manvi et al. studied microscopic features of *B. alba* roots and determined the presence of starch by microchemistry reaction [24].

Recent studies show that sugars are present in *B. alba* roots in free and bound froms. Sucrose, fructose,galactose,and glucose have been found before hydrolysis. Fructose is the most prominent of these – 25.03 mg/kg. Rhamnose, arabinose, fucose, glucose, and galactose have been found as part of other compounds, where the content of glucose (152.55 mg/kg) prevails [29].

The presenceand the quantitative contentof 16 free and bound amino acids in *B. alba* roots have also been determined. Arginine dominatesamong free amino acids, glutamic acid among bound amino acids [30].

Themacro- and microelements in *B. alba* roots have been studied too. Determination of at least 19 elements shows that *B. alba*roots do not accumulate heavy metals; K, Ca, Mg, P, and Si arethe elements found with highest content [31,32].

The aerial parts of *B. alba* are less studied. The report Ielciu et al. (2019) says that the aerial parts of *B. alba*do not contain cucurbitacins, but there are significant levels of flavonoids [33]. Different studies of flavonoids in the aerial parts of *B. alba* showed the presence of four main flavonoids: lutonarin, saponarin, isoorientin, and isovitexin [33-35].

1.3. Medicinal use

It's widely known that *Bryonia* preparations are commonly used in homeopathic medicine. The tincture of *B. alba* roots is included in many homeopathic pharmacopoeias of the world: India, Britain, USA, Germany, France [36-39]. There is some difference between the technologies usedto obtain the tincture. The Pharmacopoeia of India, for example, requires the use of dry roots of *B. alba,* whereas other pharmacopoeia use fresh roots for thetincture preparation [36-39]. *B. alba* is also used in homeopathy in the form of granules, drops, ointments, oils, and opodeldoc [6,16,40,41]. Homeopathy medicines from *B. Alba* roots are used as anti-inflammatory, antipyretic, antibacterial, and muscle relaxantpreparations, as well as to treat bronchitis, pneumonia, measles, and rheumatism [3,6,40-42].

B. alba root tincture, infusion, decoction, fresh juice, and root powder are usedin folk medicine in Europe and Asia as a painkiller, diuretic, laxative, hemostatic, and local irritant; also in small doses asa CNS suppressor [3,41,43].

B. alba roots are used in medicine of different countries. For example, *B. alba* is included in the Australian list of medicinal drugsthat are allowed for use as an active or auxiliary component [44]. We have found a report about an Armenian medicinal drug “Loshtak” (tablet form) that includes standardized powder of *B. alba* root extract. The reports say that*B. Alba* roots have adaptogenic, tonic, radioprotective, and immunomodulatory action [45-47].

There are studies of other therapeutic activities of *B. alba* roots and their substances. Extracts of *B. alba* roots and cucurbitacins exhibit high antitumor effect [12,13,16,22,27,48]. The mechanism of anticancer action of cucubitacins has been studied, and the synergistic effect of these substances with chemotherapeutic agents has been proved [11]. Cucurbitacins also have antimicrobial, antihelmintic, laxative, stimulating, and tonic effects; they can increase capillary permeability and lower blood pressure [13]. Cucurbitacin R glycoside has adaptogenic action. The mechanism of adaptogenic action has also been proved [49].

B. alba roots are used as adaptogenic agents with immunomodulatory and stress-protective properties, which increase nonspecific resistance of the organism with no genotoxicity [45,47,50,51].

The chloroform extracts of *B. alba* roots havehepatoprotective effects [24].

The hypoglycemic and hepatoprotective effects of ethanol extract of these plant roots have been proved [24,52].

The hypoglycemic activity of fatty acids of *B. alba* roots and the mechanism of their influence on the normalization of glycogen metabolism have been investigated [26]. The anti-sclerotic and antiatherogenic effects of fatty acids isolated from the roots of *B. alba*have been revealed [27].

Side effects

Poisonous compounds of *B. alba* roots are believed to be cytotoxins and microtubule modulators. Nausea, vomiting, diarrhea with blood, inflammation of

the kidneys, severe colic with gastrointestinal spasm, tachycardia, CNS lesions, and respiratory arrest are all symptoms of overdose[21,23].

Conclusions

Bryonia alba L. has important therapeutical and pharmaceutical potential.

Leaves, stems, and fruits are not well studied and need more investigation concerning the presence and content of biological active compounds. There are clearly good reason to conduct more in-depth analyses of *Bryonia alba* L. MPM to develop and standardizethe medicinal plant drugs that are based on it.

CHAPTER 2. MATERIALS AND METHODS.

2.1. Determination of the main technological parameters of Bryony root

2.1.1. Determination of weight loss on drying

Determination of loss on drying was carried out according to the State Pharmacopoeia of Ukraine [85, 87].

An excess of water in medicinal plant materials might enhance microbial growth, the presence of fungi or insects, and deteriorate the following hydrolysis. Limits for water content should therefore be set for every given plant material. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water.

The test for *weight loss on drying* determines both water and volatile matter. Drying was carried out either by heating to 100-105°C or in a desiccator over phosphorus pentoxide R under atmospheric or reduced pressure at room temperature for a specified period of time.

2.0 g of the plant material sample were cut, so that the size of the particles did not exceed 3 mm. The sample was placed into a previously dried and weighed weighing box with a lid and put in the laboratory oven heated up to 100-150°C. Drying time was measured from the moment when the temperature again reached 100-150°C.

The first weighing of raw materials was carried out in 2 hours. Drying was carried out to constant weight (constant weight was considered reached when the difference between two successive weighings after 30 min of drying and 30 min of cooling in desiccator did not exceed ±0.01 g) [82, 86].

To calculate the content of biologically active compounds and ash on the dried plant material the weight loss on drying was measured in 1 – 2 g of accurately weighed plant material taken from an analytical sample using the

abovementioned methods with the difference between weighings that did not exceed ±0.0005 g.

Weight loss on drying of plant material (*X*, %) was calculated according to the formula:

$$X = \frac{(m - m_1) \cdot 100}{m},$$

where *m* – weight of the plant material before drying, g;

m_1 - weight of the plant material after drying, g;

X – moisture of plant material, %.

2.1.2. Determination of total ash

The *total ash* method is designed to measure the total amount of MPM remaining after burning. This includes both "physiological ash" and "non-physiological" ash. "Physiological ash" is derived from the plant tissue itself. "Non-physiological" ash is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

2.0 g of crushed air-dried MPM was placed into a previously ignited and tared crucible. The plant material was spread in an even layer and burned by gradually increasing the heat to 500-600°C in the laboratory furnace until it became white, indicating the absence of carbon.

The crucible was placed down and cooled down in a suitable desiccator for 30 min. Then was weighed [82, 85, 87].

The content of total ash (*X*, %) was calculated by the formula:

$$X = \frac{(m - m_1) \cdot 100}{m},$$

where: m – weight of the crucible with the ash, g;

m_1 – initial weight of the crucible, g;

m – weight of plant material, g.

2.1.3. Determination of extractable matter

Described method determines the amount of active components of extracted with different solvents from a given amount of MPM to identify the most suitable one. This solvent can be used for obtaining extracts from the studied raw plant material.

1.0 g of the weighed crushed MPM was placed into a 200 – 250 ml conical flask where 50 ml of a solvent were added, then the flask was closed with a glass stopper, weighed with the accuracy ±0.01 g and left for 1 hour. A reflux condenser was attached to the flask and the latter was boiled slowly for 2 hours. After cooling the flask was weighed again, having covered with the same glass stopper, and then readjusted to the initial total weight with the solvent. The contents of the flask were stirred and then filtered through a dry paper filter into a dry 150 – 200 ml conical flask [33, 38].

Evaporating dish 25 ml of the filtrate were transfered into an evaporating dish. Evaporating dish previously was dried at the temperature 100 – 105°C to the constant weight. Filtrate then evaporated to dryness on a water bath. The evaporating dish with remainder was then dried at 100 – 105°C, cooled in a desiccator for 30 minutes, then weighed. The procedure was repeated until the constant weighed was achieved.

The content of extractable matter (X, %) calculated on the dried plant material was found using the formula:

$$X = \frac{m \cdot 100 \cdot 100}{m_1 \cdot (100 - w)},$$

where: m – mass of the dry residue, g;

m_1 – mass of the plant material, g;

w – weight loss on drying, % .

2.2. Determination of the main groups of BAS of Bryony root

2.2.1. General tests for glycosides

For qualitative tests on glycosides 70 % ethanol extracts of bryony root were used. 10.0 g of the plant material were placed to a conical flask, where 50 ml of 70 % ethanol was added. A reflux condenser was then attached and the mixture was boiled for 30 min with periodical stirring of the flask. The extract obtained was filtered into a conical flask. The Extraction was carried out twice more and the extracts obtained were united, concentrated and tests were carried out by the following methods [43, 33].

Test with Fehling's solution. 2 ml of bryony root extract were added in two measuring tubes; then 5 drops of concentrated hydrochloric acid were added into one of them, and an equal amount of purified water into the other one. Both tubes were heated on a boiling water bath for 15 minutes. Acid solution was neutralized by 10 % sodium hydroxide solution till pH=7 (using the indicator paper), and distilled water was added to another tube to get the same volume of the first tube. 2 ml of Fehling's solution were added to solutions in test tubes, boiled for 1 min, cooled for 10 min and the amounts of brick-red precipitates of copper oxide, indicating the presence of carbohydrates, were compared.

2.2.2. Identification of polysaccharides

5.0 g of accurately weighed crushed plant material were placed into glass conical flasks with 50 ml capacity where 30 ml of water were added. Flask was heated on a water bath for 1 hour with a reflux condenser attached. Water extract was filtrated and concentrated to the volume of 5 ml [43, 26].

15 ml of 96 % ethanol were added to the concentrated extract, stirred and left in a refrigerator for 12 hours.

2.2.3. Amino acids identification

Amino acids were identified by the means of paper chromatography. The method of multiple chromatogram development allows the solvent front to pass a longer distance in the same length of the chromatographic paper sheet. Chromatogram with substances applied was placed into the chamber with solvents and, after the solvent system passed 1/3 of the paper length, the chromatogram was taken out and thoroughly dried. The second time the procedure was repeated with the difference that the solvent system passed 1/2 of the paper length, and the third time the solvent system passed all the distance till the finish line [4, 75, 80]. The water extract obtained after polysaccharides extraction was chromatographed in the solvent system butanol-acetic acid-water (4:1:2) with triple chromatogram development in the presence of standard amino acids samples. 0,1 % ninhydrin alcohol solution was used as the detection reagent. Then the chromatogram was heated in the laboratory oven at the temperature 96°C until the amino acids spots appear. Thus the amino acid were coloured into purple or pink and purple, and yellow (proline) [84].

2.2.4. Identification of flavonoids

10 g of coarsely powdered plant material were extracted by 100 ml of 70 % alcohol on a water bath for 30 min with a reflux condenser attached. Extract was cooled and filtered through a paper filter. The filtrate was used for flavonoids identification. As a reference solution 0.1 % rutin alcohol solution was used [28, 17].

Reaction with 10 % sodium hydroxide solution. 1 ml of obtained extract was placed in a test tube, 2 drops of 10 % sodium hydroxide alcohol solution was added to the test tube.

Observation. 0.1 % rutin alcohol solution showed yellow color. In test tube with bryony root extract light yellow color appeared.

Reaction with iron (III) chloride solution. 1 ml of obtained extract was placed in a test tube, 2 drops of iron (III) chloride were added to the test tube.

Observation. 0.1 % rutin alcohol solution showed green color. In the test tubes with bryony root extracts green color appeared.

Reaction with 2 % aluminium chloride alcohol solution. 1 ml of obtained extract was placed in a test tube, 2 drops of 2 % aluminium chloride alcohol solution was added to the test tube.

Observation. 0.1 % rutin alcohol solution showed yellow color. In test tube with bryony root extract yellow-greenish color appeared.

Briant's modification of cyanidin formation test. 1 ml of obtained extract was placed in a test tube, 5 drops of hydrochloric acid and powder of metallic magnesium was added to the test tube. Colouring appeared. To the colored product from reaction of cyanidin formation 1/3 parts on volume of butanol was added, diluted by water to get 2 layers, shaken off and marked passing of pigments to the water or organic phases.

2.2.5. Determination of tannins

5 g of coarsely powdered plant material were extracted by 50 ml water on a water bath for 30 min with a reflux condenser attached. Extract was cooled. Then it was filtered through a paper filter. The filtrate was used for qualitative determination of tannins [33, 38].

Reaction with gelatin. 2 ml of obtained extract were placed in a test tube, 1 % gelatin solution by drops and 1 drop of 10 % HCl solution (to increase detection sensitivity) was added to the test tube.

Observation. Derivation of light brown flocculent precipitate was observed for bryony root extract.

Reaction with alkaloids. 2 ml of obtained extract were placed in a test tube, 1 % quinine chloride solution was added to the test tube by drops.

Observation. Derivation of light brown flocculent precipitate was observed for bryony root extract.

Reaction with iron alum solution. 2 ml of obtained extract were placed in a test tubes, 4 drops of iron alum solution were added to the test tube.

2.2.6. Determination of saponins

5 g of coarsely powdered plant material were extracted by 50 ml of 50 % ethanol on a water bath for 15 min with a reflux condenser attached. Extract was cooled and filtered through a paper filter. 25 ml of filtrate were concentrated to 10 ml and used for the froth test, some precipitation tests and determination of the chemical nature of saponins [28]. Alcohol extract of bryony root was used for the rest of qualitative tests.

Foam test. 2 ml of obtained extract were placed in a test tube, vigorously shaken during 1 min.

Precipitation tests

1. 2 ml of water extract were placed in a test tube, 3-4 drops of barium water was added.
2. 2 ml of water extract were placed in a test tube, 3-4 drops of 10 % lead acetate solution was added.
3. 2 ml of alcohol extracts were placed in a test tube, 1 ml of 1 % cholesterol alcoholic solution was added.

Colour reactions

Lafon's test. 2 ml of alcohol extract were placed in a test tube, 1 drop of 10 % copper sulfate solution and 1 ml of sulphuric acid concentrated were added and heated carefully.

Salkovski's test. 2 ml of alcohol extract were placed in a test tube, 1 ml of chloroform and 5-6 drops of sulphuric acid concentrated were added.

Determination of the chemical nature of saponins

2 graduated test tubes with plug stoppers were used for this test: 1) 5 ml of 0.1 mole/l hydrochloric acid solution was added; 2) 5 ml of 0.1 mole/l sodium hydroxide solution. 0.5 ml of water extract was added to the both test tubes. All test tubes was shaken during 1 min.

2.2.7. Determination of coumarins

Lacton test. To 2 ml of alcoholic solution of extracts of roots add 5 drops of 10% alcoholic solution of potassium hydroxide, heat in a water bath for 5 minutes. The contents of the tubes are cooled, add a 10% solution of hydrochloric acid to the acid reaction.

2.3. Quantitative determination of the main groups of BAS of Bryony root

2.3.1. Polysaccharides determination

Polysaccharides - biologically active compounds that make up to 90 % of the plant cell wall with pectin, hemicellulose, cellulose, and being the major groups [11]. They possess a wide range of biological activities, even being antioxidant and antitumor agents [34].

Determination of total polysaccharides content in the bryony root, was carried out by gravimetric method according to the well-known method [14, 19].

20 g of the crushed plant material were placed into conical flask of 250 ml capacity, then 200 ml of purified water were added and the flask was attached to the reflux condenser. The mixture was boiled on periodical stirring for 30 min on a water bath. The extraction was carried out two more times adding 200 ml of water

the first time and 100 ml – the second. The water extracts obtained were joined, centrifuged and decantated through 5 layers of gauze, placed in a glass funnel of 55 mm in diameter, to a measuring flask of 500 ml capacity (solution A).

25 ml of the solution A were placed into a centrifuge tube where 75 ml of 95% ethanol were added, the mixture was stirred and warmed up to 30°C on a water bath during 5 min. In 1 hour the contents of the tube were centrifuged for 30 min with the rotational speed 5000 RPM. The liquid was then filtered through a dried to the constant weight glass filter of 40 mm in diameter. The sediment was quantitatively placed on the filter and then consequently washed with 15 ml of the alcohol-water solution (95% ethanol and water in correlation 3:1) and 10 ml of ethyl acetate. The filter with the sediment was dried in the air and then at the temperature 100-105°C dried in the drying box to the constant weight.

The content of polysaccharides calculated on the dried plant material in percents (X, %) was calculated using the formula:

$$X = \frac{(m_2 - m_1) \cdot 500 \cdot 100 \cdot 100}{m \cdot 25 \cdot (100 - w)},$$

where m_1 – mass of the filter, g;

m_2 – mass of the filter with the sediment, g;

m – mass of the plant material, g;

w – weight loss on drying, %.

2.3.2. Polysaccharides fractionation

Previously, the raw material was degreased by exhaustive extraction with chloroform in a Soxhlet apparatus. The raw material was dried and then sequentially extracted with various solvents: 82% ethanol - obtained alcohol-soluble substances; purified water - WSPS; hot mixture of 0.5% solutions of

ammonium oxalate and oxalic acid - PS; 7% solution of sodium hydroxide - HC A and HC B [68, 87, 243, 281, 328, 389].

100 g of MPM obtaining afterextraction with 82% ethanol was extracted by purified water at the ratio of 1:20 (raw material-extractant). The extraction was performed at a temperature of 30–35 □C for 3.5 h with constant stirring. Re-extraction of polysaccharides was performed at a raw material-extractant ratio of 1:10. The resulting hoods were separated, combined and evaporated on a rotary evaporator to a minimum volume under vacuum. WSPS was precipitated with five times the volume of 96% alcohol, the precipitates were washed successively with hot 96% ethanol and acetone, dried in an oven to constant weight and weighed. After extraction, the vegetable raw materials were dried.

Shrot remaining after the removal of WSPS was used to isolate PS. Extraction of air-dry meal was carried out with a mixture in the ratio (1: 1) of 0.5% oxalic acid solution and 0.5% ammonium oxalate solution. The extraction was carried out in the ratio of raw material-extractant 1:20 twice at a temperature of 70 □C for 2 h with constant stirring at a ratio of raw material: extractant 1:20. The resulting extracts were separated from the feed, combined, concentrated and precipitated with five times 96% ethanol. A PR precipitate formed which was filtered off, washed successively with hot 96% ethanol and acetone, dried in an oven to constant weight and weighed.

From the meal that remained after the allocation of PS, was allocated shopping centers. Extraction was performed twice with 7% sodium hydroxide solution in the ratio of raw material-extractant 1: 5 at room temperature for 12 hours. The alkaline extract was filtered off. The filtrate was acidified with glacial acetic acid until a precipitate formed. The precipitate was filtered off, dried to constant weight and weighed. Thus, HC A was obtained. To the filtrate was added twice the amount of 96% ethanol, in which case a precipitate formed which was

filtered off with hot 96% ethanol, acetone, dried in drying cabinet to the constant weight and weighed. At the same time received fractions of the shopping center B.

The content of each fraction (X,%) in terms of absolutely dry raw materials was calculated by the formula:

$$X = \frac{(m_2 - m_1) \cdot 100000}{m \cdot (100 - W)},$$

where m1 is the mass of the filter, g;

m2 is the mass of the filter with sediment, g;

m - mass of raw materials, g;

W is the loss in mass during drying,%.

CHAPTER 3. RESULTS AND DISCUSSIONS

The plant material of the bryony roots, was collected in September 2021 in Rivne region.

3.1. Determination of the main technological parameters of Bryony root

3.1.1. Determination of weight loss on drying

The results of weight loss on drying determination statistical analysis in bryony root are given in the table 3.1.

Table 3.1

Results of the statistical analysis of the weight loss on drying of bryony root mean value

m	N	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval	ε, %
5	4	7,910 7,920 7,900 7,930 7,890	7,91	0,00025	0,0070711	0,95	2,78	7.91 ± 0.2	0,2485

It was determined that weight loss on drying of bryony root was 7.91±0.2%.

3.1.2. Determination of total ash

The results of total ash determination statistical analysis in bryony root are given in the table 3.2.

Table 3.2

Results of the statistical analysis of the total ash of bryony root

m	N	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval	ε, %

5	4	4,790 4,750 4,780 4,760 4,770	4,77	0,000250	0,00711	0,95	2,78	4.77	±	0.02	0,4121

It was determined that total ash of bryony root was 4.77 ± 0.02 %.

3.1.3. Determination of extractable matter

The results of extractable matter determination statistical analysis of bryony root with different solvents are given in tables 3.3 – 3.6.

Table 3.3

Results of the statistical analysis of extractable matter determination in bryony root (water)

m	N	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval			ε, %
5	4	9,13 9,11 9,1 9,14 9,12	9.12	0,00025	0,0070711	0,95	2,78	9.12	±	0.02	0.2156

Table 3.4

Results of the statistical analysis of extractable matter determination in bryony root (30% ethanol)

m	N	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval			ε, %
5	4	9,17 9,13 9,14 9,16 9,15	9.15	0.00025	0.0070711	0.95	2.78	9.15	±	0.02	0.2148

Table 3.5

Results of the statistical analysis of extractable matter determination in bryony root (50% ethanol)

m	N	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval	ε, %
5	4	9,17 9,21 9,2 9,18 9,19	9,19	0,00025	0,0070711	0,95	2,78	9.19 ± 0.02	0.2139

Table 3.6

Results of the statistical analysis of extractable matter determination in bryony root (70% ethanol)

m	N	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval	ε, %
5	4	13,75 13,85 13,65 13,7 13,8	13,75	0,00625	0,03535	0,95	2,78	13.75 ± 0.098	0.7148

Therefore, the dry residue of the water extract was 9.12±0.02%, of the 30% ethanol – 9.15±0.02%, 50% ethanol – 9.19±0.02%, and 70% ethanol – 13.75±0.098%.

The experiment has shown that the most suitable extragent for the extract on the bryony root basis obtaining was 70 % ethanol since it had the highest dry residue thus allowing extracting the highest amount of biologically active compounds from the plant material.

Moreover, we can assume that the plant material studied contains more lipophilic compounds (e.g., terpenoids.) since the highest yield of the dry residue was detected in 70% and 50% ethanol, and the lowest one – using 30% ethanol.

3.2. Determination of the main groups of BAS of Bryony root

3.2.1. General tests for glycosides

Observation. Volume of copper oxide precipitates after acid hydrolysis was higher than before hydrolysis for the plant material studied.

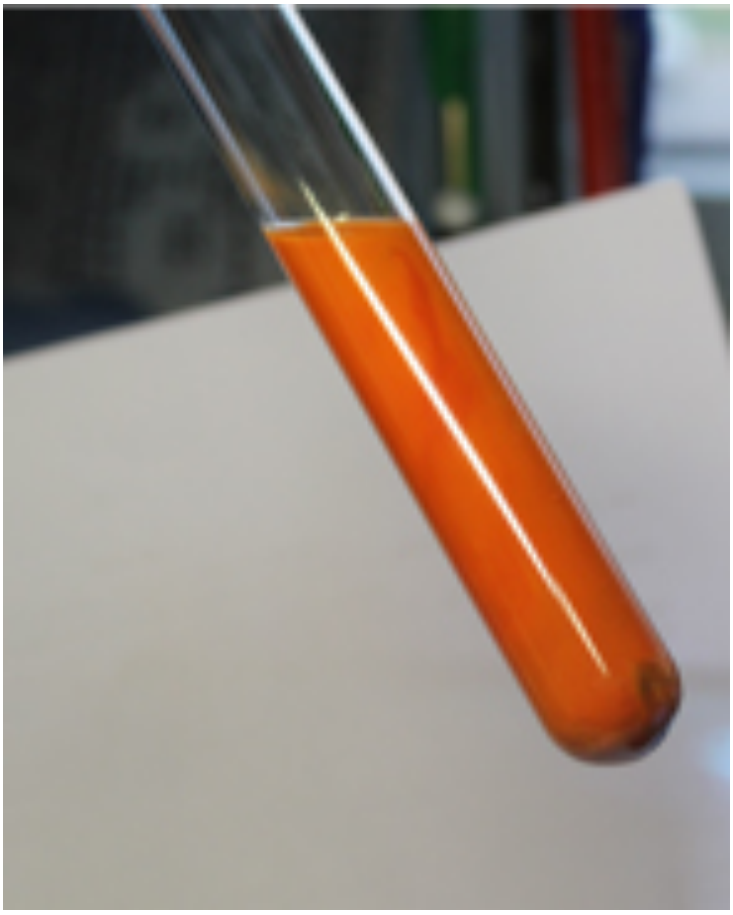


Figure. 3.1. General tests for glycosides from *B. alba* roots

Conclusion. The results of tests conducted confirm the presence of glycosides in bryony root.

3.2.2. Identification of polysaccharides

Observation. Derivation of abundant light brown flocculent precipitate was observed.

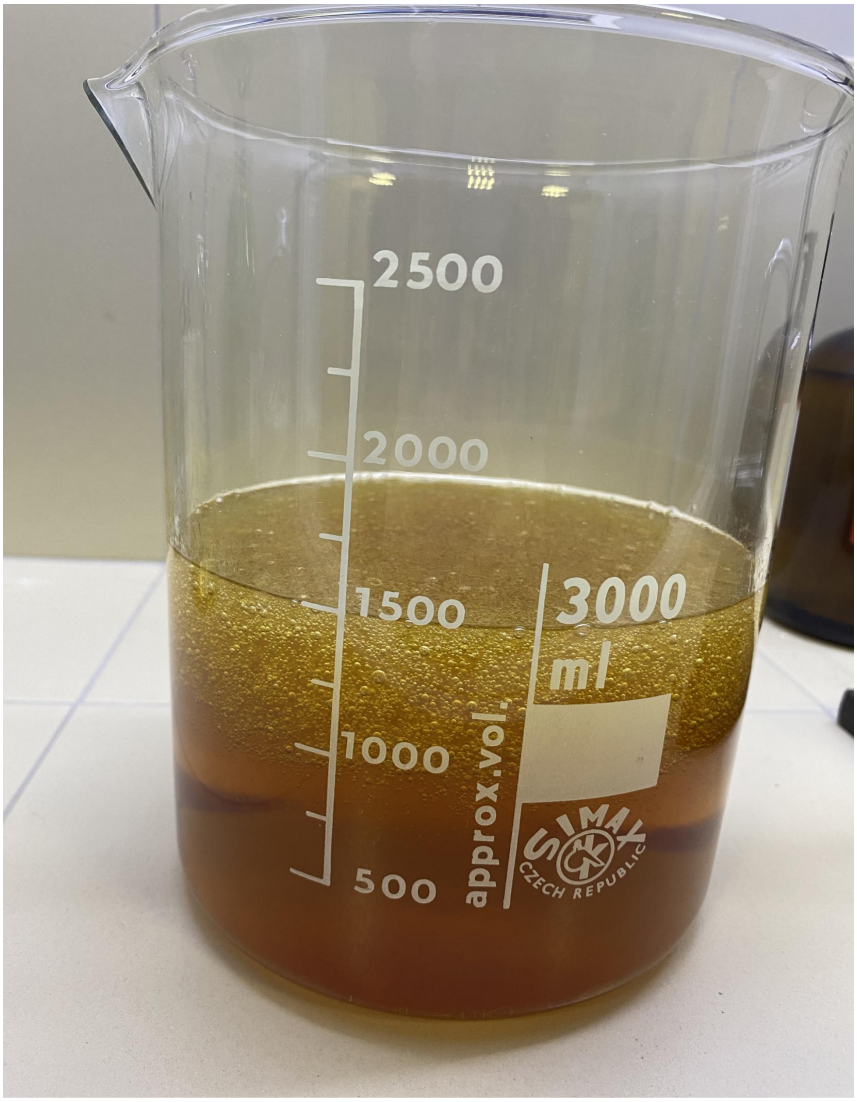


Figure. 3.2. The identification of polysaccharides of *B. alba* roots

Conclusion. The presence of polysaccharides was determined in bryony roots.

3.2.3. Amino acids identification

The scheme of the chromatogram is given in the figure 3.3.

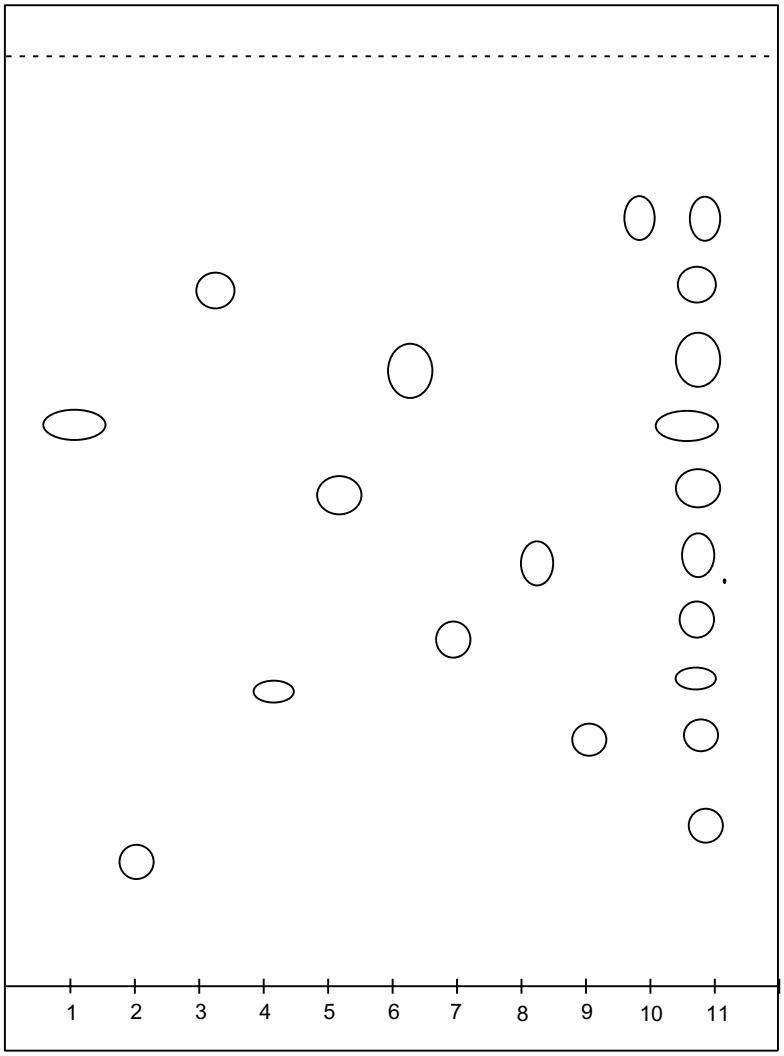


Fig. 3.3. Scheme of the chromatogram of amino acids identification in bryony root, where: 1 – proline, 2 – alanine, 3 – methionine, 4 – arginine, 5 – valine, 6 – tryptophan, 7 – aspartic acid, 8 – glutamic acid, 9 – histidine, 10 – leucine, 11 – bryony root water extract. *Solvent system*: *n*-butanol–acetic acid–water (4: 1: 2). *Detection reagent*: 0,1 % ninhydrin alcohol solution, t^0.

Thus, chromatographic analysis allowed us to detect 10 amino acids in bryony root, 4 of which are essential – valine, leucine, methionine and arginine.

3.2.4. Identification of flavonoids

Observation. 0.1% rutin alcohol solution showed pink color in water layer. In test tube with bryony root extract the colouring was brighter in water layer.

Conclusion. Presence of flavonoids was proven in bryony root raw material. The tests showed that the presence of glycosides in bryony root dominated over the presence of aglycones.

3.2.5. Determination of tannins

Observation. Derivation of green color precipitate was observed for bryony root extract.

Fig. 3.4. *B. alba* roots extract green color precipitate

Conclusion. Presence of tannins was determined in bryony root extract.

3.2.6. Determination of saponins

Foam test

Observation. Water extract gave a layer of foam in aqueous solution after 15 min standing.

Fig. 3.5. *B. alba* roots extract Foam test

Precipitation tests

1. With barium water.

Observation. Derivation of light precipitate was observed.

2. With lead acetate solution.

Observation. Derivation of light precipitate was observed.

3. With 1% cholesterol alcoholic solution.

Observation. Derivation of light precipitate was observed.

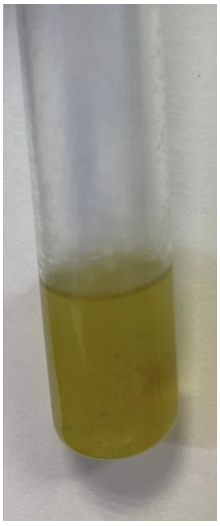


Fig. 3.6. *B. alba* roots extract with 1% cholesterol alcoholic solution

Colour reactions

Lafon's test

Observation. Derivation of bluish –green color was observed.

Salkovski's test.

Observation. Derivation of reddish-yellow color was observed.

Fig. 3.7. *B. alba* roots extract with Salkovski's test.

Determination of the chemical nature of saponins

Observation. Water extracts gave a layer of foam in aqueous solution after 15 min standing. The heights of foam were equal in both test tubes for bryony root plant material.

Conclusion. Prevalence of triterpenoidal saponins was determined in bryony root.

3.2.7. Determination of coumarins

Observation. Occurrence of opalescence, turbidity and sediment in both tubes, there is a yellow color

Fig. 3.8. Lacton test of *B. alba* roots

Conclusion. The result inticates the presence of coumarins in the raw material of of *B. alba* roots

3.3. Quantitative determination of BAS of Bryony root

3.3.1. Polysaccharides determination

The results of polysaccharides determination statistical analysis in bryony root are given in the table 3.7.

Table 3.7

Results of the statistical analysis of the polysaccharides content in bryony root mean value

m	N	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval	ε, %
5	4	4,15 4,09 4,11 4,13 4,12	4,12	0.0005	0.01	0.95	2.78	4.12 ± 0.028	0.675

It was determined that the polysaccharides content in bryony root was 4.12± 0,028 %.

3.3.2. Polysaccharides fractionation

The results of polysaccharides fractionation statistical analysis in bryony root are given in the table 3.8.-3.11.

Table 3.8

Results of the statistical analysis of WSPS determination in bryony root

m	N	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval			ε, %
5	4	5.13 5.11 5.05 5.14 5.02	5.30	0,00025	0,0070711	0,95	2,78	5.30	±	0.24	0.2156

Table 3.9

Results of the statistical analysis of PS determination in bryony root

m	N	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval			ε, %
5	4	5,25 5,13 5,14 5,16 5,05	5.18	0.00025	0.0070711	0.95	2.78	5.18	±	0.24	0.2148

Table 3.10

Results of the statistical analysis of HC A determination in bryony root

m	N	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval	ε, %
5	4	5.97 5.86 5.96 5.87 5.90	5.92	0.00025	0.0070711	0.95	2.78	5.92 ± 0.21	0.2139

Table 3.11

Results of the statistical analysis of HC B determination in bryony root

m	N	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval	ε, %
5	4	18,15 18,09 18,15 18,17 18,05	18,11	0,00625	0,03535	0,95	2,78	18.11 ± 0.72	0.7148

Conclusions

1. The most important technological parameters of the bryony root were determined.
2. The weight loss on drying of the bryony root was found to be 7.91 ± 0.2 %, total ash – 4.77 ± 0.02 %.
3. The extractable matter determination was carried out which allowed to discover 70 % ethanol to be the most suitable extragent for the extract on the bryony root basis obtaining since it allows to extract the highest amount of biologically active compounds from the plant material.
4. Qualitative identification of biologically active compounds in bryony root was carried out. The presence of glycosides, polysaccharides, aminoacids, flavonoids, coumarins, tannins, saponins was proven.
5. Determination of the content of the main biologically active groups of compounds in bryony root was carried out.
6. The content of polysaccharides in bryony root was 4.12 ± 0.028 %.
7. Polysaccharides fractionation shows the cocntent of WSPS - 5.30±0.24%, PS - 5.18±0.24%, HC A - 5.92±0.21%, HC B - 18.11±0.71%

GENERAL CONCLUSION

1. The study of the literature sources concerning bryony root and its products has been carried out.
2. Qualitative identification of biologically active compounds in bryony root was carried out. The presence of glycosides, polysaccharides, ckumarins, flavonoids, tannins, saponins was proven.
3. Chromatographic analysis has allowed to detect at least 10 free amino acids.
4. Determination of the content of the main biologically active groups of compounds in bryony root was carried out.
5. The content of polysaccharides in bryony root was 4.12±0.028%; fractionation of polysaccharides shows the cocntent of WSPS - 5.30±0.24%, PS - 5.18±0.24%, HC A - 5.92±0.21%, HC B - 18.11±0.71%
6. The weight loss on drying of the bryony root was found to be 7.91 ± 0.2 %, total ash –4.77 ± 0.02 %.
7. The extractable matter determination was carried out which allowed to discover 70 % ethanol to be the most suitable extragent for the extract on the bryony root basis obtaining since it allows to extract the highest amount of biologically active compounds from the plant material.

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