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DETERMINATION OF THE CONTENT OF BIOLOGICALLY ACTIVE SUBSTANCES IN SOME AQUATIC HIGHER PLANTS

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Abstract

The article identifies the following biologically active substances in the surface and the underground parts of *Eichhornia crassipes, Pistia Stratiotes, and Lemna minor* aquatic plants (roots, stems, leaves): alkaloids, anthraquinones, proteins, tannins, flavonoids, phenolic compounds, polysaccharides, anthraquinones, and coumarins. A brief overview of scientific works has been given for certain types of biologically active substances and their biological activity, importance, biosynthesis, and genetic transfer. The results of this research show that high amount of biologically active substances (BAS) was found in *Eichhornia crassipes* aquatic plant in terms of tannins, which in the roots amounted to 7.476%, and in the above-ground part — to 6.73%. The content of polysaccharides was 5.907%, and in the roots — 2.642%. By the amount of BAS detected in the composition of *Pistia stratiotes* aquatic plant, polysaccharides content in the aerial part was 3.073%, and in the roots — 4.881%, the content of flavonoids in the aerial part was 4.833%, and in the roots — 3.716%. Among BAS in *Lemna minor* water plant, the content of flavonoids was 5.463%.

Key words: Biologically active substances (BAS), Aquatic plants, Tannins, Flavonoids.

Introduction

Currently, a comprehensive study of many biologically active compounds is becoming an urgent issue in science and biotechnology. These compounds are made with the use of primary or secondary metabolites. The obtained products are widely used in various important fields, such as medicine, veterinary, food products, and agriculture. There are at least 12,000 species of secondary metabolites, and 10% of them are widely used in production (Paredes-López *et al.*, 2010). Helpful phytochemicals may be classified into several groups: phenols (flavonoids, tannins, coumarins, lignans), terpenes (monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes, sterols) and also nitrogen and sulphur containing compounds, such as alkaloids, glucosinolates, atropines. The composition of BAS, depending on the natural features of the plants, has economic and ecological importance. Some of them are consumed in the diet of humans, and other species have medicinal value and are an important resource of minerals and vitamins (Szajdek & Borowska, 2008). The metabolic activity of organical compounds mainly depends on the activity of oxygen forms. Various studies have shown that the rate of tolerance to adverse biotic and environmental factors depends on the level of BAS in plants and their antioxidant properties. Plants contain various important types of secondary metabolites that don't participate in vital metabolic function of cells and tissues. However, the formation of secondary metabolites in plants in response to the stress caused by physical factors (radiation, flood, salinity, drought, alkalinity, etc.) or damage by insects, pests and microbes, is a natural process. Moreover, phenolic compounds help protect from degenerative diseases. Plant phenolic compounds are a chemically heterogeneous group: some phenolic compounds are soluble only in organic solvents, some are glycosides or carboxylic acids soluble in water, whereas others are large, insoluble polymers. The concentration of phenolic compounds in the plants depends on the variety and the species of the plants, climatic factors, ripening processes, and periods of crop harvesting and storage. For example, the grapes contain a chemical compound called resveratrol which decreases fungi development. Researchers have found that the plants grown at low temperatures in the soil without fertilizers and pest control treatment contain higher amounts of polyphenolic compounds (Tulipani *et al.*, 2008). In this regard, brief characteristics of some types of BAS and their biological functions, importance, biosynthesis, and genetic transport are given.

Anthocyanins are an important group of polyphenolic compounds. Many anthocyanins are found in berries and fruits. Anthocyanins derived from flavonol are in the form of anthocyanidin glycosides and acylated anthocyanins, therefore, their chemical structure is comprised of flavylium ions and various sugar compounds. In berries, anthocyanins are found in the form of mono-, di-, or triglycosides, in which residues of glycosides are usually transferred with C3, or rarely, C5 or C7 (Viskelis *et al.*, 2009). The activity of the jasmonic acid (JA) influences the content of anthocyanins in plant. The most important components of the WD-repeat/bHLH/MYB transcription complexes are transcription factors (TFs), such as PAP1, EGL3, GL3, MYB75, and TT8, which are involved in the biosynthesis of anthocyanin. Recent biochemical and genetic data have shown that these are the TF JAZ (Jasmonate ZIM Domain (JAZ) proteins) objects; thus, JA is the mechanical basis for anthocyanins formation (Wasternack & Hause, 2013).

Tannins are the most important BAS contained in plants. Based on their structural characteristics, tannins are classified into four groups: gallotannins, ellagitannins, condensed tannins and complex tannins. Tannins have signifivant impact on general defence ability of plants. They

are responsible for changing the flavor and color of vegetable fruit. Besides, tannins are found in the leaves of woody plants in the amount of up to 5 – 10%, and have a protective function in the form of toxicity to herbaceous insects. The toxicity of tannins is the result of producing high levels of reactive forms of oxygen. As a result of feeding animals with plants that are rich in tannins, a decrease occurrs in the digestion of proteins, which, in turn, indicates toxicity. Damage of some plants by insects can have a strong stimulating effect on tannins production, which contributes to the process of induced protection of tannins synthesis (Robil & Tolentino, 2015). Currently, the sensitivity of the genes responsible for the process of woody plants' affection is determined. These genes are induced by the expression of the CT, PtMYB134regulator gene. As mentioned above, ptmyb134 transgenic expression results in the adjustment of CT point; CTS are sharply accumulated but have minimal impact on the accumulation of other flavonoids. The peculiarity of this induction is in the fact that CTs are important components in plant protection (Barbehenn & Constabel, 2011). Flavonoids are low molecular polyphenolic secondary metabolic substances found in the entire plant kingdom. More than 9,000 of their derivatives are involved in the coloring of plant flowers, transport of auxin, inhibition, protection from UV rays, and allelopathic interactions. The biosynthesis of flavonoid compounds consists of condensation by synthase chalcone enzyme for the synthesis of the naringenin chalcone (2,4,6,4 tetrahydroxychalcone) of three P molecules - coumaroyl and malonyl CoA. Subsequently, this contributes to the formation of dihydrochalcones, aurones, flavanones (dihydroflavonols), isoflavones, etc. Due to the variety of physical and chemical properties, these compounds have a wide range of actions in plants, and in animal and bacterial systems. For example, they develop symbiotic relationships between legumes and useful bacteria, and increase the plant biomass (Buer *et al.*, 2007). Under adverse biotic and environmental factors, intracellular signals provide the mechanism of molecular regulation for further developmental process. The activity of the TFs belongs to the elements that contribute to and facilitate gene expression. The genes of flavonoids biosynthesis represent the main MBW (MYB-bHLH-WD40) regulatory systems, which include special organs of r2r3myb family and basic helix-loop-helix (bHLH) TF that are responsible for the relationship between the specific regulatory circuits and the WD-recurrent factors. MYB and bHLH components can create various MBW complexes with antistress action. Another important function of flavonoids is the ability to not only protect from harmful abiotic factors but also to increase the amount and the bioavailability of soil nutrients in the conditions of limited nutrient media. In the case of nutrition deficiency in the soil, flavonoids are delivered to the rhizosphere with the carriers of active forms of oxygen for binding with the metals that are required for plant growth and vital activity (Mehrtens *et al.*, 2005).

Alkaloids belong to the largest groups of secondary metabolites, they have diverse structure and ways of biosynthesis, including over 20,000 various molecules; about 20% are synthesized in plants. Alkaloids are nitrogen compounds, and contain heterocyclic rings that contain a nitrogen atom. Crop production has some advantages that contain small amounts of alkaloids for the humankind (Yang & Stöckigt, 2010).

For example, for the plant named lupine that contains alkaloids of quinolizidine, the use of pesticides is not required. Studies have shown allelopathic activity in the protection of some crops from weeds when cultivated together with alkaloid plants. Some animals use vegetable alkaloids, for example, poisonous frogs effectively use alkaloids not only for protection but also for catching the feed. Ancient Amazon Indians efficiently used bow arrows impregnated with the secretion of poisonous frogs for hunting. At the stage of larvae, *Utetheisa Bella* feeds on legumes, which include *Crotalaria* genus, which is rich in pyrrolizidine alkaloids that have protective functions from various insects. Butterfly larvae for protection from enemies emit poisonous foam from the body, which consists of pyrrolysine alkaloids, and these alkaloids are also passed to adult butterflies during the biotransformation of alkaloids. The dynamics of alkaloids accumulation in plants are directly related to the activity of primary and secondary metabolites, and the activity of response to stressful situations. The concentration of alkaloids in plants becomes high during flowering and growth of leaves (Hantak *et al*., 2013). For example, plants of *Nicotiana* sp. (*Solanaceae*) contain large amounts of nicotinic alkaloids of pyridine that play an important role in protection from various pests. The biosynthesis of nicotine starts in the roots of plants, nicotine is transported via the xylem to the leaves and other parts. Nicotine alkaloids are mainly stored in the vacuoles of the apical meristems of plants. In scientific research, processing the apical meristems of plants *Nicotiana* with auxin significantly reduces the synthesis of nicotine, and processing with dormin increases the synthesis of nicotine twice. Phytohormones of JA are also directly involved in the biosynthesis of nicotine. JA induces nicotine synthesis and its accumulation in plant tissues by activating TFs of the MYC2 and bHLH types, which directly regulates the production of alkaloids by transactivation of the alkaloid biosynthetic genes (Todd *et al*., 2010).

At present, great importance is attached to studying the plants, in which a wide range of BAS is contained, for pharmacological purposes. BAS are involved in the metabolic processes in enzymes, and in various reduction reactions in the organism. Therefore, the aim is to identify BAS in the composition of the aboveground and underground parts of aquatic plants using phytochemical methods. The accumulated experience is recommended to be used for determining the BAS content in some water plants using the optimal methods (Yernazarova *et al.*, 2018).

Methods and objects

The objects of the study were *Eichhornia crassipes, Pistia Stratiotes, Lemna minor* aquatic plants cultivated in a special nutrient medium (at 25 – 28°C), in a light culture room. BAS were identified in the aboveground (leaves and stems) and underground parts (roots) of the experimental plants: alkaloids, anthraquinones, proteins, tannins, coumarins, polysaccharides, steroids, phenolic acids, and flavonoids. The number of plants required for the analysis was weighted by 2 g for identifying a single class of BAS.

The content of alkaloids was identified by reverse titration: a weighed sample of a crushed plant was placed in a 100 ml flask, 5 ml of ammonia solution were added along with 50 ml of ethyl acetate, the mixture was infused with stirring for 2 h. The completeness of alkaloids extraction by ethyl acetate was checked by the absence of opalescence after adding potassium tetraiodomercurate to the dry residue obtained by evaporating 5 ml of the extract and adding 1 ml of 0.5 n sulfuric acid solution to the residue. The obtained extract was filtered, 10 ml of diluted hydrochloric acid were added, then it was alkalized with ammonia solution, and extracted three times with 10 ml of chloroform. The joint extract was evaporated until dry. 15 ml of 95% ethyl alcohol and 20 ml of 0.01 n solution of hydrochloric acid were added to the dry chloroform residue, and the excess of the acid was titrated with 0.01 n solution of sodium hydroxide in the presence of methyl red. The content of alkaloids in percent (X) expressed as absolutely dry raw material was calculated by the following formula:

$$X = \frac{32.74\ (20 - V)\ 100}{m\ (100 - W)}$$

where V was the volume of 0.01 n sodium hydroxide solution used for titration, in milliliters; m was the weight of the raw material sample in grams; and W was the loss of weight after drying raw material in percentage.

The content of anthraquinones was determined using the alkali-ammonia method: an exact weighed sample of the raw materials was placed in a 100 ml flask, 15 ml of 10% sulfuric acid were added, and the mixture was heated with backflow condenser on a boiling water bath for 1 h. After that, it was cooled, 50 ml of ethyl acetate were added through the condenser, and boiled for 1 h. The extract was cooled and filtered in a separatory funnel, 20 ml of alkaline ammonia solution were added, and the mixture was stirred for 5 to 7 min. After complete stratification, the red transparent bottom layer was drained. Treatment was repeated until the alkali-ammonia layer stopped coloring, the extract was stirred. The optical density of the obtained alkali-ammonia extract was measured at the wavelength of 525 nm in a cuvette with a layer thickness of 10 mm, using the alkali-ammonia solution as a reference solution. The content of anthraquinone derivatives expressed as chrysophanic acid was calculated according to the following formula:

$$X = \frac{C \cdot 100 \cdot 100 \cdot 100}{m\ (100 - W)}$$

where C was the contents of anthraquinone derivatives in 1 ml of the tested solution determined using the calibration graph, in grams; m was the weight of the sample of raw material in grams; and W was the weight loss after raw material drying in percent. The calibration curve was built by the solutions of cobalt chloride dried until a constant weight was reached, assuming that 1% solution of cobalt chloride by its optical density at the wavelength of 525 nm corresponded to 4.3 mg of chrysophanic acid in 1 l of an alkali-ammonia solution. The alkali-ammonia solution was prepared as follows: 50 g of sodium hydroxide were dissolved with stirring in 870 ml of purified water. After cooling, 80 ml of concentrated ammonia solution were added, and the solution was stirred.

The content of proteins was determined using the ninhydrine method: 20 ml of purified water was added to an exact weighed sample, the mixture was infused at room temperature for 24 h, and filtered. 10 ml of ninhydrine reagent were added to 10 ml extract, the mixture was heated for 15 min on a water bath at 80 – 85°C, then cooled noting the color of the resulting solution, and measuring its optical density with a spectrophotometer at a wavelength of 540 nm in a cuvette with the layer thickness of 10 mm. The water purified with ninhydrine reagent was used as a reference solution. Using the calibration curve, the content of proteins in the analyzed raw material was determined.

$$X = \frac{C \cdot 50 \cdot 25 \cdot 100 \cdot 100}{2 \cdot m \cdot 10 \cdot (100 - W)}$$

where C was the concentration of proteins found by the calibration curve; m was the weight of raw material in grams; and W was the weight loss after drying of the raw material in percent.

The ninhydrine reagent was prepared as follows: 4 g of ninhydrine, 76 g of tin chloride, 150 ml of dioxane, and 50 ml of acetate buffer (pH=5.0) were thoroughly mixed.

The content of tannins was determined using permanganatometry: an exact weighed sample of the raw material was placed in a conical 100 ml flask, 50 ml of hot water were added, and the mixture was heated on a boiling water bath for 2 h. The water extract was decanted, 50 ml of hot water were added to the raw material in the flask, and the raw material was re-extracted as stated above. The combined extract was filtered in a 100 ml measuring flask, topping up the solution with purified water to the mark. 10 ml of the resulting solution were transferred to a conical 500 ml flask, 100 ml of purified water, 10 ml of a solution of sulphonic acid were added, and the solution was titrated with constant stirring by a 0.02M solution of potassium permanganate until golden yellow color appeared. In parallel, 10 ml of sulphonic acid were titrated in 100 ml of purified water. 1 ml of 0.02 M solution of potassium permanganate corresponded to 0.004157 g of hydrolyzed tannins expressed as tannin. The content of tannins (X) in percent, expressed as absolutely dry raw material, was calculated using the following formula:

$$X = \frac{(V_1 - V_2) \cdot 0.004157 \cdot V \cdot 100 \cdot 100}{V_3 \cdot m \cdot (100 - W)}$$

where V_1 was the volume of 0.02 M solution of potassium permanganate used for titration of the extract, in milliliters; V_2 was the volume of 0.02 M solution of potassium permanganate used for titration in the reference experiment, in milliliters; V_3 was the volume of the extract used for titration, in milliliters; V was the volume of the extract in milliliters; m was the weight of the raw material in grams; and W was the weight loss after drying the raw material in percentage.

The solution of sulphonic acid was prepared as follows: 1 g of indigo carmine was dissolved in 25 ml of concentrated sulfuric acid and carefully topped to 1 l with purified water.

The content of coumarins was determined using the following method: an exact weighed sample of crushed material was placed in a 100 ml flask, 50 ml of chloroform were added, and the mixture was heated with stirring for 2 h on a boiling water bath with a backflow condenser, then filtered through a paper filter. 20 ml of the filtrate were placed in a separatory funnel, 1 g of sodium chloride was added, the mixture was shaken for 5 min, and filtered. The chloroform extract was evaporated on a boiling water bath until dry. The dry residue was dissolved in 10 ml of 96% ethyl alcohol and quantitatively transferred with 10 ml of 96% ethyl alcohol in a 25 ml volumetric flask, and the solution was topped up with 96% ethyl alcohol to the mark. 5 ml of the analyzed solution were placed in a 50 ml volumetric flask, the volume was topped up with 96% alcohol to the mark, and stirred. The optical density of the solution was measured at the wavelength of 272 nm in a cuvette with a layer thickness of 10 mm, using 96 % ethanol as the reference solution. The content of coumarin derivatives in the absolutely dry raw material expressed as CO in percent was calculated by the following formula:

$$X = \frac{D \cdot 25 \cdot [50] \cdot 100 \cdot [100]}{734 \cdot 20 \cdot m \cdot [5] \cdot [(100 - W)]}$$

where D was the optical density of the tested solution at λ_{max} 272 nm; 734 was the rate of coumarin CO absorption at λ_{max} 272 nm; m was the weight of the weighed sample of the raw material, in grams; and W was the weight loss after drying raw material in percent.

The content of polysaccharides was determined by the gravimetric method: an exact weighed sample of crushed material was placed in a 100 ml flask, 50 ml of purified water were added, the flask was connected to the backflow condenser and boiled with stirring on a water bath for 1 h, then cooled. Extraction with water was repeated twice within 30 min in the same conditions. The water extracts were combined and filtered into a 250 ml volumetric flask through three layers of gauze. The filter was washed with purified water, topping up the solution of purified water to the mark. 25 ml of the resulting solution were placed in a centrifuge tube, 75 ml of 95% ethyl alcohol were added, the mixture was stirred, heated on a water bath at a 60°C for 5 min. After 30 min, the contents were centrifuged at 5,000 rpm for 30 min. The supernatant was filtered under vacuum through glass filter POR 16 dried to constant weight. After that, sediment was quantitatively transferred to the same filter and washed with 15 ml of 95 % ethyl alcohol. The filter with the sediment was dried at 100 – 105°C to constant weight. The content of polysaccharides expressed as absolutely dry raw materials in percent (X) was calculated by the following formula:

$$X = \frac{(m_2 - m_1) \cdot 250 \cdot 100 \cdot (100)}{m \cdot 25 \cdot (100 - W)}$$

where m_1 was the weight of the filter, in grams; m_2 was the weight of the filter with the sediment, in grams; m was the weight of the sample of raw material in grams; and W was the weight loss after drying raw material, in percent.

The content of steroids was determined by the following method: an exact weighed sample of raw materials was placed in a Soxhlet apparatus, and degreased with 300 ml of chloroform; then the raw material was extracted with 300 ml of 80 % methanol. The extraction fluid was distilled, the extract was evaporated in vacuum until obtaining a thick extract, which was then dried in vacuum at 50 – 60°C. The dry extract was placed on column (d = 7, h = 70 cm) with aluminum oxide of activity degree III by Brockmann, elution was performed first with methanol, then with butanol saturated with water until negative reaction to steroidal saponins was reached. The eluates were joined and transferred into a pre-weighed round-bottom 100 ml flask. They were evaporated in vacuum at 55 – 60°C until dry. The resulting precipitate was dried at 100 – 105°C until constant weight was reached and weighed. The sum of steroid saponins (X) in percent, expressed as absolutely dry raw material, was calculated by the following formula:

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

where m_2 was the weight of the weighed sample of raw material in grams; m_1 was the weight of the dry residue in grams; and W was the loss of weight after drying raw material in percent.

The content of phenolic acids was determined by the following method: an exact weighed sample of crushed material was placed in a round bottom flask, 50 ml of 70 % ethyl alcohol were added, and the mixture was extracted on a boiling water bath for 2 h. Then the mixture was cooled, filtered into a 100 ml measuring flask, and topped up to the mark with ethyl alcohol. The optical density of the obtained solution was measured at the wavelength of 290 nm in a cuvette with a layer thickness of 10 mm, using 96 % ethanol as the reference solution. The content of phenolic acids in the raw material in percent (X) expressed as absolutely dry raw material was calculated by the following formula:

$$X = \frac{D \cdot V_1 \cdot 100 \cdot 100}{510 \cdot V_2 \cdot m \cdot (100 - W)}$$

where D was the optical density of the tested solution at the wavelength of 290 nm; V_1 was the volume of the tested solution in milliliters; V_2 was the volume of the aliquot of the tested solution in milliliters; m was the weight of raw material in grams; W was the weight loss after drying raw material, in percent; and 510 was the rate of CO solution absorption at the wavelength of 290 nm for gallic acid.

The content of flavonoids was determined by spectrophotometry: an exact weighed sample of crushed material was placed in a 150 ml flask with a ground glass joint, 30 ml of 90% ethyl alcohol containing 1% concentrated hydrochloric acid were added, the flask was connected to the backflow condenser, heated on a boiling water bath for 1 h, cooled to room temperature, and the mixture was filtered through a paper filter into a 100 ml volumetric flask. Extraction was repeated 2 more times as described above, filtering through the same filter into the same volumetric flask; the filter was washed with 90 % ethyl alcohol, and the filtrate was topped up with the same alcohol to the mark (solution A). 2 ml of solution A were placed in a 25 ml volumetric flask, and 1 ml of 1% solution of aluminum chloride in 95 % ethyl alcohol was added; the solution was topped up with the same solvent to the mark. 20 min later, the optical density of the solution was measured on a spectrophotometer at the wavelength of 430 nm in a cuvette with 10 mm layer thickness. The reference was the solution that consisted of 2 ml of solution A topped up with 95 % ethyl alcohol to the mark in a 25 ml volumetric flask. The content of total flavonoids expressed as quercetin and absolutely dry raw material in percent (X) was calculated by the following formula:

$$X = \frac{D \cdot 100 \cdot 100 \cdot 25 \cdot 100}{764.6 \cdot m \cdot 2 \cdot (100 - W)}$$

where D was the optical density of the tested solution; 764.6 was the absorption rate of the combination of quercetin and aluminum chloride at 430 nm; W was the weight loss after drying raw material, in percent; and m was the weight of the sample of raw material in grams (Muzychkina *et al.*, 2004; Muzychkina *et al.*, 2010).

Results and Discussion

Some plants and prokaryotes can synthesize various biologically active compounds. These may be primary metabolites, which are necessary for the vital functions of the organisms, including photosynthesis and energy consumption; there is also a wide variety of molecules called secondary metabolites. These compounds have important functions in nature, they are used for protection from predators or competitors, and have other ecological values (Šavikin *et al.*, 2009).

While the biosynthesis properties of primary metabolites are the same in eucaryotes and procaryotes, significant differences exist for second metabolites. Understanding these metabolic processes is important for regulating the phytochemical biosynthesis of the necessary substances in plants, and their efficient use in medicine and food industry (Pinto *et al.*, 2007).

The study was aimed at identifying BAS in the aboveground and underground parts of aquatic plants using phytochemical methods.

The objects of the study were BAS in the aboveground and underground parts of *Eichhornia crassipes*, *Pistia Stratiotes*, and *Lemna minor* aquatic plants (roots, stems, and leaves).

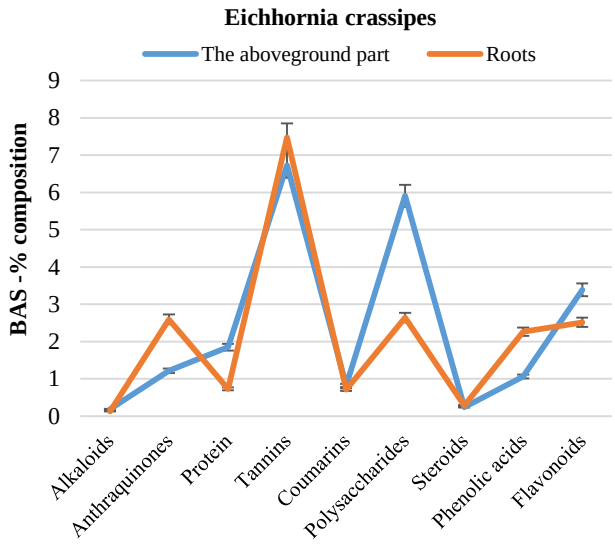

Fig. 1. The amount of BAS found in *Eichhornia crassipes* aquatic plant.

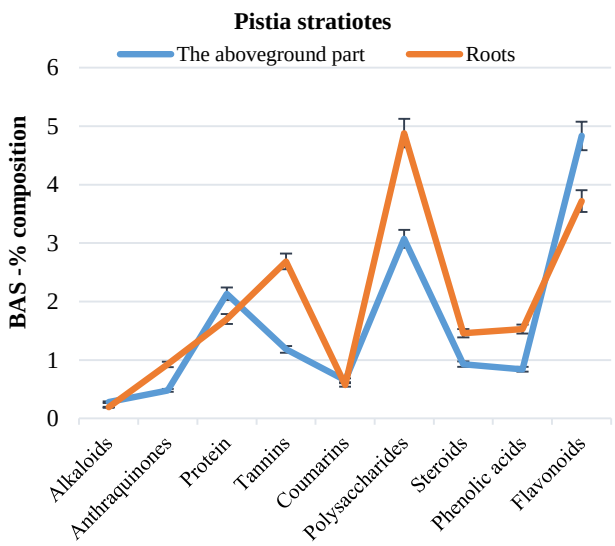

Fig. 2. The amount of BAS found in *Pistia stratiotes* aquatic plant.

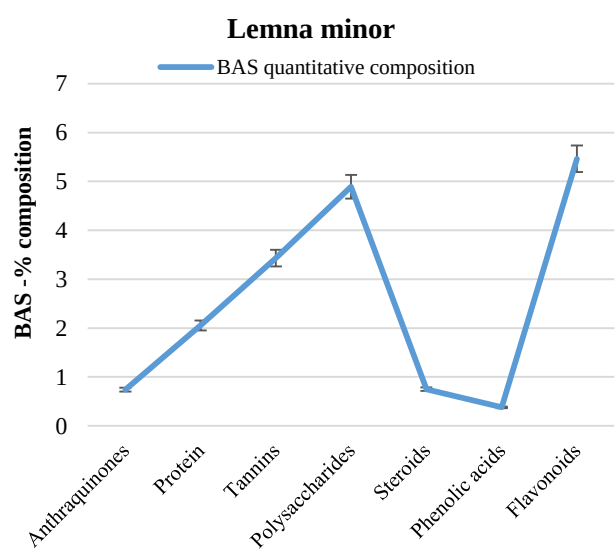

Fig. 3. The amount of BAS found in *Lemna minor* aquatic plant.

As shown in Fig. 1, in the Eichhornia crassipes aquatic plant, tannins are the most abundant; their share in the roots is 7.476%, and in the aboveground part — 6.73%. It was found that the content of polysaccharides was 5.907%, and 2.642% in the roots; in the comparable average quantities, the share of flavonoids in the aboveground part was 3.388%, and in the roots — 2.515%; the share of anthraquinones in the aboveground part was 2.594%, and in the roots — 1.216%, and the share of phenolic acids in the aboveground part was 2.263%, and in the roots — 1.064%. It was found that the minimum identified content of BAS was as follows: alkaloids in the aboveground part — 0.184%, and in the roots — 0.131%; protein in the aboveground part – 1.849%, and in the roots — 0.732%, coumarins in the aboveground part – 0.822%, and in the roots — 0.714%; steroids in the aboveground part – 0.241%, and in the roots — 0.286%. The composition of the plants of *Eichhornia crassipes* was represented by various data in the studies of scholars concerning the peculiarities of use for various useful purposes due to the presence of impurities in various BAS. Many researchers studied the antimicrobial activity of various plant extracts. Using diffuse bioanalysis, the methanol extracts of *Eichhornia crassipes* were tested against microbes (bacteria and fungi), blue-green algae, and cyanobacteria that caused pond bloom.

In practice, in *Eichhornia crassipes* with various concentrations of microelements, such as silver, copper, chromium, cadmium, mercury, nickel, lead, zinc, the following enzymes were activated within 21 days: catalase, peroxidase, superoxide dismutase, and o-deferoxamine (Malar *et al.,* 2016). The methanol extracts from the leaves of the *Eichhornia crassipes* plants were made in two different concentrations (10% and 15%) in the form of smears; the therapeutic properties were studied and tested on rats. In injuries treatment, it showed better results, compared to the variant with observation.

It was noted that in *Pistia stratiotes*, the amount of BAS also in parts of the plant was different. From the data in Figure 2 above, it was found that the greatest share in BAS was taken by polysaccharides and flavonoids. The content of polysaccharides in the aboveground part was 3.073%, and in the roots — 4.881%, and of flavonoids — 4.833% in the aboveground part, and 3.716% in the roots. According to the sources, an increased content of tannins in high quantities was observed during the flowering of plants. Tannins provide protection from phytophages, fungicides, and chemical and mutation factors. They also protect plants from various infections during spraying and fertilization. According to studies of scientists, it was found that in plants, the amount of tannins was high, since high solar radiation contributed to increasing the content of tannins. The decrease in the amount of tannins continues from the period of fertility to the end of the vegetation period (Isebe, 2016). During the study, it was found that the average content of tannins in the aboveground part was 1.182%, and 2.686% in the roots, the minimum content of alkaloids in the aboveground part was 0.192%, and 0.279% in the roots, the content of anthraquinones in the aboveground part was 0.925%, and 0.480% in the roots, the content of coumarins in the aboveground part was 0.573%, and 0.651% in the roots, the content of steroids in the aboveground part was 1.458%, and 0.928% in the roots, and the content of phenolic acid in the aboveground part was 1.53%, and 0.841% in the roots. Due to the accumulation of various active substances in the *Pistia stratiotes* plants, their medicinal properties, etc., they are cultivated specifically for obtaining various products. As the demand for medicines increases over time, there is a need for transforming traditional medicine into a modern pharmaceutical industry for the production of medicinal preparations. The allelopathic effect of the plants is the most efficient source of weed control. Besides, it may be noted about *P. Stratiotes* that this aquatic plant has a high potential in weed control and weed inhibition. Some allelochemical substances cause apoptosis of the vascular cells by producing active forms of oxygen that act as molecules that signal about changes in the hormonal balance during seed growth (Kireyeva & Kitova, 2006). *Pistia Stratiotes* is used as a substrate in the production of biogas. Given the high speed of biogas during inoculation, the average methane content is 58 – 68%, and the concentration of diethyl ketone, butyl, isobutyl, valeric and isovaleric acids is high. The inoculum significantly exceeds the efficiency of inclusion. After adding bacteria to the plant material, they may be used as biofuel, which also contributes to the reduction of water pollution, weed control, elimination of energy problems, and protection of aqueous ecosystems (Khan *et al.*, 2014).

In *Lemna minor* aquatic plant, quantitative dosages of anthraquinones, proteins, tannins, polysaccharides, steroids, phenols, and flavonoids were found.

By the results of the study in Figure 3, the BAS in *Lemna minor* water plant are flavonoids, the content of which was 5.463%, polysaccharides (the average content of 4.892%), tannins (the average content of 3.43%), proteins (the average content of 2.054%), and the minimum amounts were as follows: anthraquinones — 0.741%, steroids — 0.749%, and phenolic acids — 0.378%. The studies of the active properties of *Lemna minor* plant by scientists may be efficiently used in the production of foodstuffs with high content of protein and pectin in plants (Klimova, 2015).

Conclusion

Research results show that the highest amount of BAS was found in *Eichhornia crassipes* aquatic plant in terms of tannins, which in the roots amounted to 7.476%, and in the aboveground part — to 6.73%. The content of polysaccharides was 5.907%, and in the roots — 2.642%. By the amount of BAS detected in *Pistia stratiotes* aquatic plant, the content of polysaccharides in the aboveground part was 3.073%, and in the roots — 4.881%, the content of flavonoids in the aboveground part was 4.833%, and in the roots — 3.716%. The BAS in *Lemna minor* water plant were flavonoids, the content of which was 5.463%.

Chemical compounds with certain properties were found in the leaves and roots of aquatic plants; this contributed to the creation of new highly-effective domestic phytodrugs. Thus, higher aquatic plants are promising for further study because they represent the new source of BAS in the drug development.

References

Barbehenn, R.V. and C.P. Constabel. 2011. Tannins in plant–herbivore interactions. *Phytochem.*, 72(13): 1551-1565.

Buer, C.S., G.K. Muday and M.A. Djordjevic. 2007. Flavonoids are differentially taken up and transported long distances in Arabidopsis. *Plant Physiol.*, 145(2): 478-490.

Hantak, M.M., T. Grant, S. Reinsch, D. McGinnity, M. Loring, N. Toyooka and R.A. Saporito. 2013. Dietary alkaloid sequestration in a poison frog: an experimental test of alkaloid uptake in Melanophryniscus stelzneri (Bufonidae). *J. Chem. Ecol.*, 39(11-12): 1400-1406.

Isebe, T.I. 2016. Phytochemical composition and antibacterial activity of *Eichhornia crassipes* in Lake Victoria, Kisumu. *Int. J. Sci. & Technol. Res.*, 4(8): 45-52.

Khan, M.A., K.B. Marwat, B. Gul, F. Wahid, H. Khan and S. Hashim. 2014. *Pistia stratiotes* L. (Araceae): Phytochemistry, use in medicines, phytoremediation, biogas and management options. *Pak. J. Bot.*, 46(3): 851-860.

Kireyeva, T.B. and Y.A. Kitova. 2006. Ekologicheskiye i ontogeneticheskiye osobennosti nakopleniya dubilnykh veshchestv v trave dushitsy obyknovennoy v usloviyakh Udmurtii. Vestnik Udmurtskogo universiteta. Seriya. *Biologiya. Nauki o Zemle*, 1.

Klimova, Y.V. 2015. Issledovaniye khimicheskogo sostava ryaski maloy (*Lémna mínor*) i perspektivy ispol'zovaniya v pishchevoy promyshlennosti. *Tekhnologiya i tovarovedeniye innovatsionnykh pishchevykh produktov*, 6(35): 3.

Malar, S., S. Shivendra Vikram, P. Jc Favas and V. Perumal. 2016. Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. *Bot. Studies*, 55(1): 54.

Mehrtens, F., H. Kranz, P. Bednarek and B. Weisshaar. 2005. The Arabidopsis transcription factor MYB12 is a flavonol-specific regulator of phenylpropanoid biosynthesis. *Plant Physiol.*, 138(2): 1083-1096.

Muzychkina, R.A., D. Yu. Korulkin and Zh.A. Abilov. 2010. Metodologiya issledovaniya rastitel'nykh metabolitov. *Monografiya. Almaty*: MV-Print, 324.

Muzychkina, R.A., D.Yu. Korulkin and Zh.A. Abilov. 2004. Katchestvennyy i krupnomasshtabnyy analiz osnovnykh grupp lekarstv v farmatsevticheskikh preparatakh. Monografiya. Almaty: *Kazakhskiy Universitet*, 288.

Paredes-López, O., M.L. Cervantes-Ceja, M. Vigna-Pérez and T. Hernández-Pérez. 2010. Berries: improving human health and healthy aging, and promoting quality life - a review. *Plant Foods Hum. Nutr.*, 65(3): 299-308.

Pinto, M.S., F.M. Lajolo and M.I. Genovese. 2007. Bioactive compounds and antioxidant capacity of strawberry jams. *Plant Foods Hum. Nutr.*, 62(3): 127-131.

Robil, J.L.M. and V.S. Tolentino. 2015. Histological localization of tannins at different developmental stages of vegetative and reproductive organs in Medinilla magnifica (Melastomataceae). *Flora-Morphol. Dist. Fun. Ecol. Plants*, 217: 82-89.

Šavikin, K., G. Zdunić, T. Janković, S. Tasić, N. Menković, T. Stević and B. Dordević. 2009. Phenolic content and radical scavenging capacity of berries and related jams from certificated area in Serbia. *Plant Foods Hum. Nutr.*, 64(3): 212-217.

Szajdek, A. and E.J. Borowska. 2008. Bioactive compounds and health-promoting properties of berry fruits: a review. *Plant Foods Hum. Nutr.*, 63(4): 147-156.

Todd, A.T., E. Liu, S.L. Polvi, R.T. Pammett and J.E. Page. 2010. A functional genomics screen identifies diverse transcription factors that regulate alkaloid biosynthesis in Nicotiana benthamiana. *The Plant J.*, 62(4): 589-600.

Tulipani, S., B. Mezzetti, F. Capocasa, S. Bompadre, J. Beekwilder, C.H. de Vos, E. Capanoglu, A. Bovy and M. Battino. 2008. Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *J. Agri. & Food Chem.*, 56(3): 696-704.

Viskelis, P., M. Rubinskiene, I. Jasutiene, A. Sarkinas, R. Daubaras and L. Cesoniene. 2009. Anthocyanins, antioxidative, and antimicrobial properties of American cranberry (*Vaccinium macrocarpon* Ait.) and their press cakes. *J. Food Sci.*, 74(2): 157-161.

Wasternack, C. and B. Hause. 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Ann. Bot.*, 111(6): 1021-1058.

Yang, L. and J. Stöckigt. 2010. Trends for diverse production strategies of plant medicinal alkaloids. *Natural Prod. Reports*, 27(10): 1469-1479.

Yernazarova, G.I., A.A. Ramazanova, L.E. Anuarova, D.Y. Korulkin and B.M. Kudaibergenova. 2018. Method of use of biotechnological research (for example, biologically active substances of higher aquatic plants) in teaching Biology. *J. Edu. Sci.*, 55(2): 66-73.

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