ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Study of Amino Acid Composition and Basic Technological Parameters of Viburnum Fruits Harvested in Ukraine

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Abstract

In present material the study some parameters of viburnum fruits harvested in Ukraine. Generally, the research is carried out in order to increase the product range of medicines. However, the quality of viburnum opulus fruit as raw material plant in Ukraine was not regulated due to a lack of the monograph in SPhU 2.0. The feature of our research is that it is only a fragment of the complex system of pharmacognostic study of viburnum opulus fruit. In which raw materials series from the area of preparation on the territory of Ukraine are used. By means of HPLC the comparative research of component content of the free and linked allocation of amino acids of pericarp and a stone of fruits of *Viburnum opulus L.* is carried out. The content of free amino acids of a stone is the most various-16 connections of such nature. The largest and approximately identical content is characteristic for replaceable amino acid of proline (more that 175 μmol/kg), which is found in a free allocation in a pericarp and a stone and in the linked allocation in a pericarp. Thus, pharmacological and pharmaco-technological tests of crushed fruits of the *Viburnum opulus* were conducted and also the basic technological parameters were recognized. The results of this experiment will be considered and used to study the composition and development of industrial technology of drugs, which based on them.

Keywords: *Fruits Viburnum opulus L., Amino acids study, Pharmaco-technological investigation basic technological parameters raw materials.*

Introduction

Amino acids are an important component both in a human body, and in plants. In a plant cell this class of connections possesses several functions: regulatory, synthesizing and energetic. Regulatory function shows itself depending on development and a functional state in a plant cell-the dynamics of amino acids contest changes. An important function [1] is also biosynthesis of protein which represents the building blocks for further synthesis inside the plant.

Next but not less important the energy role of [2] amino acids is catabolism connections of this type can be considered the decisive regulative mechanism of an energy condition of a plant cell, for example in the carbohydrate deficiency conditions. Amino acids can form protein property, for example, there are some sources of information about the hydrophobicity [3] and resistance to dehydration [4].In recent years it is learnt that amino acids (AAs) are not only cellular signalling molecules, but also both regulators of gene expression [5] and a cascade of protein phosphorylation. Besides, AAs are key precursors for hormone synthesis and low-molecular nitrous substances [6], each of which has considerable biological value.

For proper maintenance of functions in an organism physiological concentration of AAs and their metabolites are required (for example, nitrogen oxide, polyamines, glutathione, taurine, thyroid hormones and serotonin). However, the increased levels of amino acids and their products (for example, ammonia, homocysteine and asymmetric dimethylarginine) are pathogens for neurological disease and cardiovascular diseases. The optimal balance between AAs in a diet is crucial for a homeostasis of all body. A growing recognition of the fact that besides they play a role of blocks of proteins and polypeptides, some AAs regulate the key metabolic pathways necessary for maintenance, growth, reproduction and the achievement of immunity. They are called functional and include arginine, cysteine, glutamine, leucine, proline and tryptophane.

Dietary supplements with one or AAs mixture can be useful to solve the health problems at different stages of life cycle (for example, foetal growth restriction, the neonatal morbidity and death rate connected with depriving intestinal dysfunction and a wasting syndrome, obesity, diabetes, cardiovascular diseases, a metabolic syndrome and infertility). The fruit of Viburnum opulus are object of active studying for decades. Scientists from many countries in their researches claim that this type of raw materials has a number of properties.

In particular, juice from the fruit of viburnum opulus has antibacterial [7], antimicrobic [8], antioxidant [8], antiurolithic [9, 10] properties. There are also data about uses of extracts in fight against cancer tumors [8, 11]. The promising development is the compound of fruit extracts and nanoparticles of metals [12, 13]. The presence of such potential and amount of useful effects can recommend the fruits of this plant for using in food and pharmaceutical industry [14].

The development of modern technological techniques allows the encapsulation of medicinal substances with different physical and chemical characteristics, including the plant origin. It contributes to a significant expansion of the nomenclature of medical forms. Justification and selection of plant raw materials is one of the most important stages of the production of phytopreparations, which will ensure its expected pharmacological action in the future.

We studied the pharmacological and technological properties of, based on the given pharmacological direction, the chemical composition and the main technological approaches to the production of the future medicinal product. Generally, the research is carried out in order to increase the product range of medicines. However, the quality of viburnum opulus fruit as raw material plant in Ukraine was not regulated due to a lack of the monograph in SPhU 2.0. Considerable numbers of national and foreign sources contain the information on various properties of these raw materials. However, in the literature studied by us we did not meet the fruit of viburnum opulus about studying of amino-acid structure, growing in the territory of Ukraine. The feature of our research is that it is only a fragment of the complex system of pharmacognostic study of viburnum opulus fruit. In which raw materials series from the area of preparation on the territory of Ukraine are used.

Materials and Methods

The fruit of Viburnum opulus were prepared in September, 2017 in Donetsk region of Ukraine. Then they were separated from fruit peduncle, dried up in the dryer at T = 60 °C to residual quantity of moisture about 40%. The fruit was divided into the components-a pericarp and a stone. Then they were crushed by cutting method to the size of particles 0.5 mm (mill laboratory LSM, Ukraine).The definition of free and linked amino acids in plant environment by HPLC method [15].

Chromatographic separation was carried out on the liquid Agilent 1200 chromatograph (Agilent technologies, USA). The column Zorbax AAA 150 mm long, with an internal diameter of 4.6 mm, diameter of a sorbent grain of 3 microns. The mobile phase A - 40 mM Na2HPO4 pH 7.8; In - ACN: MeOH: water (45:45:10, v/v/v).

The separation mode is gradient with the constant flow rate of 1.5 ml/min. The temperature of the thermostat of column is 40 0C. The pre-column derivatization was carried out in the automatic programmed mode with the use of FMOC reagent (Agilent 5061-3337) and OPA reagent (Agilent 5061-3335). The detection of derivatized amino acids was implemented by means of the fluorescent detector [16]. Sample preparation and analysis of raw materials: Free amino acids.

The batch of the medicine ground to a powdery state was placed in a viala, added 2 ml of water solution of 1N hydrochloric acid and hold on ultrasonic bath-house WUC-A03H, Daihan (South Korea) at 50 °C within

3 hours. General amino acids. The batch of medicine was placed in a viala, added 2 ml of water solution of 6N hydrochloric acid and placed in thermostat dry-air ST 150C (UOSLab, Ukraine) at 110 °C. Hydrolysis was carried out within 24 hours. 0.5 ml of fugged extract / hydrolysate vaporized on rotor evaporator of Hei-VAP Advantage HL (Germany), washing with the distilled water for hydrochloric acid removal.

Re-suspended in 0.5 ml of the distilled water and also filtered through membrane filters from the regenerated cellulose with pores 0.2 mcm. The receipts of fluorescent derivatives in the automatic programmable mode before introduction of test to the chromatographic column conducted. Identification of amino acids was carried out by comparison of hours of storage with mix of standards of amino acids (Agilent 5061-3334). The content of linked amino acids was determined as a difference between the content of free amino acids and the general content of AAs [17]. Technological parameters.

The purpose of this work was to conduct pharmacal and technological tests to establish the basic technological parameters of fruits of the Viburnum opulus L. Study the basic technological parameters of plant raw material is an important task in developing the technology of phytochemical drug. The calculation of these parameters are necessary for grinding, sifting, mixing, dosing, transportation, setting the cost standards for plant material in order to rational approach to the production process. The main technological parameters of plant raw materials include: fluidity, bulk volume and density, natural slope angle, average particle size, mass loss during the desiccation and other [18].

Testing of powdered mixture of crushed fruits of the Viburnum opulus, namely fractional composition, flowability, which is characterized by the definition of the angle of natural slope and the time of rash, humidity, bulk volume and bulk density before and after shrinking, conducted according to the methodology of the SPhU 2.0. [18, 19].The raw material was pre-grounded using a rotary mill produced by the plant «Spetstekhobladnannya», located in Kharkiv. Determination of mass loss during the desiccation of raw material was performed using the moisture analyzer "Sartorius" brand MA-150 production of Concern Sartorius AG, Germany and according to the methodology of SPhU 2.0. (2.2.32) [18-19]. The raw material in quantity about 5.0 g was dried at 120 °C for 3 h.

The bulk volume (p. 2.9.15), which shows the ability of the plant material before shrinking (V_0) and after (V_{1250} or V_{2500}), was determined at 10, 500, 1250 and 2500 displacement graduated cylinder [20]. The bulk volume, shrinkage capacity, bulk density before and after shrinking were determined using a device for vibration sealing of 545P-AK-3 powders produced by the technological equipment of Mariupol plant (Ukraine).

Flowability (clause 2.9.16) allows to determine the ability of plant material to flow in the vertical direction under the given conditions and the angle of the natural slope is an indicator that is related to intergranular friction or the resistance of the movement between the particles of the material [19]. Since the fluidity and the angle of the natural slope characterized the mobility of raw materials, so they are necessary in the solid dosage forms for the calculation of transporting means. The main technological parameters of fruits of the Viburnum opulus are given in Results and discussion.

Results and Discussion

The qualitative structure both free and linked and replaceable and irreplaceable amino acids is individual for each object. In total 16 amino acids are identified. The stone of Viburnum opulus in a free allocation also contains all of 16 amino acids. In the linked allocation 12 amino acids are identified, aspartic and glutamic acids, a lysine and proline are not found. In pericarp 14 amino acids are found in a free allocation (histidine and lysine are not found) and 13 Aas-in linked allocation (threonine is not found) (Table 1).

Table 1:Amino acids descripton

Amino acids	Pericarp		Stone	
	Free	Linked	Free	Linked
Aspartic acid	1.41	1.62	1.41	–
Glutamic acid	2.53	2.70	2.53	–
Serine	6.21	6.25	6.21	6.23

Histidine	–	–	7.44	7.45
Glycine	7.74	7.76	7.74	7.75
Threonine	7.99	–	7.98	7.99
Arginine	9.06	9.08	9.06	9.07
Alanine	9.46	9.46	9.46	9.46
Tyrosine	10.97	10.97	10.97	10.96
Valin	13.09	13.07	13.09	13.17
Methionine	13.37	13.36	13.37	13.35
Isoleucine	14.81	14.79	14.81	14.79
Phenylalanine	15.02	15.01	15.02	15.01
Leucine	15.77	15.76	15.77	15.75
Lysine	–	–	16.34	–
Proline	20.19	20.35	20.20	–
ε	147,62	140,18	171,4	130,98

Replaceable amino acids in the pericarp both in free, and in linked allocation, are presented with six identical amino acids. The qualitative content of the replaceable amino acids of a stone in a free allocation is identical with the pericarp. In linked allocation replaceable amino acids of a stone are presented with only three connections (Table 2).

Table 2: Replaceable amino acids of a pericarp and stone *Viburnum opulus* fruits

Amino acids	Pericarp		Stone	
	Free	Linked	Free	Linked
Aspartic acid	1,41	1,62	1,41	–
Glutamic acid	2,53	2,7	2,53	–
Serine	6,21	6,25	6,21	6,23
Glycine	7,74	7,76	7,74	7,75
Arginine	9,06	9,08	9,06	9,07
Proline	20,19	20,35	20,2	–
ε	47,14	47,76	47,15	23,05

The content of each amino acid is comparable in all objects.

10 amino acids are contained in the pericarp and stone in the free and linked allocation. Among them 3 are replaceable-serine, glycine and arginine.

Aspartic acid is found in the smallest quantities (10.59 µmol/kg-12.17 µmol/kg) and glutamic acid (17.20 µmol/kg-18.35 µmol/kg).

The largest content was established for proline amino acid-higher than 175 µmol/kg.

Almost the same content of phenylalanine and leucine in both objects – both in free, and linked allocation-a little bit higher than 15 µmol/kg µmol/kg µmol/kg µmol/kg. The content of isoleucine fluctuates within 14.39 µmol/kg - 14.91 µmol/kg, methionine 13.35 µmol/kg - 13.37 µmol/kg. The content of valin is slightly higher than 13 µmol/kg. (Table 3).

Table 3: Essential amino acids of a pericarp and stone *Viburnum opulus* fruits

Amino acids	Pericarp		Stone	
	Free	Linked	Free	Linked
Histidine	–	–	7,44	7,45
Threonine	7,99	–	7,98	7,99
Alanine	9,46	9,46	9,46	9,46
Tyrosine	10,97	10,97	10,97	10,96
valin	13,09	13,07	13,09	13,17
methionine	13,37	13,36	13,37	13,35
isoleucine	14,81	14,79	14,81	14,79
Phenylalanine	15,02	15,01	15,02	15,01
Leucine	15,77	15,76	15,77	15,75
Lysine	–	–	16,34	–
ε	100,48	92,42	124,25	107,93

The content of alanin is the same in all objects and made 106.17 µmol/kg. The content of argnin fluctuates within 52. µmol/kg-52.12 µmol/kg. The content of glycine and a treonin is lower. The largest total content of amino acids is established in a free allocation in a stone-171.4 µmol/kg, and the smallest one-also in a stone in the linked allocation-is 1.3 times lower -130.98 µmol/kg. The content of the total free and linked amino acids in the pericarp makes, respectively, 144.62 µmol/kg and 140.18

μmol/kg. The content of replaceable amino acids in linked allocation is twice lower than in the pericarp. And the content of irreplaceable amino acids in the free allocation in a stone is higher than in other objects. Amino-acid proline performs multifunctional biological properties in plants in the conditions of a stress (soil salinization, the shortage of water, UF-radiation, influence of heavy metals). The increasing of its content is considered as universal plant response to a stress [20].

For its part, in a human body proline promotes the development of collagen, strengthening of a cardiac muscle, improvement of connective tissue that is important for ligaments and joints, kidneys, a liver, vessels.

The results of determination of basic technological parameters crushed and dried fruits of Viburnum opulus summarized in Table 4.

Table 4: The results of determination of basic technological parameters crushed and dried fruits of Viburnum opulus (n = 5); P = 0.95

Technological parameters	Crushed and dried fruits of the Viburnum
Sample weight, g	100,08±0,06
The loss in weight on drying, %	6,25±0,04
Flowability, sec/100 g	17,5±0,4
The angle of natural slope, degree.	33,0±0,3
The bulk volume, ml. V_0 V_{10} V_{500} V_{1250} V_{2500}	 204,6±0,5 194,2±0,3 181,7±0,3 179,7±0,3 179,3±0,6
Shrinkage ability $V_{10} – V_{2500}$, ml	25,3±0,3
Bulk density, g/cm^3	0,49±0,01
Average particle size, mkm.	15,780
Hausnera Index	1,14±0,01
Compressibility index (Carra Index), %	12,36±0,05
Density after shrinkage, m/V_{2500}, g/ml	0,56±0,01

According to external features, crushed and dried fruits of the Viburnum opulus are looking like a bits, which pass through a sieve with holes of 0.2 mm. The color is from cherry to light brown with yellow inlaid. The smell is pleasant, specific. The taste is tart and sour. The results of the obtained technological properties of the investigated substance prove that the crushed fruits of the Viburnum opulus have a satisfactory value of fluidity.

This fact is confirmed by the average particle size, as well as the relatively satisfactory value of the angle of the natural slope. Indicators - the Hausnera Index (1.14) and the Carra Index (12.5%)-also indicate a satisfactory fluidity. A slight difference in the values of bulk density and density after shrinkage is indicates on stability during storage and transportation.

Conclusion

By means of HPLC the comparative research of component content of the free and linked allocation of amino acids of pericarp and a stone of fruits of *Viburnum opulus* is carried out. The content of free amino acids of a stone is the most various-16 connections of such nature. The largest and approximately identical content is characteristic for replaceable amino acid of proline (more that 175 μmol/kg), which is found in a free allocation in a pericarp and a stone and in the linked allocation in a pericarp.

Thus, pharmacological and technological tests of crushed fruits of the *Viburnum opulus* were conducted and also the basic technological parameters were recognized. The results of this experiment will be considered and used to study the composition and development of industrial technology of drugs, which based on them.

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