

Chapter

Research of Antioxidant Activity of *Aronia melanocarpa* Fruits and *Viburnum opulus* Fruits

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Abstract

The general interest and the possibility of extensive use of polyphenol compounds determines the relevance of search of new low-cost and available sources of raw material. In this aspect the *Aronia melanocarpa*, *Viburnum opulus* are promising. I decided to determine the optimal concentration of ethyl alcohol, providing the highest antioxidant activity were prepared tinctures *A. melanocarpa* fruits, *Viburnum opulus* fruits and their combination. For this I took tinctures were made 1: 5 with percolation. Alcohol (40%, 70%, 95%) was used as an extracting agent; medicinal plant material - *V. opulus* fruits, *A. melanocarpa* fruits. Study of AOA was carried using test systems and method of EPR spectroscopy. Statistical analysis of the obtained data was done with Statgraphics (n = 6). Conclusion: The highest antioxidant activity was shown tincture *V. opulus* fruits 40%. In that way the optimal concentration of ethyl alcohol, providing the highest antioxidant activity is 40%.

Keywords: antioxidant activity, *Aronia melanocarpa* fruits, *Viburnum opulus* fruits, tincture, plant

1. Introduction

The development of modern medicine makes it possible to prevent and cure many diseases. At the same time, significantly increased the number of chronic diseases has greatly increased. In search of new effective and safe drugs, clinicians and pharmacologists turn to medicinal plants.

The organism has its own system of fighting excessive amounts of free radicals, but it is weakened by the adverse effects of the environment and needs constant support from outside. Many studies have proven that flavonoids are able to bind free radicals, therefore, exhibit antioxidant activity.

The “French paradox” that caused surprise made scientists pay attention to polyphenolic compounds of plants. Polyphenols, diverse in their structure, have a diverse pharmacological effect on the human body.

The general interest and the possibility of extensive use of polyphenol compounds determines the relevance of search of new low-cost and available sources of raw material. In this aspect the *A. melanocarpa*, *V. opulus* are promising [1].

1.1 *A. melanocarpa*

Aronia prune - *A. melanocarpa* (Michx.) Elliott., family of *Rosaceae* - *Rosaceae*. A few decades ago, few people knew about Aronia prune. Since 1843, aronia has been grown only in botanical gardens. However, local residents of Altai began to taste the fruits of an unknown plant, as a result of which many began to feel much better: headaches stopped, gastrointestinal disorders passed, sleep improved. These observations formed the basis for a detailed study of the chemical composition of berries and the determination of their therapeutic effect in the clinic, which began in 1953.

A. melanocarpa is a small shrub up to 2.5 m high. Shoots are numerous with simple whole leaves obovate and saw-edged; leaves are dark green, turning red in autumn. The flowers are regular, five-membered, white or slightly pink, collected in corymbose inflorescences **Figure 1**.

1.2 The main groups of biologically active substances of *A. melanocarpa*

Aronia fruits contain a P-vitamin complex consisting of flavonoids: rutin, catechins, quercetin, hesperidin, anthocyanins, as well as a significant amount of ascorbic acid (up to 110 mg%).

Other groups of BAS:

1. tannins (upto 0.6%),
2. organic acids,
3. sugars (fructose, glucose, etc. (up to 10%)),
4. glucoside-amygdalin (5–30 mg),
5. pectin substances (up to 0.75%),
6. vitamins of group B, A, C (up to 100 mg%).



Figure 1.
Appearance of Aronia melanocarpa.

Of inorganic substances, fruits contain:

Aronia is distinguished by a large set of trace elements — it contains boron, fluorine, iodide compounds (6–10 micrograms per 100 g of fresh fruit), iron, copper, manganese, molybdenum. Se accumulates.

From the above, it can be concluded that the chemical composition of various parts of the plant is quite diverse, but the main class of biologically active substances contained are flavonoids [2].

1.3 Pharmacological properties

Aronia lowers cholesterol in the blood of patients with atherosclerosis. Fresh, dry, pureed fruits with sugar and juice of *aronia* are used for therapeutic and prophylactic purposes in various conditions accompanied by increased permeability of blood vessels — glomerulonephritis, radiation damage, rheumatism, allergies, measles, typhus, scarlet fever.

The fruits of *aronia* contain sorbitol, which is important for the nutrition of patients with diabetes mellitus, with diseases of the liver and biliary tract. Fruits and juice of *aronia*, which preserve vitamins well, increase the body's defenses, increase appetite, increase the digestibility and acidity of gastric juice.

Fresh fruits are used as a vitamin remedy for hypo- and vitamin deficiency P and hypertension of stages 1 and 11.

The juice helps strengthen the walls of blood vessels. The leaves contain substances that improve liver function, the formation and outflow of bile [3].

Recently, substances have been identified in *aronia* that increase blood pressure to normal, if it was reduced. P-active compounds of *aronia* fruits have the ability to bind and remove radioactive strontium from the body. Preparations from *aronia* have vasodilating properties.

Among many doctors, there was a fear that the use of *aronia* would cause increased thrombosis in the human body. This fear was based on the fact that *aronia* contains a large number of substances with P-vitamin properties. However, experimental and clinical studies have shown that *aronia* affects blood clotting only in cases when it is lowered, that is, with bleeding.

1.4 *Viburnum opulus*

V. opulus is a small branching shrub or a small tree 1.5–4 m high with graybrown bark. The leaves are petiolate, opposite, broadly ovate or rounded, 5–8 cm long and 5–8 cm wide, three-five-lobed **Figure 2** [1].

The main groups of biologically active substances of *viburnum vulgare*: the fruits contain carbohydrates: fructose, glucose, sucrose, mannose, galactose, xylose, arabinose, polysaccharides; pectin substances, organic acids: acetic and isovaleric (up to 3%); triterpenoids: oleanolic and hederagenic acids and their acetyl derivatives, ursolic acid; steroids, vitamin C (up to 0.09%) and carotene; phenol-carboxylic acids and their derivatives. Also flavonoids, tannins, catechins.

1.5 Pharmacological properties of *Viburnum opulus*

The infusion is used as a vitamin, diaphoretic, diuretic and disinfectant. Liquid extract and decoction, used as a hemostatic agent, mainly for uterine bleeding.



Figure 2.
Appearance of Viburnum opulus.

Flavonoids are a numerous group of phenolic compounds, the structure of which is based on a skeleton consisting of 2 benzene rings connected by a three-carbon chain [4].

1.6 Pharmacological action of flavonoids

Most flavonoids have high P-vitamin activity, that is, they are able to reduce the fragility and permeability of capillary walls. Currently, preparations with antiinflammatory and anti-ulcer effects, as well as choleretic and hepatoprotective have been obtained on the basis of flavonoids. As a result of recent studies, hypoglycemic and antiviral drugs have been obtained.

Flavonoids have an antispasmodic effect. Flavonols mainly affect the neutralizing function of the liver, the mechanism of action is associated with changes in redox processes in the mitochondria of liver cells.

Isoflavonoids have a moderate estrogen-like effect, they are sometimes referred to as phytoestrogens. Raw materials containing these compounds (species of the genus clover) are used in the production of biologically active additives, which are used in gynecology for various hormonal disorders.

Thus, it can be concluded that flavonoids have versatile pharmacological activity, which, with low toxicity, makes them a very valuable and promising group of biologically active substances [1, 2].

1.7 Antioxidant activity of plant material

Antioxidants are substances that slow down or prevent the oxidation of organic compounds. They protect our body from the bad effects of free radicals. The antioxidant creates a barrier to the destructive action of an additional electron when it combines with a free radical. The body converts the cellular oxidant into water and oxygen with the help of an enzymatic defense system.

Oxygen is a powerful oxidizer, oxidation reactions with its participation are a source of energy for many living organisms. On the other hand, oxygen compounds

are formed during metabolism, which destroy the cell structure. As a result, metabolism is disrupted not only in the cell, but throughout the body. The role of antioxidants is to bind and remove free radicals from the body.

The body has its own system for combating excessive amounts of free radicals, but it is weakened due to exposure to polluted environments, smoking, direct sunlight and needs support. It was found that many plants contain substances flavonoids - a large group of compounds with a polyphenolic structure that bind free radicals, that is, they are antioxidants [5].

1.8 The role of free radicals in a living organism

A free radical is an atom or group of atoms having an unpaired electron at the last electronic level, which makes them extremely unstable. In this state, free radicals trap vulnerable proteins, enzymes, lipids, and even whole cells. By taking an electron from a molecule, they inactivate cells, thereby disrupting the fragile chemical balance of the body. When the process happens again and again, a chain reaction of free radicals begins, while cell membranes are destroyed, important biological processes are undermined, mutant cells are created. Free radicals can reversibly or irreversibly destroy substances of all biochemical classes, including free amino acids, lipids, carbohydrates and connective tissue molecules [6].

Over the past few years, it has been proven that antioxidants are extremely useful for the body - they prevent the development of cardiovascular diseases, protect against cancer and premature aging, also increase immunity and much more. The last decade has provided a lot of evidence proving that free radicals play a role in the development of many diseases. If free radicals oxidize lipids, a dangerous form of lipid peroxide is formed. Many scientists associate the formation of lipid peroxides with cancer, heart disease, accelerated aging and immune deficiency.

Free radicals are constantly formed in the body, so there must be antioxidant protection from them, which is one of the most important components of immunity in general. It is important to supplement your diet with natural substances - antioxidants, which enhance protection against free radicals, thereby increase immunity, the body's resistance to adverse external factors, slow down the aging process [5].

The most important antioxidants are: vitamins C, E, beta-carotene, selenium, bioflavonoids (vitamin-like substances contained in the skin of plants - oranges, lemons, tomatoes, etc.). Many plant extracts, vitamins, amino acids, minerals, trace elements have antioxidant properties either directly or indirectly, as they are part of antioxidant enzymes.

About 5000 flavonoids - antioxidants with a wide range of healing effects were found in plants. They have vasodilating, antitumor, anti-inflammatory, bactericidal, immunostimulating and antiallergic properties.

Any organism can be considered as an example of a balanced and well-functioning antioxidant system consisting of many components - vitamins (vitamins C, E, P), enzymes and trace elements (selenium, zinc), polyphenolic compounds (flavonoids), and sulfurcontaining amino acids (cysteine, methionine), as well as the tripeptide glutathione. These are only some compounds that have an antioxidant effect. The chemical nature of these compounds is diverse, among them there are both water- and fat-soluble components. The main principle on which the action of the antioxidant system of a living organism is based is synergy. It consists in the fact that the components of the system work together, restoring each other and enhancing the effectiveness of the action [6].

In the course of the work, research objects and auxiliary substances were used, which corresponded in terms of qualitative indicators and quantitative content to the requirements of regulatory documentation.

2. Material and methods

Tinctures were made 1:5 with percolation. Alcohol (40%, 70%, 95%) was used as an extracting agent; medicinal plant material - *V. opulus* fruits, *Aronia melanocarpa* fruits. Study of AOA was carried using test systems and method of EPR spectroscopy. Statistical analysis of the obtained data was done with Statgraphics (n = 6).

Tinctures were obtained in a ratio of 1:5 by percolation, extractant - 40%, 70%, 95% ethyl alcohol. As medicinal plant raw materials used: *V. opulus* fructus, *A. melanocarpa* fructus, and their combination (0.5,0.5). The total content of flavonoids in terms of catechin was carried out as follows: for the preparation of the standard, 20 mg of catechin was taken and placed in a 50 ml flask, brought to the mark with purified water. 1.4 ml of purified water and 100 ml of the test sample, 60 ml of 5% sodium nitrite solution and 60 ml of 10% aluminum chloride solution were placed in the cuvette and mixed well. To prepare a reference sample, 1.4 ml of water, 100 ml of catechin solution, 60 ml of 5% sodium nitrite solution and 60 ml of 10% aluminum chloride solution are placed in a cuvette. They were kept for 5 minutes in a thermostat at 25°C, then 0.4 ml of IM NaOH was added, mixed well and the optical density was measured at 510 nm. Further calculations were carried out according to the calibration schedule [7].

The antioxidant activity was determined using the free radical 1,1-diphenyl-1-picrylhydrazyl (DPPG), using electron paramagnetic resonance (EPR). This method is based on the determination of changes in the concentration of DPPH as a result of a reaction with an antioxidant. EPR operates under the influence of a magnetic field with a constant frequency (microwave). The EPR spectrometer at the Faculty of Pharmacy of the Warsaw Medical University operates in the X band (microwave 9.4 GHz) [8].

Dilute 50 ml of each tincture with water. Add a DFGP of about 1.3 mm. Stir on a Vortex machine for 30 seconds. The solution is kept for 20 minutes in a dark place and re-mixed. The capillary is filled with the resulting solution and EPR spectra are recorded [9].

3. Research results and their discussion

The content of flavonoids (in terms of catechin) in tinctures (mg KA/ml): *Aroniae melanocarpae* + *Viburni opuli* 40% - 5.9766 ± 0.455576 ; *Aroniae melanocarpae* 70% - 1.00667 ± 0.253739 ; *Aroniae melanocarpae* + *Viburni opuli* 95% - $2.07 + 0.0496828$; *Aroniae melanocarpae* 70% - $548,667 \pm 0,339,095$; *Aroniae melanocarpae* 95% - 0.713333 ± 0.0143422 ; *Aroniae melanocarpae* 40% - 3.37 ± 0.430265 ; *Viburni opuli* 70% - $5.17333 + 0.539883$; *Viburni opuli* 40% - $6.00333 + 0.6212$; *Viburni opuli* 95% - 2.26333 ± 0.248827 **Figure 3**. Statistical processing in appendix 1 **Tables 1–3**.

Thus, the following conclusions can be drawn:

1. The quantitative content of flavonoids in tinctures based on *Viburnum opulus* fructus, *Aroniae melanocarpae* and their combinations were determined;

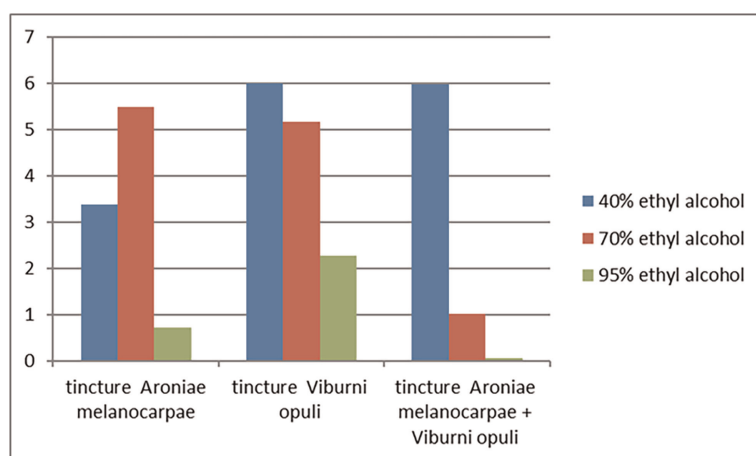


Figure 3.
The content of flavonoids in the studied tinctures.

2. The optimal alcohol concentration was determined, which provides the highest yield of flavonoids. Such concentrations are 40% and 70%:

- In tinctures based on *Aronia melanocarpa* fructus, the largest amount of flavonoids is contained in 70% concentration and is 5.48667 ± 0.339095 ;
- In a tincture based on *V. opulus* fructus, the highest content of flavonoids is manifested in 40% concentration and is 6.00333 ± 0.6212 ;
- In the combined tincture, the highest content of flavonoids is observed in 40% concentration and is 5.9766 ± 0.455576 .

3. It was found that the largest amount of flavonoids contains 40% tincture of 1 krnoaddU-JCIJS (16.00333 ± 0.6212).

Antioxidant activity (mg/ml): Aroniae melanocarpae 40% - 44.63 ± 0.08618 , Aroniae melanocarpae 70% - 13.86 ± 0.06992 , Aroniae melanocarpae 95% - 18.09 ± 0.02548 , Aroniae melanocarpae + Viburni opuli 40% - 57.76 ± 0.08455 ; Aroniae melanocarpae + Viburni opuli 70% - 71.39 ± 0.3164 ; Aroniae melanocarpae + Viburni opuli 95%- 24.55 ± 0.07154 , Viburni opuli 40% - 162.97 ± 0.0322 ; Viburni opuli 70% - 158.06 ± 0.0186 ; Viburni opuli 95%- 37.06 ± 0.08178 **Figure 4.** Statistical processing in appendix 2 **Tables 4-6.**

The results of the study were processed statistically using STATGRAPHICS Plus for Windows (n = 6).

Thus, the following conclusions can be drawn:

1. Antioxidant activity was determined in tinctures based on viburnum vulgaris, aronia melanocarpus and their combinations;

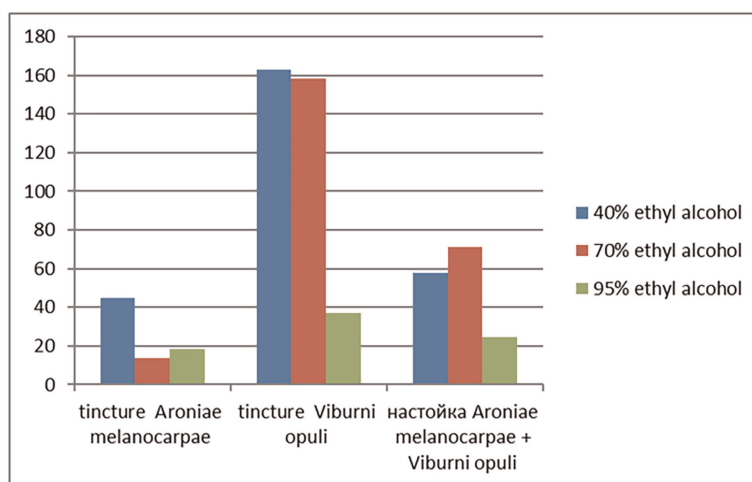


Figure 4.
Results of antioxidant activity in the studied tinctures.

2. Having determined at what concentration of alcohol the greatest antioxidant activity is manifested, we have obtained that:

- In tinctures based on *Aronia melanocarpa* fructus, the greatest antioxidant activity is manifested in 40% concentration and amounts to 44.63 ± 0.08618 ;
- In the tincture based on *viburnum* ordinary fruit, the greatest antioxidant activity is manifested in 40% concentration and is 162.97 ± 0.0322 ;
- In the combined tincture, the greatest antioxidant activity is manifested in 70% concentration and is 71.39 ± 0.3164 .

3. It was found that the greatest antioxidant activity is shown by 40% tincture of *viburnum vulgare* (162.97 ± 0.0322).

4. Conclusion

The highest antioxidant activity was shown tincture *Viburnum opulus* fruits 40%. In that way the optimal concentration of ethyl alcohol, providing the highest antioxidant activity is 40%.

A. Appendices 1

See **Tables 1–3**.

Raw material	Alcohol concentration (%)	Optical density	Content of flavonoids (divided), mg KA/ml	Content of flavonoids (concentrated), mg KA/ml	Average
A. melanocarpa fructus	40	0.220	0.16	3.47	3.37 ± 0.430265
		0.241	0.17	2.47	
		0.241	0.17	3.47	
	70	0.073	0.005	0.88	5.48667 ± 0.339095
		0.060	0.04	0.89	

Table 1.
Results of determining the quantitative content of flavonoids in the tincture of Aronia melanocarpa fructus.

Raw material	Alcohol concentration (%)	Optical density	Content of flavonoids (divided), mg KA/ml	Content of flavonoids (concentrated), mg KA/ml	Average
V. opulus fructus	40	0.220	0.16	3.17	6.00333 ± 0.6212
		0.241	0.17	3.47	
		0.241	0.17	3.47	
	70	0.364	0.26	5.37	5.17333 ± 0.539883
		0.375	0.27	5.37	
		0.345	0.25	4.94	
	95	0.149	0.11	2.16	2.26333 ± 0.248827
		0.157	0.11	2.27	
		0.163	0.12	2.36	

Table 2.
Results of determining the quantitative content of flavonoids in the tincture of Viburnum opulus fructus.

Raw material	Alcohol concentration (%)	Optical density	Content of flavonoids (divided), mg KA/ml	Content of flavonoids (concentrated), mg KA/ml	Average
Aroniae melanocarpae + Viburni opuli fructus	40	0.428	0.31	6.12	5.9766 ± 0.455576
		0.422	0.30	6.04	
		0.403	0.29	5.77	
	70	0.388	0.28	5.55	5.48667 ± 0.339095
		0.372	0.27	5.33	
		0.390	0.28	5.58	
	95	0.144	0.10	2.09	2.07 ± 0.0496828
		0.141	0.10	2.05	
		0.143	0.10	2.07	

Table 3.
Results of determining the quantitative content of flavonoids in the tincture of Aroniae melanocarpae + Viburni opuli fructus.

B. Appendices 2

See Tables 4–6.

Raw material	Alcohol concentration (%)	DDPH (mg/ml), divided	DDPH (mg/ml), concentrated	Average
<i>A. melanocarpa fructus</i>	40	2.07	41.47	44.63 ± 0.08618
		2.11	42.29	
		2.51	50.12	
	70	0.28	5.65	13.86 ± 0.06992
		1.02	20.44	
		0.77	15.48	
	95	0.09	1.85	8.09 ± 0.02548
		2.09	41.85	
		0.53	10.56	

Table 4.
Results of determination of antioxidant activity in tincture of *Aronia melanocarpa fructus*.

Raw material	Alcohol concentration (%)	DDPH (мг/мл), divided	DDPH (мг/мл), concentrated	Average
<i>V. opulus fructus</i>	40	4.14	206.89	162.97 ± 0.0322
		3.09	154.57	
		2.55	127.44	
	70	0.98	68.71	158.06 ± 0.0186
		4.21	294.82	
		1.58	110.66	
	95	0.30	14.88	37.06 ± 0.08178
		0.96	47.96	
		0.97	48.34	

Table 5.
Results of determination of antioxidant activity in tincture of *Viburnum opulus fructus*.

Raw material	Alcohol concentration (%)	DDPH (mg/ml), divided	DDPH (mg/ml), concentrated	Average
Aroniae melanocarpae + Viburni opuli fructus	40	2.96	59.18	57.76 ± 0.08455
		2.38	47.53	
		3.33	66.57	
	70	3.10	62.08	71.39 ± 0.3164
		3.88	77.64	
		3.72	74.45	
	95	1.11	22.19	24.55 ± 0.07154

Table 6.
Results of determination of antioxidant activity in tincture of Aroniae melanocarpae + Viburni opuli fructus.

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