TECHNOLOGICAL PARAMETERS OF COUNTERCURRENT EXTRACTION: DERIVING BIOACTIVE COMPOUNDS FROM PLANT RAW MATERIALS

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Abstract: Wild-growing plants produce a wide range of vitamins, macro- and microelements, mineral salts and other biologically active substances. Even in minute quantities, these constituents can mediate the healing effect on a human body. To date, the most promising method of extracting biologically active compounds from crude wild plant material is solvent extraction. The intensification of the extraction process has seen a new development in the last few years. This paper focuses on obtaining maximum yield of bioactive compounds from wild-growing plants, such as ordinary cowberry, prickly wild rose, sea-buckthorn, ordinary rowan, guelder-rose by a countercurrent extraction method, a continuous process where the plant material moves against the solvent. With that, it examines various extraction agents and their mixtures in relation to the extractives and total flavonoid content, the optimal time of the duration of the extraction. The optimal temperature of the process was found to be corresponding to the boiling point of the solvent. In the future, this study might contribute to the development of high quality new galenical preparations with maximum content of biologically active substances identical to the composition of the source plants to produce pharmaceutical drugs, food supplements, functional foods and cosmetics.

Keywords: wild-growing plant material, biologically active compounds, countercurrent extraction, extraction agent, extractives, flavonoids

INTRODUCTION

Medicinal plants remain one of the most valuable natural resources, their significance only growing in importance [1].

Medicinal plants produce many chemical compounds that are biologically active yet in other organisms, such as human or animal, and are the source for the materials used for medicinal purposes. At present, the world utilizes at least 21.000 species; the Russian Federation is home for 10% of those. 3.000 are used in folk medicine; modern medicine employs 200 species [1, 2].

Medicinal crude plant material is herbs (entire or parts) and herbal materials, harvested by different methods, and used dried or occasionally, fresh as a herbal remedy, or processed prior to formulating a finished medicinal product [3]. With that, medicinal product (also pharmaceutical drug) is a substance(-s) from plant, animal or synthetic origins pharmacologically active and allowed by an agency to be marketed to treat, cure, prevent and diagnose a human or animal disease [1].

Up to 40% of pharmaceuticals in Russian are produced from medicinal plant material. In the world market, every third drug is from plant sources [1]. Some herbs are used straightforwardly as ready-to-take remedy (in the form of total water extractions e.g. infusions, decoctions. Herbal medicines are chemically close to human body, are less intrusive, cause fewer side effects and well-tolerated if taken over extensive period of time [4].

Most medicinal plant material, though, is employed in production of pharmaceutical drugs. These may include the following:

– total ethanol extractions which are galenic preparations such as tinctures, essences;
– new galenic preparations highly purified to remove excipients and inerts;
– individual preparations on the basis of isolated biologically active constituent with a targeted action;
– combined products composed from both herbal preparation and synthetic compound [5, 6].

Plants produce a variety of chemical compounds that can be found in all embryophytes (polysaccharides, proteins, salts), as well as compounds specific to a smaller range of plants. Pharmaceutically and operationally, chemical compounds from plants are divided into active, excipient and inert [4, 7].

Acting i.e. biologically active substances provide specific treatment effect on a human body. It is the active chemicals produced by a plant that define value of each of the medicinal plant. These can be classified into major groups of alkaloids and glycosides, coumarins, vitamins, essential oils, phenols and others groups of chemicals. .

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Excipients are compounds present alongside the active constituents and share similar physical and chemical properties mainly, in regards to solubility in extraction agents. Excipients do not mediate any healing effect but are able to confer a therapeutic enhancement on the active substance (microelements, carbohydrates, etc.), to increase or otherwise, decrease absorption (for example, tannin slows down certain substances absorption) or to remain neutral.

Inert substances are known to produce sometimes undesirable effect on human body, e.g. resins, volatile oils and tannin in certain cases. Inerts may adversely affect the stability of medicines, they can easily decompose during storage, cause residue formation, and thus, reduce the shelf life of liquid herbal preparations (infusion, liquid extracts, fresh juices) [1, 5, 8, 9].

Extraction is a main technological method to derive biologically active compounds from plant or animal source.

Extraction can be defined as a method of separating the substance from solution or dry mixture by using selective solvent (also extraction agent, extractant). When extracting from plant, the primary challenge one faces is cell wall permeability for solvent's cell when passing through in and extractives upon passing through out [5].

In general, there are two extractions methods, static and dynamic. In the first case, the plant material is soaked in solvent and macerated for some period of time. Dynamic extraction involves either ongoing replacement of extracting agent or continuous circulation of the extraction agent and the plant material [8].

Parameters that define extraction ratio of biologically active compounds from herbs are as follows:

1. Origin and concentration level of solvent (extraction agent)
2. Duration of the process
3. Temperature during extraction
4. Particle fineness of the material
5. Concentration differences
6. The material load density
7. Mass fraction of the material relative to mass fraction of solvent [5, 8].

In addition to the above-listed factors, of importance, is such plant material properties as porosity or void fraction, which is a measure of void spaces either inside the plant tissue or between the particles of the material fragmented. Porosity or void fraction value defines the time those materials need to soak and swell. The swelling speed grows when the material is vacuum treated prior to soaking, and with the temperature and pressure rise [5].

To select the objects of study, we first analyzed the composition of some wild-growing plant material in Siberian federal district (*okrug*).

Ordinary cowberry (*Vaccinium vitis idaea L.*) is a short evergreen plant in the Heath *(Ericaceae)* family, of a genus *Vaccinium*. The scientific medicine uses leaves of the cowberry (*Folium Vitis idaeae*) in decoctions and infusions, macerations for disinfectant and diuretic effect as well as cowberry shoots (*Cormus Vitis idaeae*).

Cowberry leaves produce phenollic glycosides arbutin (up to 9%) and methyl arbutin, vaceinin, lycopene, hydroquinone derivatives, ursolic, tartaric, gallic, quinic and ellagic acids, tannin, hyperoside and other flavonoids. The leaves contain ash – 6.33%; macroelements (mg/g): K – 8.00, Ca – 11.00, Mn – 2.20, Fe – 0.60; microelements (BAC) Mg – 0.47, Cu – 0.90, Zn – 0.71, Co – 0.09, Cr – 0.27, Al – 0.49, Ba – 0.89, V – 0.07, Se – 3.25, Ni – 0.14, Sr – 5.96, Pb – 0.05, Ag – 8.00, B – 40.00 mcg/g. Mo, Cd, Li, Au, I, Br are not found. The leaves concentrate Fe, Cu, Zn, Se, Sr, Ag, Ba, Mn, especially Sr, Ag. They are able to accumulate Mn, Cu, Cr [3, 10].

Ordinary guelder-rose (*Viburnum opulus*) is a deciduous shrub of a genus *Viburnum* in the family *Adoxaceae*. Fruits are edible. Guelder-rose fruits (*Fructus Viburni*) and guelder-rose bark (*Cortex Viburni*) are sources of good medicinal plant material [11].

The guelder-rose fruits contain up to 9% of sugars, primarily glucose and fructose, 0.4-0.9% pectin substances known for food gelling capacity, 1.0–3.3% of organic acids. Unripe fruits have high content of quinic and caffeic acids. The following acids were found in ripe fruits: chlorogenic, malic, citric, quinic, caffeic, valeric, acetic, formic and caprylic. The fruits are rich in vitamins, such as carotenoids, ascorbic acid, vitamin E and phenolics with Vitamin P equiv. action. The fruits offer benefits of flavanols, chlorogenic acids, catechins, anthocyanins, leucoanthocyaninns. They were found to have some rutin, Viburnum glycoside and cyclic alkaloid viburnitol in the presence of protein, tannin and pigment substances. Guelder-rose fruits are rich in potassium (179.5–320 mg/100g), calcium (40.5 mg/100g), and magnesium (17.5 mg/100g), iron (6.1 mg/100g). They also contain phosphorus, manganes, zinc, copper, and some nickel, cobalt, molybdenum, titanium, vanadium, zirconium. The fruits of guelder-rose were discovered to have 13 free acids where serine, glutaminic acid and alanine prevail [10].

Ordinary sea-buckthorn (*Hippophae rhamnoides*) is a dioecious shrub or tree, genus *Hippophae* in the Oleaster (*Elaeagnaceae*) family. It is known for its pharmacological activity and is cultivated as ornamental plant. The fresh fruit (*Fructus Hippophaes rhamnoides recens*) is used for medicinal purposes.

The sea-buckthorn fruits can be described as multivitamin. They produce provitamins A (up to 10.9 mg%) and vitamins B_1, B_2, B_3, B_6, C, E, K, etc. The fruits contain 3–6% sugars (glucose and fructose), organic acids (up to 2.5%) including malic, tartaric, etc., tannins, yellow pigment – quercetin, fatty oils (9% in pulp, 12% in pits). The fruits accumulate the fatty oils composed of triacylglycerols with saturated and unsaturated fatty acids. The latter primarily include monounsaturated acids such as palmitoleic and oleic), pectin, organic acids, tannins, flavonoids, nicotinic and folic acids, macro- and microelements (boron, iron, zinc, copper, manganese, potassium, calcium), sugars and a few classes of natural antibiotics [10, 12, 13].

Prickly wild rose (*Rosa acicularis*) is a shrub, species of Wild rose genus in the Rose family (*Rossaseae)*. The fruits of a plant are a source of sugars, organic acids, vitamins, carotene, flavonoids, tannins, iron salts, salts of manganese, phosphorous salts, calcium and magnesium salts. Its fruits have the highest content of vitamin C, one of the richest plant sources available. They are known to have restorative action on human body, to enhance non-specific (innate) body resistance, to accelerate tissue regeneration, and decrease vascular permeability, to positively influence on carbohydrate and mineral metabolism, and have anti-inflammatory properties. The fruits improve cell-mediated and humoral immunity, and also have cholagogue effect due to presence of organic acids and flavonoids [10, 12].

Ordinary rowan (*Sorbus aucuparia*) is shrub or tree, species of a genus *Sorbus* in the Rose family (*Rosaceae*). Rowan fruits contain sugars (up to 5%), malic, citric, tartaric and succinic acids (2.5%), tannins (0.5%) and pectin (0.5%) substances, sorbitol and sorbose, amino acids, essential oils, salts of potassium, calcium, magnesium, sodium; carotenoids (up to 20 mg%), ascorbic acid (up to 200 mg%), flavonoids, triterpene compounds, bitters, sorbic acid. Modern medicine utilizes fruits to produce multivitamins. It is a source of carotene-containing material [13].

Rowan fruits are rich in Vitamin C (up to 160 mg%) and carotene (up to 56 mg%).

In this research, we focused on selecting a solvent, its nature and concentration, as well as duration and temperature of countercurrent extraction with a view to derive maximum bioactive compounds from wild-growing plant material. We will consider remaining parameters of countercurrent extraction in the future.

OBJECTS AND METHOD OF STUDY

For our research, we selected the following wild-growing species in Siberian federal district (*okrug)*:
– ordinary cowberry leaves (*Vaccinium vitis idaea L.*);
– ordinary guelder-rose fruits (*Viburnum opulus*);
– sea-buckthorn fruits (*Hippophae rhamnoides*);
– prickly wild rose fruits (*Rosa acicularis*);
– ordinary rowan fruits (*Sorbus aucuparia*).

Extractives content

We determined extractives content in plant material extracts using the following method. Approx. 50 ml of extract (accurate volume) was placed in a 200–250 ml capacity cone flask, closed by stopper and weighed (measurement error ±0.01 g). Then, the flask was connected to reflux condenser and heated to light-boil for 2 hours. After cooling, we plugged the flask with the same stopper, weighed it with the content inside and compensated for mass loss with solvent. Then, the flask content was thoroughly shaken and filtered through dry paper filter into a dry 150–200 ml flask. We used pipette to transfer 25 ml of filtrate to porcelain cup (7–9 cm in diameter), which was previously desiccated to constant mass at 100–105ºC, and accurately weighed those. Then, the filtrate was double-boiled dry. After drying the solids in the cup to constant mass at 100–105ºC, we cooled the cup for 30 min in desiccator containing anhydrous calcium chloride on the bottom, and weighed immediately. Content of extractives % (X) on an absolute dry matter basis was found by the equation:

$$X = \frac{m \cdot 200 \cdot 100}{m_1 \cdot (100 - W)},$$

where m is the mass of dry solids (g); m_1 is the mass of extract (g); W is the mass loss upon drying (%).

Total flavonoid content

The method involves the use of spectrophotometer to determine total flavonoid content in extracts on rutin basis. We placed 1 ml of plant extract in a 10 ml volumetric flask, added 2 ml of a 5% aluminum chloride alcoholic solution and topped up to 95% mark with ethanol. In 30 minutes we measured optical density of the solution using the spectrophotometer UV 1800 (Shimadzu, Japan) within maximum absorption at wavelength 410 nm in cuvettes with a 10 mm layer thickness. To compare, we used a solution of 2 ml 5% aluminum chloride alcohol solution in 8 ml 95% ethanol. Total flavonoid content on rutin basis is calculated based on the rutin standard test piece calibration curve.

RESULTS AND DISCUSSION

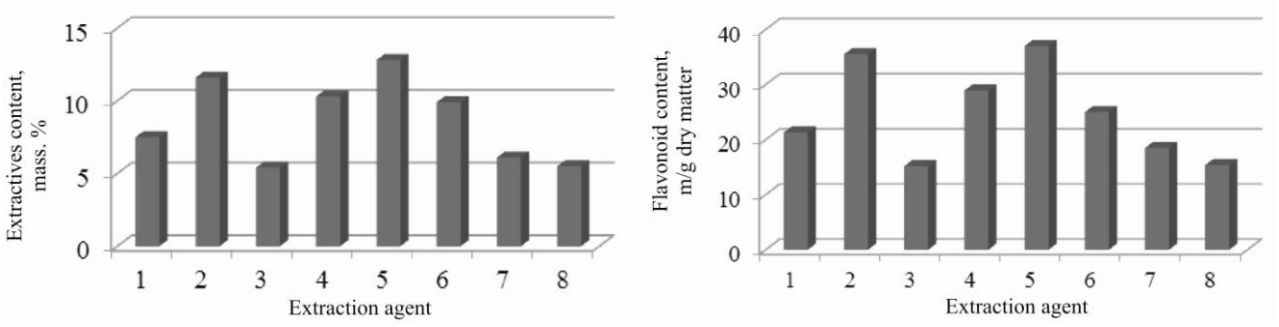
The following solvents and their mixtures are considered in this research as extraction agents (Table 1): chloroform, hexane, benzene, benzene – ethanol mixture, acetone, ethanol-water mixture, water.

We determined values of the quantities extracted from two considerations: extractives content value and value of total flavonoid content on rutin basis.

Having in mind the selection of the optimal extractant, we performed the extraction of wild-growing plant material by the solvents and mixtures selected, under identical conditions (at room temperature, duration for 2 hours, mass fraction of the material relative to mass fraction of solvent – 1 : 10, fineness of the material particles – 1–2 mm, material load density – 0.2 g/cm^3).The results of the above considerations are displayed in Figs. 1–5.

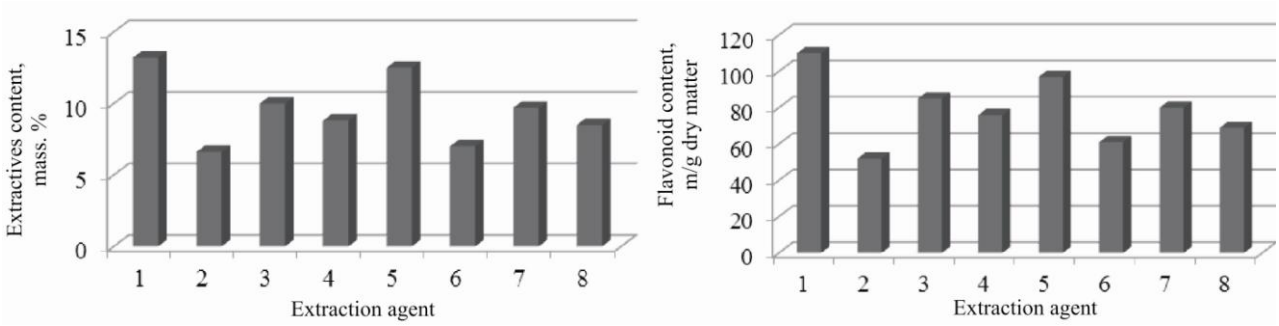
Table 1. Extraction agents

No.	Solvent	Permittivity, ε	Dipole moment, D	Concentrations, %
1	Chloroform	4.8	1.15	97.0
2	Hexane	1.9	0.08	94.0
3	Benzene	2.3	0	91.0
4	Benzene-ethanol mixture	10.4	–	1:2
5	Acetone	20.7	2.84	88.0
6	Ethanol	25.2	1.68	96.0
7	Ethanol-water mixture	57.0	–	1:2
8	Water	78.3	1.84	–



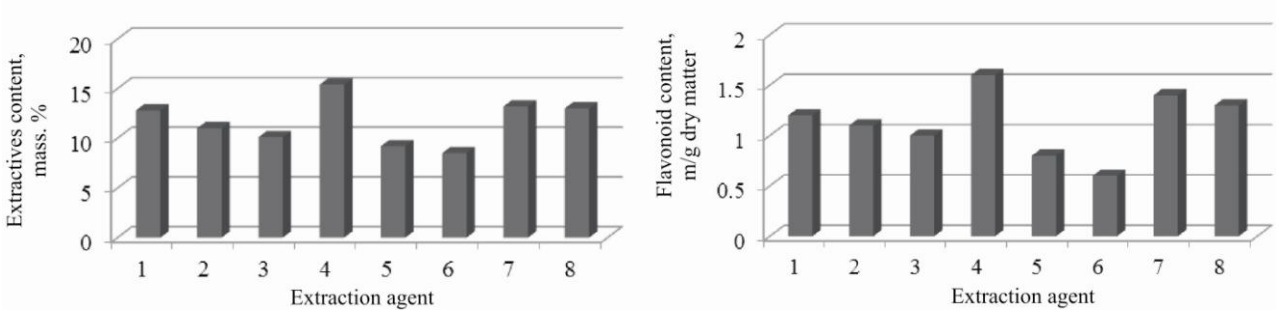
(a) (b)

Fig. 1. Extractives content (a) and total flavonoid content (b) in ordinary cowberry leaves (*Vaccinium vitis idaea L.*) extracts: 1 – chloroform; 2 – hexane; 3 – benzene; 4 – benzene-ethanol mixture 1 : 2; 5 – acetone; 6 – ethanol; 7 – ethanol-water mixture 1:2; 8 – water.



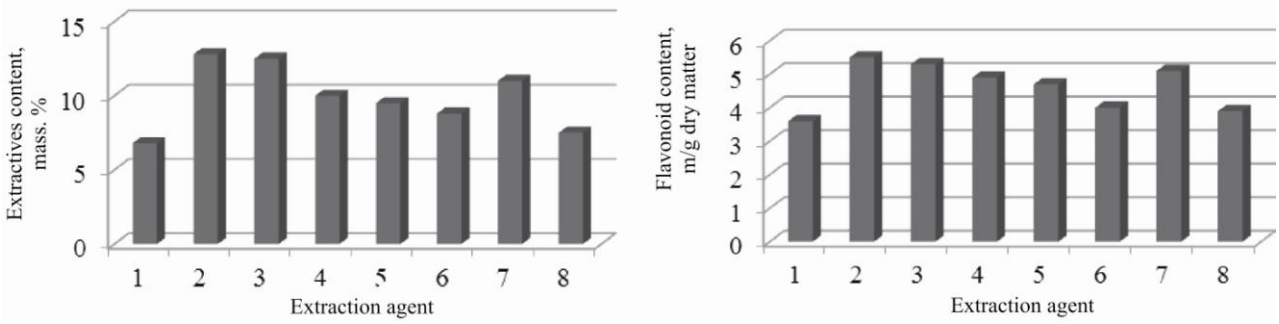
(a) (b)

Fig. 2. Extractives content (a) and total flavonoid content (b) in ordinary guelder-rose fruits (*Viburnum opulus*) extract: 1 – chloroform; 2 – hexane; 3 – benzene; 4 – benzene-ethanol mixture 1 : 2; 5 – acetone; 6 – ethanol; 7 – ethanol-water mixture 1:2; 8 – water.



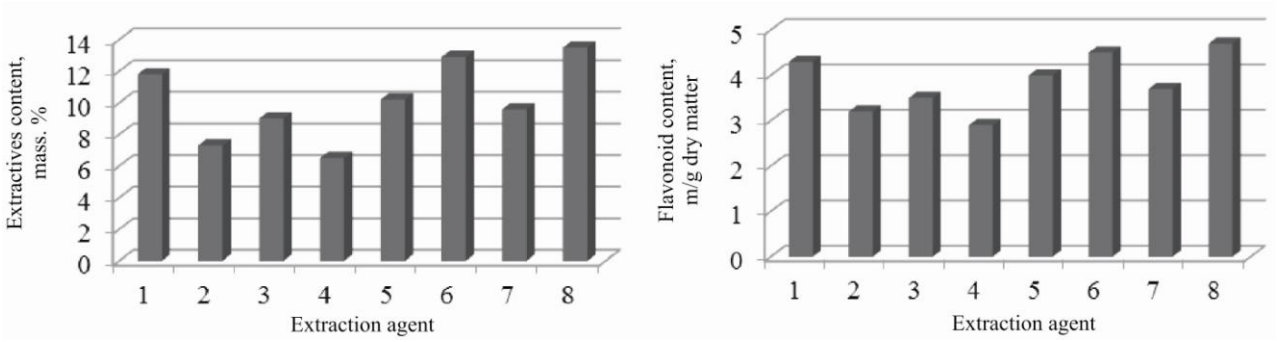
(a) (b)

Fig. 3. Extractives content (a) and total flavonoid content (b) in sea-buckthorn fruits (*Hippophae rhamnoides*) extract: 1 – chloroform; 2 – hexane; 3 – benzene; 4 – benzene-ethanol mixture 1 : 2; 5 – acetone; 6 – ethanol; 7 – ethanol-water mixture 1:2; 8 – water.



(a) (b)

Fig. 4. Extractives content (a) and total flavonoid content (b) prickly wild rose fruits (*Rosa acicularis*) extract: 1 – chloroform; 2 – hexane; 3 – benzene; 4 – benzene-ethanol mixture 1 : 2; 5 – acetone; 6 – ethanol; 7 – ethanol-water mixture 1:2; 8 – water.



(a) (b)

Fig. 5. Extractives content (a) and total flavonoid content (b) in ordinary rowan fruit (*Sorbus aucuparia*) extracts: 1 – chloroform; 2 – hexane; 3 – benzene; 4 – benzene-ethanol mixture 1:2; 5 – acetone; 6 – ethanol; 7 – ethanol-water mixture 1:2; 8 – water

Figs. 1–5 reveal that in the case of ordinary cowberry leaves the optimal agents are acetone (extractives content 12.8 mass %, flavonoid content 37.0 mg/g) and hexane (extractives content 11.6 mass %, flavonoid content 35.5 mg/g); in case of ordinary guelder-rose fruits those are chloroform (extractives content 13.2 mass %, flavonoid content 110.0 mg/g) and acetone (extractives content 12.5 mass %, flavonoid content 97.0 mg/g); in the case of sea-buckthorn fruits the optimal extraction agents appear to be benzene-ethanol mixture 1 : 2 (extractives content 15 mass %, flavonoid content 1.6 mg/g) and ethanol-water mixture 1 : 2 (extractives content 13.2 mass %, flavonoid content 1.4 mg/g); for prickly wild rose fruits (*Rosa acicularis*) those are hexane (extractives content 12.8 mass %, flavonoid content 5.5 mg/g) and benzene (extractives content 12.5 mass %, flavonoid content 5.3 mg/g); in the case of ordinary rowan fruits (*Sorbus aucuparia*) those are water (extractives content 13.5 mass %, flavonoid content 4.7 mg/g) and ethanol (extractives content 12.9 mass %, flavonoid content 4.5 mg/g).

Further, we selected the optimal temperature to extract the plant material by the solvents selected. For the purposes, the experiment involved the following identical parameters: duration of extraction 2 hours, mass fraction of the material relative to mass fraction of solvent – 1:10, fineness of the material particles – 1–2 mm, material load density – 0.2 g/cm^3). The range investigated: extraction temperatures varied from room temperature to boiling temperatures specific to each solvent). The experimental data obtained are shown in Table 2.

It is evident from the experimental data presented in Table 2 that in the case of each extractant in question the maximum efficiency, for example, for carotenoids, is achieved at temperature corresponding to the boiling temperature of the solvent. The exception is chloroform, which yields maximum result at room temperature.

Table 2. The resulting efficiency of biologically active compounds extractions from wild-growing plant materials at variable temperatures

Plant material	Solvent	Extraction temperature, ºC	Extractives content, mass. %	Flavonoid content, mg/g of dry matter
Ordinary cowberry leaves	Acetone	25.0	12.8 ± 0.6	37.0 ± 1.9
		56.1	13.5 ± 0.7	40.1 ± 2.0
	Hexane	25.0	11.6 ± 0.6	35.5 ± 1.8
		68.0	12.8 ± 0.6	38.4 ± 1.9
Ordinary guelder-rose fruits	Chloroform	25.0	13.2 ± 0.7	110.0 ± 5.5
		61.2	12.0 ± 0.6	102.5 ± 5.1
	Acetone	25.0	12.5 ± 0.6	97.0 ± 4.9
		56.1	14.2 ± 0.7	108.6 ± 5.4
Sea-buckthorn fruits	Benzene-ethanol mixture 1:2	25.0	15.4 ± 0.8	1.6 ± 0.1
		67.9	16.7 ± 0.8	2.0 ± 0.1
	Ethanol-water 1 : 2	25.0	13.2 ± 0.7	1.4 ± 0.1
		78.1	15.1 ± 0.8	1.8 ± 0.1
Rose hips (*Frūctūs Rosae)* of prickly wild rose	Hexane	25.0	12.8 ± 0.6	5.5 ± 0.3
		68.0	14.3 ± 0.7	6.7 ± 0.3
	Benzene	25.0	12.5 ± 0.6	5.3 ± 0.3
		80.1	13.2 ± 0.7	5.9 ± 0.3
Ordinary rowan fruits	Water	25.0	13.5 ± 0.7	4.7 ± 0.2
		85.0	15.3 ± 0.8	5.5 ± 0.3
	Ethanol	25.0	12.9 ± 0.6	4.5 ± 0.2
		78.4	13.8 ± 0.7	5.2 ± 0.3

The process duration is one of the most important extraction factors. Therefore, we continued with varying the duration of extraction (from 1 to 8 hours) at the temperatures selected: for acetone – 56.1ºC, for hexane – 68.0ºC, for chloroform – 25.0ºC, for benzene-ethanol mixture 1 : 2 – 67.9ºC, for ethanol-water mixture 1 : 2 – 78.1ºC, for benzene – 80.1ºC, for water – 85.0ºC, for ethanol – 78.4ºC. Figs. 6–10 represent the data sought.

Fig. 6 reveals that when extracting ordinary cowberry leaves the 4 hours process provides maximum content of extractives and total flavonoids with both acetone and hexane. If continued, further extraction fails to considerably change the amount of the substances extracted in relation to the values of a 4 hours process.

Experimentally, Fig. 7 provides that the optimal duration of ordinary guelder-rose fruits extraction will be 2 hours at a room temperature of 25ºC when extracted by chloroform, and it will be 4 hours at 56.1ºC if extracted by hexane.

We analyzed Figs. 6–10 to determine the optimal values of the process duration for the plant material under consideration.

In doing so, we discovered the most appropriate parameters applicable to the method of countercurrent extraction of biologically active compounds that would provide maximum efficiency and effectiveness:

– Ordinary cowberry leaves extraction by acetone : temperature – 56.1ºC, duration – 4 hrs;

– Ordinary cowberry leaves extraction by hexane : temperature – 68ºC. duration – 4 hrs;

– Ordinary guelder-rose fruits extraction by chloroform: temperature – 25.0ºC, duration – 2 hrs;

– Ordinary guelder-rose fruits extraction by acetone: temperature – 56.1ºC, duration – 4 hrs;

– Sea-buckthorn fruits extraction by benzene-ethanol mixture 1:2: temperature – 67.9ºC, duration – 2 hrs;

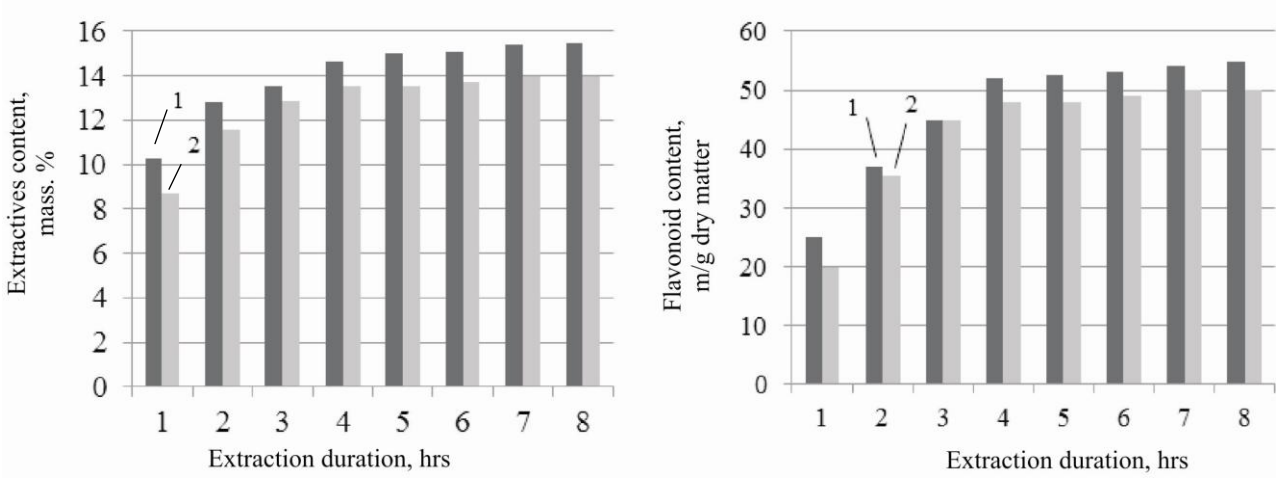
– Sea-buckthorn fruits extraction by ethanol-water mixture 1:2: temperature – 78.1ºC, duration – 2 hrs;

– Prickly wild rose fruits extraction by hexane: temperature – 68.0ºC, duration – 5 hrs;

– Prickly wild rose fruits extraction by benzene: temperature – 80.1ºC, duration – 4 hrs;

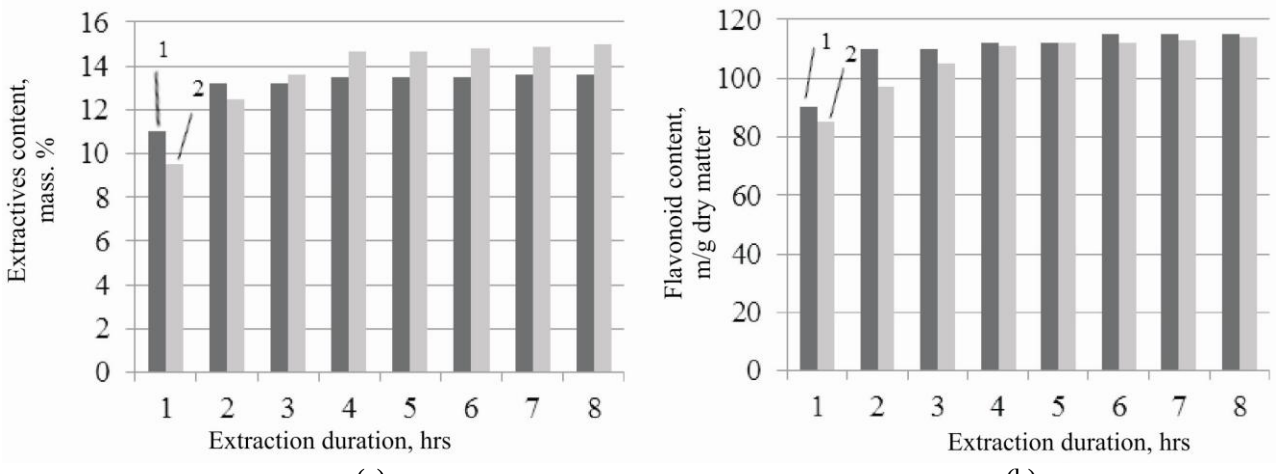
– Ordinary rowan fruits extraction by water: temperature – 85.0ºC, duration – 4 hrs;

– Ordinary rowan fruits extraction by ethanol: temperature – 78.4ºC, duration – 3 hrs



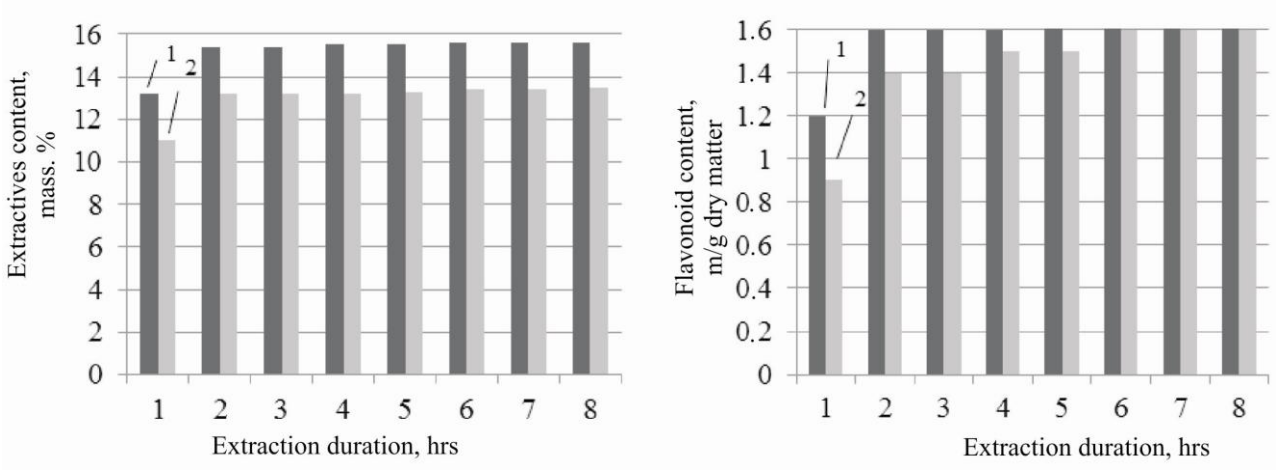
(a) (b)

Fig. 6. Extractives content (a) and total flavonoid content (b) in ordinary cowberry leaves extracts: 1 – acetone, 2 – hexane.



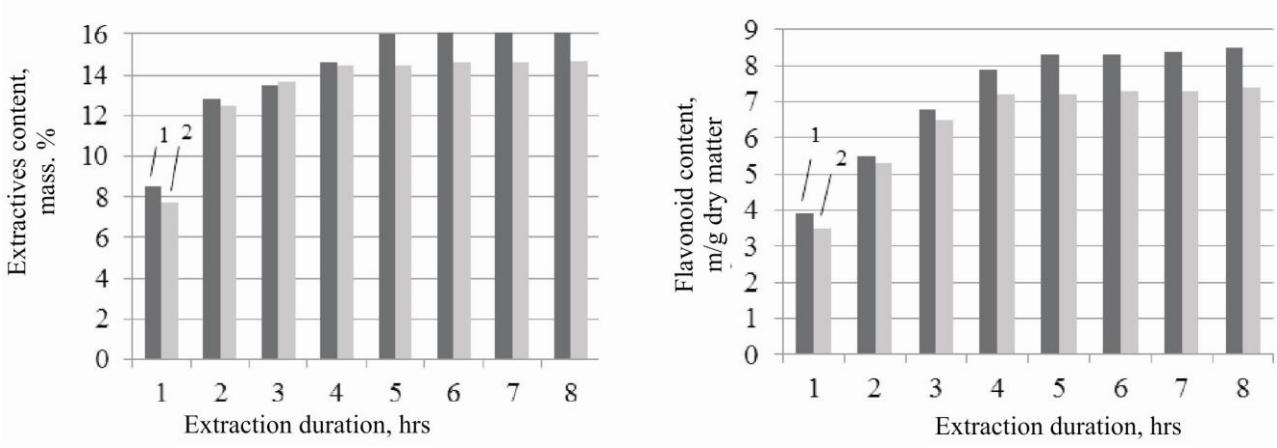
(a) (b)

Fig. 7. Extractives content (a) and total flavonoid content (b) in ordinary guelder-rose fruit extracts: 1 – chloroform, 2 – acetone.



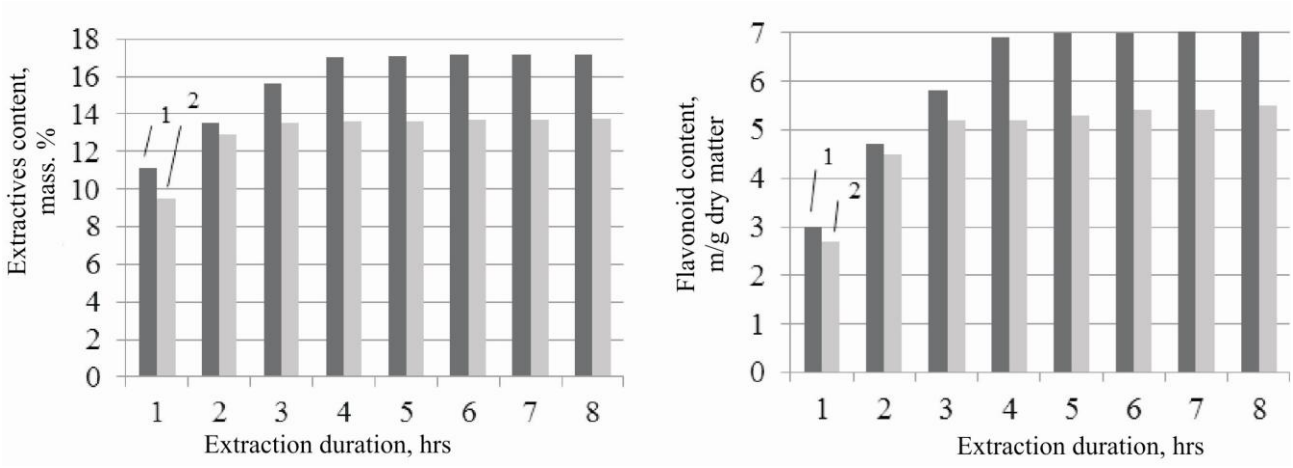
(a) (b)

Fig. 8. Extractives content (a) and total flavonoid content (b) in sea-buckthorn fruit extracts: 1 – benzene-ethanol mixture 1 : 2, 2 – ethanol-water mixture 1 : 2.



(a) (b)

Fig. 9. Extractives content (a) and total flavonoid content (b) in prickly wild rose fruits (*Rosa acicularis*) extract: 1 – hexane, 2 – benzene.



(a) (b)

Fig. 10. Extractives content (a) and total flavonoid content (b) in ordinary rowan fruits extract: 1 – water, 2 – ethanol.

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