

QUALITY ASSESSMENT OF *RHAPONTICUM CARTHAMOIDES* (WILLD.) ILJIN AS MEDICINAL RAW MATERIAL BY THE BROMIC ANTIOXIDANT CAPACITY ESTIMATION

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ABSTRACT

The possibility of an express assessment of quality medicinal raw materials *Rhaponticum carthamoides* (Willd.) Iljin is investigated, at various stages of harvesting and storage by method Bromic Antioxidant Capacity Estimation (BACE), after extraction of sample in an aqueous solution. Revealed that phytoecdysteroids *R. carthamoides* extracted in aqueous solution at level of ethanol, have the temperature resistance. Among the investigated 17 species of industrial medicinal plants value *R. carthamoides* has the greatest value BACE, exceeding values of other species from 2-3 up to 5-12 times.

Change BACE of leaf organs during the growing season was correlated with the dynamics of the concentration ecdysteroid 20-hydroxyecdysone, as defined by method of the reversed-phase highly effective liquid chromatography (RP-HPLC). Higher values for both indicators were also true for the early phases of vegetation. Established a connection between the loss of active substances and reduction value BACE during storage.

On the safety of 20-hydroxyecdysone and the value BACE strongly influenced by the presence of impurities in raw materials, infected microflora. Medicinal raw materials from the leaf parts of over 10 years to keep their consumer quality and satisfy to normative requirements on the content of ecdysteroids. The crushed underground plant parts (rhizomes with roots) within 1 year of storage lost their original properties.

Keywords: *Rhaponticum carthamoides*, leuzea, medicinal raw material, antioxidant properties, ecdysteroids, 20-hydroxyecdysone

INTRODUCTION

The modern medicine requires plant drugs of wide biological activity which would render obviously expressed effect on the functions of an organism weakened for whatever reasons, correct the development of secondary immunodeficiency and disadaptation, the obvious and latent forms of chronic diseases. Such therapeutic composition should be harmless and nontoxic, with soft action, without any dangers of development of accustoming and predilection, by-effects and negative after-action, with significant breadth of optimum dosages, it is good to be combined with classical medicamentous means.

One of sources of such preparats is *Rhaponticum carthamoides* (Willd.) Iljin, a rare endemic plant growing in high-mountainous areas of Siberia, Central Asia and China, at height of 1200-3000 m above sea level [1]. By results of long clinical researches the plant and preparations on its basis are brought in the State pharmacopoeia of USSR of IX-XI editions (1961, 1968, 1987, 1990). Pharmacotherapeutic use of this species in official medicine includes the following indication list: functional frustration of central nervous system, asthenic and depressive conditions, cardiovascular infringements, hyperglycemia, hyperlipoidemia, an anemia. This pharmaceutical substance can exert anaesthetic, hemorheologic, hypotensive, hyperglycemic, anticoagulative, antitoxic, antineoplastic, anti-inflammatory action. Preventive use *R. carthamoides* is appointed at muscular exhaustion, impotence, a premenstrual syndrome, secondary barrenness, and alcoholism. It can also be used as tonic, stimulating, wound healing, antibacterial, anabolic, nootropic, antidepressant, polyvitaminic polymicroelementic plant preparation [2-5].

Vital form of *R. carthamoides* is large-grassy perennial plant (fig. 1); duration of its life can reach 50-75 years. Its above-ground part has vegetative rosette shoots (75-100 % on individual share) and generative cauline forming a bush 50-110 cm in diameter and height 90-150 (sometimes 50-250) cm. The maximal sizes of rosette leaves can reach 80-120 x 28-43 cm. The under-

ground part of plants is located in 0-30 cm soil layer; it consists of rhizome and the main root with numerous rigid thin adventitious and lateral roots in length till 25-40 cm.



Fig. 1. *R. carthamoides*: at the left - the above-ground part consisting of rosulate shoots, on the right – root system (a rhizome with adventitious roots)

Because the long evolution dated for genesis of mountain systems passed in extreme conditions of life, on the basis of ancient vegetation on along the edges of glaciers, a floristic complex of pleistocene relicts became owner's special forms of a metabolism. The specificity of their secondary metabolism is phytoecdysteroids biosynthesis [6.] *R. carthamoides* can concentrate ecdysteroids on all vertical structure (inflorescences, leaves, rhizomes and roots). The level of these metabolites is very high, it exceeds their content in other species in 10-100 thousand times [7]. The maximal concentration of a major component *20-hydroxyecdysone* (20E) in separately taken elements of a biomass can reach 0.5-1.0 % in above-ground parts, 0.05-0.15 % in underground organs [3, 8].

The major substances of the increased level of synthesis are flavonoids, phenolcarboxylic acids, polyphenols, sesquiterpene lactones, and also polyacetylene substances, characteristic for underground parts [9]. Proceeding from the literary data, the integral content of them makes: flavonoids – in leaves of 1-2 %, in rhizomes of 0.1-0.2 %; phenolcarboxylic acids – in leaves of 0.8-1.5 %, in roots of 0.5-0.8 % [10]; flavonols – 0.7-1.9 % (leaves); tannins – in leaves of 9 %, rhizomes of 9-14 % [11-12]. It is necessary to note also the high content of vitamin C (ascorbic acid) in leaves (up to 0.1 %). For these groups of chemical compounds high jet ability to an inactivation of the free radicals, revealed in modelling experiences and confirmed in special experiments on the person is generalizing [5, 13-15].

Various parts *R. carthamoides* (roots, rhizomes, leaves, flowers, etc.) can be used as elements of medicinal raw material. Nothing but rhizomes with the roots prepared on wild-growing or cultivated plantations were used until recently in a pharmaceutical industry. The raw material was collected in the autumn, cleared of the rests of above-ground parts and the soil particles, washed out and have been dried up. "Know-how" of preparations on the basis of rhizomes had some disadvantages. First, the sources of vegetative raw material are not renewed, second, all operations on a plant underground parts harvesting, clearing of pollution, washing, drying and storage are too complex and laborious. As compared to a leaf part, the medicinal raw material is characterized by the relatively low content of active substances and their big losses during storage and processing.

According to sanitary-and-epidemiologic regulations and specifications of the Russian Federation (N 2.3.2.1153-02), since January 1, 2003 it is authorized to use all parts *R. carthamoides*, their extracts and products of processing in pharmaceutical composition structures and biologically active additives to food. Among active substances in medicinal raw material it should be detected *20-hydroxyecdysone*. Its content should be not less than 0.1 %; and extractive substances yield should be not less than 13 % [16].

High mobility and non-uniform distribution of active substances within of various organs and their elements is the prominent feature of manufacturing ecdysteroids containing medicinal raw material from above-ground parts [7, 17]. Therefore operative quality assurance of *R. carthamoides* at a stage of manufacture of the medicinal raw material, called to provide preparation of elements of a phytomass with increased content of ecdysteroids, is very actually. Modern method of the phytoecdysteroids analysis based on high-performance liquid chromatography (HPLC) is too

complex [18, 19]. This method can be applied in the presence of highly skilled experts and can be performed in conditions of the specialized laboratories only.

The purpose of our researches was the assessment of an potential of rapid quality control method of ecdysteroids containing medicinal raw material *R. carthamoides* based on integrated coulometric antioxidant capacity of water extracts estimation [20], comparison of their results to results of the analysis of the phytoecdysteroids determined by reversed-phase HPLC method. Use of a standard coulombmeter as titrates of bromine (Br^{3-} , Br^{2-} , Br^{\bullet}) is caused not only comparative simplicity of analytical procedures, but also its ability to enter reactions radical, oxidation-reduction, electrophilic replacements and connections on multiple bonds. It has been shown earlier that ability of the electrogenerated bromine to cover from a solution various water-soluble biologically active compounds of various nature, possessing strong antiradical properties concerning plant polyphenols, flavonoids and an ascorbic acid [21].

OBJECT AND METHODS

Object. Plants *R. carthamoides* cultivated in conditions of agropopulations on the European North (Russia, the Arkhangelsk area, Koryazhma-town) were used as object of researches. Geographical coordinates are 61°20' NL, 47°00' EL. The territory belongs to a subzone of a middle taiga and it is a part of the European-Siberian taiga-forest bioclimatic area. The conditions of growing season here are cold-temperate. Soils are sod-podzol, semi-tame, sandy. No mineral and organic fertilizers were applied on objects within last 5 years.

Plants under investigation were collected on the stage of middle generative period (8-11th years of a life). Selective harvesting of plant raw material was carried out during the growing season of 2005 including the basic phases of plant development. Above-ground parts of plant were collected from May till July and underground ones in October. We divided an above-ground biomass into morphologically diverse organs: innovation shoots, rosette leaves, stem leaves, a flower stalk, inflorescences, seeds; in underground parts we distinguished a rhizome, the main root, adventitious roots.

The vegetative material grouped on constituents of a biomass was dried at temperature 23-25 °C, air relative humidity 25-40 %. The drying was carried out in a shadow on racks with the cellular network positioned on height 40-60 cm from a floor level. Thickness of the raw material layer was about 2-3 cm. A material dried up in an integral kind excepting for the thickest petioles and flower stalks splitted on 2-3 parts. The residual humidity of the air-dried raw material determined by a method of accelerated drying at 130 °C averaged 10-13%.

Ecdysteroids chemical analyses. Biochemical analyses of plant samples on the ecdysteroids content were performed in the laboratory of the Botanical garden Institute of biology Komi Science Centre, Ural Division of the Russian Academy of Sciences (Syktyvkar). Samples for 20-hydroxyecdysone estimation in medicinal raw material were prepared from air-dried mass by the quartering method. Up to the analysis samples were stored in polyethylene packages at room temperature.

Concentration of ecdysteroids in various organs and biomass elements was estimated by the method of the reversed-phase highly effective liquid chromatography (RP-HPLC) combined to the internal standard method [19]. The equipment and modes of their work: liquid microcolumn chromatographer "Milichrome-5-3", a column 80x2 mm; sorbent Nucleosil 130 C18, with the size of particles 5 microns (Open Company "Medicant", Orel, Russia); an eluent water-EtOH-BuOH 75:24.2:0.8.

Elution speed is 0.1 ml/min, pump NRR 4001 (Czechia). Detector UV-VIS LCD 2536 (Czechia); $\lambda = 242$ nm. Average arithmetic values of two biological and three analytical replications are resulted in work. Concentration of phytoecdysteroids in work is resulted in recalculation on air-dry substance.

Estimation of antioxidant capacity. Initial samples were crushed up to particles of 1-3 mm in diameter. Selected sample in weight 5 g were completely dried at 120 °C. Extracts were received by means of insisting on distilled water in the ratio 1:10 on dry weight with the subsequent boiling in a water bath during 15 min and cooling within 45 min.

Coulombmeter "Expert - 006" of Open Company "Econix-expert" (Moscow) was used for electrogeneration of bromine and coulometric titration. We entered 25 ml of a background solution and into an electrochemical cell of the device (200 ml in volume) placed there the worker, ancil-

lary and tracer electrodes. Into the cell we entered an aliquot (0.1 ml) of extract under estimation and mixed within 100 seconds.

Coulometric titration was carried out in galvanostatic mode ($i=5$ mA). Bromine was generated from 0.2 M water solutions KBr on a background of 0,1 M H_2SO_4 . The glass-carbonic electrodes were used as generating and ancillary ones. The cathodic chamber where the ancillary electrode was located has been separated from analite by a semipermeable wall. The final point of coulometric titration was estimated amperometrically with two polarized needle platinum electrodes ($\Delta E=300$ mV). The platinum wire electrodes were used for establishment of the final titration point. A level of measurement is 100 mV, a current strength of measurement is 52.10 mA.

Quantity of an electricity spent on titration solution obtained from 100 g of absolutely dry extract (kC/%), calculated as follows: $Q_C = 100(I \times t) / V_{al}$; where I - current strength (0.0521 A); t - time of achievement of a final titration point (s); V_{al} - volume of an aliquot (25 ml). The results of calculation are expressed in coulombs.

Humidity of samples was controlled by the hydrometer MX-50. The content of dry substances in extracts was estimated at 180 °C; weight of sample an extract made 1-5 g. The received results were exposed to statistical processing. The average arithmetic values and relative standard deviation S_r were used at results evaluation.

RESULTS AND DISCUSSION

Accordingly to data from literature, high antiradical activity is characteristic for *R. carthamoides*. Among 215 plants of the Kaunas Botanical garden belonging to 163 genera and 60 families, 12 medicinal and aromatic plants are selected [12]; the most active among them were species of a sage (*Salvia officinalis*, *S. glutinosa*), prairieweed (*Potentilla fruticosa*) and *R. carthamoides*.

When the modelling antioxidative system on inactivation of free 2,2-diphenyl-1-picrylhydrazil radical activity were used (DPPH-method) [15], the methanol extracts of these plant species correlated with the sum of phenolic substances to the greatest degree (fig. 2). Activity values 93.9 % and 92.3 % were observed for *P. fructosa* and *S. officinalis* at concentration of the sum of phenols in an extract 37.9 mg/g and 22.6 mg/g. Comparable activity value for *R. carthamoides* (87.6 %) was shown at concentration 13.3 mg/g what is in 2-3 times smaller. Further, at detailed research, flavonoids, phenolic acids, and also ecdysteroids have been identified among substances determining the antiradical activity of *R. carthamoides* [22].

Before starting studying the importance of ecdysteroids in antiradical activity of *R. carthamoides* determination, the capability of phytoecdysteroids to be extracted from plant raw material by ethanol and water solutions, and also their stability to heating in a range of temperatures from 20 up to 100 °C were investigated in laboratory conditions. It is established, that phytoecdysteroids *R. carthamoides* can be extracted both water solutions and ethanol solutions (0.44 and 0.45 % counting upon dry substance of a sample respectively); they also possess temperature stability at heating (fig. 3).

Comparative tests of bromic antioxidant capacity have been carried out at the second stage of investigation. Two ecdysteroids containing plant species (*R. carthamoides* and *Serratula coronata*) and other herbs that are received from a chemist's shops network and not containing ecdysteroids were under comparison. The water extracts were made from various organs of plants (seeds, leaves, inflorescences, a grass with stalks, a bark and fruits). It has allowed us to determine that substances of the antiradical nature can be presented by a various set and to subdivide the herbs into 3 groups based on their composition (tab. 1).

The best antioxidative activity (AA) is recorded for the plants of 1-st group being super-concentrators of ecdysteroids especially for seeds (43.6 kC/%) and leaves *R. carthamoides* (38.3 kC/%), seeds *S. coronata* (26.1 kC/%). It is necessary to emphasize that high parameters of seeds bromic AC are caused mainly by the presence of ecdysteroids in high amounts (1.05 and 0.70% 20-hydroxyecdysone correspondingly). As against leaves and rhizomes, seeds do not contain many phenols and tannins, but they are rich with fats (18-24%) and starch (27%). Enhancement of antiradical activity of leaves *R. carthamoides* at much smaller concentration of ecdysteroids (0.45% 20E) is determined, most likely, by the contribution of phenolic acids and water-soluble fractions of flavonoids.

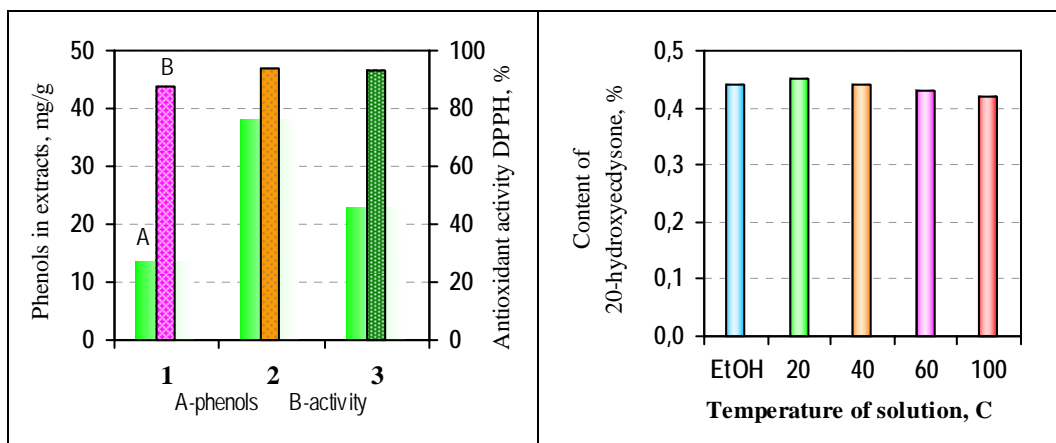


Fig. 2. Antioxidant activity:
1 - *R. carthamoides*; 2 - *P. fructosa*;
3 - *S. officinalis*; on [15] with changes

Fig. 3. Capability of phytoecdysteroids to be extracted from above-ground plant biomass *R. carthamoides* at various extraction modes

Table 1

Antioxidant capacity of water extracts of herbs

Plant species	Source of medicinal raw material	20E, %	AC, kC/%	S _r
1 group				
<i>Rhaponticum carthamoides</i> (leuzea)	seeds	1.05	43.6	0.05
<i>Rhaponticum carthamoides</i> (leuzea)	leaves	0.45	38.3	0.01
<i>Serratula coronata</i> (klasea)	seeds	0.70	26.1	0.02
2 group				
<i>Salvia officinalis</i> (garden sage)	leaves	–	17.5	0.01
<i>Helianthus tuberosus</i> (earth apple)	leaves	–	16.5	0.04
<i>Hypericum perforatum</i> (common St. John's wort)	grass	–	13.8	0.03
<i>Camellia chinensis</i> (tea-plant)	shoots	–	12-15	0.02
<i>Quercus robur</i> (english oak)	a bark	–	9.1	0.03
3 group				
<i>Betula verrucosa var. pendula</i> (drooping birch)	buds	–	6.2	0.02
<i>Leonurus quinquelobatus</i> (motherwort), <i>Chelidonium majus</i> (greater celandine)	grass	–	5.5-7.0	0.04
<i>Plantago major</i> (common plantain)	leaves	–	4.6	0.09
<i>Humulus lupulus</i> (hop), <i>Matricaria matricarioides</i> (rayless camomile), <i>Tanacetum vulgare</i> (common tansy), <i>Calendula officinalis</i> (calendula), <i>Achillea millefolium</i> (yarrow)	inflorescences	–	3.3-7.5	0.02
<i>Rosa cinnamomea</i> (cinnamon brier)	fruits	–	3.5	0.05

Activity of medicinal raw material elements at plants of 2-nd group which are rich of phenolic compounds (leaves of garden sage (*Salvia officinalis*) and earth apple (*Helianthus tuberosus*), grass of common St. John's wort (*Hypericum perforatum*) also, but can not synthesize ecdysteroids, is significant (2-3 times) below as compared to plants of 1-st group. The oak bark (*Quercus robur*) and tea-plant (*Camellia chinensis*) shoots containing significant amounts of highly active polyphenols (18-25% tannins) conceded to *R. carthamoides* on parameter AC 3-5 times.

The antiradical activity of medicinal raw material in 3-rd group of plants is 5-12 times less than values of *R. carthamoides* (3.3-7.5 kC/%). Most likely that low AC parameters are caused in one case by presence of tarry substances which are slightly soluble in water – the birch buds (*Betula verrucosa* var. *pendula*), otherwise it is the matter of low vitamin C activity – *Cinnamon brier* (*Rosa cinnamomea*), depletion of monophenolic compounds and absence of phytoecdysteroids. Thus, proceeding from a qualitative chemical composition of the water extracts investigated, the greatest value of bromic AC is characteristic of ecdysteroids containing plant *R. carthamoides*.

Interrelations between the ecdysteroids concentration dynamic and changes of their antioxidant capacity during growing season were investigated at the third stage. According to our long-term researches, the phytoecdysteroids contents in adult leaf organs of rosette shoots *R. carthamoides* during the growing season is reduced three times (tab. 2). Concentration of 20-hydroxyecdysone in rosette polycyclic shoots reaches 0.45-0.55 % in the beginning of the regrowth phase, it makes 0.35-0.40 % in 20 days of vegetation. Concentration of 20-hydroxyecdysone in adult leaves of vegetative shoots is equal 0.27-0.33 % in the period of mass raw preparation. It is reduced gradually up to 0.17 % towards the end of a procuring season (60 days of vegetation).

Similarly to ecdysteroid dynamic, the antioxidant capacity in leaves of rosette shoots was characterized by higher parameters in earlier phases of vegetation. It was reduced eventually end smoothly: in the beginning of shoots regrowth phase it was equal 36.88 kC/%, during a budding phase 25.67 kC/%, during a flowering phase of 22.27 kC/%.

Table 2

Ecdysteroids dynamic and change of 20-hydroxyecdysone antioxidant capacity in plant raw material of *R. carthamoides* during growing season

Date	A phase of development	Plant organs	Yield of extractive substances, %	Content of 20E, %	AC, kC/%	S _r
16.04	regrowth	rosette leaves	17.5	0.45-0.55	36.88	0.01
15.05	regrowth	rosette leaves	29.9	0.35-0.40	26.62	0.03
25.05	budding	rosette leaves	23.6	0.25-0.30	25.67	0.04
14.06	flowering	rosette leaves	32.0	0.15-0.20	22.27	0.02
25.05	budding	top stem leaves	27.0	0.25-0.30	30.87	0.01
25.05	budding	top part of flower stalk	13.8	0.20-0.25	22.87	0.03

As we have showed it earlier for reproductive shoots [23], the distribution of ecdysteroids on a vertical structure is strongly differentiated depending on a phase of development; it varies from 0.02 up to 0.40 % for the plants of 6-th year of life. The gradient of distribution of ecdysteroids concentration for plants 11-15-th years of life is similar, in budding phase it grows from bottom of metameres to apical as follows: 0.10 % in the bottom part of a stalk, 0.16 % on the average part, 0.25 % in the top part and 0.58 % in apical part. The antioxidant capacity for the top stem leaves was high also (30.87 kC/%). For the top part of flower stalk it was equal 22.87 kC/%

It is necessary to note that leaf organs have significant advantage of stalks on extractive substances yield, 24-32 % against 13-14 %. The low yield of extracts from generative shoots (11.5 %) is fixed in Lithuania, too [22]. Most likely, it is consequence of saturation of stalks by water-insoluble structural carbohydrates (40-60 % crude cellulose in stems against 15-18 % in leaves).

Influence of storage period of medicinal raw material on concentration of ecdysteroids and size of AC were studied at the fourth stage. Accordingly to requirements to quality of raw material from the underground organs, fixed in pharmacological asset N 42-2707-90, presence of mineral impurity (the soil rests) up to 4 %, an organic impurity (other plant kinds) up to 1 %, the rests of stalks up to 2 % are acceptable for it [16].

Proceeding from results of our researches (fig. 4, 5), the crushed roots with rhizomes can lose quality within several months as they include necrotic parts and the soil particles infected with

microflora (up to 10-15 %). As a rule, underground organs do not satisfy to normative requirements under the content of the basic active substances in 1 year of storage, because 20E level in then decreases from 0.10 % up to 0.05 %; and in 2 years ecdysteroids are present at them in minute amounts. The parameter of antioxidant capacity within one year of storage fell from 35.1 up to 15.6 kC/%

As the practical experience shows, raw material from above-ground parts, which can include necrotic parts of the plants, infected with epiflora and rhizospheric microflora similar to rhizomes, can lose quality within several months; the contents of ecdysteroids in it is reduced from 0.2-0.3 % up to 0.004-0.03 %. And on the contrary, the plant material subjected to preprocessing three times at different stages of preparation and processing, carefully cleaned from mineral and organic impurities, is capable to keep the consumer qualities for a long time.

Concentration of *20-hydroxyecdysone* in raw material after 5 years of storage in crushed kind has made 0.15-0.18 %; after 10 years 0.10-0.12 % that did satisfy to normative requirements, too. The antioxidant capacity of initial raw material makes 26.6 kC/%; it varies during storage period as follows: 25.6 kC/% in 1 year, 22.3 kC/% in 5 years, 21.0 kC/% in 10 years. Thus, relation between losses of active substances and decrease in antioxidant capacity of medicinal raw material at storage is clearly recognized; and the falling of *20-hydroxyecdysone* concentration is stronger the decrease in AC parameter is more significant.

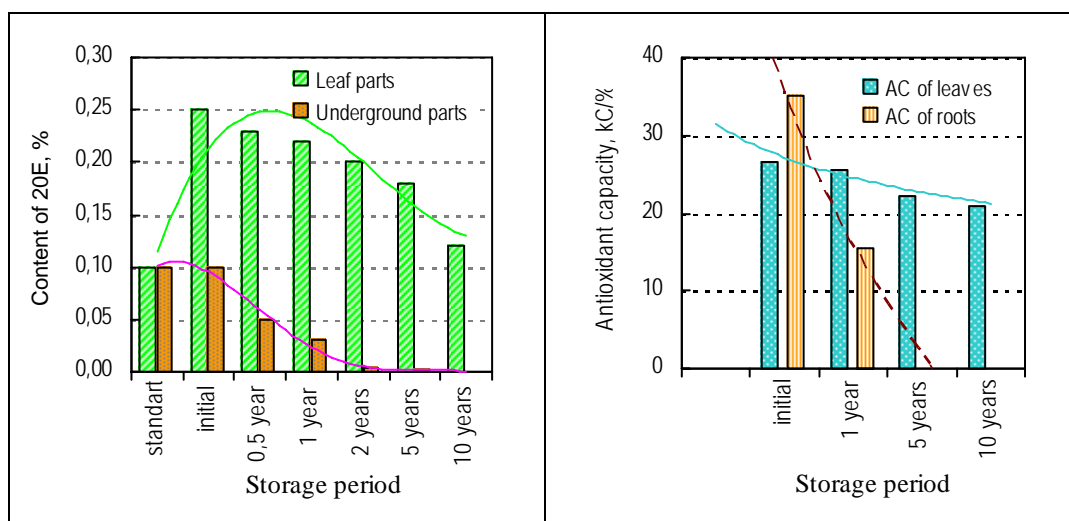


Fig. 4. Dynamic of ecdysteroids in medicinal raw material *R. carthamoides* during a long storage period

Fig. 5. Change of antioxidant capacities (AC) of medicinal raw material *R. carthamoides* in a storage time

CONCLUSION

Proceeding from versatile laboratory researches, dependence of antioxidant capacity of water extracts *R. carthamoides* from concentration ecdysteroids is revealed. Presence of correlation between antioxidant capacity and dynamic of major ecdysteroid *20-hydroxyecdysone* in leaf organ is established during the growing season and long storage periods of raw material.

By results of the additional expanded researches, the method of an express estimation of bromic antioxidant capacity of medicinal raw material can be used for quality assurance of stored and processed production *R. carthamoides* in pharmaceutical and the food-processing industry.

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