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JUSTIFICATION OF SAPONARIA OFFICINALIS (S. OFFICINALIS) CULTIVATION PERIOD IN THE SOIL AND CLIMATIC CONDITIONS FOR PRIMORSKY REGION (RUSSIA) AND ANALYSIS OF SAPONIN ROOT EXTRACTS

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Abstract

The purpose of this study was to explore the possibility ofboth soapwort (S. officinalis L.) and bouncing Bet,or double-flowered soapwort (var.floreplene hort.) cultivation in the soil and climate conditions of PrimorskyKrai (Russia); to study the effects ofS. officinalis vegetation time on the composition and micellar properties of saponin root extracts for their use as highly effective natural surfactants. The dynamics of the saponin accumulation in the root, depending on the cultivation time, is studied. The values of the surface tension and the critical micelle concentration indicators of saponins and water root extracts are established for both S. officinalis types and different vegetation time.The influence of different technological factors on the micellar parameters of saponin roots for both S. officinalis types is investigated.It was found that highersaponin parameters of the bouncing Bet S. officinalis type allows recommending it as a highly promising source of natural surfactants, on a pair with commercial Quillajasaponin by its micellar parameters.The established pattern in hemolytic activity changes of the extracts from the vegetative stage S. officinalis roots allowsexpanding the sphere of their application.

Keywords: Biological activity, cultivation period, emulsifiers, micellar parameters, Saponaria roots (*S. officinalis*), saponin extracts, soil and climatic conditions, surfactants, triterpene glycosides

I. Introduction

Today, the problem of refusal of synthetic surface-active agents (surfactants) and their replacement with natural ones occupies an important place in a variety of industries around the world. In this context, one of the most urgent tasks is to discover natural surfactants and provide rationale for their wide practical use as foaming agents, emulsifiers and solubilizers.

In this regard, plant triterpene glycosides are promising, which, thanks to amphiphilic structure oftheir molecules (they contain both fat-soluble aglycone and water-soluble carbohydrates), belong to the class of high-molecular colloidal surfactants. They are capable of forming strong bilayer elastic adsorption layers on the phase boundary, thereby causing a high stability of foams and emulsions, and also capable of forming micelles in an aqueous solution and dissolve hydrophobic substances(Trapeznikov et al., 1970; Abramzon, 1981, pp. 239-250; Mitra and Dungan, 1997, 2000; Yang et al., 2013).

Moreover,saponinsare capable of not only forminga necessary intended structure of aggregatively unstable food system, but alsoproviding theirfunctional orientationthrougha wide spectrum of biological and physiologic actions (Sparg et al., 2004; Kuznetsova et al., 2014), it makes it possibleto considerthem as complex food additives (Güçlü–Üstündag and Mazza, 2007; Tamura et al., 2012; Tsybulko et al., 2004).

Numerous *in vitro* and *in vivo* studies have convincingly proved that hypocholesteremic and anti carcinogenic effects of plant saponinsaredue to their abilityto form stable complexes with cholesterol land bile acids, with subsequent excretion from the body (Rao and Sung, 1995; Lacaille-Dubois and Wagner, 2000; Mitra and Dungan, 2001; Gurfinkel and Rao, 2003; Kim et al., 2003; Güçlü–Üstündag and Mazza, 2007; Man et al., 2010).

It is now believed that saponins are able toprotect humanity from two global problemsof the century, associated with inappropriate nutrition andexcess blood cholesterol – ischemic heart disease and bowel cancer.

Triterpene glycosides are secondary plant metabolites wide spread in flora, they have been discoveredin more than 90 families, among which,first and foremost, pink family (*Caryophyllaceae*), ginseng family (*Araliaceae Juss.*),legumes (*Leguminosae/Fabaceae*), rose family (*Rosaeceae*),from 900 to 2,000 plant species in each, should be mentioned. Members of these families, such ascommon licorice (*Glycyrrhizaglabra*L.), Manchurian aralia (*Araliamandshurica* Rupr. etMaxim.), babies'-breath (*Gypsophilapaniculata*), Turkestan soapwort *(Acanthophyl lumgypsophiloides*R.), red soapwort root, soapwort *(Saponariaofficinalis*L.), as well as soap tree, orsoapbark (*Quillajasaponaria Molina),*etc.,that are commercially important, are of interest for searching newefficient producers of triterpenesaponins. Saponins have been detectedin different plants'organs,their content depends onplant genus,ranging between 0.5 and 30%.

For the moment, *quillaja* and *yucca* saponins of *Quillajasaponaria* and*Yucca schidigera* barks have been most intensively studied (Mitra and Dungan, 1997; Cheeke, 2000; Mitra and Dungan, 2000, 2001; Patra et al., 2012)*,*their water extracts having been recognized as safe additives (having the GRAS

(GenerallyRecognizedAsSafe)status) andused in many industries, including functional food production(Summary of All GRAS Notices, 2002, pp. 1–6).

To date, choiceof domestic natural food surfactants with highmicellarparameters in the food industryis limited.One of the reasons impeding development of the production of saponin-containing food emulsifiers in Russia is the lack of domestic raw material base and, therefore, scientifically based technologies of saponin-containing plant extracts production.

The precondition for this research waspractical inaccessibility of *A. gypsophiloides*roots, since the plant is an endemic of Central Asia listed in Red Book because of its uncontrolled harvesting.In contrast, *S. officinalis*grows singly or as little spinneyin many climatic zones in Russia, it is easy to grow, beinga promising object for cultivation(Motkhin and Sukach, 1970).

Furthermore, the maintriterpene glycosides of bouncing Bet – *saponaroside* A and B – are highly similar in their structure to commercial *quillaja*saponinsof the *Quillajasaponaria*tree bark, which are practically the only ones permitted in the United States, Japan and some European countries for use in the food industry as an emulsifier, foaming agent and solubilizer (Masahiro et al., 1984; Yukio et al., 1985; Ninomiya et al., 1996; Cheeke, 2000; Wen-Teng et al., 2007).Saponarosides and *quillaja*s aponins have identicaglycones (quillaic and gypsogenic acids) and twobranched carbon chains of close structure. Structural kinship of the saponinscan probably determine their similar micellar properties and biological activity.

This worktherefore aims to study the possibility of cultivation Saponaria officinalis (*S. officinalis*L.)in the soil and climate conditions of Primorsky Krai (Russia), justify a cultivation periodin order to obtain commercial quantities of saponins and investigate their functional and technological properties to be usedin technologies of colloidal unsustainable food systemsas highly effectivesurfactants.

The objectives of the study were:

- to study dynamics of root mass and saponins accumulation, depending on the cultivation period of *S.officinalis*of two types (soapwortanddouble-flowered soapwort);

- to research influence of time periods and vegetative stage of Saponariaofficinalison chemical composition of the extract from the roots of both types;

- to describe micellar parameters (surface tension andcritical concentration for micelle formation) ofsaponins and water extracts from Saponariaofficinalis (*S. officinalis*) of different forms and vegetation periods;

-to studythe influenceof different technological factorson micellar parameters of saponins from the roots of both Saponariaofficinalis;

-to investigate the chemical structureof the bouncing Bet roots(var.floreplene *hort.*)of the second vegetation year.

II. Materials and Methods

Saponins Eduction

To educesaponins from the plant roots, a standard method was used (Dekanosidze et al., 1982, p. 151). It is basedon an exhaustive three-stage extractionof dry rootswith70% methanol at boiling temperatures of the solvent (in a Soxhletextractor). Mixed extracts were boiled downin the rotaryevoperatorand dissolved in water, thensaponins were deposited by acetone. The precipitate was allowed to stand 10 h at –17°C, then it was separated by centrifugation and lyophilized. The saponin content was calculated on the weight of the roots.

Low- and High-Polarsaponins Fractionation

Low-polar saponins (LPS), such as mono- and bidesmosides with short bonds, were educed from water extractsunder study by butanol extraction.This is one of the most widely used approaches (Dekanosidze et al., 1982, p. 151;Ladygina et al., 1983, pp. 41–56).Subsequent subsidenceof the water extractwith acetone made it possible to allocate high-polar bidesmosides (HPB). This methodallowsto estimate the LPScontent due to the presence of phenolic glycosides inbutanol fraction. Related polysaccharides (PS) impedingthe saponin definition, were pre-precipitated with 70% ethanol. Completeness of the saponinsprecipitation was controlled by a test forfoamability. This is a sensitive and specific method for saponins identification.

Quantitative Estimation of Polysaccharides and Saponinsin the Roots Extracts

To precipitate polysaccharides in the extract aliquot (concentration 9-10%),2 volumes of 98% ethanol were added in portions under vigorous stirring. Then test-tubes were left for 4-5 h at–17°C for a precipitate to form.The precipitate (gel) was centrifuged at 5,000 rpm for 10 minutes, washed with 70% ethanol and air dried to constant weight at 80°C. The supernatant and washing liquid were mixed together in a flask and evaporated using a rotary evaporator to dryness. The dried pellet was dissolved in water and quantitatively transferred to the weighted centrifuge tube (total volume of the solution being 1 ml).Saponins were precipitated with a fivefold volume of cooled acetone. Then pellet (saponins) was left for 4-5 h at–17°C, centrifuged and air dried to constant weight at 80°C. The content of polysaccharides and saponins was calculated as a percentage of the mass fraction of soluble solids.

CCM Definition by the Method of Fluorescent Probes

The method is based on imbedding8-anilino-1-naphthalenesulfonic acid (ANS, Sigma) into the formed micelles (Horowits, 1977). To an aliquot (0.5ml) after dilution series of the studied extracts (concentration of 0.03–90 $mg*ml^{-1}$), an equal volume of ANS (1.6 $ug*ml^{-1}$) was added, and the mix was incubated in the dark for 1 h at 25°C. The fluorescence of the resulting solutions was measured at 360 nm at 540 nm excitation.The value of the fluorescence for each concentration was calculated as the average of three parallel measurements.

Capillary Electrophoresis

The method is based on abilityofmonosaccharide's having 1,2-*cis*-hydroxyl groupsto formcomplexes with borate (Tava et al., 2000). Electrophoretic mobility of saponins depended on total positive charge determined by the size of the carbohydrate chains, quantity of1,2-*cis*-OH groupsand free carboxyl groupsin the molecule. The electrophoresis was madein 150 m Mborate buffer (pH 9.3), that contains 15% methanol, at 40 °C and 20 kV,on the Capillary ElectrophoresisSystem 270A (AppliedBiosystem), using a glass capillary 40 cm*50 m.

Study of Saponins HemolyticActivity in Vitro

Hemolytic activity was investigated on human red blood corpuscles of blood group O (Anisimov and Chirva, 1980). Red blood corpuscles were washed by saltphosphate buffer (0.01 M phosphate buffer pH7.2, 0.9% NaCL), then saponin solutionof the calculatedconcentration (0.05-7.0%) was added; the mixture was shaken and incubated for 20 minat37°C. Unlysed red blood corpuscles were centrifuged for 10 minat 3,000 rpm. The color intensityoflysedred blood corpuscleswas determined at 540 nm on the Specol-11 spectrophotometer (Carl Zeiss-Jena, Germany). The hemolysispercentage was determined by the following formula:

$$X(\%) = \frac{A \times 100}{k_1}, \tag{1}$$

Where*A* isoptical density of the studied extract;

k_1 – optical density of lysed erythrocytes.

Results and Discussion

Plantations of Saponariaofficinalis(*S. officinalis*) were set upin the territoryofPrimorskyFruit and Berry Experiment Station(Vladivostok, Russia). As aseed grain soboles of the roots *S. officinalis*of two types were used – soapwort, picked in a suburbanarea of PrimorskyKrai, and selectively breeded double-floweredsoapwort(var.floreplene*hort.*),picked from the territoryof the formerbotanical collection of theAll-Russian Scientific Research Institute of Medicinal and Aromatic Plants (Vladivostok, Russia), recognized for itsstrong stems, large leavesanddouble flowers.

All plants of the first year planting blossomedin the period of July 20–25 (bud stage); blossom-timecontinued until frosts. By the endof the firstvegetation period in September (fruiting stage)a height of creeperwas 20–25 cm; some shortabove-ground sprigs were formed in the bushes. The 100% overwintering of the plants was marked in the spring. It was observed in the second year of the plants cultivation that they took roots andproliferated; overgrowth was noticed onApril20–25 (the height of shoots was 10–15 cm); intensivegrowth of the planting was observedstarting from the second decade of June; a massive florificationbeganin the period ofJuly 20–25. The height of the plantingreached its maximum in September; alsoin this period, intensive growth of side shoots was observed. The average height of the plants reached 75 cm, the number of stems of the one bush varied from 35 to 65 pieces.Phenological observations and biometric surveys confirmed the possibility and breeding perspectiveness of two types of*S. officinalis*in the soil and climate conditions of the region.

The mass of the root system and dynamics of saponins accumulation depending on the plant cultivation period has been studied to justifyoptimal terms of harvesting(Table1).

Table 1:Yield and saponincontent of the S. officinalis roots depending on the cultivation period

Cultivation period	Mass of the root system per 1 m^2, kg		Saponins content, %	
	double-flowered soapwort	soapwort	double-flowered soapwort	soapwort
1st year	0.4	0.4	–	–
2nd year	1.0	0.7	30	23
3rd year	1.2	0.8	35	26

It has been foundthatthe root system ofboth Saponariaofficinalisgrows most actively during the second year of vegetation.By the end of thethird yearthere was a strongexpansion of the root system, which made it difficult to prepare raw material – part of the roots remained between the ridges, therefore, their total weight did not differ significantly from the weight of the roots of the 2nd year of cultivation.

The saponin content in 2-year roots of double-flowered soapwort was higher (30%)thanin the soapwort roots (23%).With the further cultivation of up to 3 years, there was almost no growth of the saponin content – 35 and 26%, respectively, which suggestedthat the cultivation period of S. officinalisshould be reduced to two years. The results of*A. gypsophiloides* R. cultivation in Turkmenistan showed that maximum accumulation of saponins in the roots of the plant (20%) was observed only in the fifth year of cultivation, while the yield of roots was almost 2 times lower than the yield S. officinalis(Gladyshev and Mishchenko, 1990, p. 100).

The feasibility of reducing the cultivation period was also confirmed by a comparative study of the chemical composition of the root extracts of the cultured soapwort (ES), double-floweredsoapwort(EDFS) and wild forms of the perennialSaponaria (EWS).

For preparing extracts from *S. officinalis*, the method of preparationwater extractof the roots*A. gypsophiloides* was adopted as a basis, this plant being officially permitted for use as a foamerin the halva (boiling the roots at 100°C at a liquor ratio 1:20 to extractives content 9-11%).

The analysis of water extracts of roots collected in the main periods of saponin accumulation –at the bud stage (July) and at the fruiting stage (September) –has shownthatthe dominant components of the investigated extracts were saponins(Table 2).

Table 2:Influence of vegetation phase of the plant on the composition and properties of *S. officinalis*water root extracts

Water extract	PS, %	TF, %	PC, %	PC_{50}ug*ml^{-1}
EWS (September)	15.0	55.0	3.4	250
ES (July)	11.9	58.6	1.7	80
ES (September)	12.3	57.6	1.0	250
EDFS (July)	4.6	73.1	1.5	50
EDFS (September)	9.1	66.6	1.1	150

PS – polysaccharides; TF – total fraction of saponins; PC – phenolic compounds; PC_{50} –Hemolytic activity – concentration required to achieve 50% lysis of erythrocytes

Their content ranges from 55 to 73% depending on the type of Saponaria. Double-flowered soapwort extracts had a highersaponinscontent(67–73%)against the extracts of the cultured soapwort(58–59%) and wild forms of perennialSaponaria(55%).

Aregularity in the change of the content of saponins and polysaccharides – the main components of the extract associated with the phenological cycle of the plant – has been detected. Thus, with the increase in the period of vegetation,a decrease in the proportion of saponin and an increase in the proportion of polysaccharides was observed(Table2).More clearly this trend was viewed for the wild roots extract, which can be considered as perennial roots of the soapwort, as they served seed for the latter.

Polysaccharide contentincreased up to 15%, and saponin content decreased to 55% as compared with extracts of the roots of the common type of the second year of cultivation collected in the same phenological phase. A similar but less pronounced tendency to accumulate polysaccharides was also observed depending on the vegetative phase of the plant development – in extracts of the roots of both species collected in autumn, there werean increasing proportion of polysaccharides and the decreasing proportion ofsaponins.

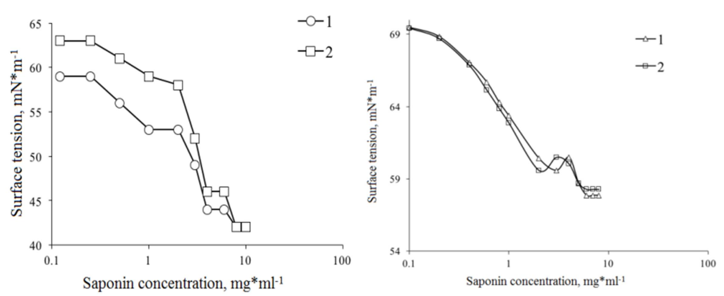
Apparently, the increase in polysaccharides is due toaccumulation ofreserve polysaccharide in the rootsduring the preparationfor winter anddecrease inthe relative weight of the cortex, which is known to be the first place to accumulatesaponins as a result of the age-related roots lignification.

The vegetative phase did not substantially influence the saponin content in the extracts, becausethe total contain of saponins according to harvesting periodvaried little (Table2). However, there was a significant change in the hemolytic activity of the extracts–the extracts of summer rootshad greater activity (50–80 μg*ml^{-1}) in comparison with autumn roots (150–250 μg*ml^{-1}), which, apparently, is an evidence of high content of more toxic low polar saponins in the roots of the summer plants(Oda et al., 2000).

Consequently, a highsaponin contentin the extractsof the roots of the second and third years of cultivation andthe observed decreasing trend in saponincontentin the extractsof perennialroots make it possible toreduce the cultivation period to two years. For the final justification ofthe roots harvestingterms,a comparative study of physical and chemical properties of the extracts from the roots of Saponariaofvarious forms and terms of vegetation was conducted.

Physical and chemical propertiesof the extractswere estimated bymainaspectsthat characterizefunctional and technological efficiency of the emulsifier: surface (interfacial) activity, whichdetermines the ability of the extractsto be adsorbedon theinterfacial surfaceandreduce the surface tension at the interface; micellar parameters, which includecritical micelle concentration – a quantitative characteristic of the efficiency of an emulsifier, the degree of hydrophobicity of the formed micelles. These parameters of the extracts were assessed in comparison with parameters of the saponinsextracted from *S. officinalis*roots with the standard method (Dekanosidze et al., 1982, p. 151).

The interfacial tension is one of the main parametersthat determine the dispersibility of a two-phase system – low superficial tension contributes to the formation of stable fine emulsion systems.The isothermof saponins surface tension (Figure 1A) bears evidenceof theirhigh interfacial activity, becausewith the increase in concentration, there is a reduction ofthe surface tension at the interface to the minimum value of 41–43 mN*m^{-1} (depending on the type ofthe roots).



A

B

Fig. 1: Surface tension isotherms: A – saponins of soapwort roots (1) and double-flowered roots (2); B – extracts of soapwort roots (1) and double-flowered (2) rootsof*S. officinalis* (water, 22°C, by the drop counting method), mN*m^{-1}.

Interfacial activity of saponins of the roots *S. Officinalis* is commensurate with activity of commercial *quilajal*saponin (by the Sigma company), for which this indexis36–37 mN*m^{-1}(Mitra and Dungan, 1997).

Root extractsshowedlowerinterfacial activity, while theisotherms were almost identical to these ofsaponins (Figure 1B). The increase in the minimum surface tension up to 55–58 mN*m^{-1} appears to be caused by the presence of related plant components.

An observable character of isotherm curves –passingthrough the minimum – is connected with a high heterogeneity ofthe investigatedsaponins. According to the results of capillary electrophoresis, saponins of double-flowered roots *S. officinalis*are a blend ofat least seven saponins of different polarity (Figure 2) and different degree of the hydrophilic-lipophilic balance,and, as a result, different adsorptionrateat the phase interface.

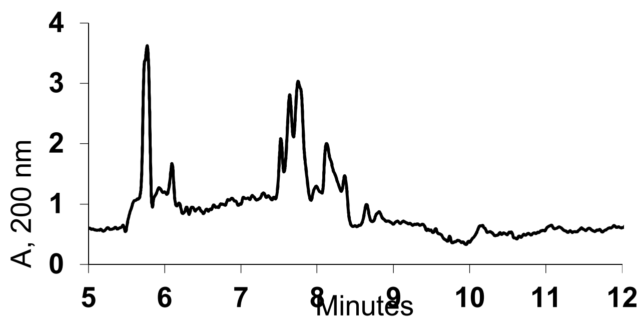


Fig. 2: Capillary electrophoresis of the saponinsof double-flowered soapwort *S. officinalis* (150 mM borate buffer pH 9.3, 15% methanol; Capillary Electrophoresis System 270A, Applied Biosystem).

A similar nature of isotherms caused by saponins' complexity has been earlier shownby Trapeznikovet al. (1970).

Because theemulsifying ability of surface-active agents depends on their micellar characteristics, micellarproperties of the extracts from two types of roots of cultivated *S. officinalis*are crucial characteristics when using them as food emulsifiers (additives). Colloidal surface-active agents demonstrate their emulsifying properties with a concentration higher than CCM, therefore this value can serve asa quantitativeparameter designatingemulsifiers' efficiency (Abramzon et al., 1984).

Determination of CCMwas carried out by the fluorescence probes method. Figure 3 displays the influence of saponin concentration in roots of soapwort and double-flowered soapwort (summer harvest) of *S. officinalis* on probes incorporation.

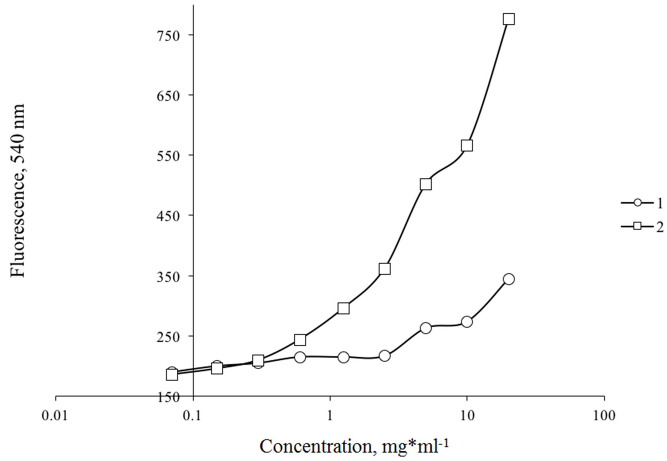


Fig. 3: Influence of saponin concentration on ANS incorporation in saponin micelles of soapwort (1) and double-flowered soapwort (2)of*S. Officinalis.*

The fluorescence increase was higher than CCM value, whichgave evidence of thebeginning of micelles formation. Saponins of double-flowered soapwort and soapwort had differentCCM values, because a linear increase in fluorescence was registered after reaching a critical concentration of 0.68 and 1.20 mg*ml^{-1} (in the water), respectively. The slope of the fluorescence curves thatis determined by the amount of the included probe pointed toa higherlevelof the micelles hydrophobicity formed by the double-flowered soapwort saponins.

Micellar characteristics of the double-flowered soapwort saponins coincided with characteristics of commercial samples of the saponins*quillaja*(by the Sigma andAcrosPenco companies), the CCM value of which ranged from 0.56 to 0.75 mg*ml^{-1} (Mitra and Dungan, 1997).

Comparison with the properties of traditionally used food emulsifiers, such as lecithin (E322), mono- and diglycerides (E 471), seems to benot correct, because they are water-insoluble and have another mechanism of hydrocarbyladsorptionon the interface.

Micellar characteristics of aqueous extracts, as well as saponin characteristics, depended on thetype of the plant (Figure 4).

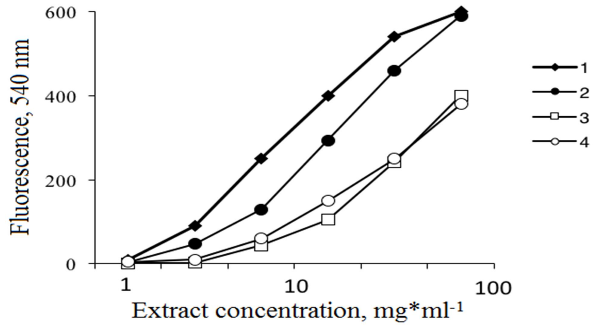


Fig. 4: Influence of cultivation time on CCM values of the extracts: 1, 2 – from double-flowered soapwort roots, 3,4 – from soapwortroots (1,3 – the third year; 2,4 – the second year).

An increasein fluorescence of the double-flowered soapwort roots extracts,testifying for thebeginning of micelle formation, was also observed at a lower concentration – 1.24 mcg*ml^{-1}, while for the extract of soapwort it was observed at the concentration of 3.64 mcg*ml^{-1} (summer harvest roots). The slope of the fluorescence curves also pointed to a higher hydrophobicity of the micelles formed by the bouncing Bet extract. The higherCCM values as compared with the values for saponins, are, apparently, associated with related plant components (polysaccharides and phenolic compounds) they contain.

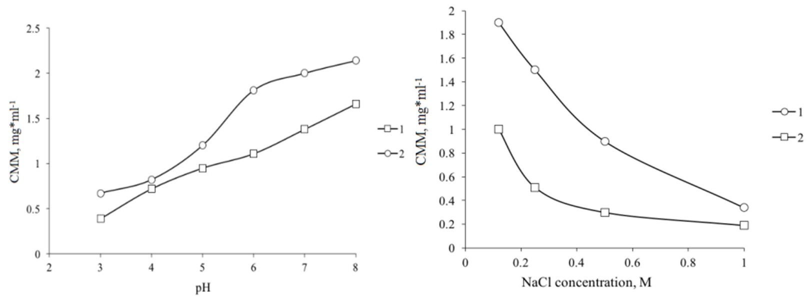
The roots age did not have any essential effect on the character of fluorescence curves. The extracts from the roots of the second and third years of cultivation had a similar slope of the fluorescence curves, and micelles formationbegan at the same mass fraction of soluble solids (Figure 4).

ThepH value and ionic strengthof watermediumare generally known to influence micellar characteristics of ionic surface-active agents.

Sincethere are carboxyl groupsin the saponins of *S. officinalis*structure (oneinthe aglycone, another one – in thecarbohydrate chains), it was necessary to reveal the influence of this factors on saponinmicellar characteristics (Figure 5).

The research results have shown thatCCM values of saponins gradually increased with the decreaseinpH values.

Ionization of carboxyl groups in an alcaline medium (pH 8) heightenedelectrostatic repulsive forces, which resulted in the micelle formation having been observed at a higher saponin concentration.



A B

Fig. 5: Influence of pH of the medium (A) and salt concentration (B) on CCM of the saponins of *S. officinalis*: 1 – of soapwort; 2 – of bouncing Bet types (22°C).

Saponinof *S. officinalis* have shown a regularity in the CCM changes depending on the NaCl concentration, which istypical of all ionic surface-active. Thus, in solutions of weak ionic force (0.12 M), as a result of electrostatic repulsive forces of carboxyl groups, the micellar were formed at the highest saponin concentration. With the increase in sault concentration, significant decrease in CCM values was observed,which is attributed to a partial screening of electrostatic repulsive forces.

Sharp decrease in the values was observed at the sault concentrationof 0.5 M. A further increase in the concentration did not influence significantly theCCM value. The CCM values of the bouncing Betsaponins were lower as compared to the soapwortsaponinsin all of experiments bellow (Table3).

Table 3:CCM values of saponinsof *S. officinalis*depending onthe aqueous phaseconditions, mg*ml^{-1}

Population	pH					NaCl, M, pH 7.0			
	pH 3	pH 4	pH 5	pH 7	pH 8	1	0.5	0.25	0.12
Soapwort (*S. officinalis*L.)	0.67	0.82	1.20	1.81	2.14	0.34	1.11	1.51	2.27
Double-flowered soapwort (var.floreplene*hort.*)	0.39	0.72	0.95	1.38	1.66	0.19	0.47	0.51	1.01

Morphological traitsof the *S. officinalis*roots structure, their branched rhizome formed with little offshoots and absence of woody coredeterminetheir highcontent of extractives. Thus, more than 50% extractives of different nature were educed from the roots of double-flowered soapwort *S. officinalis* of the second vegetation year using the standard method, which is widely used for educing secondary metabolites of different classes (Ladygina et al., 1983, pp. 41–56) (Table 4).

Table 4:Chemical composition of the roots of double-flowered *S. officinalis*of the second year of vegetation

Content, %						
High-polar saponins(HPS)	Low-polar saponins (LPS)	Water-soluble polysaccharides	Phenolic glycosides	Proteic substances	Lipids	Minerals
29	8	5.5	3	0.9	0.3	5

Major secondary metabolites of two-year roots were saponins, sincethe total content was38% by weightof dry root, which is equivalent of79% of the mass fraction of allextractives. Of these, high-polar saponins dominated(29%),while a quantity of low-polar saponins was lower (8%). Besides saponins, the roots containedwater-soluble polysaccharides (5.5%) and phenolic glycosides (3%). The albumens and lipidscontentdid not exceed 0.9%and 0.3%, respectively, which is typical ofplant raw materials.A high contentof mineral substances (5%) detected in the rootsapparently was causedby the roots' ability to selectively accumulate micro- and macronutrients (Lovkova et al., 2001).Roots ofthe cultivated *S. officinalis* are "super"-concentrators of the some biologically important for any living organism micro- and macronutrients. (Table 5).

Table 5:Mineral composition of the cultivated double-flowered soapwort *S. officinalis*

Content, μg*g^{-1}								
K	Ca	Mg	P	Al	Fe	Mn	Cu	Co
9477	7717	556	2713	444	222	23.1	2.1	0.4

Their contentis well over average values typical of the plants of theareal.

III. Conclusions

To sum up, the shortest possible cultivationperiod(2 years), high yields(10 tonnesper 1 ha)of the roots containing 26–35% saponins make it possible to regard*S. Officinalis*introduced in the soil and climate conditions of PrimorskyKraiasa perspective commercial source of saponins.Theestablishedregularityof hemolytic efficiencychangesin the extracts from the *S. officinalis* rootsfrom the vegetative stage to the plantdevelopment makes it possible to expand the area of roots' application: it is reasonable to use autumn harvest roots (period of a low toxic level) in production of food emulsifier, and flower bud stage roots (period of thelargesttoxic level) – inmedicine and cosmeticsproduction.

A highsurface activity and CCM values of saponinsof the *S. officinalis*determine a functionally technological efficiencyof aqueous extracts from two root typesof

*S. officinalis*cultivatedinPrimorskyKrai. However, higherparametersof bouncing Bet (double-flowered soapwort)saponins allowsto recommend this type asa promising source of highly effective natural surfactants, which are not inferior to commercialsaponins*quillaja*bymicellar parameters.

A high content of mineral substances (5%) has been found in the roots. Roots of the cultivated*S. officinalis*are ”super”-concentratorsof the somebiologically importantmicro- and macronutrients for living organisms.

A decrease in CCM valuesunder acidic condition in the presence of salt makes it possible to modify the emulsion preparation technologyusing saponins as a natural emulsifier by varying acidity and salt concentration.

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