PRELIMINARY DATA REGARDING BIOACTIVE COMPOUNDS AND TOTAL ANTIOXIDANT CAPACITY OF SOME FLUID EXTRACTS OF *LONICERA CAERULEA* L. BERRIES

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Lonicera caerulea L., (blue honeysuckle, blue honeyberry or haskap) is an Eastern Siberian shrub with fruits known to have potential medicinal applications. The aim of this paper was to investigate the physico-chemical properties, composition and the correlation to the total antioxidant capacity of some fluid extracts of haskap berries for their use as nutraceuticals. The dried fruits were collected from Moldavia region in North-Eastern Romania and the extracts were prepared at a concentration of 10% (w/v) in ethanol concentrations of 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v), respectively. The total content of main bioactive compounds, such as polyphenols, flavonoids, tannins, anthocyanins, carotenoids, lycopene, lutein, vitamin C and carbohydrates was determined by UV-Vis spectrophotometric assays. Total antioxidant capacity of extracts was assessed by photochemiluminescence method, in comparison to Trolox®, an analogue of vitamin E, used as antioxidant standard. The obtained results emphasized that the hydroalcoholic extract in 70:30 (v:v) ethanol had significantly ($p<0.01$) higher content of vitamin C, carbohydrates and carotenoids than the other two extracts, and similar quantities of total phenolics, flavonoids, lycopene and lutein, In accordance, the same extract had the highest total antioxidant activity and close to

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the values of resveratrol, a known antioxidant polyphenolic compound. In conclusion, hydroalcoholic extracts of Lonicera caerulea L. berries could be used as valuable products for the formulation of novel nutraceuticals with significant antioxidant activity.

Keywords: *Lonicera caerulea* L., berries, fluid extracts, bioactive compounds, total antioxidant capacity

1. Introduction

Lonicera caerulea L., commonly known as honeyberry, blue honeysuckle, sweetberry honeysuckle or haskap, belongs to the honeysuckle family (Caprifoliaceae) and is closely related to decorative honeysuckles from *Lonicera* genus. It is a deciduous shrub that grows up to 2 m, having opposite, oval and waxy leaves on its branches. Flowers are 1-2 cm long, yellow-white color and have five equal petals. Fruits are dark blue berries, 1-2 cm long, cylindrical in shape with a flat end. Taste can vary from sweetish to bitter [1, 2].

It is a resistant shrub, native to circumpolar regions, having various local varieties in Northern and Central Asia, North America and Eastern Europe. One particular variety, *Lonicera caerulea* L. var. *kamtschatica* (Sevast.) (Fig. 1), native to Eastern Siberia and Northern Japanese Archipelago is the most cultivated throughout the world for its known applications in folk medicine or as a "super-food". Blue honeysuckle was introduced in Romania since 1998 and proved to be a special plant for its fruits with valuable biochemical content in polyphenols, anthocyanins, vitamin C, which indicating their use as functional food with an increased antioxidant action [3, 4]. Typical products derived from haskap berries are jams, juices, candies, canned fruits, infusions, and even wine through fermentation [5].

In recent years, *Lonicera caerulea* L. was actively cultivated in various countries with temperate climate, being a high-quality, blue-berried plant valued for ultra early fruit ripening, with multiple uses in medicine, cosmetics, food industry and agriculture [3, 4].

In Eastern Asian folk medicine, haskap berries are used against allergies, angina, infections, eye illnesses and periodontal disease. Studies on *Lonicera* berries extracts have shown various anti-diabetic and anti-aggregating activity, while also enhancing night vision, protecting the cardiovascular system or limiting the damage of vascular endothelia [1,4]. Also, they have been reported to exhibit strong antioxidant properties, along with antimicrobial [7], antitumor, anti-inflammatory, immunomodulatory, antiviral and anti-allergic activities [3, 4, 8].

Its diverse and high content of phytochemicals also provided hepatoprotective activity and potential benefits in neurodegenerative diseases [9]. Haskap berries have an increased content of anthocyanins, in particular of

cyanidin-3-*O*-glucoside, compared to other berries, such as strawberries, blueberries, cranberries and chokeberries [6].

A phenolics-rich extract of haskap demonstrated the capacity to suppress the pro-inflammatory factors in an experimental model *in vitro* on RAW264.7 macrophage cells and to attenuate the levels of serum transaminases and antioxidant enzymes in an *in vivo* model of adjuvant-induced arthritis in rats [10]. In addition, polyphenols from blue honeysuckle could inhibit lipid accumulation, a highly valuable effect in the most common, life-threatening diseases associated with obesity [11].

Recent studies approached the mechanisms by which microbiota and metabolites might shape host health in normal, infectious, allergic or asthmatic state. The potential use of the studied extracts in order to support dysbiosis could be also confirmed by numerous studies that reported the anti-inflammatory action of *Lonicera* extracts [12, 13, 14]. Further interdisciplinary research grouping asthma immunology, microbiome, nutraceuticals, metabolomics, translational pulmonary are needed in order to identify specific knowledge gaps and challenges and potential therapeutic effects of nutraceuticals [12, 13].

Carotenoids as bioactive compounds are common pigments in fruits, essential for melanin and retinol production, thus, for skin and eye health, while they also possess antitumor properties [5]. Phenolic acids, flavonoids and tannins as bioactive principles with protective functions against microbial and fungal pathogens [9], acting as radical scavengers, reducing agents and lipid oxidation inhibitors [10, 15, 16], also, present anti-inflammatory, antiproliferative and antiviral actions [9].

The aim of this paper was to assess the physico-chemical properties, the content of the main bioactive principles and the correlation to the total antioxidant capacity of some fluid extracts of indigenous blue honeysuckle *Lonicera caerulea* L. var. *kamtschatica* (Sevast.) berries, in regard of their use in biomedical formulations as nutraceutical products.

2. Experimental

The vegetal product of the fruit tree blue honeysuckle *Lonicera caerulea* L. var. *kamtschatica* (Sevast.) used in this study was represented by its berries with whitish dark blue surface and red-purple pulp, collected from Moldavia region in North-Eastern of Romania (Fig. 1), in the period April-May 2022, when they reached maturity.

Haskap berries were oven-dried at 45 °C, for 72 h, ground to a fine powder using an electric grinder and analyzed for dry biomass percentage. The berries extracts were prepared by ultrasonication of 10 g dried berries powder in 100 mL ethanol solvent, at concentrations of 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v),

respectively, using an ultrasonic processor (Hilscher, UP200HT), 200 W, at ambient temperature.

Fig. 1. Blue honeysuckle *Lonicera caerulea* L. var. *kamtschatica* (Sevast.) and its berries

The obtained hydroalcoholic extracts were filtered through Whatman blue band filter paper, at vacuum.

The physico-chemical parameters *p*H, conductivity and redox potential of the hydroalcoholic extracts of *Lonicera caerulea* L. berries were determined using a multi-parameter analyzer (Consort C831, *p*H electrode SenTix 82, WTW, temperature sensor, 0 - 14 *p*H, 0 - 100 °C, conductivity cell, SK10B, Consort, 0 - 80 °C, cell constant of 1.056, electrode redox potential SenTix ORP WTW, 0 - 100 °C).

The total phenolic content of fluid extracts was determined using an UV-Vis spectrophotometric version of the Folin-Ciocâlteu assay. Briefly, an aliquot of 1 mL of extract was reacted with 5 mL Folin-Ciocâlteu reagent (10%) and 4 mL sodium bicarbonate solution (7.5%) for 30 min. Spectrophotometric absorbance was read against a blank at 765 nm (Cecil Super Aurius CE 3021 Scanning UV-Vis Spectrophotometer, range 190-1100 nm, Cecil Instruments Limited, UK, PC software DataStream 3COD 9000). A calibration curve was built using different concentrations of gallic acid [17 - 19]. The results of total phenolic content were expressed as mg gallic acid equivalent (GAE)/kg dry weight (DW) [23 - 25].

For flavonoids content determination, an aliquot of 0.5 mL fruits extract was diluted in 4 mL water and 8 mL methanol p.a., and spectrophotometric absorbance of the mixture was read at a wavelength of 340 nm, against a blank of methanol [18, 21 - 25]. Tannins content was determined using the same procedure, after gelatin precipitation [18, 20].

The anthocyanins content determination was performed by reading the absorbance of the original extracts at 520 and 700 nm wavelengths. Conversion to concentration values was done according to the literature [26].

For determining total carotenoids content, the spectrophotometric absorbance was read at 470 nm, 647 nm and 663 nm wavelengths, against a blank

of 80% (v/v) acetone. Absorbance values were used to calculate carotenoids concentration, according to the specific trichromatic equations [22 - 25, 27].

Lycopene content was determined after dilution in acetone:petroleum ether (2:3, v/v) by absorbance reading at 453, 505, 645 and 663 nm wavelengths [28].

For lutein dosing, the extracts were diluted in petroleum ether and the absorbance reading was performed at 446 nm wavelength [29].

Vitamin C (total ascorbic and dehydroascorbic acids) content was determined by each extract reaction with 5% sulfuric acid and 5% ammonium molybdate, and absorbance reading at 494 nm wavelength [30].

The content of total soluble carbohydrates was determined by each extract reaction with sulfuric acid and 5% phenol, and spectrophotometric absorbance reading at 490 nm wavelength [31].

The determination of total antioxidant capacity of lipid soluble substances (ACL) of haskap extracts was performed by photochemiluminescence method using a Photochem apparatus (Analytik Jena AG, Germany) [32]. For analysis, each berries hydroalcoholic extract (coded stock solution) was diluted in 1:10 and 1:20 molar ratios, respectively, with kit reagent R_1, according to ACL procedure (Analytik Jena AG). Aliquots of 5 and 10 µL of each sample were taken from the supernatant and exposed to external radiation from a Hg lamp lined with phosphorous that provides the maximum energy at 351 nm wavelength, in the presence of a photosensitive reagent, producing free superoxid anion radicals in the analysed sample and a photochemical reaction. The free radicals were partially eliminated by the antioxidants present in the sample. The residual radicals' luminescence was measured as an electrical signal for 120 s and converted to concentration values. For analyses, the standard reagents kit (Analytik Jena, Germany) of ACL procedure was used: R_1 (dilution solvent), R_2 (buffer reagent), R_3 (photosensitive reagent), R_4 (reagent sized). Mixtures were prepared as presented in Table 1. A solution of 10% (w/v) resveratrol (3,4′,5-trihydroxy-trans-stilbene, 5-[(1E)-2-(4-hydroxyphenyl) ethenyl]-1,3-benzenediol) (Sigma Aldrich), a non-flavonoid polyphenolic compound widely found in berries skin, was prepared in 96% (v/v) ethanol and served as positive control. The calibration curve was built using a series of standard solutions containing 0.5, 1.0, 2.0, 3.0 nM Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a derivative of vitamin E (Fig. 2). The determinations were performed in triplicate and expressed as nM Trolox equivalents (TE)/µL sample, respectively mg (TE)/kg sample [33 - 35].

Table 1.

Working scheme of ACL procedure [33 - 36]

Reagents kit	R_1 (µL)	R_2 (µL)	R_3 (µL)	R_4 (µL)	Sample (µL)
Blank	2300	200	25	0	0
Calibration curve	2300	200	25	5	0
Measurement sample	2300	200	25	0	5; 10

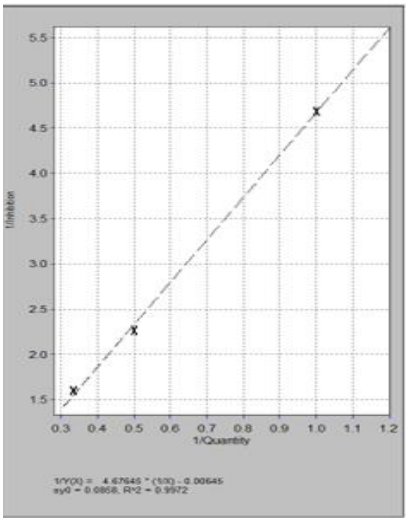

Fig. 2. Calibration curve using Trolox® as standard
(photochemiluminescence method, ACL procedure, Analytik Jena AG)

Data analysis has been performed by ANOVA-Single Factor test from Microsoft EXCEL. This tool performs a simple variance analysis of data obtained for two or more samples. The analysis provides a test of the hypothesis that each sample is drawn from the same underlying probability distribution against the alternative hypothesis that underlying probability distributions are not the same for all samples. Significant statistical difference was considered at $p<0.01$.

3. Results and Discussion

The analyzed hydroalcoholic extracts of *Lonicera caerulea* L. berries were prepared using ultrasonication, a green technique that significantly improves the extraction yield of bioactive compounds from plants and seeds and it is currently used for the production of pharmaceuticals and nutraceuticals.

The physico-chemical parameters analysed for the hydroalcoholic extracts of *Lonicera caerulea* L. berries were *p*H, conductivity and redox potential. The obtained results are presented in Table 2. The data highlighted that samples emphasize an acidic *p*H with values between 3.22 and 4.05. Lower *p*H values but close, were registered in the case of berries extracts in 50:50 (v:v) and 70:30 (v:v) ethanol concentration, which indicated an optimal extraction of the bioactive compounds. In addition, it was observed that hydroalcoholic extracts of *Lonicera caerulea* L. in ethanol 50:50 (v:v) presented higher values of electrical conductivity (0.398 mS/cm) and redox potential (317 mV), compared to the extracts in 96:4 (v:v) ethanol concentration.

The composition of the fluid extracts of *Lonicera caerulea* L. was analysed by determination of the main bioactive compounds content.

Table 2

Physico-chemical parameters of *p*H, electrical conductivity and redox potential for *Lonicera caerulea* L. berries extracts in 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v) ethanol concentrations

Sample	*p*H			Electrical conductivity [mS/cm]			Redox potential [mV]		
Ethanol concentration, v:v	96:4	70:30	50:50	96:4	70:30	50:50	96:4	70:30	50:50
Lonicera caerulea L. berries extracts, 10 g/100 mL	4.05	3.35	3.22	0.132	0.371	0.398	253	309	317

The results of total extractable phenolic compounds showed variation between 47.399-55.355 mg/kg (reported to the initial fruit dry weight), with the highest value registered in the 50:50 (v:v) hydroalcoholic extract (Fig. 3). Of these, 5.574-14.966 mg/kg were flavonoids, with highest value in the 70:30 (v:v) hydroalcoholic extract (Fig. 4) and 424-685 mg/kg were anthocyanins, with the highest value in the 50:50 (v:v) hydroalcoholic extract (Fig. 5). The tannins content was upmost in extracts of 70:30 (v:v) and 96:4 (v:v) hydroalcoholic extracts (Fig. 4).

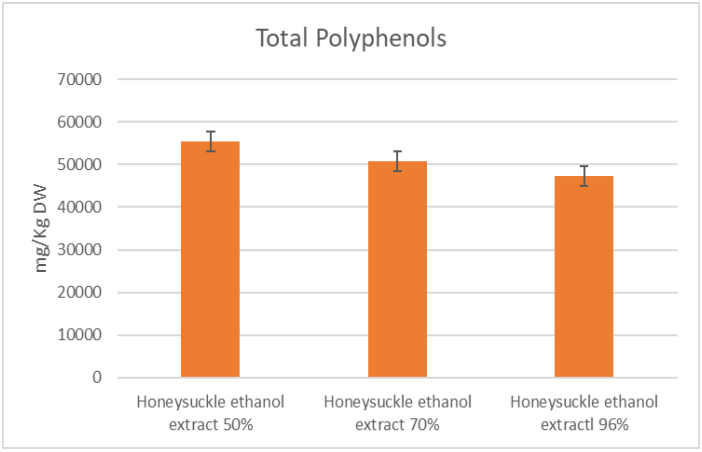

Fig. 3. Total phenolic content (mg/kg DW) of *Lonicera caerulea* L. berries extracts in 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v) ethanol concentrations

It was previously showed that the phenolics content of haskap berries extracts varied according to the solvent and the method of extraction [9]. The presence of a high content of phenolics is important for both food and biomedical applications, due to their involvement in free radicals scavenging and minimization of the lipid peroxidation chain propagation [36].

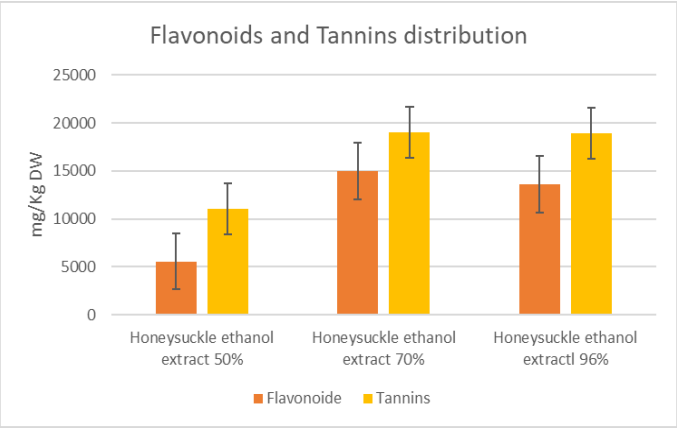

Fig. 4. Flavonoids and tannins content (mg/kg DW) of *Lonicera caerulea* berries extracts in 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v) ethanol concentrations

In regard of tannins-rich extracts consumption, other reports showed that, besides their sensorial properties, they provided several benefits for the human health, such as production of short chain fatty acids and positive modulation of gut microbiota [37].

Extracted carotenoids amounted for 77-207 mg/kg, with the highest average value in 96:4 (v:v) extract (Fig. 5). However, the upmost content of lycopene was registered in 70:30 (v:v) extract, while that of lutein in 50:50 (v:v) extract (Fig. 6).

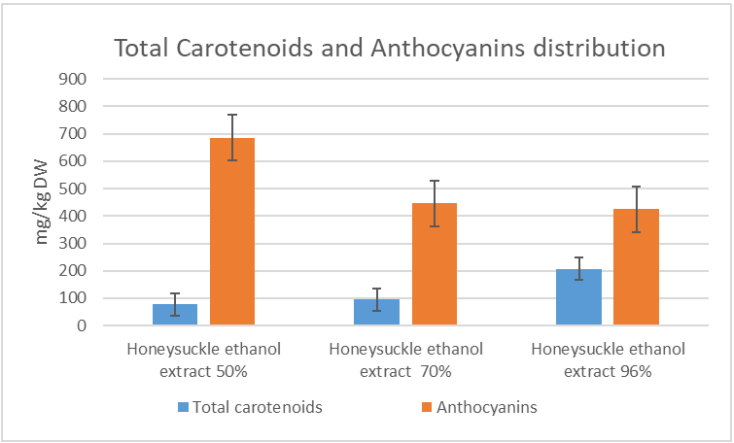

Fig. 5. Total carotenoids and anthocyanins content (mg/kg DW) of *Lonicera caerulea* berries extracts in 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v) ethanol concentrations

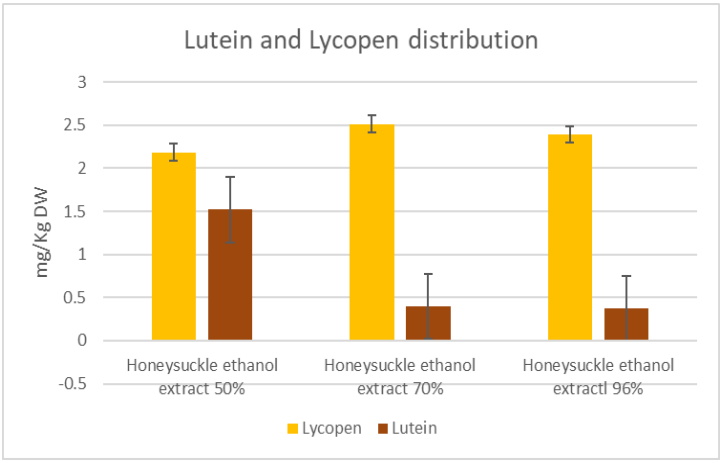

Fig. 6. Lutein and lycopene content (mg/kg DW) of *Lonicera caerulea* berries extracts in 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v) ethanol concentrations

It was previously mentioned that carotenoids had antioxidant and anti-inflammatory properties in normal cells, regulating the production of reactive oxygen species and pro-inflammatory cytokines [38]. In tumoral cells, carotenoids acted as pro-oxidant agents and regulators of the apoptosis process [39].

Total carbohydrates content of haskap berries extracts had values between 6100-7900 mg/kg DW. The hydroalcoholic extracts in 50:50 (v:v) ethanol had higher content of vitamin C than the other two extracts.

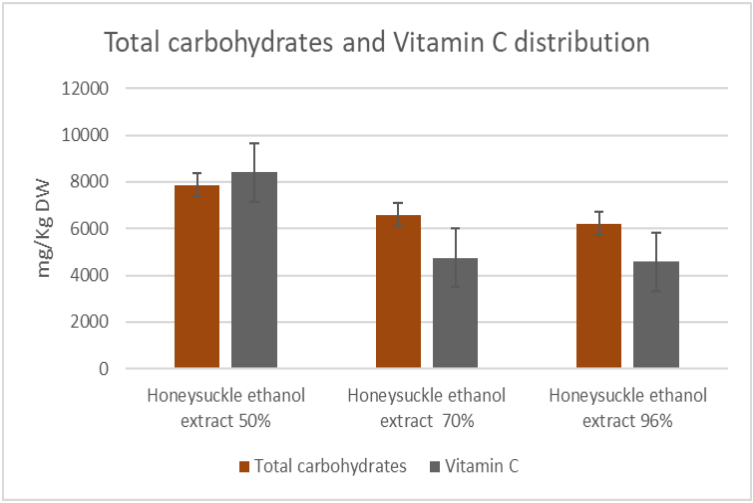

Fig. 7. Total carbohydrates and vitamin C content (mg/kg DW) of *Lonicera caerulea* berries extracts in 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v) ethanol concentrations

Previous studies have reported that values of ascorbic acid in *Lonicera caerulea* berries were higher when cultivated in wet climates with lower

temperatures, but also varied according to the solar radiation, stage of ripeness, cultivation conditions and harvest period [9].

In order to analyse these data representing constituents quantitation of haskap berries extracts at different concentrations of ethanol, i.e. 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v), respectively, the One-Way ANOVA statistical test has been used (Table 3). For an independent variable with *k* groups, the *F* statistic evaluated whether the group means were significantly different. According to the test results applied between two sets of data, it came out significant statistical differences ($p<0.01$) between the extraction method using different concentration of ethanol and the following substances: total carotenoids, tannins, anthocyanins, total carbohydrates and vitamin C. There were no significant statistical differences ($p>0.01$) found in relation to lycopene, lutein, flavonoids and total polyphenols. According to the determined values of bioactive compounds, the haskap berries extract in 70:30 (v:v) ethanol concentration was the most appropriate for our experimental setup.

Table 3.

Statistical analysis of *Lonicera caerulea* L. berries extracts in 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v) ethanol by ANOVA test

Groups	*Source of variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Total carotenoids	Between Groups	704.7042	1	704.7042	12.12533	0.003077	4.493998
	Within Groups	929.8938	16	58.11836			
	Total	1634.598	17				
Lycopene	Between Groups	0.49038	1	0.49038	0.606541	0.447459	4.493998
	Within Groups	12.93579	16	0.808487			
	Total	13.42617	17				
Lutein	Between Groups	3.449934	1	3.449934	7.778409	0.013136517	4.493998
	Within Groups	7.096431	16	0.443527			
	Total	10.54636	17				
Flavonoids	Between Groups	1928465	1	1928465	1.67821	0.213541926	4.493998
	Within Groups	18385916	16	1149120			
	Total	20314381	17				
Total polyphenols	Between Groups	653627.9	1	653627.9	0.007111	0.93384493	4.493998
	Within Groups	1.47E+09	16	91923921			
	Total	1.47E+09	17				
Tannins	Between Groups	6.99E+10	1	6.99E+10	18.23028	0.000587	4.493998
	Within Groups	6.14E+10	16	3.83E+09			
	Total	1.31E+11	17				
Anthocyanins	Between Groups	216330.1	1	216330.1	1447.733	4.02238E-17	4.493998

	Within Groups	2390.828	16	149.4267			
	Total	218720.9	17				
Total carbohydrates	Between Groups	7151074	1	7151074	39.73945	1.04943E-05	4.493998
	Within Groups	2879184	16	179949			
	Total	10030258	17				
Vitamin C	Between Groups	60280200	1	60280200	71.43667	2.69857E-07	4.493998
	Within Groups	13501235	16	843827.2			
	Total	73781435	17				

Total antioxidant capacity of *Lonicera caerulea* L. berries extracts in 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v) ethanol and resveratrol reference substance in 96:4 (v:v) ethanol, compared to Trolox® standard, was quantified according to the ACL procedure (Analytik Jena AG). The free radicals inhibition curves are presented in Fig. 8.

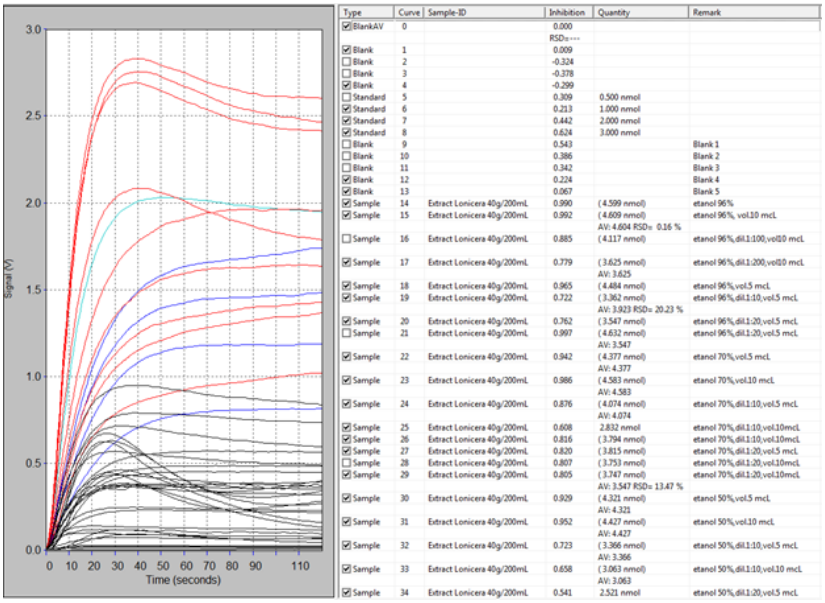

Fig. 8. Free radicals inhibition curves of *Lonicera caerulea* L. berries extracts in 50:50 (v:v), 70:30 (v:v) and 96:4 (v:v) ethanol and resveratrol reference substance in 96:4 (v:v) ethanol, compared to Trolox® standard, according to ACL procedure (Analytik Jena AG)

The quantitative results of total antioxidant capacity of *Lonicera caerulea* L. berries hydroalcoholic extracts and resveratrol as reference substance, expressed in mg TE/kg DW, are presented in Table 4. The obtained results emphasized that all analyzed hydroalcoholic extracts of *Lonicera caerulea* L. berries presented an increased total antioxidant capacity. It was observed that sample dilution to 1:10 and 1:20 molar ratios, respectively, and the minimum

working volume of 5 μL decreased the maximum inhibition of free radicals due to an increase of the *TEAC* Quantity (mg TE/kg DW).

Table 4.

Free radicals maximum inhibition and total antioxidant capacity (*TEAC* Quantity) of *Lonicera caerulea* L. berries hydroalcoholic extracts and resveratrol as reference substance

No.	Sample / dilution / working volume	Free radicals Max. Inhibition	Total antioxidant capacity (nM TE/μL)	*TEAC* Quantity means (mg TE/kg DW)
1.	10% *Lonicera* extract in 96:4 (v:v) ethanol / stock sol. / 10 μL	0.992	4.604	1152.381
2.	10% *Lonicera* extract in 96:4 (v:v) ethanol / dil. with ethanol 1:10 / 10 μL	0.885	4.117	10304.851
3.	10% *Lonicera* extract in 96:4 (v:v) ethanol / dil. 1:20 / 10 μL	0.779	3.625	18146.750
4.	10% *Lonicera* extract in 96:4 (v:v) ethanol / stock sol. / 5 μL	0.965	4.484	2244.690
5.	10% *Lonicera* extract in 96:4 (v:v) ethanol / dil. with ethanol 1:10 / 5 μL	0.722	3.362	16830.172
6.	10% *Lonicera* extract in 96:4 (v:v) ethanol / dil. with ethanol 1:20 / 5 μL	0.762	3.547	35512.564
7.	10% *Lonicera* extract in 70:30 (v:v) ethanol / stock sol. / 10 μL	0.986	4.583	1147.124
8.	10% *Lonicera* extract in 70:30 (v:v) ethanol / dil. with ethanol 1:10 / 10 μL	0.816	3.794	9496.382
9.	10% *Lonicera* extract in 70:30 (v:v) ethanol / dil. with ethanol 1:20 / 10 μL	0.807	3.753	18787.518
10.	10% *Lonicera* extract in 70:30 (v:v) ethanol / stock sol. / 5 μL	0.942	4.377	2191.126
11.	10% *Lonicera* extract in 70:30 (v:v) ethanol / dil. with ethanol 1:10 / 5μL	0.876	4.074	20394.444
12.	10% *Lonicera* extract in 70:30 (v:v) ethanol / dil. with ethanol 1:20, 5 μL	0.820	3.815	38195.780
13.	10% *Lonicera* extract in 50:50 (v:v) ethanol / stock sol. / 10 μL	0.952	4.427	1108,0781
14.	10% *Lonicera* extract in 50:50 (v:v) ethanol / dil. with ethanol 1:10 / 10 μL	0.658	3.063	7666.689
15.	10% *Lonicera* extract in 50:50 (v:v) ethanol / dil. with ethanol 1:20 / 10 μL	0.794	3.693	18487.158
16.	10% *Lonicera* extract in 50:50 (v:v) ethanol / stock sol. / 5 μL	0.929	4.321	2163.093
17.	10% *Lonicera* extract in 50:50 (v:v) ethanol / dil. with ethanol 1:10 / 5 μL	0.723	3.366	16850.196
18.	10% *Lonicera* extract in 50:50 (v:v) ethanol / dil. with ethanol 1:20 / 5 μL	0.541	2.521	25240.252
19.	10% Resveratrol (Reference substance) in 96% ethanol / stock sol. / 5 μL	0.999	4.701	2353.321
20.	10% Resveratrol (Reference substance) in	0.966	4.488	2246.693

	96:4 (v:v) ethanol / dil. with ethanol 1:1 / 5 µL			
21.	10% Resveratrol (Reference substance) in 96:4 (v:v) ethanol / dil. with ethanol 1:2 / 5 µL	0.958	4.455	4460.346
22.	10% Resveratrol (Reference substance) in 96:4 (v:v) ethanol / dil. with ethanol 1:4 / 5 µL	0.843	3.921	7851.410
23.	10% Resveratrol (Reference substance) in 96:4 (v:v) ethanol / dil. with ethanol 1:6 / 5 µL	0.840	3.908	11738.069
24.	10% Resveratrol (Reference substance) in 96:4 (v:v) ethanol / dil. with ethanol 1:8 / 5 µL	0.777	3.617	14485.362
25.	10% Resveratrol (Reference substance) in 96:4 (v:v) ethanol / dil. with ethanol 1:10 / 5 µL	0.772	3.595	17996.570

The highest value of *TEAC* Quantity (38195.78 mg TE/kg DW) was registered for 5 µL of 10% *Lonicera caerulea* L. berries extract in 70:30 (v:v) ethanol, diluted 1:20. Lower *TEAC* Quantity value (25240.25 mg TE/kg DW) was registered for the extract in 50:50 (v:v) ethanol, in the same working conditions.

It was observed that the *TEAC* Quantity values of 5 µL extract in 96:4 (v:v) ethanol (2244.69 mg TE/kg DW) were similar to those registered for the analytical reference substance, resveratrol (2353.32 mg TE/kg DW), in the same working conditions. The high *TEAC* Quantity values could be also correlated to the high bioactive compounds content found in *Lonicera caerulea* L. berries extract in 70:30 (v:v) ethanol concentration. In accordance to these results, strong correlation was previously reported between the total phenolics and total carotenoids content and the antioxidant activity of *Lonicera caerulea* L. berries extracts [9]. Moreover, polyphenolic components of berries extracts have exerted an effect of free radicals scavenging in biological systems, as natural antioxidant agents and several biological properties involved in the anti-aging processes, such as inflammation, oxidative stress, cell apoptosis [40].

4. Conclusions

The hydroalcoholic extracts of *Lonicera caerulea* L. var. *kamtschatica* (Sevast.) berries obtained in different concentrations of ethanol presented a high diversity of bioactive compounds. In particular, the 70:30 (v:v) hydroalcoholic extract was rich in flavonoids, tannins, carotenoids, carbohydrates and vitamin C. Statistical analysis indicated that no significant differences were found between the extracts, in regard of total phenolics, flavonoids, lycopene and lutein content. The 70:30 (v:v) hydroalcoholic extract had the highest total antioxidant activity, determined by photochemiluminescence method and close values to a known

antioxidant polyphenolic compound, resveratrol, were determined. All these results demonstrated that the hydroalcoholic extracts of haskap berries could be valorized as nutraceuticals with antioxidant activity. Future studies will investigate the loading of *Lonicera caerulea* L. berries extracts in natural matrices for protection of components against oxidation and their biological activity.

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