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Polyphenolic Compounds in the Stems of Raspberry (*Rubus idaeus*) Growing Wild and Cultivated

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Abstract: The stems of *Rubus idaeus* L., a by-product of the fruit-food industry, are known sources of bioactive compounds. The main objective of this study was to investigate the composition of the polyphenolic compounds in *R. idaeus* stems. 7 cultivated raspberry varieties, 13 garden samples, including five well-known raspberry varieties and 13 wild raspberry samples from different locations in Estonia were analyzed. The HPLC-MS method detected 62 substances, of which 42 were identified, 12 were tentatively identified, and 8 compounds remained unknown. Protocatechuic acid pentoside was dominant in most varieties and in all garden and wild raspberry samples. Dihydroxybenzoic acid hexoside 1, *p*-coumaroyl quinic acid 1, quercetin 4'-glucuronide and *p*-coumaric acid glycoside were found in significant quantities. Correlations between the contents of individual compounds have been established. When studying the dynamics of polyphenolic compounds accumulation on the example of the GR1 sample during the year, it was found that the largest amount of them in raspberry stems accumulates in April, slightly less in January and October. Investigating the dependence of the accumulation of polyphenols on the parts of the stem, it was found that the upper parts have the highest phenolics content. Therefore, it is recommended to harvest approximately the upper third of the stem.

Keywords: *Rubus idaeus*; stems; polyphenolic compounds; HPLC/MS analysis

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1. Introduction

Rubus idaeus L., or raspberries, or red raspberries, of the *Rosaceae* family, is a well-known plant with a natural habitat in Europe, Asia, and North America, introduced in other regions of the world. It is one of the most famous cultivated berry crops [1,2]. In 2022, world production of raspberries was 1,43 billion kg. The main producers were Mexico (18.33% of the world total), Serbia (12.25%), Poland (11.07%), the United States (8.07%) and Ukraine (3.54%) [3].

This fruit provides the vitamins, minerals, fatty acids [4,5], proteins, polyphenolic compounds [6–8], especially ellagitannins [9] and anthocyanins [10], carbohydrates and dietary fibre [11] needed for healthy nutrition in humans and animals [12–15]. Adding raspberries to starch-based foods has not altered the glycemic response [16].

Antioxidant [17–23], anti-inflammatory [24,25], antihypertensive [26], vasorelaxation [27], neuroprotective [28] and antimicrobial [29] activity have been established for raspberry fruits. Its potential in modulating the risk of metabolic diseases, especially cardiovascular disease, diabetes, obesity, and Alzheimer's disease – all of which have critical metabolic, oxidative, and inflammatory connections have been confirmed [20]. It has been found that raspberry polyphenols may be a dietary route to slow down or alleviate neurodegenerative dysfunctions [21]. The flavonoids of *R. idaeus* had a good therapeutic effect on the perimenopausal mouse model after administration of its high, medium, and low doses for some time [22].

The chemical composition of raspberry leaves has been extensively studied. It was discovered polyphenolic compounds [30–37], mainly hydrolyzable tannins (2.6% to 6.9%) [38], including gallotannins which are esters of gallic acid and D-glucose [38,39], dimeric and tetrameric ellagitannins have also been identified; flavonoids such as kaempferol, Kaempferol hexosides, quercetin and quercetin glycosides [38]; phenolic acids such as chlorogenic, gallic, ferulic, and caffeic acids [40]. In addition, terpenes such as oxygenated monoterpenes, 1,8-cineole (50.8%), α-terpineol (5.2%), terpinyl acetate (3.7%), and camphor (2.9%) and others [40], carotenoids [8,35], Vitamin C and E, and minerals such as calcium, magnesium and zinc have been identified. A monograph on Raspberry Leaf (ref.:2950) was newly included in Ph. Eur. [41].

In the EC, the dry extract of *R. idaeus* leaves (solvent water) is a herbal remedy for the symptomatic relief of minor spasms associated with menstrual periods, for the symptomatic treatment of mild inflammation in the mouth or throat and for the symptomatic treatment of mild diarrhoea [42,43].

Raspberry leaf extract can significantly modulate platelet reactivity in whole blood: it affects platelet aggregation, possibly through modulation of redox state, which depends on the oxidative activity of neutrophils [44]. Fatty acids and terpenoids account for the antifungal effect of raspberry leaves and stems against *Candida albicans* [45]. Experimental studies show that red raspberry leaf extract has antioxidant, antibacterial, and anti-inflammatory effects [46,47].

Stems are studied less than fruits, probably because of the wide use of fruit in human nutrition. However, the antioxidant, antimicrobial and neutrophil-modulating activity of extracts of the herb *R. idaeus* have been established [48,49]. The crude aqueous extracts from the aerial part of raspberries exhibit antiparasitic activity against *Toxoplasma gondii* [50]. Antioxidant activity of raspberry stem and bark extracts was found [51]. Research has shown that ethanolic extracts from the fruits, roots, stems, seeds, leaves, unripe fruits, and inflorescences of raspberry "Polka" are effective against *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Bacilus subtilis*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* [52]. Raspberry stem extract has also been found to inhibit the activity of α-amylase and α-glucosidase, and exhibit anti-AGE activity [53].

Raspberry stems have traditionally been used in Estonia as tea to relieve symptoms of colds and reduce fever. In addition, raspberry stems have played an important role in relieving various pains (including rheumatism, joint, head and abdominal pain), cough, menstrual ailments, diarrhoea, indigestion, intestinal inflammation, internal bleeding and anaemia [54,55]. Tea of the stems and leaves taken from the barn helps with acute respiratory diseases, with a decoction of the leaf and stem, the throat should be rinsed for angina and laryngitis [56]. Baths made from stems and twigs have been used for rheumatic pains, skin inflammations and eczema [57].

In Estonian folk traditions, it is recommended to use different forms of raspberry plant primarily to lower fever in case of a cold, and this is precisely because of their sweating effect. Raspberry stems were the most common, followed by fruits and jam made from them [58]. In addition to lowering the fever, the old people considered raspberry stalk tea a good treatment for cough (especially closed, unproductive cough), sore throat, bronchitis and runny nose. It was said that when suffering from tuberculosis, one had to drink tea made from coarse raspberry stems. Also, for diabetes, raspberry stem tea was recommended, which was supposed to be drunk 1 litre per day. Raspberry stalk tea was also a good remedy for relieving abdominal pain. In addition to the above, raspberry stem tea was important for women with painful menstruation. It was suggested that rather younger shoots be used [58]. Since raspberries raise diuresis, they were also considered useful for bladder problems. Raw raspberries were eaten half a litre daily for nervous diseases and fever [58].

It is known that the content of polyphenolic compounds and their composition differ in wild and garden raspberries, as well as their different varieties, and, in addition, depend on the stage of development and environmental conditions [51,59–65].

In farms that cultivate raspberries, pruning and thinning raspberry bushes are regular agrotechnical means [2]. Removed stems and shoots are production waste and will not be used

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further. But, considering the experience of their use in traditional medicine, they can be an additional source of valuable biologically active compounds (BAC).

The aim of the study was to analyze the qualitative and quantitative content of polyphenolic compounds in stems of 1) raspberry cultivars (RC), garden raspberry (GR) and wild raspberry (WR); 2) in different parts of raspberry stems (five parts from top to bottom); and also 3) to establish dynamics of the content of polyphenolic compounds in stems within 12 months.

2. Results

The results of the HPLC analysis of the raspberry stems are presented in Table 1 and Figure 1. For identification, the m/z of fragments of the MS/MS spectra of the substances were compared with literature data [66,67] and data of standard substances.

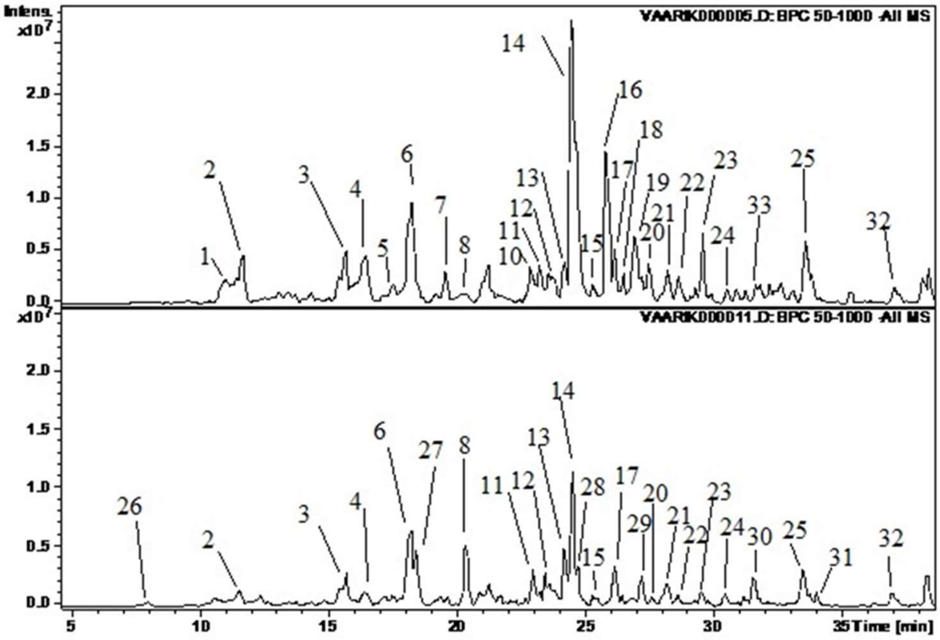

Figure 1. Illustrations of chromatograms (Base Peak Chromatogram). The upper graph represents the sample VA 4, the lower WR 1. Substances corresponding to peaks: 1 – Dihydroxybenzoic acid hexoside 2; 2 – Protocatechuic acid pentosidetechic acid; 3 – Chlorogenic acid; 4 – Procyanidin B(2); 5 – Procyanidin B(3); 6 – Epicatechin; 7 – *p*-Coumaric acid glycoside; 8 – *p*-Coumaroyl quinic acid 2; 10 – Quercetin glucorhamnoside; 11 – Quercetin pentoside 1; 12 – Ellagic acid; 13 – Hyperoside; 14 – Quercetin 4′-glucuronide; 15 – Quercetin 7-glucuronide; 16 – Kaempferol glucoside; 17 – Isorhamnetin glucoside, 18 – Quercetin-3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside); 19 – Kaempferol glucuronide; 20 – Isorhamnetin/rhamnetin; 21 – Unknown 6; 22 – Acetylxyloside of ellagic acid; 23 – Isorhamnetin rhamnoside; 24 – Isorhamnetin rhamnosideive 2; 25 – Unknown 10; 26 – Dihydroxybenzoic acid glucoside 1; 27 – p-Coumaroyl quinic acid 1; 28 – Isoquercetin; 29 – Isorhamnetin rhamnosideive 1; 30 – Chlorogenic acid rhamnoside; 31 – Neochlorogenic acid rhamnoside; 32 – Isorhamnetin C-hexoside 2.

Table 1. Phenolic compounds detected in raspberry stems.

Rt	M/z	M/z of main collision fragments	Compound
8.1	315	153;109	Dihydroxybenzoic acid hexoside 1
11.8	315	297;153;109	Dihydroxybenzoic acid hexoside 2
12	285	153;109	Protocatechuic acid pentoside
13.8	299	179;137;135	Hydroxybenzoic acid hexoside
14.7	577	559;451;425;407;289	Procyanidin B(1)
14.8	357	195;339	Dihydroxyferulic acid glucoside
15.1	289	245;205;179;125	Catechin

16	353	191;179;135	Chlorogenic acid
16.7	577	559;451;425;407;289	Procyanidin B(2)
17.7	577	559;451;425;407;289	Procyanidin B(3)
18.2	353	191;179;135	Neochlorogenic acid
18.5	639	463;301	Quercetin 3-glucuronide-glucoside
18.6	289	245;205;179;125	Epicatechin
18.8	337	191;163;173;301	*p*-Coumaroyl quinic acid 1
19.6	325	163;119;289	*p*-Coumaric acid hexoside
20.7	337	191;163;173;301	*p*-Coumaroyl quinic acid 2
23.1	609	301;302;431;179	Quercetin glucorhamnoside
23.2	595	463;343;301;300;179	Quercetin pentohexoside alias rumarin
23.4	433	301;300;151	Quercetin pentoside 1
23.9	301	229;257;185;284	Ellagic acid
23.9	433	300;302;387;161	Quercetin pentoside 2*
24.4	463	301;179;343;271	Quercetin galactoside alias hyperoside
24.6	477	301;179	Quercetin 4`-glucuronide
24.6	567	341;329;521;279	Unknown 1
24.6	609	301;343;271;179	Quercetin rutinoside alias rutin
24.8	499	475;463;489	Unknown 2
24.9	463	301;271;179;355;161	Quercetin glucoside alias Isoquercetin
25.6	477	301;323;221;179;161	Quercetin 7-glucuronide
26.0	433	300;301;151;179	Quercetin pentoside 3
26.2	447	285;255	Kaempferol hexoside
26.2	505	463;301;300;271	Quercetin acetylhexoside 1
26.2	607	463;301;151;545;505	Quercetin 3-[6''-(3-hydroxy-3-methylglutaroyl)-hexoside] 1
26.4	477	315;153;433	Isorhamnetin hexoside 1
26.8	447	315;300	Isorhamnetin pentoside 1
26.8	607	463;301;151;545;505	Quercetin 3-[6''-(3-hydroxy-3-methylglutaroyl)-hexoside] 2
26.9	505	461;301;300;271;179	Quercetin acetylhexoside 2
27.0	461	285;323;357;175	Kaempferol glucuronide
27.2	475	301;300;315;153	Isorhamnetin rhamnosideive 1
27.3	477	301	Quercetin 3-glucuronide
27.4	567	521;179;559;341;390	Dicaffeic acid derivative *
27.5	447	315;300	Isorhamnetin pentoside 2
27.7	315	300;301;271;153	Rhamnetin or isorhamnetin *
28.2	505	323;389;301;179;161	Acetyl hexoside
28.3	475	415;300;301;185	Ellagic acid acetylarabinoside *
28.3	571	523;345;357;195;493	Quercetin-3-glucuronide
28.7	475	300;301;323	Ellagic acid acetylxyloside*
28.7	515	353;191;179;317;299	Dicaffeoyl quinic acid
29.6	461	301;315;159;179;151	Isorhamnetin rhamnoside 1*
29.8	571	523;345;357;195;493	Unknown 3
30.4	489	315;429;300	Isorhamnetin rhamnosideive 2*
31.6	499	353;173;203;255	Chlorogenic acid rhamnoside *
31.7	489	315;429;300	Isorhamnetin rhamnosideive 3*
31.8	301	151;179;257;211	Quercetin
32.1	517	300;457;179	Unknown 4
32.6	489	315;429;300	Isorhamnetin rhamnosideive 4*
33.4	585	537;359;330;223	Unknown 5
33.5	517	300;457;179	Unknown 6

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33.8	585	537;359;330;223	Unknown 7
34.1	499	353;173;460;256	Unknown 8
35.8	531	471;300;314;411;456	Isorhamnetin C-hexoside 1*
36.9	531	315;300;411;471	Isorhamnetin *C*-hexoside 2*1
38.0	531	315;300;471;411	Isorhamnetin C-hexoside 3*

* In the case of these substances, doubts arose due to the non-overlap of some fragments, the retention time, or the identity of the substance came mainly from the literature.

The content of identified phenolic compounds in the analyzed raspberry stems was from 180.5 mg% in sample GR11 to 2246.2 mg% in sample GR12 (Figures 2 and 3, Table 2 and 3). The content of polyphenols in the raspberry stems growing in the wild (WR1-WR13) is presented in Table 4.

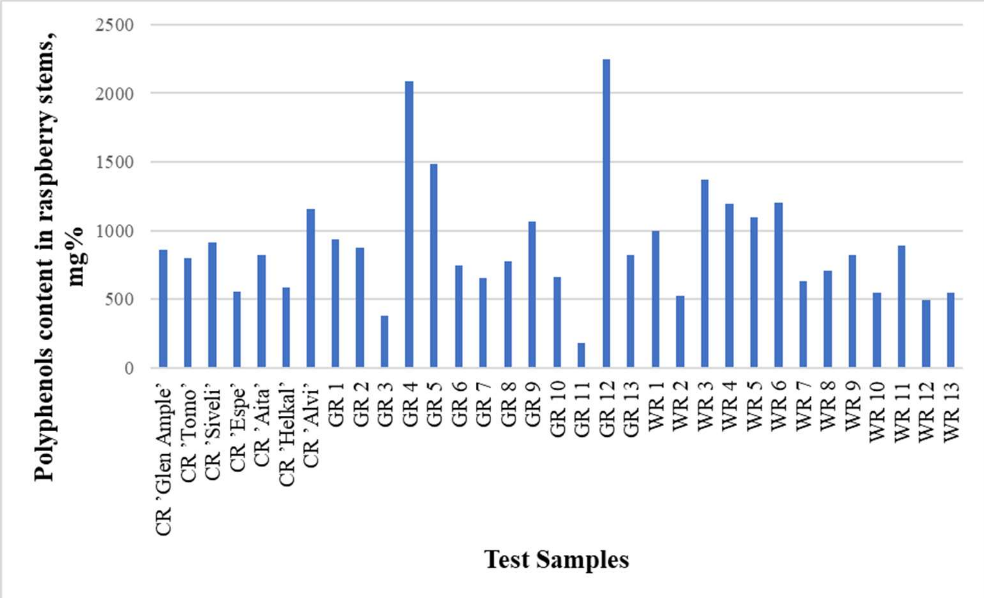

Figure 2. Graph for comparing the total concentrations of all the substances studied in all 33 samples.

In addition, a couple of samples were analyzed by positive ionization, which detected the presence of cyanidin hexoside, apparently either a glucoside or a galactoside. The mass of the corresponding positive molecular ion was 449, the main fragment had a mass of 287.

Table 2. Polyphenolics in the stems of raspberry cultivars, mg%.

Compound	CR ’Glen Ample’	CR ’Tomo’	CR ’Siveli’	CR ’Espe’	CR ’Aita’	CR ’Helkal’	CR ’Alvi’
Dihydroxybenzoic acid hexoside 1	41.5	29.3	36.1	-	80.5	-	17.5
Dihydroxybenzoic acid hexoside 2	18.1	49.9	35.7	18.5	118.5	53.5	51.1
Protocatechuic acid pentosidetechuic acid	153.6	135.1	375.0	89.8	254.8	242.7	448.8
Hydroxybenzoic acid hexoside	8.2	-	-	-	45.3	8.3	-
Procyanidin B(1)	2.4	-	-	-	-	-	-

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Dihydroxyferulic acid glycoside	-	-	2.0	-	-	-	-
Catechin	7.8	7.7	15.2	2.3	7.8	6.8	2.7
Chlorogenic acid	5.6	-	3.0	7.2	1.2	1.5	5.2
Procyanidin B(2)	16.3	-	8.9	-	3.6	-	-
Procyanidin B(3)	4.8	5.9	3.2	2.2	8.6	5.1	2.5
Neochlorogenic acid	1.0	5.9	1.0	1.0	-	0.8	1.0
Quercetin 3-glucuronide-glucoside	< 0.1	0.1	-	< 0.1	0.6	-	< 0.1
Epicatechin	2.9	2.0	2.8	0.4	2.3	2.6	1.5
P-Coumaroyl quinic acid 1	261.5	175.9	138.5	220.9	14.7	57.8	296.8
P-Coumaric acid glycoside	-	8.4	10.5	34.7	-	2.2	9.5
p-Coumaroyl quinic acid 2	139.2	112.1	98.0	114.1	14.5	37.1	127.4
Quercetin glucoramnoside	< 0.1	1.5	0.1	-	1.3	< 0.1	0.1
Quercetin pentoxoside	-	< 0.1	-	-	0.1	-	-
Quercetin Pentoside 1	2.1	1.9	2.3	0.8	2.4	1.5	1.5
Ellagic acid	15.1	27.34	28.2	14.2	19.6	26.8	22.0
Quercetin Pentoside 2	4.6	5.6	6.2	4.0	3.8	5.8	5.1
Hyperoside	0.6	2.6	1.2	1.3	2.0	1.2	1.4
Quercetin rutinoside alias rutin	24.3	4.1	1.6	2.2	5.8	2.2	3.1
Quercetin 4′-glucuronide	65.7	85.9	34.4	7.8	84.9	49.6	74.0
Isoquercetin	4.3	13.3	6.1	0.4	14.9	3.1	2.8
Quercetin 7-glucuronide	0.1	0.1	0.2	-	< 0.1	< 0.1	-
Quercetin Pentoside 3	0.1	39.8	17.3	< 0.1	51.9	27.7	16.2
Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 1	0.4	3.1	1.0	5.6	5.5	2.8	< 0.1
Kaempferol hexoside	1.6	-	1.1	0.2	-	0.5	0.9
Quercetin hexoside malonate	0.5	-	-	< 0.1	< 0.1	< 0.1	0.5
Isorhamnetin hexoside 1	< 0.1	< 0.1	< 0.1	< 0.1	0.2	< 0.1	< 0.1
Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 2	-	2.5	0.1	-	2.5	0.5	< 0.1
Isorhamnetin Pentoside 1	5.8	-	< 0.1	< 0.1	< 0.1	< 0.1	-
Kaempferol glucuronide	3.6	1.2	0.5	0.2	1.8	0.6	1.4
Isorhamnetin rhamnosideive 1	2.4	3.4	3.5	1.1	4.6	2.3	2.7
Dicaffeic acid derivative	7.0	14.2	18.0	-	20.0	7.3	-

Isorhamnetin Pentoside 2	< 0.1	1.3	0.2	< 0.1	1.3	< 0.1	0.8
Rhamnetin/isorhamnetin	<0.1	0.6	0.4	0.2	0.2	0.3	0.2
Ellagic acid acetylarabinoside	37.9	38.9	39.0	13.6	36.8	24.5	28.6
Acetylxyloside of ellagic acid	12.2	6.6	10.1	0.3	2.8	4.5	17.3
Dicafeoil quinic acid	1.3	1.2	1.2	1.6	1.2	-	1.4
Isorhamnetin rhamnoside	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	-	< 0.1
Isorhamnetin rhamnosideive 2	1.5	2.6	2.6	0.7	2.6	1.3	2.2
Chlorogenic acid rhamnoside	3.3	2.1	1.6	7.8	1.2	1.1	2.9
Isorhamnetin rhamnosideive 3	0.7	1.2	1.2	0.3	1.2	0.4	0.6
Quercetin	0.1	0.8	0.2	0.1	0.2	0.2	0.8
Neochlorogenic acid rhamnoside	1.7	1.3	1.1	3.4	-	-	1.5
Isorhamnetin rhamnosideive 6	3.2	3.7	4.1	0.6	4.2	1.6	2.5
Isorhamnetin rhamnosideive 7	< 0.1	-	< 0.1	-	< 0.1	-	0.8
Total:	862.8	799.1	913.0	557.1	825.1	584.0	1155.0

Table 3. Polyphenols in the raspberry stems growing in home gardens (GR1-GR13), mg%.

	Garden raspberry												
Compound	**GR 1**	**GR 2**	**GR 3**	**GR 4**	**GR 5**	**GR 6**	**GR 7**	**GR 8**	**GR 9**	**GR 10**	**GR 11**	**GR 12**	**GR 13**
Dihydroxybenzoic acid hexoside 1	350.7	132.5	-	93.6	124.6	48.2	35.4	55.0	307.7	63.1	-	212.7	49.2
Dihydroxybenzoic acid hexoside 2	58.0	66.3	-	88.7	-	28.8	27.2	28.9	47.4	32.4	-	226.8	32.7
Pentozide of protocatechic acid	62.2	373.2	134.3	1233.7	1077.7	-	278.3	297.4	101.5	262.1	-	1052.6	402.8
Hydroxybenzoic acid hexoside	31.7	35.4	-	45.3	-	24.2	-	52.0	31.4	-	29.4	111.1	22.4
Procyanidin B(1)	7.2	-	-	4.1	-	4.4	2.8	2.5	3.1	4.8	4.1	3.5	12.6
Dihydroxyferulic acid glycoside	19.7	5.3	-	24.1	9.3	15.4	2.6	27.8	8.4	3.0	-	22.7	-
Catechin	0.8	0.7	0.3	1.7	0.5	2.5	1.4	0.8	2.4	10.8	1.4	7.1	10.1
Chlorogenic acid	6.2	2.0	1.2	11.2	1.2	8.6	2.8	1.4	5.5	1.0	-	1.4	0.8

Procyanidin B(2)	35.1	20.0	2.2	62.6	16.7	72.9	11.7	32.8	27.6	50.6	6.4	53.6	88.5
Procyanidin B(3)	6.9	4.8	-	16.3	5.6	30.3	2.8	7.0	6.0	15.4	8.2	15.3	13.0
Neochlorogenic acid	1.2	1.0	0.9	1.7	-	2.0	1.0	-	1.2	0.8	-	0.9	-
Quercetin 3-glucuronide-glucoside	1.0	< 0.1	< 0.1	7.9	< 0.1	2.7	< 0.1	< 0.1	4.0	0.2	-	1.2	-
Epicatechin	22.3	8.1	1.1	34.7	11.7	46.6	4.8	26.6	15.0	42.2	2.7	54.7	65.6
p-Coumaroyl quinic acid 1	15.2	11.6	49.7	18.3	1.2	53.3	21.7	1.5	48.1	6.0	-	7.6	-
p-Coumaric acid glycoside	24.2	6.2	4.2	10.6	2.8	27.6	7.8	3.1	52.0	38.3	6.2	51.8	4.6
p-Coumaroyl quinic acid 2	15.5	9.2	32.4	12.5	1.7	39.0	17.8	1.8	27.7	5.6	-	3.7	-
Quercetin glucoramnoside	1.9	1.2	0.3	4.2	1.0	1.2	0.1	2.1	2.8	< 0.1	-	0.4	-
Quercetin pentoxoside	0.9	-	-	0.6	0.3	0.9	< 0.1	1.0	1.8	-	-	< 0.1	-
Quercetin pentoside 1	3.4	3.0	0.8	4.5	2.6	1.6	2.1	2.4	3.3	2.4	0.3	6.0	2.2
Ellagic acid	36.9	19.9	35.5	44.4	29.5	24.00	21.4	25.1	26.8	15.1	92.8	67.2	40.2
Quercetin pentoside 2	7.0	4.7	6.10	9.2	6.9	5.6	5.9	6.4	6.6	2.6	14.9	10.0	7.2
Hyperoside	2.7	1.9	2.3	4.0	6.4	2.3	2.1	7.4	3.7	< 0.1	-	< 0.1	0.1
Quercetin rutinoside alias rutin	4.1	1.9	1.5	4.2	22.7	15.1	16.7	8.1	7.5	-	-	-	1.0
Quercetin 4'-glucuronide	61.1	32.8	45.9	105.5	54.6	93.2	54.5	36.8	100.6	-	< 0.1	107.9	1.1
Isoquercetin	5.0	4.8	1.9	28.5	7.1	48.1	5.0	4.3	19.9	8.0	-	10.5	0.2
Quercetin 7-glucuronide	0.6	1.0	-	2.3	1.1	2.5	-	0.8	2.1	57.0	< 0.1	2.8	0.4
Quercetin pentoside 3	21.5	14.6	11.9	33.9	0.6	0.1	5.4	< 0.1	43.6	< 0.1	-	< 0.1	-

Quercetin 3-(6''-(3-hydroxy-3-methyl-glutaroyl)hexoside 1	2.2	3.1	1.3	2.4	10.4	2.2	3.5	1.2	4.4	< 0.1	< 0.1	0.4	< 0.1
Kaempferol hexoside	< 0.1	-	-	8.4	1.3	0.8	0.6	0.5	< 0.1	0.5	0.1	3.5	1.8
Quercetin hexoside malonate	1.2	0.1	0.2	5.5	2.8	3.4	2.4	1.8	5.1	1.2	-	1.8	< 0.1
Isorhamnetin hexoside 1	2.3	0.8	-	3.2	2.0	6.7	0.2	1.5	5.3	0.6	< 0.1	3.0	0.6
Quercetin 3-(6''-(3-hydroxy-3-methyl-glutaroyl)hexoside 2	0.8	1.8	0.4	3.7	0.3	< 0.1	-	-	< 0.1	-	< 0.1	0.8	-
Isorhamnetin Pentoside 1	2.1	1.2	0.6	4.7	2.7	5.1	1.7	2.6	2.4	0.4	0.3	-	-
Kaempferol glucuronide	1.9	0.5	1.0	5.6	3.3	6.7	2.1	2.3	2.6	1.5	-	2.5	< 0.1
Isorhamnetin rhamnosideive 1	3.5	< 0.1	1.9	3.4	3.3	1.7	4.1	3.5	2.8	3.1	0.3	6.9	2.5
Dicaffeic acid derivative	29.7	59.0	10.2	19.9	5.7	37.9	4.0	32.2	30.8	10.4	4.0	66.4	6.4
Isorhamnetin Pentoside 2	0.4	< 0.1	0.8	< 0.1	-	-	-	< 0.1	0.5	-	-	< 0.1	0.8
Rhamnetin/isorhamnetin	0.6	0.3	1.4	1.4	0.9	1.4	0.5	0.4	0.5	0.2	3.9	1.9	1.4
Ellagic acid acetylarabinoside	31.1	21.8	18.7	50.5	28.6	28.6	46.0	53.1	34.1	12.9	3.1	67.6	19.4
Acetylxyloside of ellagic acid	42.3	19.7	6.7	43.5	30.3	30.1	44.8	35.0	48.6	4.2	1.3	34.8	25.3
Dicafeoil quinic acid	2.3	1.5	1.2	4.2	1.5	1.6	1.4	1.2	2.8	-	-	1.2	-
Isorhamnetin rhamnoside	0.7	0.3	-	6.6	1.6	7.6	< 0.1	0.6	2.8	0.1	< 0.1	2.1	1.3
Isorhamnetin rhamnosideive 2	3.1	1.2	1.5	3.6	2.3	0.9	2.6	2.5	2.67	2.1	0.3	8.5	1.9
Chlorogenic acid rhamnoside	2.2	1.3	1.6	3.7	1.1	3.9	2.2	-	2.8	-	-	1.1	-
Isorhamnetin rhamnosideive 3	1.5	0.5	0.3	1.0	0.6	0.3	1.3	1.2	1.0	0.6	0.1	4.0	1.2

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Quercetin	1.7	0.1	0.6	1.8	0.7	0.9	0.2	0.1	1.9	0.2	0.1	0.4	< 0.1
Neochlorogenic acid rhamnoside	1.5	1.2	-	1.6	-	1.8	1.4	-	1.6	-	-	-	-
Isorhamnetin rhamnosideive 6	3.3	2.5	1.78	3.2	2.0	1.3	3.3	2.9	3.4	3.5	0.4	7.5	-
Isorhamnetin rhamnosideive 7	< 0.1	< 0.1	0.2	3.7	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.5	2.4
Total:	936.9	878.6	382.6	2089.6	1486.9	743.8	653.8	775.4	1063.4	662.8	180.5	2246.2	819.2

Table 4. Polyphenols in the raspberry stems growing in the wild (WR1-WR13), mg%.

Compound	Wild raspberry												
	WR 1	**WR 2**	**WR 3**	**WR 4**	**WR 5**	**WR 6**	**WR 7**	**WR 8**	**WR 9**	**WR 10**	**WR 11**	**WR 12**	**WR 13**
Dihydroxybenzoic acid hexoside 1	141.0	23.4	-	98.5	123.3	106.1	-	74.0	15.9	22.7	55.7	17.2	-
Dihydroxybenzoic acid hexoside 2	36.5	76.4	176.5	59.8	36.4	74.8	-	84.6	-	19.5	21.3	13.8	32.0
Pentozide of protocatechic acid	456.9	130.6	530.1	517.7	742.6	793.5	199.4	294.1	457.3	185.3	597.1	224.2	175.9
Hydroxybenzoic acid hexoside	-	72.3	300.8	41.0	-	40.8	-	-	-	-	-	-	-
Procyanidin B(1)	2.1	-	2.3	-	-	-	3.6	-	5.7	3.0	1.7	-	-
Dihydroxyferulic acid glycoside	9.8	1.4	10.2	19.1	-	-	-	1.9	-	1.5	-	2.0	-
Catechin	0.5	0.8	0.5	1.4	0.7	0.3	1.6	4.1	1.5	6.0	0.7	0.8	0.6
Chlorogenic acid	6.1	1.4	2.4	3.2	1.0	0.8	-	1.2	-	1.4	0.8	2.4	2.1
Procyanidin B(2)	18.2	4.5	25.4	27.9	6.7	9.5	56.8	14.6	93.0	24.3	20.5	4.2	2.8
Procyanidin B(3)	8.2	3.2	7.8	9.9	3.6	4.2	9.6	7.1	15.6	9.8	4.7	3.0	2.2
Neochlorogenic acid	1.8	-	0.9	0.9	-	-	-	-	-	-	-	0.9	-
Quercetin 3-glucuronide-glucoside	< 0.1	< 0.1	1.2	3.6	< 0.1	< 0.1	-	0.1	-	< 0.1	-	-	-
Epicatechin	19.1	3.0	13.6	25.8	4.2	3.0	18.1	17.8	81.9	17.8	7.9	6.5	2.1
p-Coumaroyl quinic acid 1	65.8	15.4	2.7	2.3	4.3	-	-	7.4	-	16.4	-	37.4	109.8
p-Coumaric acid glycoside	-	6.3	11.3	39.4	48.4	2.1	43.2	6.1	21.9	36.5	16.5	39.5	78.3
p-Coumaroyl quinic acid 2	62.9	9.4	2.5	3.1	3.7	-	-	3.7	-	12.3	-	29.2	60.0
Quercetin glucoramnoside	-	< 0.1	0.5	8.4	-	< 0.1	-	-	0.1	< 0.1	< 0.1	-	-

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Quercetin pentoxoside	0.4	< 0.1	< 0.1	2.7	-	-	-	< 0.1	< 0.1	< 0.1	-	-	-
Quercetin Pentoside 1	1.2	2.1	3.1	3.5	1.1	2.6	4.3	1.3	2.2	2.6	2.5	1.5	1.0
Ellagic acid	24.9	18.6	31.2	21.0	14.6	27.2	82.8	26.8	48.4	24.2	31.3	27.2	15.9
Quercetin Pentoside 2	1.4	1.9	6.5	4.8	4.0	4.9	23.1	4.7	7.2	5.0	6.4	5.4	2.5
Hyperoside	5.3	6.3	16.6	12.2	3.5	7.4	0.1	2.8	0.5	< 0.1	-	5.9	6.2
Quercetin rutinoside alias rutin	4.1	4.9	10.2	6.1	2.5	4.2	-	1.7	-	2.1	0.5	4.5	4.0
Quercetin 4′-glucuronide	17.5	40.0	63.7	83.1	12.5	21.3	0.9	67.2	3.8	67.4	12.7	6.7	16.9
Isoquercetin	4.0	4.9	7.1	16.5	2.3	2.8	1.9	4.7	3.0	14.9	1.6	2.3	1.9
Quercetin 7-glucuronide	0.8	-	1.5	2.3	< 0.1	0.4	1.4	-	1.4	-	1.0	< 0.1	-
Quercetin Pentoside 3	< 0.1	< 0.1	< 0.1	22.8	3.9	< 0.1	-	16.6	< 0.1	-	-	0.1	< 0.1
Quercetin 3-(6''-(3-hydroxy-3-methyl-glutaroyl)hexoside) 1	5.3	3.9	24.6	9.1	0.4	6.9	0.2	2.1	0.1	< 0.1	< 0.1	2.4	8.9
Kaempferol hexoside	< 0.1	1.0	0.5	0.4	0.1	0.1	-	< 0.1	0.9	1.3	3.0	1.6	1.2
Quercetin hexoside malonate	< 0.1	1.3	1.5	3.5	0.5	0.8	< 0.1	0.6	< 0.1	2.8	< 0.1	0.3	0.1
Isorhamnetin hexoside 1	3.0	< 0.1	< 0.1	1.6	0.2	0.7	3.7	0.1	3.0	< 0.1	0.7	< 0.1	< 0.1
Quercetin 3-(6'''-(3-hydroxy-3-methyl-glutaroyl)hexoside) 2	< 0.1	-	0.8	4.3	< 0.1	< 0.1	-	0.4	< 0.1	2.6	0.8	-	< 0.1
Isorhamnetin Pentoside 1	0.8	1.4	1.5	4.8	1.5	0.9	1.9	1.1	< 0.1	-	-	1.1	0.7
Kaempferol glucuronide	0.8	2.2	2.5	7.3	1.0	0.8	< 0.1	1.5	0.4	1.5	0.3	0.6	1.1
Isorhamnetin rhamnosideive 1	1.9	4.0	2.9	3.5	2.8	3.7	4.6	2.2	1.0	4.0	3.0	2.3	1.8
Dicaffeic acid derivative	38.5	7.4	9.6	41.1	11.1	12.5	12.3	-	2.2	4.9	23.5	10.0	2.7
Isorhamnetin Pentoside 2	0.1	< 0.1	0.4	1.9	1.0	0.1	1.0	0.3	0.5	< 0.1	2.8	0.8	< 0.1
Rhamnetin/isorhamnetin	0.8	0.2	0.4	0.9	0.3	1.1	2.7	0.2	2.9	0.6	1.6	0.6	1.0
Acetylarabinoside of ellagoic acid	26.8	48.7	48.1	40.3	39.0	43.2	80.8	34.4	26.2	38.9	41.0	24.4	13.2
Acetylxyloside of ellagoic acid	16.6	14.0	34.3	19.9	11.7	19.2	69.1	12.8	25.7	9.9	23.2	4.8	< 0.1
Dicafeoil quinic acid	2.2	-	2.3	1.7	-	-	-	1.1	-	-	-	1.1	-

Isorhamnetin rhamnoside	1.1	< 0.1	2.1	3.6	< 0.1	0.8	1.8	0.1	1.2	< 0.1	0.3	-	-
Isorhamnetin rhamnosideive 2	1.5	2.8	2.2	3.8	2.7	2.8	2.9	1.8	0.5	3.4	3.2	1.9	1.3
Chlorogenic acid rhamnoside	5.4	1.3	1.4	1.1	-	-	-	-	-	-	-	1.4	1.3
Isorhamnetin rhamnosideive 3	0.5	1.2	0.9	1.0	0.9	1.6	2.3	0.6	0.4	1.3	0.8	0.5	0.4
Quercetin	0.8	0.2	0.6	0.9	-	< 0.1	0.2	0.3	0.3	0.4	< 0.1	0.2	0.1
Neochlorogenic acid rhamnoside	2.8	-	-	-	-	-	-	-	-	-	-	-	-
Isorhamnetin rhamnosideive 6	2.0	4.6	3.4	3.1	3.0	4.2	3.9	3.0	0.6	5.1	2.1	1.6	1.7
Isorhamnetin rhamnosideive 7	-	-	< 0.1	2.0	1.9	< 0.1	< 0.1	0.8	-	< 0.1	1.4	0.4	< 0.1
Total:	999.3	520.8	1368.4	1192.8	1097.4	1205.2	634.1	705.9	825.0	549.1	890.3	490.5	549.7

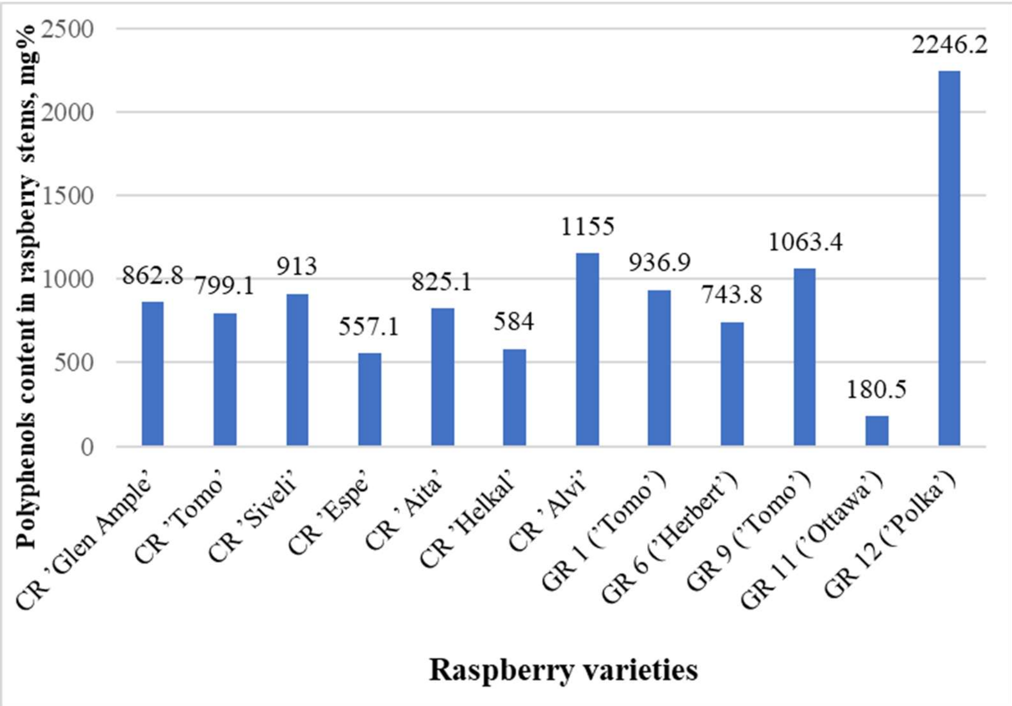

Figure 3. Graph for comparison of the total concentrations of all substances in samples of known raspberry varieties.

It has been established that for most raspberry varieties, the dominant components are: Protocatechuic acid pentosidetechuic acid (5 cultivars), *p*-coumaroyl quinic acid 1 (3 cultivars), *p*-coumaroyl quinic acid 2, Dihydroxybenzoic acid hexoside 1 and 2, and Quercetin 4′-glucuronide (Figure 4).

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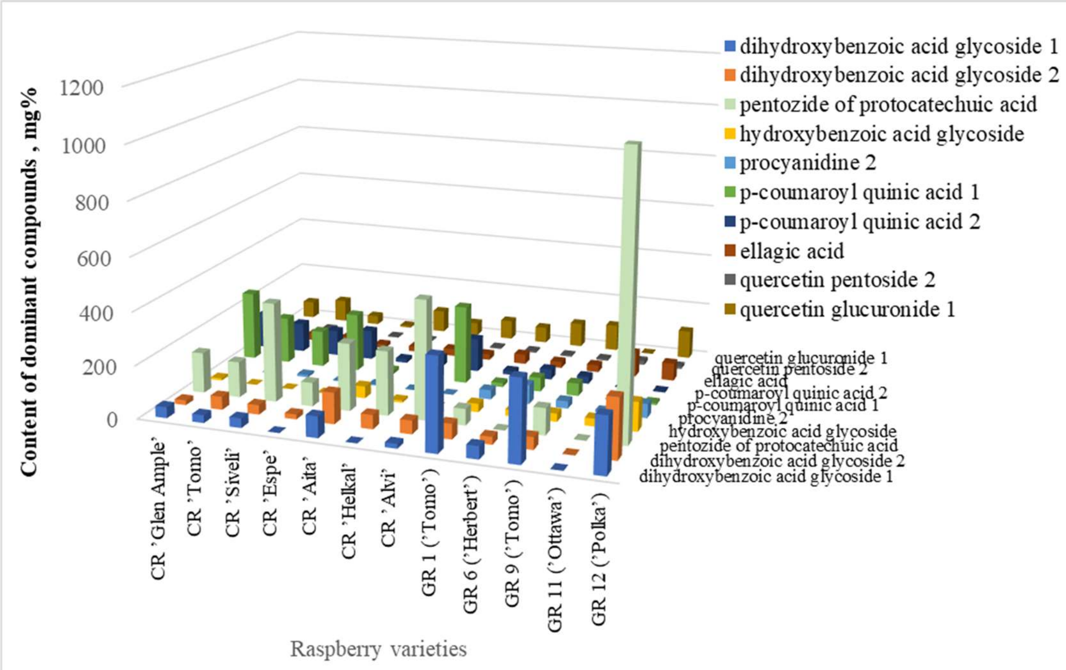

Figure 4. Comparison of dominant polyphenolic compounds in Raspberry cultivars.

In all garden and wild raspberry samples, Protocatechuic acid pentosidetechuic acid is the absolute dominant. Dihydroxybenzoic acid hexoside 1, quercetin 4′-glucuronide and *p*-coumaric acid glycoside are in significant quantities.

The raspberry bush used to study content dynamics year-round was also a sample GR 1. GR 1 was collected in mid-July 2016, and the July sample was collected in early July 2017. Interestingly, when comparing the two samples with each other, the difference is significant (936.9 mg for GR 1 (Figure 3), and 222.4 mg for July (Figure 5). This difference probably comes primarily from the fact that, for some reason, in all the samples of the dynamics of the year, dihydroxybenzoic acid hexosides 1 and 2, protocatechuic acid pentoside, dihydroferulic acid glycoside, and hydroxy-benzoic acid glycoside, which were present in the sample in GR 1 and most others in fairly large quantities, are missing (Appendix A).

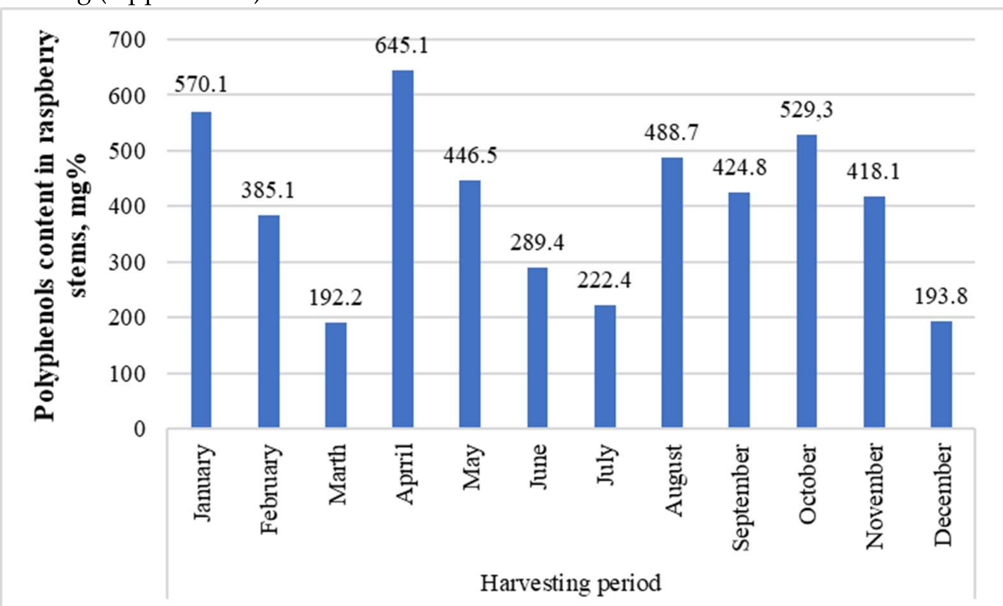

Figure 5. Total content dynamics of polyphenolic compounds in GR1 sample over the year.

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When studying the dependence of the accumulation of polyphenolic compounds in different parts of the stem on the example of GR 12, GR 13 and OCT samples (Appendix A, Table A1), it was found that the upper parts differ in their highest content (Figure 6). Therefore, harvesting about the upper third of the stem is advisable.

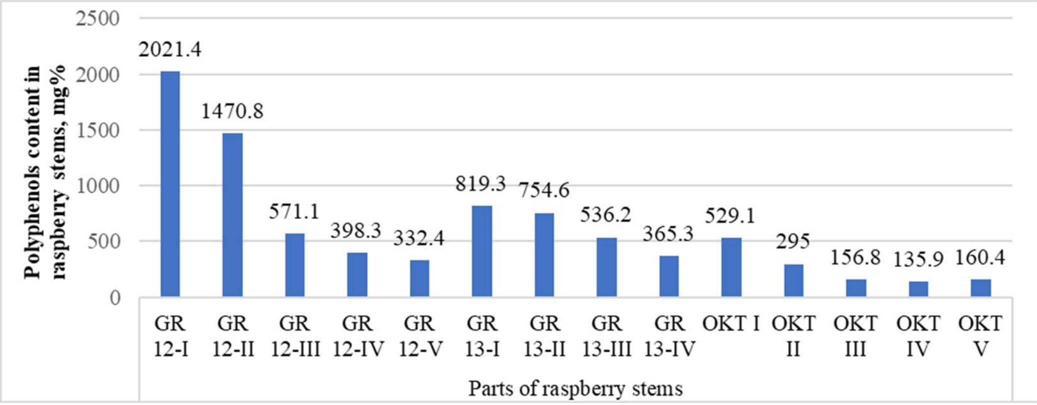

Figure 6. The content of polyphenolic compounds in different parts of raspberry stems, mg%. The parts of the stem, starting from the apex, are marked with numbers I to IV.

When studying the correlation between the content of individual compounds in raspberry stems, a number of regularities were established (Appendix B, Tables A2 and A3).

3. Discussion

As a result of the HPLC analysis, 39 polyphenolic components were found in raspberry stems. In addition, 12 substances were found, the identification of which gave grounds for suspicion, and 11 unknown substances were fixed. In total, the peaks of 62 substances were detected. The total content of polyphenolic compounds for individual cultivars such as Glen Ample and Polka differs from data from other researchers [52,61].

The largest species composition is distinguished by samples GR 1, GR 4 and GR 9, where all 62 substances listed in the previous table were present. In addition to these, more than 56 substances (more than 90%) could be found in samples WR 1, WR 3, WR 4, GR 2, GR 4, GR 5, GR 6, GR 7, GR 8, GR 12, CR ’Aita’, CR ’Glen Ample’, and CR ’Siveli’ (Tables 2-4). Less than 43 substances (< 70 %) were found in sample GR 11 ('Ottawa'). Epicatechin, catechin, ellagic acid, ellagic acid 4-acetylarabinoside and acetylxyloside, quercetin, quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)-hexoside 1, quercetin pentosides 1 and 2, rhamnetin/isorhamnetin and Isorhamnetin rhamnosideives 1, 2, and 3, were detectable in all samples.

DiHydroxybenzoic acid hexosides 1 and 2, protocatechic acid pentoside, chlorogenic acid, procyanidins 2 and 3, *p*-coumaroyl quinic acid 1 and 2, *p*-coumaric acid glycoside, dicaffeic acid derivative, hyperoside, Quercetin rutinoside alias rutin, Quercetin 4’-glucuronide, isoquercetin, quercetin pentoside 3, quercetin hexoside malonate, Kaempferol hexoside and glucuronide, isorhamnetin pentosides 1 and 2, isorhamnetin rhamnoside, Isorhamnetin hexoside 1, and Isorhamnetin rhamnosideives 5 and 6, were detectable in over 80% of the samples. The detection of Quercetin 3-glucuronide and quercetin glucosylrhamnoside (rutin) is consistent with previously published data on their presence in raspberry leaves [37]. The dominance of ellagic acid, the presence of protocatechic and chlorogenic acids, hyperoside, quercetin-3-O-glucuronide, isoquercetin, monomeric catechin and epicatechin, as well as dimeric proanthocyanidins – procyanidin B1 and B2 in raspberry shoots is confirmed by other scientists [51,52,60,61]. Hydroxybenzoic acid glucoside and neochlorogenic acid rhamnoside were present in less than 50% of the samples studied.

In general, the fluctuations between the months seemed to be considerably large, apparently due to weather conditions precisely in spring and autumn (melting snow and freezing), the low concentration in June and July in summer can be explained by the fact that the energy of the plant is primarily focused on the ripening of fruits.

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It should be noted that for some reason, all samples of the dynamics of the year do not contain glycosides of dihydroxybenzoic acid 1 and 2, protocatechic acid pentoside, dihydroferulic acid glycoside and hydroxy-benzoic acid glycoside, which were present in the sample in GR 1 and most others in sufficiently large quantities.

Procyanidin B(1) (2.6 – 13.5 mg%), procyanidin B(2) (47.0 – 271.0 mg%, highest in April, lowest in December) and procyanidin B(3) (4.3 – 71.0 mg%, exceptionally high in August), catechin (1.9 – 23.9 mg%, highest in October) and epicatechin (24.3 – 66.8 mg%, highest in May, April and February), *p*-coumaric acid glycoside (2.0 – 32.8 mg%, highest in January and April) were consistently found during the year, quercetin pentoside 1 (0.7 – 2.0 mg%), ellagic acid (10.8 – 33.0 mg%), quercetin pentoside 2 (1.7 – 2.9 mg%), isoquercetin (1.3 – 49.0 mg%, highest in September, January and October, lowest in December), Isorhamnetin hexoside 1 (0.3 – 3.3 mg%), isorhamnetin pentoside 1 (0.3 – 1.4 mg%), Isorhamnetin rhamnosideive 1 (6.9 – 19.8 mg%), rhamnetin/isorhamnetin (0.2 – 1.4 mg%), ellagic acid acetylarabinoside (13.7 – 35.5 mg%, highest in April), ellagic acid acetylxyloside (9.4 – 32.2 mg%, highest in April), Isorhamnetin rhamnosideive 2 (0.7 – 2.3 mg%) and Isorhamnetin rhamnosideive 6 (1.1 – 3.9 mg%).

Of the other substances, chlorogenic acid and neochlorogenic acid, which were found in low concentrations, can be singled out only from August to November (chlorogenic acid also in January). Concentrations of *p*-coumaroyl quinic acids 1 and 2 were higher from August to November and January, with the remaining months remaining several times lower. A similar phenomenon occurred with quercetin rutinoside alias rutin, from August to October and higher concentrations in January. The concentration of quercetin 4′-glucuronide was lowest in July, February and March. Quercetin Pentoside 3 was found more in January and September. An interesting sample was collected in July, which turned out to be the only one in which *p*-coumaroyl quinic acids 1 and 2, quercetin pentoside 3, quercetin hexoside malonate, chlorogenic acid rhamnoside, quercetin and quercetin 4′-glucuronide were not detectable.

Also, with most individual substances, a smooth decrease in concentrations is noticeable, deficient in several cases near the stem. For samples GR 13 and GR 12, protocatechic acid pentoside content decreases from the top of the stem to the bottom. However, it was not detected at all in the OKT sample. Of the more significant changes, it would be pointed out that in the sample GR 13, the largest amount of diHydroxybenzoic acid hexoside was found in the II quarter (almost 3 times higher than the next), followed by III and I, the lowest was still close to the stem. However, for the same substance in the GR 12 sample, the lowest level was in the middle part (III) and then rose slightly as it moved to both sides. Hydroxybenzoic acid hexoside was uniformly around 20 mg% in the first three parts of GR 13, with 8.7 mg% in the stem part. For the same substance in the GR 12 sample, the highest level was in the stem part of II; the lower I and IV, and III and V were undetected. In the second part of the stem, GR 12 also had higher levels of both catechin and epicatechin, but GR 13 and OKT decreased evenly as we moved from the apex to the stem. A kind of dynamics appeared with procyanidins, which were the highest in Part II of the GR 12 sample, and the OKT sample smoothly when it fell but then rose again in parts IV and V. The level in the GR 13 samples was relatively constant in each section but still slowly decreasing. Differences may have arisen in parts of the stem due to the different lengths of the stems.

For the remaining substances, the changes were either barely noticeable or decreased according to the expected dynamics, being the highest at the peak and the lowest near the stem. Apparently, in the lower part of the stem, substances have a lower concentration since on the stem side, the stem is woodier. Many substances, which were also not originally present in very high concentrations, are absent when close to the strain.

As a result of the data analysis (Table 2-4, Appendix B: Tables A2 and A3), quite strong correlations between the content of biologically active substances and the Pearson coefficients confirm this. Thus, the correlation coefficients between the content of procyanidins and catechins are: r = 0.60-0.93; procyanidins and flavonoids: r=0.60-0.73; derivatives of benzoic and ellagic acids: r=0.60-0.70; individual hydroxycinnamic acids: r=0.70-0.97; hydroxycinnamic acids and flavonoids: r=0.60-

1.00; benzoic acid derivatives and flavonoids: r=0.62-0.84; ellagic acid derivatives and flavonoids: r=0.61-0.88; individual flavonoids: r=0.60-0.97.

An absolute positive correlation was established between the content of neochlorogenic acid rhamnoside – isorhamnetin rhamnosideive 7 (r = 1.0). A very strong correlation (r = 0.97) has been established for pairs of compounds such as quercetin 3-glucuronide-glucoside – isorhamnetin rhamnosideive 7, *p*-coumaroyl quinic acid 1 – *p*-coumaroyl quinic acid 2, chlorogenic acid rhamnoside – neochlorogenic acid rhamnoside; dicaffeoyl quinic acid – isorhamnetin rhamnosideive 7 (r = 0.94); procyanidin B(2) – epicatechin (r = 0.93); quercetin 3-glucuronide-glucoside – quercetin hexoside malonate, isoquercetin – isorhamnetin rhamnoside and quercetin – isorhamnetin rhamnosideive 7 (r = 0.92); isorhamnetin rhamnosideive 1 – isorhamnetin rhamnosideive 6 (r = 0.91).

There is a strong inverse correlation between pairs of compounds, such us quercetin pentoside – isorhamnetin rhamnosideive 7 (r = -1.0), Isorhamnetin rhamnosideive 1 – neochlorogenic acid rhamnoside (r = -0.82), neochlorogenic acid – quercetin pentoside (r = -0.80) and a moderate inverse correlation between pairs of compounds, such as neochlorogenic acid rhamnoside – Isorhamnetin rhamnosideive 6 (r = -0.78) and Hydroxybenzoic acid hexoside – Isorhamnetin rhamnosideive 7 (r = -0.74) (Appendix B).

Phenolic compounds are known to have an adaptive function in plant life. Many works are devoted to studying the relationship between the accumulation of phenolic compounds and the duration of the light period, the elemental composition of the soil, humidity, and altitude above sea level. We took the average data on the content of biologically active substances in 33 different cultivars of the species *R. idaeus*. Therefore, the revealed correlations between different groups of biologically active substances characterise the genotypic correlations of substances of the species.

The presence of positive strong correlations indicates the conjugated biosynthesis and accumulation of these compounds in 33 samples of stems of *R. idaeus* L. varieties, which confirms the genotypic relationships of these compounds, being a characteristic of this species.

4. Materials and Methods

4.1. Raw Materials

The work considers both garden varieties of raspberries in homes and specific varieties of crops (Table 1). The varieties of stems obtained from people's home gardens were largely unknown. A brief description of the varieties studied in this work (EMÜ, 2017, Neeva Garden, 2014) and their photos are given in Appendix C.

The raspberry stems used in the research were collected in the summer of 2016: 7 raspberry cultivated varieties (CR1-CR7) from the Polli garden of the EEC Horticultural Research Center, 13 from different home gardens (GR1-GR13), including five known raspberry varieties and 13 samples from wild raspberries (WR1-WR13) from different benefits in Estonia. Thus, a total of 33 samples of raspberry stems from different places of growth were analyzed. Most of the samples were from Southern Estonia. 19 samples were collected from Viljandi County, 4 from Lääne County, 4 from Valga County, 3 from Tartu County, 2 from Ida-Viru County and 1 from Rapla County (Table 5, Appendix C). A top part 20 cm long was collected on the stems for examination. To study the dynamics of the content of polyphenolic compounds during a year, one sample was collected every month from one and the same bush (sample GR1, apex parts 20 cm long). The content of the substances to be determined in different parts of the raspberry stems was also determined using the example of three raspberry bushes (GR12, GR13, and the October sample). From the bushes, stems of as similar lengths as possible were cut from the ground, and divided into five parts of equal length. The collected material was stored in a refrigerator at -18 ^{o}C and analyzed immediately after defrosting.

Table 5. Origin of samples.

Sample	Origin
CR 1 ('Glen Ample')	EMÜ Centre for Horticultural Research, Polli, Karksi parish, Viljandi County
CR 2 ('Tomo')	EMÜ Centre for Horticultural Research, Polli, Karksi parish, Viljandi County
CR 3 ('Siveli')	EMÜ Centre for Horticultural Research, Polli, Karksi parish, Viljandi County
CR 4 ('Espe')	EMÜ Centre for Horticultural Research, Polli, Karksi parish, Viljandi County
CR 5 ('Aita')	EMÜ Centre for Horticultural Research, Polli, Karksi parish, Viljandi County
CR 6 ('Helkal')	EMÜ Centre for Horticultural Research, Polli, Karksi parish, Viljandi County
CR 7 ('Alvi')	EMÜ Centre for Horticultural Research, Polli, Karksi parish, Viljandi County
GR 1 ('Tomo')	Simmi farm, Kivilõppe village, Tarvastu parish, Viljandi County
GR 2	Iisaku, Iisaku parish, Ida-Viru County
GR 3	Kadarbiku village, Taebla parish, Lääne County
GR 4	Kadarbiku village, Taebla parish, Lääne County
GR 5	Vanamõisa farm, Kolila village, Ridala parish, Lääne County
GR 6 ('Herbert')	Soe village, Tarvastu parish, Viljandi County
GR 7	Soe village, Tarvastu parish, Viljandi County
GR 8	Paeküla, Märjamaa parish, Rapla County
GR 9 ('Tomo')	Rüssa farm, Kivilõppe village, Tarvastu parish, Viljandi County
GR 10	Raudtee street, Tõrva city, Valga County
GR 11 ('Ottawa')	Raudtee street, Tõrva city, Valga County
GR 12 ('Polka')	Rebase Street, Tartu, Tartu County
GR 13	Rebase Street, Tartu, Tartu County
WR 1	Paju otsas, Simmi farm, Kivilõppe village, Tarvastu parish, Viljandi County
WR 2	Simmi Forest, Kivilõppe village, Tarvastu parish, Viljandi County
WR 3	Härma quarry, Helme parish, Valga county
WR 4	Palu mets, Järveküla, Tarvastu parish, Viljandi County
WR 5	Iisaku Forest, Iisaku Parish, Ida-Viru County
WR 6	Vanamõisa lakeside, Tõrva city, Valga county
WR 7	Kadarbiku village, Taebla parish, Lääne County
WR 8	Vasara village, Viljandi parish, Viljandi County
WR 9	Kolila village, Ridala parish, Lääne County
WR 10	Lake Võrtsjärve, Kivilõppe village, Tarvastu parish, Viljandi County
WR 11	Rüssa Forest, Kivilõppe village, Tarvastu parish, Viljandi County
WR 12	Rulli village, Põdrala parish, Valga County
WR 13	Ahimäe village, Karksi parish, Viljandi County

CR – cultivar raspberry, GR – garden raspberry, WR – wild raspberry.

4.2. Preliminary Test to Determine a Suitable Solvent

Preliminary tests were conducted with different ethanol concentrations (20-80%), methanol and distilled water to find the most suitable solvent for extracting the phenolic compounds under investigation. In doing so, the base area of the HPLC UV chromatogram was estimated at 280 nm, where most of the phenolic substances absorb radiation, and it was concluded, based on both the qualitative and quantitative content of the substances, that it is optimal to use 60 % ethanol for the study of polyphenols (Figure 7).

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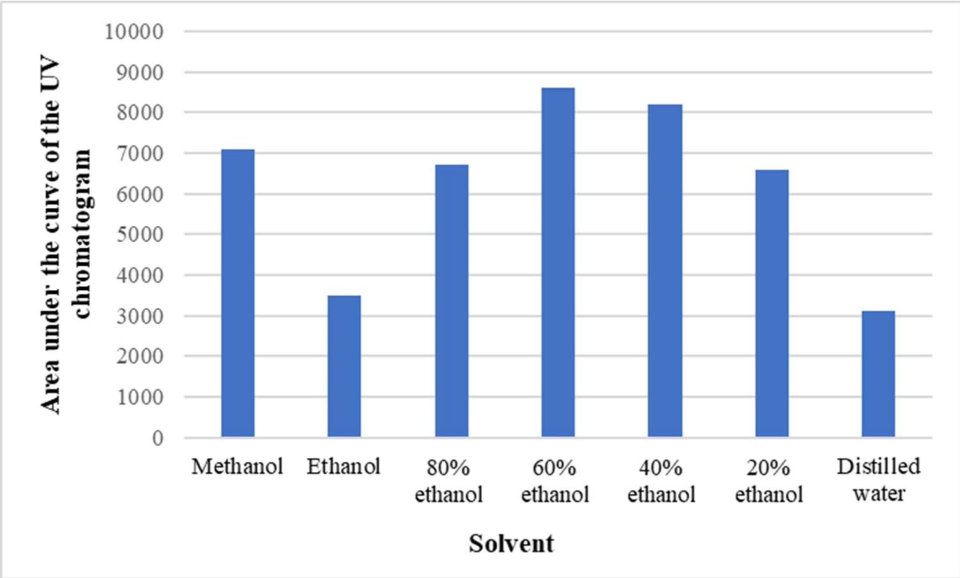

Figure 7. Areas of UV chromatograms obtained with different solvents.

4.3. Extraction and HPLC/MS Analysis of Polyphenolic Compounds in Raspberry Stems

To extract polyphenols, raspberry stems were chopped into 1-2 mm long pieces with scissors, 0.50 g was weighed into a test tube and 60% ethanol/water (v/v) was added to 10 mL. The samples were then allowed to stay for 24 hours with occasional slight shaking then the samples were filtered through a paper filter and centrifuged at 6000 rpm for 10 minutes

Agilent 1100 Series LC/MSD Trap-XCT with ESI ionization unit was used. Blocks: autosampler, solvent degasser, binary pump, column in thermostat and UV-Vis diode array detector. Column: Zorbax 300SB-C18 (2.1 mm × 150 mm), with a particle diameter of 5 µm. HPLC 2D ChemStation software was used in combination with the ChemStation Spectral SW module to control the process. 5 µL of the test solution was injected into the column, the elution time was 50 minutes, the UV-Vis diode detector operated at the wavelength range of 190-530 nm, the temperature of the column was kept at 35 °C. The analytes were separated using a C18 reversed-phase column and an ascending linear gradient of an aqueous 0,1 % formic acid solution (eluent A) and acetonitrile (eluent B). Polyphenols were identified by an ion trap with an MS/MS detector using negative ionization mode (Table 2, Figure 2). The particle mass-to-charge ratio range (m/z) under study was 50-1700, with a target m/z of 1000. The flow rate was 0.3 ml/min.

To determine the quantitative content of polyphenols, solutions of a certain concentration of 96% ethanol were prepared from the standard substances and chromatographed under the same conditions as rhubarb stem extracts, with the difference that the target mass of the characteristic substances was 700 m/z. With the help of a computer program, the base areas of the characteristic peaks were determined, and a calibration graph was prepared for each standard substance. Standards used: quercetin glucoside (Sigma-Aldrich), ≥ 90 % HPLC purity, quercetin galactoside (Sigma-Aldrich), ≥ 97 % HPLC purity, myricetin (Sigma-Aldrich), ≥ 96 % HPLC purity, kaempferol (Sigma-Aldrich), ≥ 90 % HPLC purity, quercitrin (Alpha-Aesar), caffeic acid (Sigma-Aldrich). A similar methodology has been used in our previous studies [68]

By comparing the basal areas of the characteristic peaks with those of raspberry, the content of substances in 1 g of herbal drug was calculated. Since some of the standard polyphenols were in the form of aglycones (for example, myricetin and kaempferol), but in the plant material they were present as glycosides, a coefficient was used for the aglycone, with the help of which the concentration of glycoside was obtained. The coefficient (x) was calculated according to the formula:

$$x = \frac{glycoside\ molecular\ weight}{aglycone\ molecular\ weight}$$

The content of a particular substance in the dried herbal drug was calculated according to the straight formula of the calibration graph of the characteristic substance:

$$x = \frac{(y \times b)}{m} \times z \times 20$$

where:
x – substance content in dried herbal drug (μg/g)
y – area under the peak of the test substance (PÜ)
b – straight intersection with the y-axis
m – straight ascent
z – coefficient

5. Conclusions

The composition of the stems of wild and garden raspberries has been compared for the first time in this work. The HPLC-MS method detected 62 substances, of which 42 compounds were identified, 12 were suspected, and 8 were unknown.

The largest amount of polyphenolic compounds was found in the garden raspberry sample GR12 ('Polka') - 2246,2 mg% and in the sample GR4 - 2089,6 mg%.

The main polyphenolic ingredients of raspberry stems are Protocatechuic acid pentosidetechuic acid, *p*-coumaroyl quinic acid 1, *p*-coumaroyl quinic acid 2, diHydroxybenzoic acid hexosides 1 and 2, and Quercetin 4'-glucuronide. There are no significant differences in the chemical composition of garden and wild raspberries.

The raspberry variety and its place of growth significantly impact the composition of the substances contained in the stems. During the year, the largest amount of them accumulates in raspberry stems in January (570.1 mg%), April (645.1 mg%) and October (529.3 mg%). Therefore, these months are the most optimal for procuring raw materials.

When studying the correlation between the content of individual compounds in raspberry stems, a number of regularities were established. An absolutely positive correlation was established between the content of neochlorogenic acid rhamnoside and Isorhamnetin rhamnosideive 7 (r = 1.0); an inverse correlation between quercetin pentoxide and Isorhamnetin rhamnosideive 7 (r = -1.0).

Different phenolic substances are more numerous at the apex of the raspberry stalk, than near the stem, and the concentration of these substances is also higher at the apex.

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Appendix A

Table A1. Polyphenols in different parts of raspberry stems, mg%.

Compound	Parts of raspberry stems													
	GR 12 I	GR 12 II	GR 12 III	GR 12 IV	GR 12 V	GR 13 I	GR 13 II	GR 13 III	GR 13 IV	OC T I	OC T II	OCT III	OCT IV	OC T V
Dihydroxybenzoic acid hexoside 1	212.7	139.2	38.5	47.9	56.1	49.2	146.8	55.8	34.5	-	-	-	-	-
Dihydroxybenzoic acid hexoside 2	22.6	67.1	-	18.1	-	32.6	28.6	21.1	16.4	-	-	-	-	-
Pentozide of protocatechic acid	1052.6	516.4	173.9	98.4	85.5	402.8	292.2	170.6	72.4	-	-	-	-	-
Hydroxybenzoic acid hexoside	11.1	42.5	-	8.2	-	22.4	17.8	20.6	8.7	-	-	-	-	-
Procyanidin B(1)	3.5	8.2	6.2	3.5	3.2	12.6	9.8	5.9	4.2	5.0	5.1	2.5	-	-
Dihydroxyferulic acid glycoside	22.7	14.3	1.5	-	-	-	-	-	-	-	4.0	2.1	-	-
Catechin	7.1	16.3	6.3	1.9	1.7	10.1	5.4	4.8	4.1	23.9	8.3	2.4	2.5	2.6
Chlorogenic acid	1.4	1.0	-	-	-	0.8	-	-	-	2.4	1.4	-	-	-
Procyanidin B(2)	53.6	150.2	110.7	69.8	64.4	88.5	85.5	85.2	83.5	109.4	91.3	53.2	59.2	80.0
Procyanidin B(3)	15.3	32.1	7.8	4.7	3.5	13.0	9.3	8.9	6.9	13.1	9.2	5.2	5.3	7.6
Neochlorogenic acid	0.9	-	-	-	-	-	-	-	-	0.9	-	-	-	-
Quercetin 3-glucuronide-glucoside	1.2	0.5	-	-	-	-	-	-	-	0.1	< 0.1	-	-	-
Epicatechin	54.7	124.5	87.5	60.1	52.1	65.6	64.0	64.7	58.7	42.4	38.0	25.0	25.0	31.0
p-Coumaroyl quinic acid 1	7.6	2.0	-	-	-	-	-	-	-	46.9	18.1	4.0	-	-
p-Coumaric acid glycoside	51.8	19.7	4.6	2.4	2.4	4.6	2.5	2.0	2.0	23.4	14.3	4.6	2.6	1.9
p-Coumaroyl quinic acid 2	3.7	1.3	-	-	-	-	-	-	-	31.4	13.0	2.3	1.3	-
Quercetin glucorhamnoside	0.4	< 0.1	-	-	-	-	-	-	-	0.2	< 0.1	-	-	-
Quercetin pentoxide	< 0.1	< 0.1	-	-	-	-	-	-	-	< 0.1	0.2	-	-	-
Quercetin pentoside 1	6.0	4.5	3.4	3.1	2.5	2.2	2.2	2.2	2.0	0.7	0.9	1.3	1.1	1.1
Ellagic acid	67.2	47.5	27.6	19.9	13.1	40.2	21.8	24.0	18.2	10.8	12.1	14.9	15.0	11.9
Quercetin entoside 2	10.0	6.6	4.7	3.7	3.0	7.2	5.4	5.5	4.8	2.0	2.0	2.4	2.2	2.3
Quercetin rutinoside alias rutin	-	-	-	-	-	-	-	-	-	0.6	0.5	-	-	1.0
Hyperoside	10.5	17.7	2.0	0.6	0.2	1.0	0.5	< 0.1	< 0.1	26.9	1.8	< 0.1	-	-
Quercetin 4′-glucuronide	108.0	82.3	6.0	0.1	< 0.1	1.1	0.1	< 0.1	< 0.1	43.1	24.2	1.8	< 0.1	< 0.1
Isoquercetin	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.2	< 0.1	< 0.1	< 0.1	47.0	3.2	0.4	< 0.1	< 0.1
Quercetin 7-glucuronide	2.8	2.4	0.4	0.1	< 0.1	0.4	< 0.1	< 0.1	< 0.1	0.5	0.1	< 0.1	< 0.1	< 0.1
Quercetin pentoside 3	< 0.1	0.1	< 0.1		-	-	-	-	-	12.3	6.9	1.1	< 0.1	-
Quercetin 3-(6''-(3-hydroxy-3-methyl-glutaroyl)hexoside) 1	0.8	0.3	< 0.1	< 0.1	< 0.1	< 0.1	-	< 0.1	-	0.6	0.3	-	-	-
Kaempferol hexoside	3.5	1.1	1.6	1.3	1.1	1.8	1.6	2.0	1.6	< 0.1	-	-	-	-
Quercetin hexoside malonate	0.1	0.1	-	-	-	< 0.1	-	-	-	14.8	0.7	< 0.1	-	-
Isorhamnetin hexoside 1	3.0	2.2	0.2	< 0.1	< 0.1	0.6	< 0.1	< 0.1	< 0.1	1.1	0.6	< 0.1	< 0.1	< 0.1
Quercetin 3-(6'''-(3-hydroxy-3-methyl-glutaroyl)hexoside) 2	0.4	0.3	< 0.1	< 0.1	-	-	-	-	-	0.2	< 0.1	-	-	-
Isorhamnetin Pentoside 1	-	1.2	-	-	-	-	-	-	-	0.3	0.3	0.4	0.2	0.3
Kaempferol glucuronide	2.5	1.8	< 0.1	< 0.1	-	< 0.1	-	-	-	7.5	0.1	-	-	-
Isorhamnetin rhamnosideive 1	2.7	2.1	0.8	0.6	0.6	2.5	2.0	2.2	2.2	13.1	0.9	0.8	0.6	0.4
Dicaf acid derivative	66.4	28.4	16.0	7.1	3.0	6.4	6.6	3.9	-	-	-	-	-	-
Isorhamnetin Pentoside 2	< 0.1	< 0.1	0.1	0.1	< 0.1	0.8	0.5	0.7	0.7	< 0.1	0.1	0.1	0.2	0.2
Rhamnetin/isorhamnetin	1.9	0.8	0.5	0.3	0.1	1.4	0.7	0.8	0.6	0.2	0.3	0.3	0.2	0.2

Acetylarabinoside of ellagoic acid	115.8	71.3	37.3	25.7	22.3	19.4	16.8	18.3	12.7	21.6	15.1	16.8	13.4	11.0
Acetylxyloside of ellagic acid	67.6	42.8	24.9	15.8	13.8	25.3	29.8	31.6	27.3	17.5	14.9	12.1	6.1	8.1
Dicaffeoyl quinic acid	1.2	-	-	-	-	-	-	-	-	1.4	1.2	-	-	-
Isorhamnetin rhamnoside	2.1	1.5	-	-	-	1.3	0.5	-	-	0.2	-	-	-	-
Isorhamnetin rhamnosideive 2	8.5	4.6	2.7	1.5	1.2	1.9	1.5	1.9	1.2	1.1	0.7	0.4	0.3	0.2
Chlorogenic acid rhamnoside	1.1	-	-	-	-	-	-	-	-	2.5	1.7	-	-	-
Isorhamnetin rhamnosideive 3	4.0	2.6	1.7	0.8	0.5	1.2	1.0	1.3	0.9	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Quercetin	0.4	5.3	0.5	-	-	0.2	0.1	-	-	0.1	0.3	< 0.1	-	-
Neochlorogenic acid rhamnoside	-	-	-	-	-	-	-	-	-	1.4	1.8	1.1	-	-
Isorhamnetin rhamnosideive 6	1.9	0.9	0.3	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	2.2	< 0.1	< 0.1	< 0.1	-
Isorhamnetin rhamnosideive 7	9.9	7.3	3.6	2.5	2.1	2.4	1.8	2.4	1.7	-	1.4	1.0	0.6	0.7
Total:	2021.4	1470.8	571.1	398.3	332.4	819.3	754.6	536.2	365.3	529.1	295.0	156.8	135.9	160.4

Appendix B

Table A2. Positive correlation between the content of individual compounds in Raspberry stems.

Pairs of compounds	**Correlation coefficient, r**
Procyanidin - catechine	
Procyanidin B(1) - Epicatechin	0.60
Procyanidin B(1) - Procyanidin B(2)	0.64
Procyanidin B(3) - Epicatechin	0.76
Procyanidin B(2) - Procyanidin B(3)	0.78
Procyanidin B(2) - Epicatechin	0.93
Procyanidin - flavonols	
Procyanidin B(2) - Isorhamnetin rhamnosideive 7	0.60
Procyanidin B(3) - Isorhamnetin hexoside 1	0.62
Procyanidin B(3) - Isorhamnetin rhamnoside	0.71
Procyanidin B(3) - Isoquercetin	0.73
Benzoic acid derivates - Ellagic acids derivates	
Dihydroxybenzoic acid hexoside 2 - Ellagic acid	0.62
Ellagic acid acetylarabinoside - Acetylxyloside of ellagic acid	0.64
Dihydroxybenzoic acid hexoside 2 - Hydroxybenzoic acid hexoside	0.70
Hydroxycinnamic acids derivates	
Chlorogenic acid - Dicafeoyl quinic acid	0.70
Chlorogenic acid - Neochlorogenic acid	0.79
Chlorogenic acid - Chlorogenic acid rhamnoside	0.73
Chlorogenic acid rhamnoside - Neochlorogenic acid rhamnoside	0.97
p-Coumaroyl quinic acid 1 - p-coumaroyl quinic acid 2	0.97
Hydroxycinnamic acids derivates - catechin – Procyanidin	
Dihydroferulic acid glycoside - Epicatechin	0.61
Dihydroferulic acid glycoside - Procyanidin B(2)	0.63
Dihydroxybenzoic acid derivates - flavonols	
Dihydroxybenzoic acid hexoside 2 - Isorhamnetin rhamnosideive 6	0.62
Dihydroxybenzoic acid hexoside 2 - Isorhamnetin rhamnosideive 1	0.63
Dihydroxybenzoic acid hexoside 1 - Quercetin	0.64
Dihydroxybenzoic acid hexoside 2 - Isorhamnetin rhamnosideive 2	0.64

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Dihydroxybenzoic acid hexoside 2 - Quercetin Pentoside 1	0.66
Dihydroxybenzoic acid hexoside 2 - Isorhamnetin rhamnosideive 3	0.66
Dihydroxybenzoic acid hexoside 2 - Quercetin Pentoside 3	0.72
Hydroxybenzoic acid hexoside - Hyperoside	0.78
Hydroxybenzoic acid hexoside - Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 1	0.84
Ellagic acid derivates - flavonols	
Quercetin 3-glucuronide-glucoside - Acetylxyloside of ellagic acid	0.61
Quercetin hexoside malonate - Acetylxyloside of ellagic acid	0.64
Acetylxyloside of ellagic acid - Isorhamnetin rhamnosideive 7	0.65
Quercetin Pentoside 1 - Acetylxyloside of ellagic acid	0.66
Ellagic acid acetylarabinoside - Isorhamnetin rhamnosideive 6	0.70
Quercetin Pentoside 1 - Ellagic acid acetylarabinoside	0.71
Ellagic acid acetylarabinoside - Isorhamnetin rhamnosideive 3	0.76
Isorhamnetin rhamnosideive 1 - Ellagic acid acetylarabinoside	0.78
Ellagic acid - Rhamnetin/isorhamnetin	0.87
Ellagic acid - Quercetin Pentoside 2	0.88
Hydroxycinnamic acids derivates - flavonols	
Dihydroferulic acid glycoside - Isorhamnetin rhamnosideive 7	0.60
Neochlorogenic acid - Quercetin hexoside malonate	0.60
Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 2 - Neochlorogenic acid rhamnoside	0.60
Dihydroferulic acid glycoside - Rhamnetin/isorhamnetin	0.61
Chlorogenic acid - Isorhamnetin rhamnosideive 7	0.61
Chlorogenic acid - Quercetin	0.62
Neochlorogenic acid - Isorhamnetin rhamnoside	0.64
Chlorogenic acid rhamnoside - Isorhamnetin rhamnosideive 7	0.64
Chlorogenic acid - Isorhamnetin Pentoside 1	0.64
p-Coumaroyl quinic acid 1 - Isorhamnetin hexoside 1	0.68
Dihydroferulic acid glycoside - Isorhamnetin Pentoside 1	0.68
Ellagic acid acetylarabinoside - Isorhamnetin rhamnosideive 2	0.68
Neochlorogenic acid - Isorhamnetin hexoside 1	0.70
Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 1 - Neochlorogenic acid rhamnoside	0.68
Chlorogenic acid - Isorhamnetin hexoside 1	0.73
Quercetin hexoside malonate - Dicafeoyl quinic acid	0.73
p-Coumaric acid glycoside - Quercetin pentoxoside	0.76
Dicafeoyl quinic acid - Quercetin	0.78
Kempferol glycoside - Dicafeoyl quinic acid	0.78
Chlorogenic acid - Isorhamnetin rhamnoside	0.78
Chlorogenic acid - Quercetin 3-glucuronide-glucoside	0.81
Neochlorogenic acid - Isorhamnetin rhamnosideive 7	0.87
Quercetin 3-glucuronide-glucoside - Dicafeoyl quinic acid	0.86
Dicafeoyl quinic acid - Isorhamnetin rhamnosideive 7	0.94
Neochlorogenic acid rhamnoside - Isorhamnetin rhamnosideive 7	1.00
Flavonols - flavonols	
Quercetin Pentoside 1 - Kempferol glycoside	0.60
Quercetin 4′-glucuronide - Isorhamnetin rhamnoside	0.62
Quercetin glucoramnoside - Isorhamnetin rhamnosideive 7	0.64

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Quercetin pentoxoside - Hyperoside	0.64
Isoquercetin - Isorhamnetin Pentoside 1	0.64
Isoquercetin - Isorhamnetin rhamnosideive 7	0.64
Quercetin glucoramnoside - Isorhamnetin Pentoside 1	0.65
Quercetin 4′-glucuronide - Kempferol glucuronide	0.65
Isorhamnetin rhamnoside - Isorhamnetin rhamnosideive 7	0.65
Quercetin 3-glucuronide-glucoside - Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 2	0.66
Quercetin 4′-glucuronide - Isorhamnetin Pentoside 1	0.66
Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 2 - Kempferol glucuronide	0.67
Quercetin 3-glucuronide-glucoside - Kempferol glucuronide	0.68
Quercetin 4′-glucuronide - Isoquercetin	0.68
Isoquercetin - Isorhamnetin hexoside 1	0.68
Quercetin hexoside malonate - Isorhamnetin hexoside 1	0.68
Quercetin Pentoside 1 - Isorhamnetin rhamnosideive 6	0.69
Quercetin hexoside malonate - Kempferol glucuronide	0.69
Quercetin hexoside malonate - Quercetin	0.69
Quercetin 3-glucuronide-glucoside - Quercetin	0.70
Quercetin glucoramnoside - Isorhamnetin Pentoside 2	0.70
Quercetin Pentoside 3 - Isorhamnetin rhamnosideive 7	0.70
Kempferol glycoside - Quercetin	0.70
Quercetin Pentoside 2 - Rhamnetin/isorhamnetin	0.71
Quercetin 4′-glucuronide - Quercetin hexoside malonate	0.71
Quercetin hexoside malonate - Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 2	0.71
Quercetin glucoramnoside - Kempferol glucuronide	0.72
Isorhamnetin hexoside 1 - Isorhamnetin rhamnoside	0.72
Quercetin 3-glucuronide-glucoside - Isorhamnetin Pentoside 1	0.73
Isoquercetin - Quercetin hexoside malonate	0.73
Quercetin 3-glucuronide-glucoside - Isorhamnetin rhamnoside	0.74
Isoquercetin - Kempferol glucuronide	0.75
Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 2 - Isorhamnetin rhamnoside	0.75
Isorhamnetin Pentoside 1 - Isorhamnetin Pentoside 2	0.75
Quercetin hexoside malonate - Isorhamnetin rhamnoside	0.76
Isorhamnetin rhamnoside - Isorhamnetin rhamnosideive 7	0.77
Quercetin Pentoside 1 - Isorhamnetin rhamnosideive 1	0.78
Quercetin Pentoside 1 - Isorhamnetin rhamnosideive 2	0.78
Quercetin Pentoside 1 - Isorhamnetin rhamnosideive 3	0.78
Quercetin 3-glucuronide-glucoside - Kempferol glycoside	0.79
Quercetin glucoramnoside - Quercetin pentoxoside	0.80
Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 2 - Isorhamnetin rhamnosideive 7	0.81
Hyperoside - Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 1	0.82
Isoquercetin - Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 2	0.83
Isorhamnetin Pentoside 1 - Isorhamnetin rhamnosideive 7	0.83
Quercetin glucoramnoside - Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 2	0.84
Kempferol glucuronide - Isorhamnetin rhamnoside	0.84

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Isorhamnetin rhamnosideive 2 - Isorhamnetin rhamnosideive 6	0.84
Quercetin hexoside malonate - Isorhamnetin rhamnosideive 7	0.85
Quercetin 3-(6''-(3-hydroxy-3-methylglutaroyl)hexoside) 2 - Isorhamnetin Pentoside 1	0.85
Isorhamnetin rhamnosideive 7 - Isorhamnetin rhamnosideive 6	0.85
Isorhamnetin Pentoside 1 - Isorhamnetin rhamnoside	0.86
Isorhamnetin Pentoside 1 - Kempferol glucuronide	0.87
Isorhamnetin rhamnosideive 1 - Isorhamnetin rhamnosideive 2	0.88
Isorhamnetin rhamnosideive 1 - Isorhamnetin rhamnosideive 3	0.88
Isorhamnetin rhamnosideive 2 - Isorhamnetin rhamnosideive 3	0.89
Isorhamnetin rhamnosideive 1 - Isorhamnetin rhamnosideive 6	0.91
Quercetin 3-glucuronide-glucoside - Quercetin hexoside malonate	0.92
Isoquercetin - Isorhamnetin rhamnoside	0.92
Quercetin - Isorhamnetin rhamnosideive 7	0.92
Quercetin 3-glucuronide-glucoside - Isorhamnetin rhamnosideive 7	0.97

Table A3. Negative correlation between the content of individual compounds in Raspberry stems.

Pairs of compounds	Correlation coefficient, r
Hydroxybenzoic acid hexoside - Isorhamnetin rhamnosideive 7	-0.74
Neochlorogenic acid rhamnoside - Isorhamnetin rhamnosideive 6	-0.78
Neochlorogenic acid - Quercetin pentoxide	-0.80
Isorhamnetin rhamnosideive 1 - Neochlorogenic acid rhamnoside	-0.82
Quercetin pentoxoside - Isorhamnetin rhamnosideive 7	-1.0

Appendix C

Table A4. Short descriptions of cultivated varieties *Rubus idaeus*.

Variety	Place of selection	The cross was made	Fruit	Bush
Aita		seedling of Johannes Parksepp Nr. 2–64–24 × 'Glen Clova'.	early maturing, light red, big (average 3.7 g), round, druplets cohering firmly, easy cropping	moderately growing, young canes light green with weak spines; fruiting-canes are light brown
Alvi	Polli Horticultural Research Centre, Estonia	seedling 67-60-12 × 'Novost Kuzmina'.	ather late, dark red, bright, big (average 3.5 g), conical, druplets cohering firmly, with good quality	moderately growing, young canes light green with few spines; fruiting-canes are greyish brown
Helkal		seedling of breeder 67-60-12 ('Golden Queen' × 'Spirina Belaja') × 'Novost Kuzmina'.	midseason, orange yellow, big (average 3.5 g), round conical, druplets cohering firmly	moderately strong, produces numerous erect canes, which are light green, covered thickly with spines; fruitingcanes are light brown
Espe		'Deutschland' and 'Novost Kuzmina'	Red blunt-cone-shaped fruits are medium-ripening	Erect stems are high, their stems slightly curled. Light green shoots are strong, have

			and medium in size (an average of 2.5 g). The partial fruits are well joined and firmly attached to the base of the flower.	single weak spikes. The stems of the second year are light brown
Томо		‘Superlative’ × ‘Novost Kuzmina’.	midseason, dark red, medium, round or oblate, druplets cohering firmly	moderately growing, produces medium or numerous erect canes, which are light green with few weak spines; the fruiting-cane is light brown with grey tinge
Siveli		'Golden Queen' × 'Spirina belaja' x 'Novost kuzmina'	Red fruits are medium-sized rounded or broad-rounded, partial fruits well joined, relatively resistant to collapse	The height of the erect stem is average. The shoots are light green with weak spikes, which are more sparsely located at the top of the stem. In the second year, the stems are light brown with a grayish tinge
Polka			dark red, large and conical	medium-growing, upright and high-yielding
Glen Ample	Scotland	by crossbreeding, 'Glen Rosa', 'Meeker'	large and conical, bright red berries that can weigh up to 3gm	stems strong and erect, spine-free
Herbert	Canada		The fruits are round	The growth of stems is moderate, the shoot with a slightly purple bark, on the branch a lot of sharpening spikes just in the ladva part. Bright red spikes are very sharp.

Variety ’Aita’

Variety ’Alvi’

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Variety 'Espe'	Variety 'Glen Ample'
	
Variety 'Helkal'	Variety 'Herbert'
	
Variety 'Ottawa'	Variety 'Polka'

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Variety 'Siveli'	Variety 'Tomo'

Figure A1. Illustrations of the studied raspberry varieties.

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