Acta Societatis Botanicorum Poloniae

DOI: 10.5586/asbp.3539

Publication history
Received: 2016-07-05
Accepted: 2017-02-07
Published: 2017-03-31

Handling editor
Grzegorz Jackowski, Faculty of Biology, Adam Mickiewicz University in Poznań, Poland

Authors' contributions
RB carried out the laboratory experiments; AS and CP designed the method; CP made GC-MS analyses and interpreted the obtained data; AS collected the samples, participated in laboratory experiments, wrote and edited the manuscript

Funding
The study was carried out with the support of the project financing agreements POIG.02.02.00-14-024/08-00 (CePT).

Competing interests
No competing interests have been declared.

Citation
Becker R, Pączkowski C, Szakiel A. Triterpenoid profile of fruit and leaf cuticular waxes of edible honeysuckle *Lonicera caerulea* var. *kamtschatica*. Acta Soc Bot Pol. 2017;86(1):3539. https://doi.org/10.5586/asbp.3539

ORIGINAL RESEARCH PAPER

Triterpenoid profile of fruit and leaf cuticular waxes of edible honeysuckle *Lonicera caerulea* var. *kamtschatica*

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Abstract

Edible honeysuckle (honeyberry) *Lonicera caerulea* is becoming popular as a novel berry crop with several useful features such as early fruit ripening and exceptional hardiness, particularly resistance to pests and diseases as well as severe frosts in winter and droughts in summer. The triterpenoid profile of fruit and leaf cuticular waxes of edible honeysuckle (a Russian cultivar Chernichka) was analyzed by GC-MS. The major compounds identified were the tetracyclic triterpenoids campesterol, cholesterol, cycloartanol, cycloart-23-ene-3,25-diol, 24-methylenecycloartanol (only in leaves), sitosterol, stigmasta-3,5-dien-7-one, and stigmasterol; and the pentacyclic triterpenes: α-amyrin, β-amyrin, hop-22(29)-en-3-one, oleanolic acid, and ursolic acid. Several remarkable features of the analyzed triterpenoid contents were revealed, including the relatively low abundance of triterpenoids in fruit waxes (6.5% of wax extract) compared to leaf waxes (22%), and a particularly high proportion of tetracyclic triterpenoids (tetracyclic to pentacyclic compound ratios of 4:1 in fruits and almost 7:1 in leaves). These rare features distinguish the triterpenoid profile of the cuticular waxes of *L. caerulea* var. *kamtschatica* from the majority of triterpenoid profiles in plant cuticular waxes investigated to date. To our knowledge, this is the first quantitative compositional study on triterpenoid compounds in the cuticular waxes of edible honeysuckle, supplementing the knowledge of cuticular triterpenoid diversity and distribution.

Keywords

Lonicera caerulea; edible honeysuckle; cuticular wax; sterols; triterpenoids

Introduction

The genus *Lonicera* (Caprifoliaceae) consists of approximately 200 species of vines and shrubs distributed throughout the Northern Hemisphere, mostly in temperate regions. A number of these plants are well known as ornamental shrubs prized for their sweet scented flowers, some (e.g., *Japanese honeysuckle*) are important traditional medicines [1], while others, that bear edible fruits, are becoming popular as novel berry crops with several useful features such as early fruit ripening and exceptional hardiness [2–5].

Up to 17 species of edible honeysuckle belonging to the *Caerulea* Red. section have been recognized including the most popular ones: *Lonicera caerulea* var. *edulis*, *L. c.* var. *kamtschatica*, and *L. c.* var. *altaica* [6,7]. The first report mentioning edible honeysuckle as a horticultural plant was in 1894 [2], and it subsequently spread widely in private and commercial gardens in Russia and Japan. More recently, breeding

programs have been carried out in Europe, the USA, and Canada [3,6,8]. Edible honeysuckle (also known as blue honeysuckle, sweet berry honeysuckle, honeyberry, haskap, haskappu) is one of the most hardy fruiting plants, being resistant to pests and diseases as well as severe droughts in summer and temperatures below −40°C in winter [6,8]. Moreover, the expanded flowers have proven resistant to injury even when exposed to a temperature of −8°C [4,7].

The fruits of edible honeysuckle, maturing several weeks before strawberries, are elongated, cylindrical or elliptical in shape, blue with dark-purple flesh and a flavor resembling that of bilberry, although with a characteristic bitterness. They have been used in folk medicine in northern Russia, China, and Japan, and several pharmacological activities of honeysuckle berry extracts have recently been reported, including antioxidant, antiinflammatory, and antibacterial properties ascribed mainly to their high phenolic content [5,7,9]. Over the last decade, the phenolic profile of edible honeysuckle berries has been thoroughly studied, but in contrast to other edible fruits, its triterpenoid content has not yet been well characterized. Therefore, the aim of this study was the profiling and quantitative determination of triterpenoids in chloroform extracts from fruit and leaf cuticular waxes of honeysuckle *Lonicera caerulea* L. var. *kamtschatica* by GC-MS.

Triterpenoids represent a large group of plant metabolites synthesized from isopentenyl diphosphate via the C30 precursor hydrocarbon squalene [10,11]. These polycyclic compounds include steroids and triterpenes of high structural and functional diversity [12,13]. In their free and esterified forms, they are commonly found in cuticles, particularly plant surface waxes [14]. However, before the development of suitable analytical methods, triterpenoid content of cuticular waxes was considered as rather not interesting and poor in contrast to the rich composition of these compounds occurring in the entire organs. The lack of appropriate methods and standards caused the misleading opinion that only a few structures are present in the cuticular waxes of diverse plant taxa [15]. The recent intensive studies on the occurrence of triterpenoids in surface waxes covering plant organs have demonstrated that the chemical composition of cuticles shows great variability, not only among different plant species, but also between different organs of an individual plant. Therefore, the studies on triterpenoid content in plant waxes require thorough case-by-case investigation.

The presence of triterpenoids in the surface layer is of interest due to their potential role in the protection against biotic stresses, and in the case of fruits – in the modulation of the mechanical toughness and the post-harvest quality [16,17]. It is commonly assumed that the protection against water loss is not a primary function of triterpenoid constituent. However, as recently demonstrated for *Fuyu persimmon* fruit, triterpenoids might enhance the mechanical resistance of the cuticle by filling the gaps in the cutin matrix with low-ordered crystals [16]. The new data about triterpenoid content in edible fruits also supplement the current knowledge of the potential health benefits ascribed to their consumption. Berry fruits particularly are regarded as a functional mixture of water-soluble compounds from the fruit flesh and lipophilic constituents of the cuticle [18]. Moreover, the acquired data on the triterpenoid profile of cuticular waxes give some indication of the main triterpenoid skeletons synthesized by the plant and provide the basis for further investigations of the triterpenoid content of whole plant organs.

Material and methods

Plant material

Young plants of the Russian honeysuckle cultivar *L. caerulea* var. *kamtschatica* "Chernichka" were purchased from Polish Vegetable Seed Farming and Nursery Enterprise PNOS and cultivated in a small plantation in Stare Bosewo, central Poland (52°460 N, 21°332 E). Fruit and leaf samples were collected from 10-year-old bushes in May 2015.

Chemicals and standards

Analytical grade solvents, i.e., chloroform, diethyl ether, methanol (Chempur, Poland) were used for extraction, chromatography, and sample preparation for GC-MS analysis. Authentic standards of α-amyrin, ursolic acid methyl ester (Roth, Germany), β-amyrin, campesterol, cholesterol, sitosterol, stigmasterol, and oleanolic acid (Sigma-Aldrich, Germany) were used for chromatography and mass spectrometry. NaOH (POCH, Poland), nitrosomethylurea (Sigma-Aldrich, Germany) were used for alkaline hydrolysis and methylation. Preparative thin layer chromatography was performed on 20 × 20 cm glass plates coated with a 0.25 mm layer of silica gel 60H (Merck).

Extraction of cuticular waxes

Fruits (samples of weigh of approximately 6 g) and leaves (samples of approximately 2 g) of honeysuckle were extracted by short (30 s) immersion in chloroform (volume of 25 mL and 40 mL for fruits and leaves, respectively) at room temperature. All experiments were done in three replicates. The obtained extracts were decanted, filtered, evaporated to dryness under a gentle stream of nitrogen, and weighted.

Fractionation of wax extracts

Extracts were fractionated by adsoption chromatography as described previously [19–22] on silica gel plates in the solvent system $CHCl_3$/MeOH (97:3, v/v). Fractions were localized on plates by comparison with standards (oleanolic acid, α-amyrin, and sitosterol) and eluted from the gel in diethyl ether. Three fractions, i.e., triterpene acids, combined fraction of free (non-esterified) triterpene alcohols and sterols (referred to as neutral triterpenoids), and triterpenoid esters (extracted from the line of the solvent) were obtained. The fractions containing free triterpene alcohols and sterols were directly analyzed by GC-MS without derivatization, whereas prior to this analysis, fractions containing triterpene acids were methylated with the solution of diazomethane in diethyl ether at 2°C for 24 h, and those containing triterpene esters were subjected to alkaline hydrolysis with 10% NaOH in 80% MeOH at 80°C for 3 h [19–21].

Identification and quantification of triterpenoids by GC-MS

An Agilent Technologies 7890A gas chromatograph was applied for qualitative (MS) and quantitative (FID) analyses with the use of a 5975C mass spectrometric detector and a flame ionization detector, respectively. Samples were dissolved in 1–4 µL of a 5:1 diethyl ether : methanol mixture and applied by split injection 1:10 to a HP-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 µm). A carrier gas was helium at a flow rate of 1 mL/min. The other parameters were employed as follows: column temperature – 280°C, inlet and FID temperature – 290°C, MS transfer line temperature – 275°C, quadrupole temperature – 150°C, ion source temperature – 230°C, EI 70 eV, m/z range – 33–500, FID gas (H_2) flow rate – 30 mL/min (hydrogen generator), air flow rate – 400 mL/min. Identification of individual compounds was achieved by comparing their mass spectra with library data from Wiley 9th ed. and NIST 2008 Lib. SW (databases version 2010), and where available, by comparison of their retention times and corresponding mass spectra with those of authentic standards [19–22]. Quantification was based on measuring the area of peaks in the GC-FID chromatograms and performed with the use of external standard method, i.e., calibration curves prepared separately for known concentrations of standards belonging to main triterpenoid classes (α-amyrin for triterpene alcohols, oleanolic acid methyl ester for acids, sitosterol for sterols). Final data were counted as the means ± standard deviation of three independent samples.

Results

Representative GC chromatograms of the fractions containing triterpene alcohols, ketones, and sterols obtained from chloroform extracts of cuticular waxes covering honeysuckle fruits and leaves are shown in Fig. 1. The peaks identified as being associated with triterpenoid compounds are numbered from the shortest to the longest retention time (Tab. 1). Not numbered peaks were in most cases identified as aliphatic compounds, e.g., the significant peaks of R_T 7.86, 9.39, 11.51, 17.13 and 28.19 min were associated with 1-tetracosanol $C_{24}H_{50}O$, 1-heptacosanol $C_{27}H_{56}O$, 1-octacosanol $C_{28}H_{58}O$, 17-pentatriacontene $C_{35}H_{70}$, and oleyl palmitate, respectively.

The principal peaks identified as triterpenoid compounds on the chromatogram of this fraction from fruit wax, i.e., peaks numbered 1, 4, 8, and 11 in Fig. 1a, had mass spectra of cholesterol, sitosterol, stigmasta-3,5-dien-7-one and pentacyclic triterpene ketone A′-neogammacer-22(29)-en-3-one [i.e., hop-22(29)-en-3-one], respectively. Other, smaller peaks were also identified: Peaks 2 and 3 – the typical phytosterols, campesterol and stigmasterol, Peaks 5 and 10 – tetracyclic triterpene cycloartanol and its derivative with an additional hydroxyl group, cycloart-23-ene-3,25-diol, and Peaks 6 and 7 – pentacyclic triterpene alcohols α-amyrin and β-amyrin. The chromatogram of the respective fraction from leaf wax (Fig. 1b) differed markedly from that of fruit wax in the intensity of some of the identified peaks (e.g., 7, 8, 11) as well as in the presence of the predominant Peak 9, identified as 24-methylenecycloartanol, which was not detected on chromatogram showed in Fig. 1a.

The fractions supposed to contain free triterpene acids were analyzed by GC-MS after derivatization. However, only very small peaks identified as associated with methyl esters of oleanolic and ursolic acids were detected on the obtained chromatograms

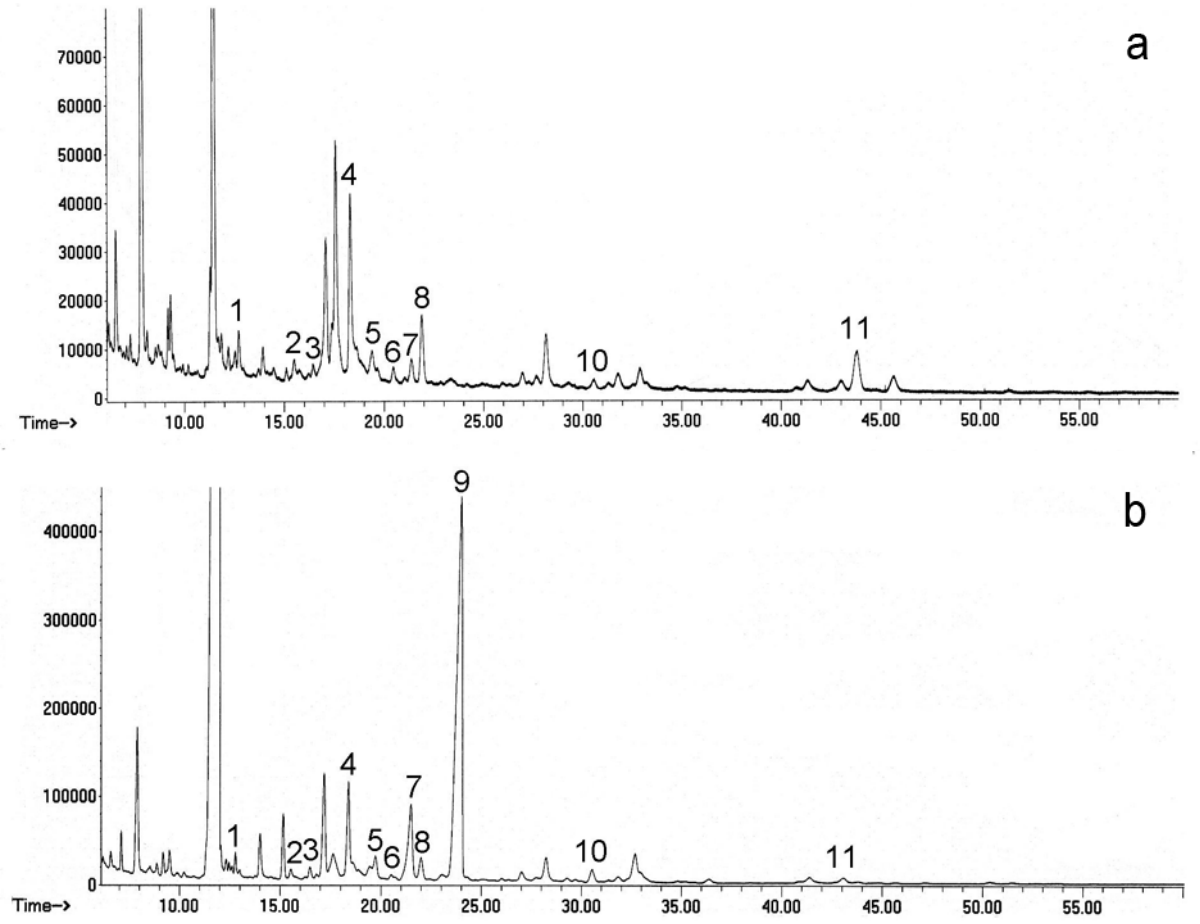

Fig. 1 GC-FID chromatograms of the fractions containing steroids and triterpenes obtained from chloroform-soluble cuticular waxes of edible honeysuckle fruits (**a**) and leaves (**b**). Retention times and MS spectra of peaks associated with the identified triterpenoids are shown in Tab. 1.

Tab. 1 GC-MS data of triterpenoids identified in fruit and leaf cuticular waxes of edible honeysuckle. Compounds are arranged in the elution order on GC. Peaks are numbered on the chromatogram of the fractions containing steroids and triterpenes.

Peak number	Retention time (min)	Identified compound	Mass spectrum *m/z* (relative intensity)
1	12.61	Cholesterol	386 (26), 107 (50), 105 (48), 91 (57), 81 (54), 79 (46), 69 (47), 57 (87), 55 (73), 43 (100), 41 (55)
2	15.54	Campesterol	400 (30), 107 (51), 105 (55), 95 (49), 83 (45), 81 (64), 71 (62), 57 (77), 55 (77), 43 (100), 41 (52)
3	16.48	Stigmasterol	412 (36), 145 (64), 107 (52), 95 (100), 83 (66), 81 (90), 78 (60), 69 (67), 67 (85), 55 (69)
4	18.39	Sitosterol	414 (29), 145 (54), 107 (59), 105 (60), 95 (54), 91 (49), 81 (57), 57 (68), 55 (70), 43 (100)
5	19.43	Cycloartanol	428 (4), 205 (60), 109 (98), 95 (100), 93 (64), 81 (69), 69 (78), 57 (73), 55 (82), 43 (89), 41 (67)
6	20.51	*β*-Amyrin	426 (27), 219 (18), 218 (100), 203 (49), 189 (17), 135 (11), 109 (13), 105 (12), 95 (15), 81 (18), 69 (14)
7	21.50	*α*-Amyrin	426 (4), 219 (18), 218 (100), 203 (20), 189 (19), 135 (17), 133 (15), 122 (16), 119 (15), 95 (16),
8	21.99	Stigmasta-3,5-dien-7-one	410 (32), 187 (27), 174 (100), 161 (37), 159 (26), 91 (28), 57 (28), 55 (37), 43 (44), 41 (28)
9	24.01	24-Methylenecycloartanol	440 (5), 121 (60), 119 (55), 109 (62), 107 (76), 105 (57), 95 (98), 93 (64), 81 (72), 69 (99), 55 (100)
-	27.32	Oleanolic acid methyl ester	470 (1), 203 (100), 262 (44), 189 (22), 202 (21), 133 (179), 204 (16), 119 (16), 69 (14), 105 (14), 207 (13)
10	30.54	Cycloart-23-ene-3,25-diol	442 (10), 203 (48), 121 (73), 109 (100), 107 (82), 95 (75), 81 (91), 69 (54), 55 (62), 43 (77)
-	31.02	Ursolic acid methyl ester	470 (1), 262 (100), 203 (96), 133 (81), 119 (35), 207 (34), 189 (29), 55 (22), 105 (22), 69 (21), 43 (20)
11	43.83	Hop-22(29)-en-3-one	424 (33), 205 (29), 189 (100), 107 (39), 95 (56), 93 (29), 81 (35), 69 (36), 55 (27), 41 (21)

(not shown). GC-MS analysis of the fractions containing esters, which had been subjected to alkaline hydrolysis to release free triterpenoid compounds, revealed the presence of some small peaks: the chromatograms of the fruit wax fraction contained peaks representing cholesterol, stigmasterol, and sitosterol, whereas peaks representing sitosterol and both amyrins were present on chromatograms of the leaf wax fraction (chromatograms not shown).

Therefore, according to GC-MS analysis (Tab. 1), the main triterpenoid profile of honeysuckle cuticular waxes is composed of the tetracyclic compounds campesterol, cholesterol, cycloartanol, cycloart-23-ene-3,25-diol, 24-methylenecycloartanol (only in leaves), sitosterol, stigmasta-3,5-dien-7-one, and stigmasterol and the pentacyclic compounds *α*-amyrin, *β*-amyrin, hop-22(29)-en-3-one, oleanolic acid, and ursolic acid.

The results of the quantitative determination of individual triterpenoids identified in cuticular waxes of the fruits and leaves of honeysuckle are presented in Tab. 2. The total triterpenoid content was much lower in fruits than in leaves, accounting for 62.51 and 219.75 μg mg^{-1} of wax extract mass, respectively. Tetracyclic triterpenoids were dominant in the extracts of both fruit and leaf wax, comprising (including esters) 80% of all triterpenoids in the former and 87% in the latter. The principal triterpenoid in fruit wax was sitosterol (42% of all triterpenoids, 57% of tetracyclic compounds), whereas in leaf wax, 24-methylenecycloartanol was predominant (57% of

Tab. 2 Contents of triterpenoids (mean ±*SD* of triplicate assays) in honeysuckle fruit and leaf cuticular waxes.

Compound	Fruit wax	Leaf wax
	μg mg^{-1} of wax extract	
Free		
Tetracyclic triterpenoids		
Campesterol	2.92 ±0.12	3.56 ±0.10
Cholesterol	5.09 ±0.13	5.64 ±0.18
Cycloartanol	3.46 ±0.16	5.62 ±0.12
Cycloart-23-ene-3,25-diol	0.97 ±0.08	4.21 ±0.04
24-Methylenecycloartanol	n.d.	126.27 ±4.14
Sitosterol	26.58 ±0.71	34.56 ±0.64
Stigmasta-3,5-dien-7-one	6.27 ±0.10	4.74 ±0.11
Stigmasterol	1.32 ±0.17	3.32 ±0.23
Sum	46.61	187.92
Pentacyclic triterpenoids		
α-Amyrin	3.63 ±0.06	20.23 ±1.46
β-Amyrin	2.27 ±0.07	2.76 ±0.02
Hop-22(29)-en-3-one	4.49 ±0.10	1.72 ±0.03
Oleanolic acid	0.36 ±0.03	0.70 ±0.05
Ursolic acid	1.46 ±0.08	0.74 ±0.04
Sum	12.21	26.15
Esters		
Cholesterol	0.84 ±0.07	n.d.
Stigmasterol	1.10 ±0.06	n.d.
Sitosterol	1.75 ±0.12	3.05 ±0.10
α-Amyrin		2.07 ±0.07
β-Amyrin		0.56 ±0.06
Sum	3.69	5.68
Total	62.51	219.75

all triterpenoids, 66% of tetracyclic compounds). A considerable amount of cholesterol, which has been reported as a minor constituent of surface waxes of some other plants [23,24], was detected in both the fruit and leaf waxes of honeysuckle. In the fruit wax, cholesterol accounted for 8% of all triterpenoids, i.e., 11% of all tetracyclic compounds, whereas in the leaf wax, its level was markedly lower (2.6% of all triterpenoids, i.e., 3% of tetracyclic compounds). Among the pentacyclic compounds, hop-22(29)-en-3-one was predominant in the fruit wax, where it accounted for 37% of this fraction, while *α*-amyrin was the most abundant in the leaf wax, constituting 77% of the pentacyclic triterpene fraction. Oleanolic acid and ursolic acid were detected in waxes of both organs in relatively small amounts: together these compounds accounted for 15% of the triterpenoid fraction in fruit wax, and for only 5.5% in leaf wax. Triterpenoid esters present in fruit wax were mainly those of sterols, whereas in leaf wax, besides sitosterol esters, esters of both amyrins were also detected.

Discussion

There have been a number of attempts to use profiles of cuticular waxes as taxonomic markers for species, or species groups, reflecting both ecological and genetic relationships [25,26]. Many of these studies were based primarily on the analysis of aliphatic compounds, which are essential for wax composition and function. However, the utility of chemotaxonomic analyses based on aliphatic compounds is debatable, since the predominant alkane molecules are ubiquitous in higher plants [27]. Therefore, it is necessary to not only identify these compounds but also to establish their proportions and the characteristic ratios of linear alkanes of different chain length, but this still does not always permit the differentiation of species. Therefore, the presence and diversity of triterpenoids in cuticular waxes may be of great systematic significance, even if in some species these compounds are present only in trace amounts. The fruit cuticular wax of edible honeysuckle contains a strikingly low amount of triterpenoids (only 6.25% of the total wax extract), which is a feature that clearly distinguishes this berry (a member of the Caprifoliaceae) from edible berries of plants belonging to other families, e.g., bilberry *Vaccinium myrtillus* L. [20], cowberry *V. vitis-idaea* L. [19,28], and cranberry *V. macrocarpon* Ait. of the Ericaceae [29] or grapevine *Vitis vinifera* L. of Vitaceae [21,22].

There are both quantitative and qualitative differences between the triterpenoid contents of the fruit and leaf cuticular waxes of honeysuckle, e.g., the higher level of hop-22(29)-en-3-one in fruits and the occurrence of 24-methylenecycloartanol only in leaves. It has frequently been demonstrated that waxes from different organs of the same plant can differ substantially in their chemical composition [24,28,30]. However, the characteristic profile of dominant triterpenoids, which reflects the pathway

of their biosynthesis in the plant, remains the same in each organ, regardless of such fluctuations. Pentacyclic triterpenes with oleanane, ursane, and neogammacerane skeletons are found in the cuticular waxes of both the fruit and leaves of honeysuckle. The striking abundance of tetracyclic triterpenoids (ratios of tetracyclic to pentacyclic compounds of 4:1 in fruits and 6.7:1 in leaves), which comprise both common phytosterols and also remarkable amounts of cholesterol and cycloartanol derivatives, namely: cycloart-23-ene-3,25-diol and 24-methylenecycloartanol (the latter only in leaves), distinguishes the triterpenoid profile of the cuticular waxes of *Lonicera caerulea* var. *kamtschatica* from the majority of other such profiles investigated to date.

The relative abundance of triterpenoids in cuticular waxes can also have other implications concerning the form in which these compounds occur in the plant. Ericaceae species accumulate significant amounts of tritepenoids in a free and esterified form in cuticular waxes, while they synthesize very small amounts of their glycoside conjugates called saponins found in other tissues [20,28,31]. In contrast, many plants belonging to the genus *Lonicera* are known for high levels of saponins, mainly of oleanolic acid and hederagenin [1], or lupane-type triterpenes as aglycones [32]. Thus, the low abundance of free-form triterpenoids in cuticular waxes might indicate their occurrence as saponins accumulated inside the plant organs. If this presumption would be confirmed in the future research, the evaluation of the plant material as a source of triterpenoids in either free or glycosidic forms can be significantly simplified and based exclusively on the analysis of the content of triterpenoids in cuticular waxes of the plant organs (leaves, flowers, fruit). Consequently, a fast screen for triterpenoid profile in cuticular waxes might permit to presume the form and composition of these compounds in the entire plant organ.

References

1. Shang X, Pan H, Li M, Miao X, Ding H. *Lonicera japonica* Thunb.: ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. J Ethnopharmacol. 2011;138(1):1–21. https://doi.org/10.1016/j.jep.2011.08.016
2. Plekhanova MN. Blue honeysuckle (*Lonicera caerulea* L.) – a new commercial berry crop for temperate climate: genetic resources and breeding. Acta Hortic. 2000;538:159–164. https://doi.org/10.17660/ActaHortic.2000.538.25
3. Hummer KE. Blue honeysuckle: a new berry crop for North America. Journal of the American Pomological Society. 2006;60(1):3–8.
4. Ochmian I, Skupień K, Grajkowski J, Smolik M, Ostrowska K. Chemical composition and physical characteristics of fruits of two cultivars of blue honeysuckle (*Lonicera caerulea* L.) in relation to their degree of maturity and harvest date. Not Bot Horti Agrobot Cluj Napoca. 2012;40(1):155–162.
5. Kula M, Majdan M, Radwańska A, Nasal A, Hałasa R, Głód D, et al. Chemical composition and biological activity of the fruits from *Lonicera caerulea* var. *edulis* 'Wojtek'. Academia Journal of Medicinal Plants. 2013;1(8):141–148.
6. Ochmian I, Grajkowski J, Skupień K. Field performance, fruit chemical composition and firmness under cold storage and simulated "shelf-life" conditions of three blue honeysuckle cultigens (*Lonicera caerulea*). Journal of Fruit and Ornamental Plant Research. 2008;16:83–91.
7. Jurikova T, Rop O, Mlcek J, Sochor J, Balla S, Szekeres L, et al. Phenolic profile of edible honeysuckle berries (genus *Lonicera*) and their biological effects. Molecules. 2012;17(1):61–79. https://doi.org/10.3390/molecules17010061
8. Chaovanalikit A, Thompson MM, Wrolstad RE. Characterization and quantification of anthocyanins and polyphenolics in blue honeysuckle (*Lonicera caerulea* L.). J Agric Food Chem. 2004;52(4):848–852. https://doi.org/10.1021/jf030309o
9. Wojdyło S, Jáuregui PNN, Oszmiański J, Golis T. Variability of phytochemical properties and content of bioactive compounds in *Lonicera cearulea* L. var. *kamtschatica* berries. J Agric Food Chem. 2013;61(49):12072–12084. https://doi.org/10.1021/jf404109t

10. Mahato SB, Nandy AK, Roy G. Triterpenoids. Phytochemistry. 1992;31(7):2199–2249. https://doi.org/10.1016/0031-9422(92)83257-Y
11. Phillips DR, Rasbery JM, Bartel B, Matsuda SP Biosynthetic diversity in plant triterpene cyclization. Curr Opin Plant Biol. 2006;9(3):305–314. https://doi.org/10.1016/j.pbi.2006.03.004
12. Patlolla JM, Rao CV. Triterpenoids for cancer prevention and treatment: current status and future prospects. Curr Pharm Biotechnol. 2012;13(1):147–155. https://doi.org/10.2174/138920112798868719
13. Podolak I, Janeczko Z. Pharmacological activity of natural non-glycosylated triterpenes. Mini Rev Org Chem. 2014;11(3):280–291. https://doi.org/10.2174/1570193X1103140915105546
14. Szakiel A, Pączkowski C, Pensec F, Bertsch C. Fruit cuticular waxes as a source of biologically active triterpenoids. Phytochem Rev. 2012;11(2–3):263–284. https://doi.org/10.1007/s11101-012-9241-9
15. Jetter R, Kunst L, Samuels AL. Composition of plant cuticular waxes. In: Riederer M, Müller C, editors. Biology of the plant cuticle. Oxford: Blackwell Publishing; 2006. p. 155–157. (Annual Plant Reviews; vol 23). https://doi.org/10.1002/9780470988718.ch4
16. Tsubaki S, Sugimura K, Teramoto Y, Yonemori K, Azuma J. Cuticular membrane of *Fuyu persimmon* fruit is strengthened by triterpenoid nano-fillers. PLoS One. 2013;8:e75275. http://dx.doi.org/10.1371/journal.pone.0075275
17. Lara I, Belge B, Goulao LF. The fruit cuticle as a modulator of postharvest quality. Postharvest Biol Technol. 2014;87:103–112. https://doi.org/10.1016/j.postharvbio.2013.08.012
18. Seeram NP. Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. J Agric Food Chem. 2008;56(3):627–629. https://doi.org/10.1021/jf071988k
19. Szakiel A, Pączkowski C, Koivuniemi H, Huttunen S. Comparison of the triterpenoid content of berries and leaves of lingonberry *Vaccinium vitis-idaea* from Finland and Poland. J Agric Food Chem. 2012;60:4994–5002. https://doi.org/10.1021/jf300375b
20. Szakiel A, Pączkowski C, Huttunen S. Triterpenoid content of berries and leaves of bilberry *Vaccinium myrtillus* from Finland and Poland. J Agric Food Chem. 2012;60(48):11839–11849. https://doi.org/10.1021/jf3046895
21. Pensec F, Pączkowski C, Grabarczyk M, Woźniak A, Bénard-Gellon M, Bertsch C, et al. Changes in the triterpenoid content of cuticular waxes during fruit ripening of eight grape (*Vitis vinifera*) cultivars grown in the Upper Rhine Valley. J Agric Food Chem. 2014;62(32):7998–8007. https://doi.org/10.1021/jf502033s
22. Pensec F, Szakiel A, Pączkowski C, Woźniak A, Grabarczyk M, Bertsch C, et al. Characterization of triterpenoid profiles and triterpene synthase expression in the leaves of eight *Vitis vinifera* cultivars grown in the Upper Rhine Valley. J Plant Res. 2016;129(3):499–512. https://doi.org/10.1007/s10265-016-0797-0
23. Szafranek BM, Synak EE. Cuticular waxes from potato (*Solanum tuberosum*) leaves. Phytochemistry. 2006;67(1):80–90. https://doi.org/10.1016/j.phytochem.2005.10.012
24. Szakiel A, Niżyński B, Pączkowski C. Triterpenoid profile of flower and leaf cuticular waxes of heather *Calluna vulgaris*. Nat Prod Res. 2013;27(15):1404–1407. https://doi.org/10.1080/14786419.2012.742083
25. Medina E, Aguia G, Gomez M, Aranda, J, Medina JD, Winter K. Taxonomic significance of the epicuticular wax composition in species of the genus *Clusia* from Panama. Biochem Syst Ecol. 2006;34:319–326. https://doi.org/10.1016/j.bse.2005.10.009
26. Cordeiro SZ, Simas NK, Arruda RCO, Sato A. Composition of epicuticular wax layer of two species of *Mandevilla* (Apocynoideae, Apocynaceae) from Rio de Janeiro, Brazil. Biochem Syst Ecol. 2011;39(3):198–202. https://doi.org/10.1016/j.bse.2011.02.009
27. Medina E, Aguiar G, Gómez M, Medina JD. Patterns of leaf epicuticular waxes in species of *Clusia*: taxonomical implications. Interciencia. 2004;29:579–582.
28. Kondo M, MacKinnon SL, Craft CC, Matchett MD, Hurta RAR, Neto CC. Ursolic acid and its esters: occurrence in cranberries and other *Vaccinium* fruit and effects on matrix metalloproteinase activity in DU145 prostate tumor cells. J Sci Food Agric. 2011;91(5):789–796. https://doi.org/10.1002/jsfa.4330
29. Szakiel A, Mroczek A. Distribution of triterpene acids and their derivatives in organs of cowberry (*Vaccinium vitis-idaea* L.) plant. Acta Biochim Pol. 2007;54(4):733–740.

30. Guinda A, Rada M, Delgado T, Gutiérrez-Adánez P, Castellano JM. Pentacyclic triterpenoids from olive fruit and leaf. J Agric Food Chem. 2010;58(17):9685–9691. https://doi.org/10.1021/jf102039t

31. Murphy BT, MacKinnon SL, Yan X, Hammond GB, Vaisberg AJ, Neto CC. Identification of triterpene hydroxycinnamates with in vitro antitumor activity from whole cranberry fruit (*Vaccinium macrocarpon*). J Agric Food Chem. 2003;51(12):3541–3545. https://doi.org/10.1021/jf034114q

32. Xiang T, Tezuka Y, Wu LJ, Banskota AH, Kadota S. Saponins from *Lonicera bournei*. Phytochemistry. 2000;54(8):795–799. https://doi.org/10.1016/S0031-9422(00)00194-1