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Variation of fruits morphometric parameters and iridoid content of *Lonicera caerulea* L. germplasm collection

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The dark-blue, fleshy berries of *Lonicera caerulea* L. are used as fresh or processed in many products such as juice, smoothie, wine, compote, jam, marmalade, candies, chocolate, cake, or extracts. *L. caerulea* fruits are appreciated in the food industry and medicine mainly due to a high content of biologically active phenolic compounds, especially anthocyanins and vitamin C – in the amount up to 186 mg.100 g^{-1}, and health-promoting properties. Thirty-four genotypes of *Lonicera caerulea* species originated from germplasm collection of the Forest-Steppe of Ukraine in M.M. Gryshko National Botanical Garden of NAS of Ukraine (NBG) (Kyiv, Ukraine) and were characterized by using the morphometric traits and iridoids content. Differences between the genotypes were significant in all observed parameters. The fruits of *L. caerulea* collection varied in evaluated morphometric parameters as follows: fruit weight 0.70–1.54 g, length 15.33–26.52 mm, diameter 7.12–13.10 mm. The shape indexes of fruits varied from 1.42 to 3.65. Our results showed that the content of iridoids ranged from 97 to 314 mg.100 g^{-1} of fresh *L. caerulea* fruits. Interestingly, *L. caerulea* berries taste differs between the varieties, those with bitter or sour-bitter taste are distinguished by the highest content of iridoids (225–314 mg.100 g^{-1}), while berries without bitter taste were characterized by significantly lower contents. Thus, honeysuckle berries should be selected preferentially regarding their bitterness, due to the anti-inflammatory and antioxidative properties of iridoids.

Keywords: honeysuckle, fruits, iridoids, morphometric parameters, genotypes, Ukraine

Introduction

Fruits (berries) are extremely important and valuable components of the human diet, to maintain good health and well-being. The main goal for the food industry and food and nutrition research is to deliver to the marketplace new, safe, highly accepted by consumers and convenient food products with good nutritional and health-promoting properties. Recently, non-traditional and underutilized edible medicinal plants (Vergun et al., 2019; Buyun et al., 2021; Stefanowski

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et al., 2021), especially their fruits rich in bioactive components gained much attention (Klymenko et al., 2017; Latocha, 2017; Lachowicz et al., 2019; Zhurba et al., 2021). Among them should be emphasized fruits of honeysuckle (*Lonicera caerulea* L.).

Nowadays *Lonicera caerulea* is cultivated as an edible plant (Skvortsov, 1986; Grygorieva et al., 2021), belonging to polymorphic species and is regarded by some authors as a complex of microspecies or geographical races, including *L. altaica* Pall., *L. pallasii* Ledeb., *L. × subarctica* Pojark., *L. edulis* Turcz. ex Freyn, *L. stenantha* Pojark., *L. buschiorum* Pojark., *L. baltica* Pojark., *L. turczaninowii* Pojark. and *L. kamtschatica* (Sevast.) Pojark. It should be highlighted that significant progress has been made in the industrial cultivation of *L. caerulea* and its processing in China (Huo et al., 2005; Zhao et al., 2015). Also, numerous studies on *L. caerulea* were conducted in European countries, such as the Czech Republic, Estonia, Poland, Slovakia, Romania, and Lithuania (Smolik et al., 2010; Jurikova et al., 2012; Bieniek et al., 2021; Grygorieva et al., 2021). The fruits of *L. caerulea* are used fresh or processed in many products such as jam, marmalade, jelly, compote, cake, juice, sauce, extracts, liqueur, smoothie, and wine (Liu et al., 2010; Klymenko et al., 2017; Senica et al., 2019).

The *L. caerulea* is highly appreciated for ultra-early fruit ripening, as well a high content of biologically active compounds (Khattab et al., 215; Peng et al., 2016; Kucharska et al., 2017; Bieniek et al., 2021; Cheng et al., 2022; Piekarska et al., 2022) with strong antioxidant (Bąkowska-Barczak et al., 2007; Gruia et al., 2008; Celli et al., 2014; Gao et al., 2016; Hsu et al., 2016), anti-inflammatory (Xu et al., 2007; Hsu et al., 2016; An et al., 2020), immunomodulating (Svarcova et al., 2007), antiviral (Svarcova et al., 2007), antifungal (Palikova et al., 2008), antiallergic (Svarcova et al., 2007), antibacterial (Celli et al., 2014), and immunotropic (Piekarska et al., 2022) properties.

Iridoids are natural secondary metabolites of plants, belonging to the chemical group of heterocyclic monoterpenoids. Iridoids are widespread mainly in the green parts of plants, therefore their presence in edible fruits is scarce. There are only a few reports devoted to the content of iridoids in the genus *Lonicera*, thus the content of iridoids of *Lonicera caerulea* fruits remains to be well-studied. Thus, this study aimed to determine the iridoid content and morphometric parameters of *Lonicera caerulea* of 34 genotypes originated from germplasm collection of the Forest-Steppe of Ukraine in M.M. Gryshko National Botanical Garden of NAS of Ukraine (Kyiv, Ukraine), simultaneously pointing the best genotypes which can be successfully applied as a novel plant source of functional foods.

Material and methodology

Collection of plant material

Berries of *Lonicera caerulea* (Figure 1) were harvested in the full maturity stage and collected in 2022 from the 13–18-year-old plants growing in the Forest-Steppe of Ukraine of Department of Fruit Plants Acclimatization in M.M. Gryshko National Botanical Garden of NAS of Ukraine (NBG) (Kyiv, Ukraine). Thirty-four genotypes (LC-01–LC-34) of *Lonicera caerulea* species were evaluated.

Morphometric characteristics

Pomological characteristics of ripened fruits were conducted with four replications on a total of 120 fruits per genotype. In our experiments, only one plant was used per genotype. In total 4080 fully ripened fruits of *L. caerulea* were investigated. Morphometric parameters were evaluated as follows: fruit weight (g), fruit length (mm), and fruit diameter (mm). The length and diameter of the fruits were measured using a digital calliper Kronos KM-DSM-200 (0–200/0.01; ±0.02 MM). The fresh fruit weights were determined using an analytical balance (Kern ADB-A01S05, Germany).

Chemicals

All chemicals and reagents were of analytical grade and were purchased from Merck (Darmstadt, Germany) and HIMLABORREACTIVE (Ukraine).

Iridoids content

The content of iridoids was determined in the fresh fruit extracts of 34 *L. caerulea* genotypes. Identification of iridoids was performed by the interaction of iridoid compounds with hydroxylamine and the formation of the oxime. The resulting oxime reacts by complexation with trivalent iron cations. The maximum absorption of the complex was measured at 512 nm (photoelectrocolorimeter Zalimp KF 77; Poland), with harpagid as the control.

Extraction was carried out with chloroform: ethanol mixture (5:1), and after the removal of the solvent, the residue was extracted with water. The change in the solvent made it possible to avoid the influence of concomitant substances on the results of the

Figure 1 Variability in the shape of *Lonicera caerulea* L. fruits

hydroxame reaction (Ivanova et al., 2010). Obtained data was calculated using the following equation:

$$C_{irid} = \frac{D \times K}{56 \times m(100 - W)}$$

where: D – the optical density of the solution; V – the total amount of the extract and the average sample (ml); m – linkage, the average of the sample (g); K – conversion factor; 56 – the specific absorption index of the products of the reaction of harpagide with hydroxylamine and iron (III) chloride; W – raw material humidity

The accuracy of the method was in the range of 2.5–4.8%. The results were expressed as mg.100 g^{-1} of fresh weight (FW) in terms of anthocyanidin.

Statistical analysis

All analyses were performed in triplicate. The results were presented as mean ± standard deviation (SD). Hierarchical cluster analyses of similarity between genotypes were computed by the Bray-Curtis similarity index and were performed using PAST 2.17 software (Norway, 2001).

Results and discussion

Fruit pomological properties

It should be pointed out that there are more than 40 genotypes of seed origin from the European part of Russia, the Kuril Islands, and Canada in the germplasm collection of the M.M. Gryshko National Botanical Garden of NAS of Ukraine (Kyiv, Ukraine). The fruits of the *Lonicera caerulea* collection varied in weight,

Table 1 The variability of morphometric parameters of all *Lonicera caerulea* L. genotypes

Characteristics	n	min	max	x̄	V%
Fruit weight, g	4080	0.70	1.54	1.04	19.74
Fruit length, mm	4080	15.33	26.52	20.66	17.10
Fruit diameter, mm	4080	7.12	13.10	9.93	12.37
Shape index	4080	1.42	3.65	2.11	22.49

Note: n – the number of measurements; min, max – minimal and maximal measured values; x̄ – arithmetic mean; V – coefficient of variation (%)

shape, size, the color of fruits, and also degree of the wax coating were noted (Figure 1).

The biometric values for the weight, length, diameter, and shape index of fruits of 34 *Lonicera caerulea* genotypes are shown in Table 1. Fruit weight, which is economically the most important characteristic of fruits, ranged from 0.70 (LC-18) up to 1.54 g (LC-13). Morphological variation of fruit length varied between 15.33 mm for genotype LC-24 and 26.52 mm for genotype LC-06 (Table 1, Figure 2, 3, 4). The values of diameter varied within the interval from 7.12 mm (LC-27) to 13.10 mm (LC-16).

Fruit weight and size are primarily phenotypic features and reflect the impact of environmental growth conditions, while the fruit shape index is a genetically fixed feature. It is on this basis that some subspecies of *L. caerulea* were previously identified as distinct species (Grygorieva et al., 2021).

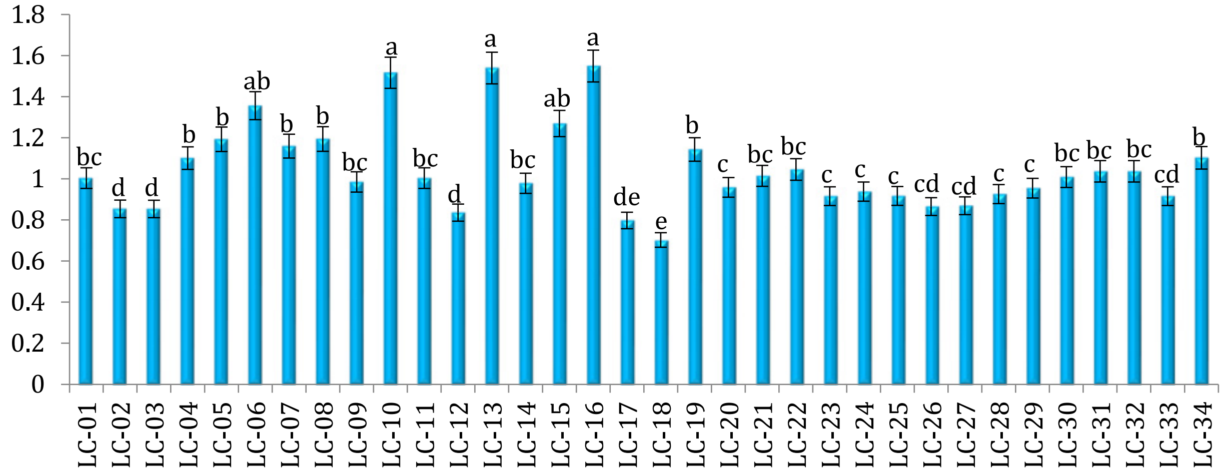

Figure 2 *Lonicera caerulea* L. fruits weight of 34 genotypes (means values ± SD); different superscripts in each column indicate the significant differences in the mean at p <0.05

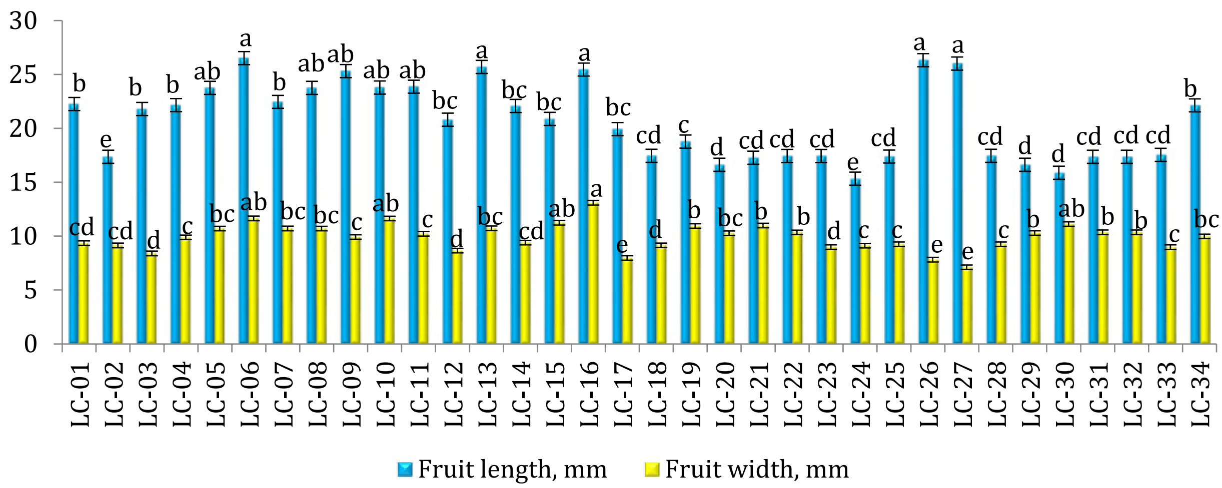

Figure 3 *Lonicera caerulea* L. fruits length and width of 34 genotypes (means values ± SD); different superscripts in each column indicate the significant differences in the mean at p <0.05

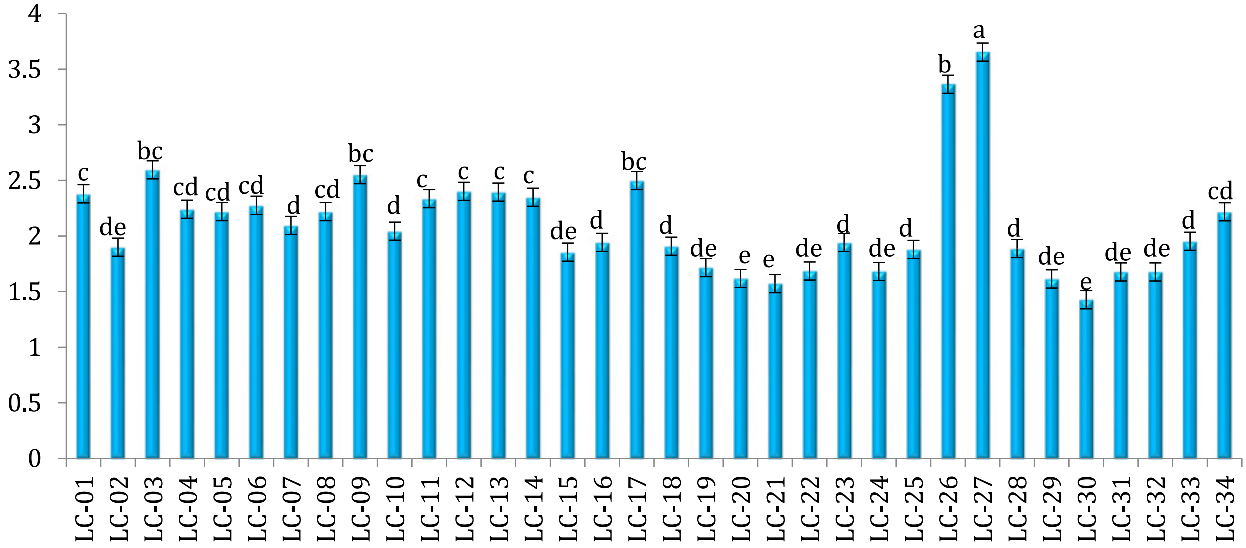

Figure 4 Comparison of shape index of *Lonicera caerulea* fruits 34 genotypes; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

The shape index average values of *Lonicera caerulea* fruits ranged from 1.42 (LC-60) to 3.65 (LC-57) (Figure 4). These results are in agreement with the study of Grygorieva et al. (2021) who determined the shape index of *L. caerulea* fruits (1.51–3.52).

Differences in the weight of *Lonicera caerulea* fruits were also previously reported by Plekhanova (2000), Thompson and Barney (2007), Fu et al. (2011), Gawronski et al. (2014), MacKenzie et al. (2018), and Holubec et al. (2019) who found the weight ranged from 0.21 to 2.70 g of different plant genotypes. A study by Fu et al. (2011) showed that the length of fruits was in the range of 11.16–19.43 mm, and Senica et al. (2018) detected values in the interval of 18.10 to 26.32 mm. Investigations by Holubec et al. (2019) established the range of fruit length of varieties from 15.50 to 20.40 mm. According to the previously studied collection of 26 *L. caerulea* genotypes (Grygorieva et al., 2021), the morphometric parameters were as follows: fruit weight from 0.73 to 1.60 g, fruit length from 16.42 to 27.29 mm, fruit diameter from 7.77 to 12.34 mm. The presented results are in accordance with the previously published studies and strongly support the statement that between *L. caerulea* genotypes exists high variability in morphometric parameters.

The analysis of the coefficient of variation showed a significant level of variability of morphological parameters between studied *L. caerulea* samples. The variation coefficients (%) ranged between 14.35 (LC-55) and 31.04 (LC-03) for fruit weight, between 7.31 mm (LC-50) and 16.35 (LC-04) for fruit length, between 6.99 (LC-60) and 18.54 (LC-04) for fruit diameter, and between 4.21 (LC-56) and 17.88 (LC-01) for the shape index. Data clearly showed that the most variable is fruit weight.

Iridoids content in *Lonicera caerulea* fruits

Iridoids are known plant-derived compounds mainly for the variety of their health-promoting properties. Pharmacological studies devoted to the isolation and application of iridoids from different plants proved their valuable effect on human health, namely antioxidative, anti-inflammatory, anti-cancer, anti-atherogenic, antidiabetic, neuroprotective, antimicrobial, diuretic, sedative, hepatoprotective, hypolipidemic, neuroprotective, and purgative activities (Tundis et al., 2008; Dinda et al., 2011; Viljoen et al., 2012).

Regarding the fact, that iridoids are mainly found in the green parts of plants, such as leaves and young stems, but only occasionally can be present in fruits and shoots (Dinda et al., 2007; Villasenor, 2007). However, there are some exceptions to this rule, e.g., fruits *Vaccinium macrocarpon* Aiton (Turner et al., 2007), *Vaccinium myrtillus* L. (Juadjur and Winterhalter, 2012), *Cornus mas* L. (Kucharska et al., 2015), *Cornus officinalis* Torr. ex Dur. (Klymenko et al., 2021).

Among the species of the genus *Lonicera*, iridoid compounds were identified mainly in the leaves of *L. caerulea* (Machida et al., 1995a, b) and different morphological parts of *L. japonica* (like e.g. flowers, buds, stem, leaves, and caulis) (Qi et al., 2009; Guo et al., 2014; Ye et al., 2014; Zhang et al., 2015). It should be highlighted that, there are published only a few reports devoted to the content of iridoids in the fruits

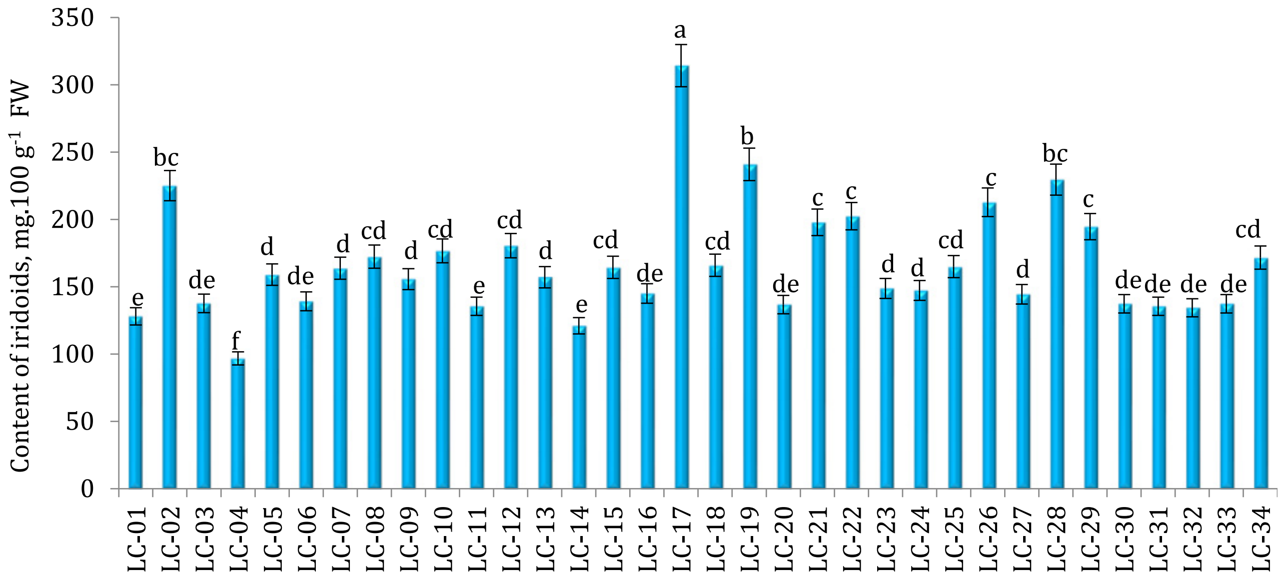

Figure 5 Content of iridoids in fruits of various genotypes of *Lonicera caerulea* L; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

of the genus *Lonicera* (Whitehead and Bowers, 2013; Kucharska and Fecka, 2016; Kucharska et al., 2017).

In our study, the iridoid content in all studied *Lonicera caerulea* genotypes ranged from 96.78 (LC-04) up to 314.32 mg.100 g^{-1} FW (LC-17) (Figure 5). Interestingly, the *L. caerulea* berries taste differs between the varieties (data not shown). Those with bitter or sour-bitter taste are distinguished by the highest content of iridoids (225–314 mg.100 g^{-1} FW), while berries without bitter taste were characterized by significantly lower contents. It should be noted that the exceptionally high content of iridoids was observed in one sample – LC-17 genotype (314 mg.100 g^{-1} FW). Sensory evaluation of these fruits revealed that LC-17 genotype fruits are characterized by extremely bitter taste (data not shown). The LC-19 genotype also represents a fairly high content of iridoids – 241 mg.100 g^{-1} FW. According to the sensory analyses, the fruits of this genotype were characterized as sour-bitter (data not shown). Genotypes LC-02 and LC-28 have an iridoid content of 225 and 230 mg.100 g^{-1}, respectively and the sensory estimation proved that these fruits were slightly bitter (data not shown). Thus, honeysuckle berries should be selected preferentially regarding their bitterness, due to the anti-inflammatory and antioxidative properties of iridoids.

The study of Kucharska et al. (2017) was devoted to the identification of the iridoids profile of 30 different honeysuckle berry cultivars and genotypes with an application of UPLC-ESI-qTOF-MS/MS combined with HPLC-PDA. It should be highlighted that some compounds, like 8-epi-Loganic acid, pentosyl-loganic acid, taxifolin 7-O-dihexoside, and taxifolin 7-O-hexoside were detected in honeysuckle berries for the first time. The Kuvshinovidnaya cultivar was distinguished by the highest content of iridoids (372 mg.100 g^{-1} FW). In the iridoid profile, loganic acid was assayed as dominated compound (even up to 73% of the total amount of quantified iridoids) in honeysuckle berries (Kucharska, et al., 2017). In the experiment of Perova et al. (2019) who analyzed 15 frozen fruit samples of *Lonicera edulis* collected in Tambov, Voronezh, Moscow regions, and Karelia, total iridoids content ranged from 78 to 342 mg.100 g^{-1}. Accordingly to literature data and the results presented in this study, we can conclude that *Lonicera caerulea* berries proved to be rich in iridoids and the content of iridoids markedly differed between *Lonicera caerulea* genotypes.

The amounts of iridoids in *L. caerulea* were generally much lower than in cornelian cherry (*Cornus mas* L.) ripe fruits, in which the content of total iridoids ranged from 86.91 to 493.69 mg.100 g^{-1} FW (Kucharska et al., 2015). Extremely rich in iridoids turned out to be fruits of *Cornus officinalis* with the content of four main iridoids in the range of 1002–3819 mg.100 g^{-1} (Liu et al., 2012). Moreover, the total iridoids content in many other fruits covered a wide range, i.e. from 89.09 (*C. mas* cv. Ekzotychnyi) to 1441.22 mg.100 g^{-1} FW (*C. officinalis*, Co-01). The average iridoids contents in the analyzed *C. mas*, *C. officinalis*, and *C. mas* × *C. officinalis* fruits were 190.11, 1117.01, and 293.47 mg.100 g^{-1} FW, respectively (Klymenko et al., 2021). Whitehead and Bowers (2013) determined

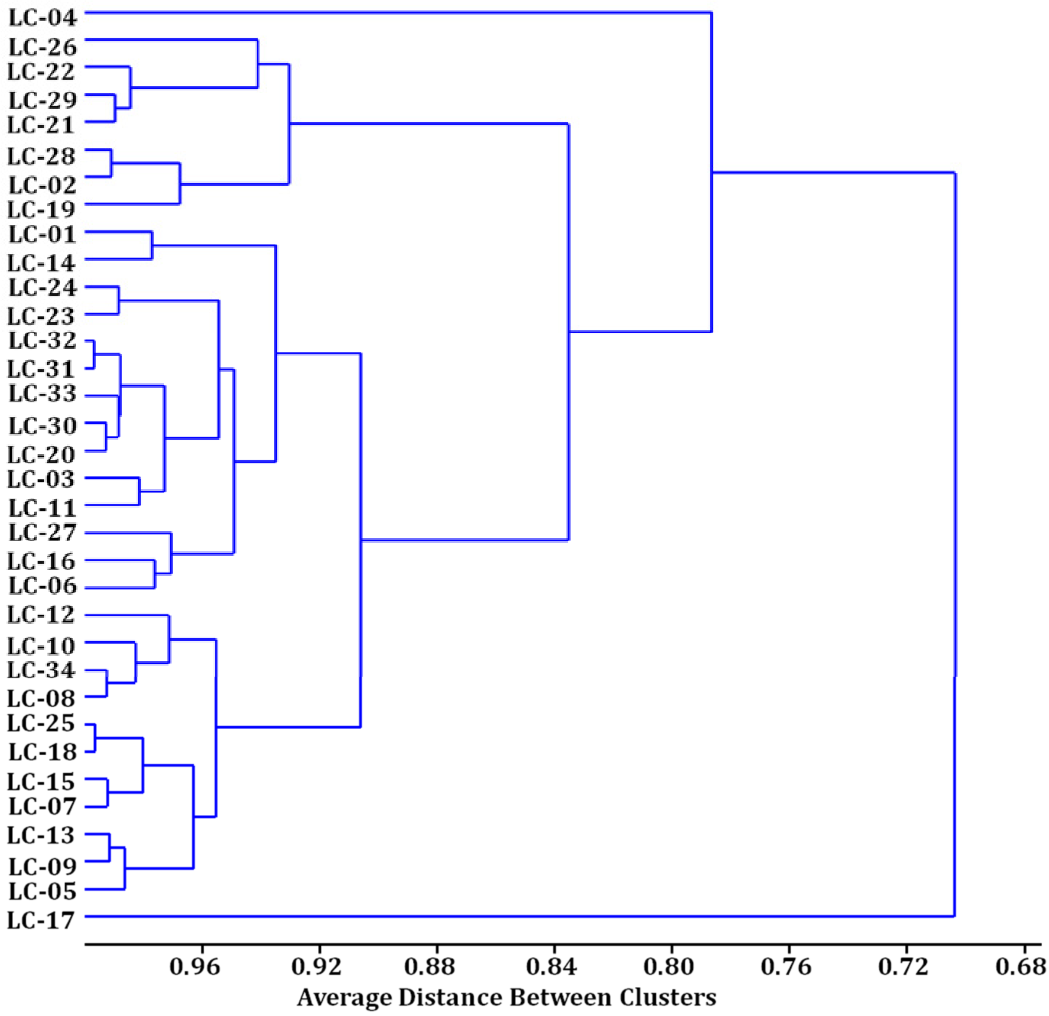

Figure 6 The cluster dendrogram analyzed on the morphometric parameters and iridoid content of 34 genotypes of *Lonicera caerulea* fruits

sixcompounds from this group in non-edible fruits from the species *Lonicera morrowii* A. Gray, *Lonicera tatarica* L., and their hybrid *Lonicera* × bella Zabel.

Based on the data obtained in our study we provided the determination of relatedness by the method of discriminant analysis (Figure 6). A comparison clearly shows the different genotypes and grouping and the significant differences between them.

Conclusions

This study demonstrates that *Lonicera caerulea* L. fruits may be regarded as a valuable plant source of biologically active compounds – iridoids (from 97 (LC-04) to 314 mg.100 g^{-1} FW (LC-17). However, it should be highlighted that studied *Lonicera caerulea* genotypes (34) available from M.M. Gryshko National Botanical Garden of NAS of Ukraine (Kyiv, Ukraine) differed significantly in all morphological parameters and iridoid contents. Interestingly, *L. caerulea* berries taste differs between the varieties, those with bitter or sour-bitter taste are distinguished by the highest content of iridoids (225–314 mg.100 g^{-1}), while berries without bitter taste were characterized by significantly lower contents. Thus, honeysuckle berries should be selected preferentially regarding their bitterness, due to the anti-inflammatory and antioxidative properties of iridoids. Moreover, the presented germplasm collection has significant genotypic potential for further selection for adaptability and improvement of fruit quality.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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