

**Research Article** 



# Content of Iridoids in the Fruits of Cornus mas L. Cultivars

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#### DOI: https://doi.org/10.15414/ainhlq.2024.0004

The genetic fund of the cornelian cherry (Cornus mas L.) cultivars at the M.M. Gryshko National Botanical Garden (NBG), National Academy of Sciences of Ukraine includes more than 100 genotypes collected from wild and cultivated plants in Ukraine, and cultivars of Bulgarian, Slovak, English, Austrian and Georgian selection. The genetic fund of the cornelian cherry of the NBG presents a rich variety of biological and economic properties. The fruits of *C. mas* are valued in the food industry and medicine mainly due to their high content of bioactive compounds such as vitamin C, carotenoids, and anthocyanins. C. mas fruits also have antioxidant activity. We have given the pomological characteristics of 21 studied cultivars of C. mas, and also determined the content of iridoids. Cultivars of C. mas with early, middle, and late fruit ripening periods were studied. To determine the content of iridoids, a spectrophotometric method was used, which consists of the interaction of an iridoid compound with hydroxylamine to form an oxime. The resulting oxime is capable of forming a coordination compound with ferric iron cations. As a result of the study, it was found that the content of iridoids in the fruits of C. mas has a significant difference in cultivars. Among the early ripening cultivars of *C. mas*, the most promising as a source of iridoids are Pervenets and Samofertylnyi, among the cultivars of C. mas with an average fruit ripening period is Yevgenia, and among the cultivars of *C. mas* with a late ripening period of fruits, the most promising cultivars are Sokoline and Yuvileinyi Klymenko. These most promising cultivars should be noted for use in the food and medical sectors of the national economy.

Keywords: Cornelian cherry, cultivar, fruits, iridoids, Ukraine

#### Introduction

Fruits 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 medicinal plants (Vergun et al., 2019; Buyun et al., 2021; Stefanowski et al., 2021), especially their fruits rich in bioactive components gained much attention (Levon and Golubkova, 2016; Klymenko et al., 2017; Latocha, 2017; Levon and Goncharovska, 2017; Lachowicz et al., 2019; Zhurba et al., 2021; Karapatzak et al., 2022; Kumari et al., 2024).

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Slovak University of Agriculture in Nitra www.uniag.sk Species of the genus *Cornus* L. belong to the group of plants whose aboveground organs contain iridoids (Deng et al., 2013; Wang et al., 2022; Li et al., 2022; Tenuta et al., 2023). The presence of iridoids along with phenolic compounds determines the resistance of cornelian cherry trees to various diseases and pests (Blagojević et al., 2021).

Iridoids belong to a class of secondary metabolites that are found in various organs of many plants and some animals and insects. These cyclopentelated monoterpenoids are synthesized from isoprene and are often intermediates in the biosynthesis of alkaloids. Iridoids are usually found in plants as glycosides and are most often associated with glucose. Iridoids are synthesized by plants primarily to protect against herbivores, insects, or microbial damage. In accordance with this function, iridoids, unlike, for example, carotenoids or flavonoids, are quite stable and do not break down during long-term storage or heat treatment of medicinal plants that contain them. Due to their bactericidal and antioxidant properties, iridoids can act as a natural preservative (Mnatsakanyan, 1986; West et al., 2012; Przybylska et al., 2022).

Currently, more than 250 iridoids have been isolated from many species belonging to the families Viburnaceae, Actinidiaceae, Ericaceae, Loganiaceae, Gentianaceae, Rubiaceae, Verbenaceae, Lamiaceae, Oleaceae, Plantaginaceae, Scrophulariaceae, Valerianaceae, Menyanthaceae, Caprifoliaceae and others (Jensen, 1992; Pérez et al., 2005; Viljoen et al., 2012; Rimpler and Timm, 2014; Levon et al., 2016; Leisner et al., 2017; Yuana et al., 2017; Kroll-Møller et al., 2017; Bello et al., 2018; Gousiadou et al., 2019; Zhang et al., 2022; Levon et al., 2022; Jaafar et al., 2024; Kim et al., 2024).

Iridoids are known plant-derived compounds mainly for the variety of their health-promoting properties. Pharmacological studies devoted on 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 and purgative activities (Inouye et al., 1974; Tundis et al., 2008; Dinda et al., 2011; Viljoen et al., 2012; Omelchenko et al., 2016; Kucharska et al., 2017; Hussain et al., 2019; Esakkimuthu et al., 2019; Ji et al., 2019; Wang et al., 2022, 2023; Bridi et al., 2023). Cornel iridoid glycoside improves memory ability (Zhao et al., 2010).

Thus, the purpose of this study was to determine the content of iridoids of *C. mas* cultivars originating from the collection of M.M. Gryshko National Botanical Garden, while pointing out the best cultivars that can be successfully used as a new plant source of functional nutrition.

# Material and methodology

# **Collection of plant material**

Plants collected from the collection of the Department of Fruit Plants Acclimatization in M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (NBG) at the stage of fructification during 2023. Samples for analysis were taken from the middle part of the plant on four sides: northern, southern, western, and eastern. Height above sea level 127 m. Cornelian cherry plantings in the National Botanical Garden are concentrated on the sod-podzolic soil with pH 7.0-8.0. Periodically, once every 2 years, we apply lime to reduce soil acidity. Adding lime to soil increases the growth of plants and increases yields. Cornelian cherry can grow on abandoned land that is not suitable for other crops. Cornelian cherry plants on fertile and moist soils develop normally with a pH of 6.7–7.0. Despite the cornelian cherry's unpretentiousness to the soil, it prefers soils of light texture and air permeability. The water table should be no closer than 1.5-2.0 metres. Cornelian cherry is a moisture and drought-resistant, light-loving, and somewhat shade-tolerant plant that is not picky about its soil, as evidenced by its natural range. However, to obtain large, juice fruits and good yields, cornelian cherry fruit very well in low, but not flooded, locations.

Biochemical analyses were carried out in the laboratory of the Department of Fruit Plants Acclimatization. Biochemical studies were carried out on fruits stored before analysis in a well-ventilated area without direct sunlight at 20–25 °C and crushed in an electric mill (Vektor HR-200). Particle size after grinding is 200 microns. The fruits of *C. mas* plant cultivars were studied with early (Radist, Nikolka, Nizhnyi, Pervenets, Volodymyrskyi, Vytivka Svitlany, Samofertylnyi, Alyosha, Uholok), average (Yevgeniia, Koralovyi, Yantarnvi, Medok, Vyshgorodskyi, Lukianivskyi, Starokyivskyi) and late (Semen, Sokolyne, Kolisnyk, Yuvileinyi Klymenko, Kozerig) (Table 1, Figure 1). All cultivars of Ukrainian selection and bred in NBG selection.

Cultivar	Fruit				
	form	color	size, length/largest diameter (mm)	weight, average (max), (g)	source of information
Radist	oval-ovoid to oval-pear-shaped	from dark red to black	29.0-31.0/17.0-18.0	5.2-5.5(6.6)	Klymenko, 2013
Nikolka	pear-shaped and somewhat asymmetrical	black-red to black	33.0-35.5/16.5-17.5	5.5-5.8(6.5)	Klymenko, 2013
Nizhnyi	pear-shaped with an elongated neck	yellow	32.0-35.5/15.0-16.5	4.5-5.5	Klymenko, 2013
Pervenets	Oval-pear-shaped with an indistinct neck	black-red to black	25.0-30.0/17.0-19.0	4.7-5.5(6.6)	Klymenko, 2013
Volodymyrskyi	oval-cylindrical, somewhat asymmetric, and flattened at both ends	from black-red to black	20.0-23.0/6.5-7.5	7.5-8.5(9.5)	Klymenko, 2013
Vytivka Svitlany	oval and somewhat elongated, truncated, and slightly concave at the top	red to dark red	20.6-24.0/14.4 - 17.3	3.0(4.0)	Klymenko & Ilyinska, 2023
Samofertylnyi	oval	black-red	24.3/16.3	3.5(4.5)	Klymenko, 2013
Alyosha	oval	bright yellow	20.0-23.0/13.0-17.0	3.5-5.0(5.7)	Klymenko, 2013
Uholok	wide-oval	black	15.9-21.4/11.6-14.9	2.2(3.1)	original data
Yevgeniia	oval	dark red to almost black	27.0-30.0/17.0-19.0	6.0-6.8(7.6)	Klymenko, 2013
Koralovyi	wide-rounded	pink-orange	21.0-22.0-15.0-17.0	3.4-4.0(4.4)	Klymenko, 2013
Yantarnyi	oval-cylindrical, liquid-like	amber yellow	19.5-21.5/14.0-16.0	3.2-3.5(4.0)	Klymenko, 2013
Medok	oval with a pronounced neck	dark red to almost black	21.8-31.1/12.4-16.5	3.3(4.4)	original data
Vyshgorodskyi	oval, somewhat asymmetrical	dark cherry	22.3-28.3/15.0-18.0	4.5-4.8(5.3)	Klymenko, 2013
Lukianivskyi	pear-shaped	dark red to almost black	30.6-34.0/16.5-19.5	6.0(7.5)	Klymenko, 2013
Starokyivskyi	pear-shaped with a narrow neck, somewhat asymmetrical	dark red	35.0-37.0/17.0-18.0	5.6-6.0(7.8)	Klymenko, 2013
Semen	broad-pear-shaped, shortened, barely ribbed	dark cherry	26.0-29.0/16.5-18.0	6.0-6.4(7.2)	Klymenko, 2013
Sokolyne	oval with a distinct short neck	dark red	25.2-34.6/12.9-20.2	5.2(7.2)	original data
Kolisnyk	pear-shaped	dark red	19.8-26.9/13.4-18.3	4.2(6.4)	original data
Yuvileinyi Klymenko	oval-pear-shaped with an indistinct neck	dark red	21.1-34.5/16.6-20.7	6.5(8.2)	original data
Kozerig	oval with a distinct neck	dark red	22.4-30.0/12.0-16.6	3.5(5.7)	original data

# **Table 1**Brief pomological characteristics of fruits of the Cornus mas L. cultivars



Figure 1 Fruits of Cornus mas L. cultivars collection of the M.M. Gryshko National Botanical Garden of the NAS of Ukraine 1 - Radist; 2 - Nizhnyi; 3 - Pervenets; 4 - Vytivka Svitlany; 5 - Samofertylnyi; 6 - Alyosha; 7 - Uholok; 8 - Koralovyi; 9 - Yantarnyi; 10 - Starokyivskyi; 11 - Yuvileinyi Klymenko; 12 - Semen; 13 - Volodymyrskyi; 14 - Yevgeniia; 15 - Medok; 16 - Vyshgorodskyi; 17 - Sokolyne; 18 - Lukianivskyi; 19 - Nikolka

#### Chemicals

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

## Iridoids content

Quantification of the amount of iridoids was carried out by UV-spectrophotometry. 3 ml of water-alcohol extracts were transferred to volumetric flasks with a capacity of 25 ml and brought to the mark with purified water and mixed. 5 ml of an alkaline solution of hydroxylamine was added to 5 ml of the obtained solution and left for 20 min. After 20 minutes, 10 ml of a 1 M solution of hydrochloric acid and 5 ml of a 1% solution of iron (III) chloride in a 0.1 M solution of hydrochloric acid were added. The obtained mixture was stirred. The optical density of the solution was immediately measured on a spectrophotometer at wavelengths of 370–386 nm in a cuvette with a layer 1 cm thick. To prepare the compensation solution, 5 ml of an alkaline solution of hydroxylamine, 10 ml of a 1 M solution of hydrochloric acid, and 5 ml of 1% solution of iron (III) chloride in a 0.1 M solution of hydrochloric acid and brought the solution up to the mark with 70% ethanol (Tsurkan et al., 2014). Similarly, the optical absorption of the aucubin standard solution was measured. The obtained data were calculated according to the formula:

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

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

The number of parallel determinations was three. The accuracy of the method was in the range of 2.5–4.8%. The results were expressed as  $mg \cdot 100 g^{-1}$  of fresh weight (FW).

The optical density of all the studied solutions was measured using an Ulab 102UV spectrophotometer (China).

## Statistical analysis

Statistically processed data is shown on histograms as arithmetic means and their standard errors. The significance level was set at  $\alpha = 0.05$ . The statistical analysis was performed with IBM SPSS Statistics, release 27.0.1.

# **Results and discussion**

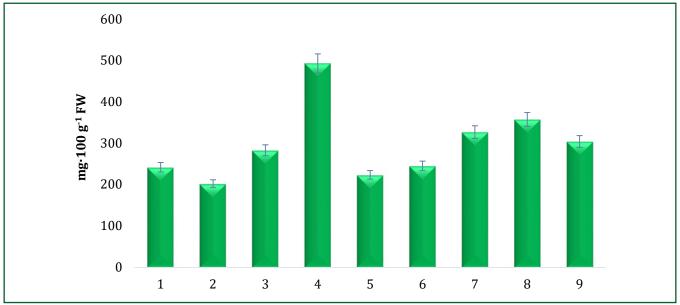
The presence of a large amount of biologically active substances in species of the genus *Cornus* not only makes them a valuable medicinal raw material but also increases their resistance to many stress factors (Ma et al., 2014; Biaggi et al., 2018; Bayram and Ozturkcan, 2020; Demir et al., 2020; Levon and Klymenko, 2021). Long-term observations have shown that species of the genus *Cornus* have extremely high resistance to both pests and adverse weather conditions, plants bloom profusely and give birth (Klymenko, 2000).

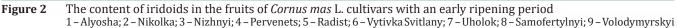
As a result of our research, it was found that the content of iridoids in *C. mas* fruits is quite wide and amounts to 111.61–492.61 mg·100 g<sup>-1</sup> FW. The content of iridoids in *C. mas* fruits with an early ripening period is in the range of 201.96–492.61 mg·100 g<sup>-1</sup> FW. The highest content of iridoids is observed in the fruits of the cultivar Pervenets 493 mg·100 g<sup>-1</sup> FW. Cv. Samofertylnyi also had a high content of iridoids 357 mg·100 g<sup>-1</sup> FW (Figure 2).

The content of iridoids in *C. mas* fruits during the average maturation period is in the range of 112–315 mg·100 g<sup>-1</sup> FW. The highest content of iridoids in fruits had the cv. Yevgeniia (315 mg·100 g<sup>-1</sup> FW) (Figure 3).

The content of iridoids in *C. mas* fruits of the lateripening period is in the range of 129–186 mg·100 g<sup>-1</sup> FW. The highest content of iridoids in fruits had the cv. Sokolyne 186 mg·100 g<sup>-1</sup> FW. Fruits of the cv. Yuvileinyi Klymenko also had a fairly high iridoid content of 172 mg·100 g<sup>-1</sup> FW (Figure 4).

We found that the fruits of *C. mas* cultivars with an early ripening period showed a higher content of iridoids. The average iridoid content of these cultivars is 297 mg·100 g<sup>-1</sup> FW. In cultivars with an average fruit ripening period, the average content of iridoids is 183 mg·100 g<sup>-1</sup> FW. *C. mas* cultivars with different fruit maturation periods have an average iridoid content of 155 mg·100 g<sup>-1</sup> FW (Figure 5). This may be due to a decrease in daylight hours, which correlates with the quantitative content of iridoids (Xiao et al., 2023).





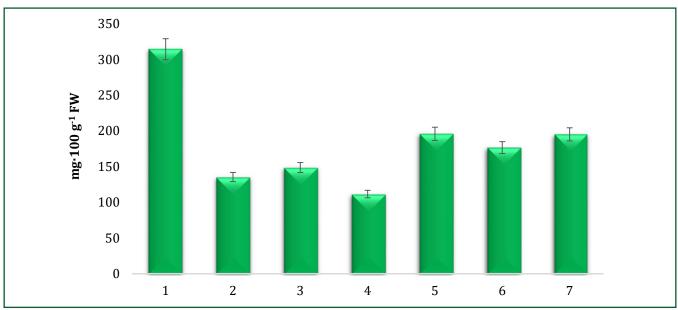
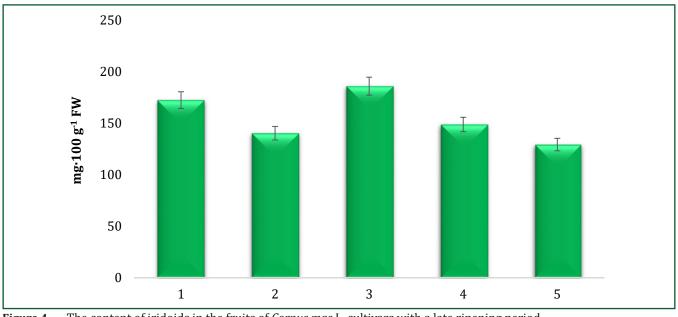
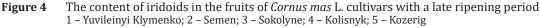


Figure 3The content of iridoids in the fruits of *Cornus mas* L. cultivars with an average ripening period1 - Yevgeniia; 2 - Koralovyi; 3 - Medok; 4 - Yantarnyi; 5 - Starokyivskyi; 6 - Lukianivskyi; 7 - Vyshgorodskyi

Multiple studies have shown that the content of iridoid compounds present in plant-based materials or extracts is directly correlated with their antioxidant potential. Consequently, iridoids are a valuable material for a number of industries including the cosmetic industry. Antioxidant potential tests confirmed the thesis that the antioxidant capacity of the solutions is directly proportional to the iridoid content (Nizioł-Łukaszewska et al., 2017).

Previously, we studied the amplitude of variability in the content of anthocyanins and flavonols in the fruits of various *C. mas* cultivars (Levon and Klymenko, 2021). Since iridoids, anthocyanins and flavonols are strong antioxidants, we conducted a correlation analysis between the content of these compounds in the fruits of *C. mas* varieties. It has been established that there is a very high correlation between the content of iridoids and anthocyanins in the fruits of *C. mas* plants of early ripening with the early period of fruit ripening (r = 0.838). There is also a very high correlation between the content of iridoids and flavonols in the fruits of *C. mas* plants with early fruit ripening (r = 0.860).





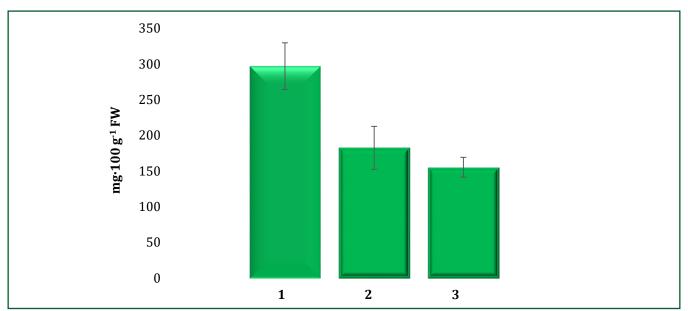


Figure 5 Comparison of the average content of iridoids and their confidence intervals (95% confidence level) in the fruits of *Cornus mas* L. cultivars with early, medium, and late ripening periods 1 – early fruiting; 2 – medium fruiting; 3 – late fruiting

Correlation analysis between the content of iridoids and anthocyanins in the fruits of *C. mas* plants with the average period of fruit ripening showed a moderate correlation (r = 0.534). There is also a moderate correlation between the content of iridoids and flavonols in the fruits of *C. mas* plants with the average period of fruit ripening (r = 0.397).

A correlation analysis between the content of iridoids and anthocyanins in the fruits of *C. mas* plants with late-ripening showed a moderate correlation (r =0.390). There is also a moderate correlation between the content of iridoids and flavonols in the fruits of *C. mas* plants with a late period of fruit ripening (r =0.658).

Similar studies were conducted by Kucharska et al. (2017) with *Lonicera caerulea* berries. A correlation was established between the content of iridoids and anthocyanins and antioxidant activity. The content of iridoids, in addition to anthocyanins, may be one of the most important parameters for characterizing fruits in terms of their nutritional and medicinal value.

A study by Kucharska et al. (2015) was devoted to identifying the iridoid profile of 26 different cultivars and 2 ecotypes of *C. mas* fruits, collected from five locations in 2007–2011. The content of total iridoids in cornelian cherry fruits covered a wide range, i.e., from 86.91 to 493.69 mg·100 g<sup>-1</sup> FW. Loganic acid was the most dominant iridoid compound identified in cornelian cherry fruits and amounted to 88–96% of total iridoids.

The literature describes an original method for determining dogwood iridoids using HPLC with UV- and MS-detection. The main iridoids of cornelian cherry fruits were found to be 2 iridoid glycosides (loganin and loganinic acid) and 2 secoiridoid glycosides (cornuside and sveroside) (Deng et al., 2013).

Iridoids also contain other species of the genus *Cornus*. Extremely rich in iridoids turned out to be fruits of *C. officinalis* Siebold & Zucc. with the content of four main iridoids in the range of  $1,002-3,819 \text{ mg}\cdot 100 \text{ g}^{-1}$  (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  $1,441.22 \text{ mg}\cdot 100 \text{ g}^{-1}$  FW (*C. officinalis*, Co-01). The average iridoid contents in the analyzed *C. mas*, *C. officinalis*, and *C. mas* × *C. officinalis* fruits were 190.11, 1,117.01, and 293.47 mg·100 g<sup>-1</sup> FW, respectively (Klymenko et al., 2021).

Four new and rare iridoid glucosides, cornusfuroside A–D, containing a furan ring were identified from the aqueous extract of *C. officinalis* fruits. The chemical structure of these compounds was determined using extensive spectroscopic analysis, including 1D and 2D NMR, IR, HRESIMS, experimental and calculated electron circular dichroism (ECD It is interesting to note the fact that this study is the first report on the isolation of four iridoid glucosidic structures with acetal functions in the sugar moiety (He et al., 2017).

Three new iridoids, cornifins A-C were obtained from EtOAc layer of leaves of *Cornus officinalis*. The structures of new compounds were elucidated based on extensive spectroscopic analyses (Le et al., 2015). Three previously undescribed iridoids, cornusfurals A-C, were isolated from the ethanolic extracts of fruits of *C. officinalis*. Their structures were elucidated by spectroscopic methods, including one-dimensional and two-dimensional nuclear magnetic resonance, infrared spectroscopy, ultraviolet spectroscopy, and mass spectrometry (Ji et al., 2019).

A new iridoid glucoside, 6'-O-acetyl-7 $\beta$ -O-ethyl morroniside, was isolated from an aqueous extract of ripe fruits of *C. officinalis* (Qiao et al., 2017; Czerwińska and Melzig, 2018). Cornsecoside A and cornsecoside B, unique novel iridoid glycosides, were discovered in a 40% ethanol elution fraction obtained from a 50% ethanol extract of *C. officinalis* fruit. Their structures were determined by spectroscopic data analysis combined with hydrolysis and ECD spectroscopy (Li et al., 2022).

Fifteen new and rare iridoid glucoside dimers, cornusides A–O were isolated from the fruit of *C. officinalis*. These new chemical structures were established through spectroscopic analysis (UV, IR, HRESIMS, 1D, and 2D NMR) (Ye et al., 2017). A new secoiridoid, demethoxy-cornuside, along with six known secoiridoids was isolated from the twigs of *C. officinalis* (Lee et al., 2021).

A new iridoid glucoside, cornusoside A, and four new naturally occurring iridoid aglycones, cornelactones A–D, were isolated from the leaves of *C. florida* L. The structure of these compounds was established by interpreting their spectroscopic data. Cornolactone B is the first naturally occurring cis-fused tricyclic dilactone iridoid containing both a five- and six-membered lactone ring. Cornelactones C and D are epimers of cornelactones A and B (He et al., 2014).

Iridoid glycosides are also found in the fruits of *C. kousa* Bürger ex Hance. The main component of this species is verbenaline (Przybylska et al., 2022). Two iridoid glucosides,  $6\alpha$ -dihydrocornic acid, and  $6\beta$ -dihydrocornic acid, were isolated from *C. capitata* Wall. adventitious roots (Tanaka et al., 2001).

The iridoid glycosides scandoside, scandoside methyl ester, monotropein, and galioside were found in *C. canadensis* L. from several widely distributed collection sites. Cornin and hastatoside were isolated from *C. nuttallii* Audubon ex Torr. & A.Gray (Tenuta et al., 2023). The iridoid glycoside geniposide has been reported to be the major iridoid of *C. suecia* L. from Denmark and is accompanied by a small amount of monotropein (Jensen et al., 1973).

The high content of biologically active substances in the fruits of the cultivars of *C. mas*, in particular iridoids, makes it possible to use the raw materials of these plants as an active preventive and curative agent.

# Conclusions

As a result of the conducted studies, it was found that the fruits of various cultivars of species of the genus Cornus have a fairly high content of iridoids. The content of iridoids in fruits ranges from 129-493 mg·100 g<sup>-1</sup> FW. It is shown that differences in the content of these compounds are determined by the genetic properties of cultivars and the ripening period of fruits. Cultivars Pervenets, Samofertylnyi, Yevgeniia, Yuvileinyi Klymenko, and Sokolyne have the highest content of these compounds. A correlation has been established between the content of iridoids and some phenolic compounds, namely anthocyanins and flavonols in the fruits of C. mas cultivars. The correlation coefficient between the content of iridoids and anthocyanins ranges from moderate (r = 0.390) in cultivars with late fruit ripening to high (r = 0.838) with early fruit ripening. The correlation coefficient between the content of iridoids and flavonols also ranges from moderate (r = 0.397) in cultivars with medium fruit ripening to high (r = 0.860) in cultivars with early fruit ripening. There was a decrease in the average content of iridoids in the fruits of *C. mas* cultivars with an average and late fruit ripening period. We assume that this may be due to a reduction in the duration of daylight hours. The study of the content of iridoids in the fruits of different cultivars of cornelian cherry as well as in other species of the genus Cornus is important when planting plantations, selecting cultivars with a high content of iridoids and according to the time timing of ripening, which is associated with the accumulation of iridoids depending on the degree of ripeness of the fruits. It is about the use of fruits for processing.

## **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.

## Acknowledgements

The publication was prepared with the active participation of researchers in the International Network AgroBio*Net*.

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