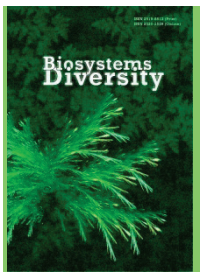

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Soluble cuticular wax composition and antimicrobial activity of the fruits of *Chaenomeles* species and an interspecific hybrid

Y. V. Lykholat*, N. O. Khromykh*, O. O. Didur*, S. I. Okovytyy*, T. V. Sklyar*, V. R. Davydov*, T. Y. Lykholat*, I. M. Kovalenko**

**Oles Honchar Dnipro National University, Dnipro, Ukraine*
***Sumy National Agricultural University, Sumy, Ukraine*

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Oles Honchar Dnipro National University, Gagarin av., 72, Dnipro, 49010, Ukraine. Tel.:+38-050-487-87-17. E-mail: khromykhn@ukr.net

Sumy National Agricultural University, Kondratyeva st., 160/5, Sumy, 40021, Ukraine. Tel.:+38-099-525-61-82. E-mail: kovalenko_977@ukr.net

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Plants of the genus *Chaenomeles* Lindl. (Rosaceae) naturally grow in Southeast Asia and represent the richest resource of biologically active compounds with beneficial properties for humans. Plants of *C. japonica* (Thunb.) Lindl. and *C. speciosa* (Sweet) Nakai species, and interspecific hybrid *C.* × *superba* (Frahm) Rehder (*C. japonica* × *C. speciosa*, Superba group) have been successfully introduced in the steppe zone of Ukraine and bear fruits. In this study, we evaluated chemical composition of fruit cuticular waxes and antimicrobial activity of fruit extracts. The soluble waxes were characterized using gas chromatography-mass spectrometry (GC-MS), and 26–36 compounds, representing 91.7–96.6% of the total soluble cuticular waxes, were identified. Waxes of *Chaenomeles* fruits belonged to six classes, namely fatty acids, alcohols, aldehydes, esters, ethers and alkanes. Aldehydes 7-hexadecenal and heptacosanal, and alkanes hexatriacontane and tetrapentacontane were the main constituents in the soluble cuticular waxes of *C. speciosa* and *C.* × *superba* fruits, accounting for more than half of the total contents. However, alkane tetrapentacontane, alcohol 8,10-hexadecadien-1-ol and heptacosanal prevailed in *C. japonica* fruit waxes. Isopropanolic fruit extracts exhibited dose-dependent antimicrobial activity against four Gram-negative bacteria, five Gram-positive bacteria and one fungal strain in the disc diffusion assay. In general, extracts from the *Chaenomeles* fruits demonstrated higher activity against Gram+ bacteria than Gram- strains. The strongest inhibiting activity was shown against *Staphylococcus epidermidis* (by the fruit extracts of *C.* × *superba* and *C. speciosa*), *Micrococcus lysodeikticus* and *Candida albicans* (both by *C.* × *superba* fruit extract). Results of the study confirmed accumulation of the bioactive compounds in the fruit waxes of different *Chaenomeles* species and antimicrobial ability of *Chaenomeles* fruits as well. These findings revealed the bioactive compounds in fruit cuticular waxes and suggested health-promoting properties of introduced *Chaenomeles* species.

Keywords: *Chaenomeles japonica*; *Chaenomeles speciosa*; *Chaenomeles* × *superba*; fruits; cuticular wax; fatty acids; aldehydes; alkanes; antimicrobial ability.

Introduction

Genus *Chaenomeles* Lindley (Rosaceae) consist of five species originated in Southern-East Asia, namely *C. speciosa*, *C. sinensis*, and *C. thibetica* which are the endemics in China, and *C. cathayensis* and *C. japonica* (Yang et al., 2015). Health-promoting abilities of *Chaenomeles* plants have long been known and used in traditional medicine to treat various diseases, such as rheumatism, cholera, dysentery, enteritis, beriberi and vitamin C deficiency syndrome (Zhang et al., 2014). In recent years, pharmacological assays of *C. speciosa* have confirmed multifaceted properties, including anti-inflammatory, antinociceptive, antimicrobial, antioxidant, immunoregulatory, antiparkinsonian, hepatoprotective and antitumour effects. Analysis of chemical composition of essential oils from *C. speciosa* dried fruits carried out by Xianfei et al. (2007) led to identification of α-terpineol, β-caryophyllene, terpinen-4-ol and 1,8-cineole as the main constituents. Miao et al. (2016) represented two common *Chaenomeles* fruits in China (*C. speciosa* and *C. sinensis*) as a rich resource of phenolic acids (vanillic, gallic, chlorogenic, ferulic and p-coumaric acids), triterpenes (oleanolic and ursolic acids), flavonoids (rutin, catechin and epicatechin) and other compounds contributing to high antioxidant capacity of *Chaenomeles* fruits.

The incredible variety of useful properties of the *Chaenomeles* plants has made them an attractive object for introduction in various regions (Rumpunen, 2002). In particular, cultivation of *C. japonica* is gaining popularity in northern European countries, especially in the Baltic Sea area. Kikowska et al. (2019) reported the development of protocol for *C. japonica* micropropagation with a confirmation of genome size stability of the *in vitro*-propagated plantlets and the high acclimatization rate. Urbanaviciute et al. (2020) tested different extraction conditions for the fruits of the new Japanese quince (*C. japonica*) cultivars by studying variability of phenolic compounds and free radical scavenging activity of plant extracts, in which isoquercitrin, rutin, (+)-catechin, (–)-epicatechin, and chlorogenic acid were identified. The leaf extracts of the same *C. japonica* new cultivars can efficiently reduce glioblastoma cell viability while preserving non-cancerous cells, and are worth further investigations as potential anticancer drugs (Zvikas et al., 2021).

In Ukraine, those plants with beneficial properties have not yet received proper attention and distribution, occupying an extremely small area of plantations (Moskalets et al., 2019). In the steppe zone, several *Chaenomeles* species and interspecific hybrids have been successfully introduced, and they bear fruits (Lykholat et al., 2019) and demonstrate the ability to accumulate the phenolic compounds. However, abundance and composition of the bioactive compounds in *Chaenomeles* fruits greatly depend on the growth conditions. Zheng et al. (2018) revealed significant differences in chemical composition and antioxidant activity of *C. speciosa* dried fruits from four production areas in China. Great variability both of total polyphenol content and the main bioactive compounds content were determined in the fresh fruits of five wild *Chaenomeles* species, namely *C. japonica*, *C. sinensis*, *C. speciosa*, *C. cathayensis* and *C. thibetica* (Du et al., 2013). The successful introduction of the *Chaenomeles*

plants in the steppe zone is largely due to the cuticle waxes, which create a protective barrier against environmental stresses and, in turn, are also affected by various environmental factors (Xue et al., 2017). The cuticular waxes contribute to the protection against insects (Rebora et al., 2020) and pathogens (Łaźniewska et al., 2012), as well as to fruit development and ripening (Trivedi et al., 2019). Despite the important role of wax in plant vitality (Lykholat et al., 2018), chemical composition of the cuticular waxes of *Chaenomeles* fruits is poorly studied. In addition, the ability of introduced plants to accumulate bioactive compounds and retain beneficial properties in a new environment has not been sufficiently observed. The aim of this work was to clarify the specific features of fruit cuticular waxes and antimicrobial activity of *Chaenomeles* plants from different regions of origin, adapted to the conditions of the steppe zone.

Materials and methods

Fruits of the *Chaenomeles* plants were taken from the Botanical Garden of Oles Honchar Dnipro National University (48°26'07" N, 35°02'34" E, Dnipro city, Ukraine). There, several plants of the genus *Chaenomeles* Lindl. Were introduced more than 25 years ago in the steppe climate with low precipitation (473 mm average, but 265 mm in dry years) and sharp temperature changes. Ripe fruits of *C. japonica* (Thunb.) Lindl., *C. speciosa* (Sweet) Nakai, and *C.* × *superba* (Frahm) Rehder (*C. japonica* × *C. speciosa*, Superba group) were collected in the first half of September 2021, packed in plastic containers and delivered to the laboratory immediately. The soluble cuticular waxes from the surface of *Chaenomeles* fruits were extracted with chloroform (Chloroform Pharm, Ukraine) according to Buschhaus et al. (2007) by immersing the fruit in a solvent for 60 seconds, followed by solvent evaporation in 40 ºC using rotary evaporator (IKA® RV 10, Germany). Obtained solid fraction was stored in 4 ºC; for GC-MS analysis, dry residue was dissolved in chloroform and filtered through a syringe filter.

Chloroformic extracts were subjected to gas chromatography – mass spectrometry (GC-MS) analysis using Shimadzu GCMS-QP 2020 El equipped with Rxi®-5 ms column (30 m × 0.25 mm, film thickness 0.25 µm) containing 5% diphenyl/95% dimethyl polysiloxane as a fixed liquid phase. The column temperature was 50 °C, with 5 min initial hold, and then the programmed temperature gradient was increased to 300 °C at the rate of 15 °C per min, and kept constant in 300 °C for 10.5 min. The carrier gas helium passed at the flow rate of 54 mL/min. Injector temperature was 300 °C; sample volume was 1 µL. Mass Spectrum Library 2014 for GC-MS (O2125401310) was used to identify the separated compounds by comparing the mass spectra obtained with those stored in the library database (National Institute of Standards and Technology library similarity index, NIST14.lib, NIST14s.lib). The content of individual compounds of cuticular waxes was estimated according to the area of the corresponding peak and expressed as a percentage of the total.

Plant extracts for biological assays were prepared using 80% isopropanol (INEOS, Germany). Then, 2.0 g weighed portion of fresh fruit (peel and flesh) was triturated with 20 mL of isopropanol and kept for 24 hours at room temperature in dark with occasional shaking. Then, the extracts were filtered through the paper filters and dried at 45 °C using rotary evaporator IKA® RV 10 (Germany). For bioassays, solid residue was dissolved in isopropanol.

Antibacterial activity of isopropanolic extracts of *Chaenomeles* fruits was determined by the disc diffusion method (Bhimba et al., 2012). Test cultures of microorganisms were taken from the culture collection of Microbiology, Virology and Biotechnology Department of Oles Honchar Dnipro National University. Four Gram-negative bacteria, namely *Erwinia dissolvens* (strain 170), *Escherichia coli* (strain B 906), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and five Gram-positive bacteria, namely *Micrococcus lysodeikticus*, *Staphylococcus aureus* (strain B 904), *S. aureus* (strain B 209), *S. epidermidis* (strain ATCC 149), *S. epidermidis* (strain 919), and diploid fungus *Candida albicans* were tested. Petri plates containing MPA medium (Meat Peptone Agar RM1049 HiMedia Laboratories Pvt. Limited, India) were inoculated with 10^9 CFU (colony forming units) suspension of microorganisms. Sterile paper discs (6 mm diameter) were impregnated with 10 µL of crude isopropanolic fruit extracts and placed on the agar surface, followed by incubation at 37 °C for 24 h. Ofloxacin (5.0 µg per disc) was used as the positive control in all cases. Inhibition zones produced by plant fruit extracts around the discs were measured along with disc diameter, and antibacterial activity was expressed as the diameter of the inhibition zone (mm). Photographs of Petri plates were taken using a camera Canon IXUS 185 Silver (Japan).

All bioassays were carried out in triplicate. Statistical processing of the experimental results was based on the analysis of variance (ANOVA). The obtained data were expressed as the mean ± standard deviation (x ± SD), and the differences between the means were tested with Tukey's HSD. All differences were considered to be statistically significant at $P < 0.05$.

Results

Gas chromatography – mass spectrometry assays showed a similar distribution of phytochemicals on the chromatograms of chloroformic extracts of the soluble cuticular waxes from the fruits of three different *Chaenomeles* species (Fig. 1). The study of phytochemicals carried out by GC-MS analysis of chloroformic extracts revealed a total of 53 compounds in fruit waxes, particularly 36 components in *C. japonica*, 26 in *C. speciosa* and 29 components in *C.* × *superba* soluble waxes (Table 1).

The compounds of fruit cuticular waxes of all studied plant species were classified into six chemical classes, namely fatty acids, aldehydes, alcohols, alkanes, esters, and ethers. Results of GC-MS identification showed different distribution of different chemical classes in the fruit cuticular wax of *Chaenomeles* species (Fig. 2).

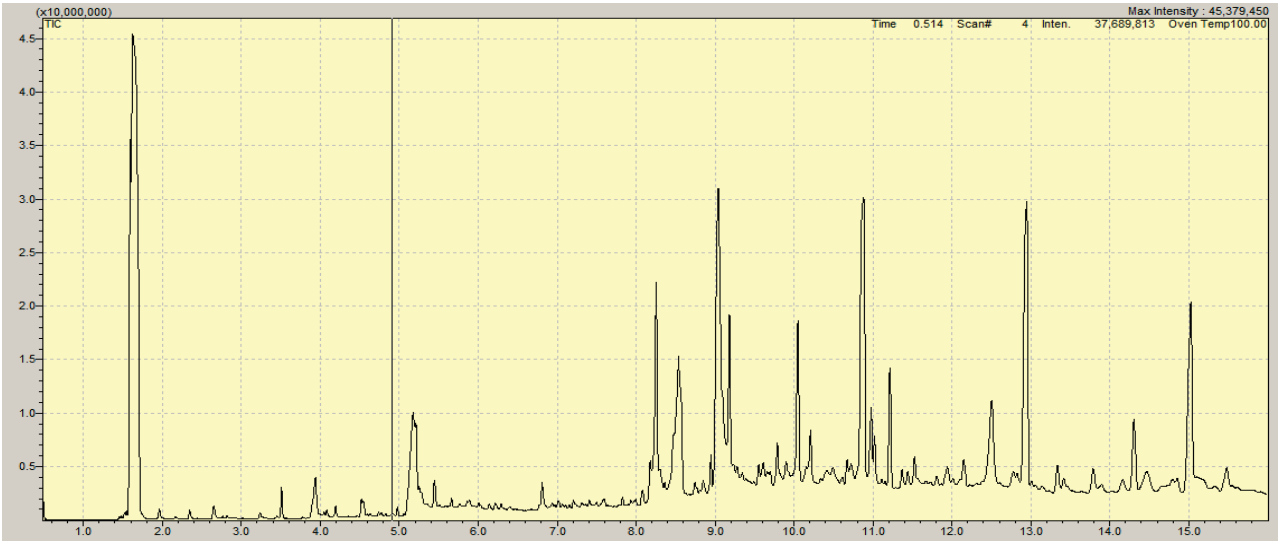

Fig. 1. GC-MS chromatogram showing the components of the soluble cuticular waxes from *Chaenomeles* fruits

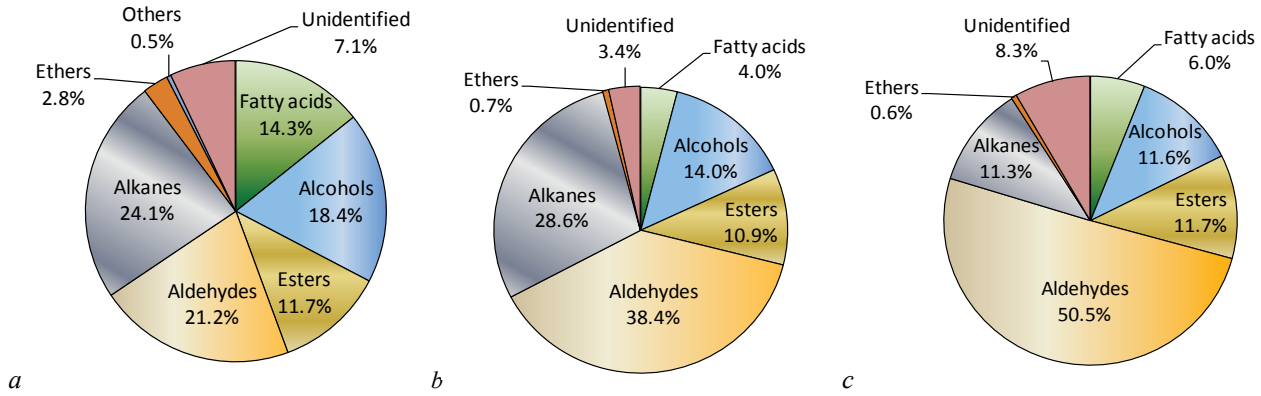

Fig. 2. Relative content (% of total) of different chemical classes in soluble cuticular wax of *Chaenomeles* fruits:
a – *C. japonica*, *b* – *C. speciosa*, *c* – *C.* × *superba*

Fraction of fatty acids in the fruit cuticular waxes consisted of 12 compounds with an even and odd number of carbon atoms in the range $C_6 - C_{25}$. The greatest content of fatty acids and their diversity was found in the soluble cuticular waxes of *C. japonica* fruits (14.27% of total amount of the compound was represented by nine different fatty acids). Share of long-chain fatty acids (C_{16} and more) was the largest in *C. japonica* fruit waxes (10.62% of total amount of the compound compared with 3.61% in *C. speciosa* fruits and 4.97% in *C.* × *superba* fruits), with dominating hexadecanoic acid in waxes of all species.

The most abundant alcohol fraction was found in *C. japonica* cuticular waxes, consisting of primary (seven compounds in the range $C_{10} - C_{41}$), secondary (two compounds), and tertiary alcohols (one compound), but the latter were not identified in the waxes of two other species. Share of primary alcohols was the highest in all fruit waxes and reached 17.32% in *C. japonica*, 13.29% in *C. speciosa* and 9.58% in *C.* × *superba*. Among the primary alcohols, 1-hentetracontanol prevailed in the cuticular waxes of *C. speciosa* and *C.* × *superba* fruits, while the highest content of 8,10-hexadien-1-ol was in *C. japonica* waxes.

Aldehydes in the fruit cuticular waxes were represented by 18 compounds with an even and odd carbons number in the range $C_7 - C_{60}$. The geeatest amount of long-chain aldehydes was identified in the cuticular waxes of *C.* × *superba* fruits (49.22% of total amount versus 19.97% in *C. japonica* and 37.69% in *C. speciosa* fruits). Heptacosanal was the main compound in the aldehydes fraction of all fruit waxes; however, only cuticular waxes of *C. speciosa* and *C.* × *superba* fruits contained the significant amount of hexadecenal.

The alkane's fraction in the soluble cuticular waxes of *Chaenomeles* fruits consisted of three long-chain compounds ($C_{27} - C_{54}$) dominated by hexatriacontane in *C. speciosa*, and tetrapentacontane in *C. japonica* and *C. speciosa* fruits.

The fraction of fatty acid esters was represented by six different compounds with an even and odd carbons number in the range $C_{10} - C_{25}$. Among them, diisooctyl phthalate content was the highest in the fruit cuticular waxes of all species. The content of another fatty acid esters was the highest in *C. speciosa* fruit waxes (2.80% of total amount compared with 1.29% in *C. speciosa* and 1.35% in *C.* × *superba* fruits).

In the fruit cuticular waxes of all species, the ethers content was the lowest, representing only three long-chain compounds ($C_{25} - C_{35}$), which in total accounted for 2.77% of *C. japonica* fruits. Overall content of primary alcohols and esters was 29.03% in the cuticular waxes of *C. japonica* fruits, 24.15% in *C. speciosa* fruit waxes and 21.24% in *C.* × *superba* fruits. Aldehydes and alkanes in total accounted for 44.07% in fruit waxes of *C. japonica*, 66.31% in *C. speciosa* fruits, and 60.51% in *C.* × *superba* fruit waxes.

Results of the disc diffusion assay showed bioactivity of the isopropanolic extracts of all *Chaenomeles* fruits. Bacteriostatic and fungistatic effects on the tested strains varied depending on plant species and extract concentration (Table 2).

Table 1
Components of chloroform extracts of the *Chaenomeles* fruit cuticular waxes as identified by GC-MS assay

Compound name	Formula	RT, min	Peak area, %		
			C. japonica	*C. speciosa*	*C.* × *superba*
Heptanal	$C_7H_{14}O$	2.349	0.13	0.10	0.17
Hexanoic acid (syn. Caproic acid)	$C_6H_{12}O_2$	2.662	0.19	–	–
Octanoic acid (syn. Caprylic acid)	$C_8H_{16}O_2$	2.683	–	0.12	0.22
Octanal	$C_8H_{16}O$	2.880	–	–	0.07
Nonanal	$C_9H_{18}O$	3.418	–	0.26	0.44
3,7-Dimethyl-1,6-octadien-3-ol (syn. Linalool)	$C_{10}H_{18}O$	3.488	0.35	–	–
Benzoic acid	$C_7H_6O_2$	4.044	2.15	–	–
Octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	4.091	–	0.08	–
2-Decenal	$C_{10}H_{18}O$	4.255	0.26	–	–
Nonanoic acid (syn. Pelargonic acid)	$C_9H_{18}O_2$	4.376	0.17	0.28	0.31
3-Decyn-2-ol	$C_{10}H_{18}O$	4.524	0.16	–	–
Undecanoic acid	$C_{11}H_{22}O_2$	4.659	–	–	0.16
2H-1-Benzopyran (syn. 2H-Chromene)	C_9H_8O	4.975	0.48	–	–
9-Decenoic acid	$C_{10}H_{18}O_2$	5.134	0.78	–	0.32
2,6-Octadiene-1,8-diol (syn. Hydroxynerol)	$C_{10}H_{18}O_2$	5.351	0.19	–	0.06
3-Phenylprop-2-enal (syn. Cinnamaldehyde)	C_9H_8O	5.467	–	–	0.59
2-Propenoic acid, 3-phenyl (syn. Cinnamic acid)	$C_9H_8O_2$	5.646	0.36	–	–
Benzaldehyde-4(1-methylethyl)	$C_{10}H_{12}O$	6.957	0.23	–	–
1-Heptatriacontanol	$C_{31}H_{64}O$	7,019	0.21	0.32	0.29
10,12-Pentacosactiynoic acid	$C_{25}H_{42}O_2$	7.200	0.14	–	–
9-Tetradecanal (syn. Myristylaldehyde)	$C_{14}H_{26}O$	7.560	–	0.10	–
1-Pentadecanal	$C_{15}H_{30}O$	7.596	0.61	–	–
Hexadecanal	$C_{16}H_{32}O$	7.612	1.89	–	1.86
1-Hexadecanol	$C_{16}H_{34}O$	7.900	–	–	0.34
9-Tetradecenal	$C_{14}H_{26}O$	8.107	–	0.20	–

Compound name	Formula	RT, min	Peak area, %		
			C. japonica	*C. speciosa*	*C. × superba*
cis-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	8.173	–	–	0.44
Octadec-9-enoic acid (syn. Oleic acid)	$C_{18}H_{34}O_2$	8.191	0.53	–	–
Hexadecanoic acid (syn. Palmitic acid)	$C_{16}H_{32}O_2$	8.268	7.97	3.61	4.38
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	8.358	0.68	–	–
8,10-Hexadecadien-1-ol	$C_{16}H_{30}O$	8.456	15.48	–	0.36
7-Hexadecenal	$C_{16}H_{30}O_2$	8.846	0.90	12.31	20.83
8-Hexadecenal-14-Methyl	$C_{17}H_{32}O$	8.957	–	0.12	–
9,12-Octadecadienoic acid (syn. Linoleic acid)	$C_{18}H_{32}O_2$	9.089	1.98	–	0.15
Ethyl-9-octadec-9-enoate (syn. Ethyl oleate)	$C_{20}H_{38}O_2$	9.123	0.27	–	–
Undec-10-ynoic acid, tetradecyl ester	$C_{25}H_{46}O_2$	9.463	–	1.21	1.35
Benzenpropanoic acid, 4-pentadecyl ester	$C_{23}H_{38}O_2$	9.822	1.85	–	–
Hexadecyl nonyl ether	$C_{25}H_{52}O$	10.027	0.81	0.69	0.62
Eicosanal	$C_{20}H_{40}O$	10.128	–	1.56	–
Henicosanal	$C_{21}H_{42}O$	10.200	2.97	3.84	1.77
1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	10.216	0.54	0.75	1.97
Diisooctyl phthalate	$C_{24}H_{38}O_4$	10.874	8.91	9.57	10.31
Hexacosanal	$C_{26}H_{52}O$	10.935	–	1.15	–
1-Heptacosanol	$C_{27}H_{56}O$	12.135	–	3.19	2.26
Eicosyl nonyl ether	$C_{29}H_{60}O$	12.179	1.20	–	–
Tricosanal	$C_{23}H_{46}O$	12.488	1.86	2.32	0.54
Octacosanol	$C_{28}H_{58}O$	12.812	1.47	0.68	0.95
2-Methylhexacosane	$C_{27}H_{56}$	13.842	0.67	3.01	–
Heptacosanal	$C_{27}H_{54}O$	14.138	10.47	16.39	23.04
Hexatriacontane	$C_{36}H_{74}$	14.226	–	23.74	–
Hexacosyl nonyl ether	$C_{35}H_{72}O$	14.821	0.76	–	–
Tetrapentacontane	$C_{54}H_{110}$	14.912	23.43	1.87	11.29
1-Hentetracontanol	$C_{41}H_{84}O$	15.048	–	9.10	5.38
Triacosanal	$C_{60}H_{30}O$	15.457	1.88	–	1.18
Total, %			92.93	96.57	91.65
Unidentified, %			7.07	3.43	8.35

Note: RT – retention time; data on compounds content are expressed as peak area (% of the total).

Table 2

Diameter of inhibition zone (mm) of the *Chaenomeles* fruits isopropanolic extracts

Test-culture	*C. japonica* extracts		*C. speciosa* extracts		*C. × superba* extracts		Ofloxacin
	235.0 μg/μL	45.0 μg/μL	235.0 μg/μL	45.0 μg/μL	235.0 μg/μL	45.0 μg/μL	5.0 μg/disc
E. dissolvens	11.67 ± 0.31^{a}	11.33 ± 0.35^{ah}	18.01 ± 0.26^{a}	11.13 ± 0.21^{a}	16.73 ± 0.49^{abc}	14.13 ± 0.47^{ab}	30.17 ± 0.55^{a}
E. coli	18.47 ± 1.45^{b}	14.67 ± 0.59^{b}	15.57 ± 0.35^{b}	13.50 ± 0.51^{b}	17.03 ± 0.42^{adf}	13.57 ± 0.51^{a}	30.13 ± 0.65^{a}
P. aeruginosa	13.17 ± 0.15^{c}	10.60 ± 0.56^{ac}	9.53 ± 0.25^{c}	9.23 ± 0.35^{c}	17.67 ± 0.15^{ae}	10.47 ± 0.25^{c}	21.4 ± 0.44^{b}
M. lysodeikticus	15.30 ± 0.10^{d}	14.23 ± 0.25^{bd}	14.70 ± 0.20^{d}	9.97 ± 0.21^{c}	21.53 ± 0.40^{g}	11.40 ± 0.20^{de}	19.27 ± 0.25^{c}
K. pneumoniae	14.90 ± 0.36^{def}	6.53 ± 0.06^{g}	16.20 ± 0.30^{be}	13.03 ± 0.12^{b}	16.13 ± 0.31^{bf}	10.90 ± 0.36^{cdf}	27.90 ± 0.44^{d}
S. aureus B904	17.02 ± 0.17^{bg}	15.23 ± 0.25^{b}	16.87 ± 0.15^{e}	14.67 ± 0.42^{d}	12.47 ± 0.15^{h}	7.37 ± 0.06^{g}	26.63 ± 0.31^{d}
S. aureus B209	13.60 ± 0.17^{ce}	13.07 ± 0.25^{ef}	18.87 ± 0.15^{f}	15.53 ± 0.42^{de}	14.30 ± 0.60^{i}	11.43 ± 0.45^{ef}	25.03 ± 0.51^{e}
S. epidermidis ATCC149	15.93 ± 0.25^{dg}	13.6 ± 0.26^{de}	19.03 ± 0.12^{g}	15.83 ± 0.21^{e}	17.67 ± 0.21^{cde}	14.01 ± 0.26^{a}	23.63 ± 0.12^{f}
S. epidermidis B919	13.77 ± 0.31^{cef}	10.23 ± 0.15^{c}	29.60 ± 0.17^{h}	19.43 ± 0.25^{f}	33.33 ± 0.15^{j}	16.30 ± 0.26^{h}	32.20 ± 0.56^{g}
C. albicans	15.10 ± 0.20^{df}	12.23 ± 0.20^{fh}	18.43 ± 0.15^{af}	11.17 ± 0.25^{ag}	19.93 ± 0.06^{k}	14.93 ± 0.25^{b}	NA

Note: * – the diameter of the inhibition zones (mm), including the disc diameter (6 mm), are given as mean ± SD of triplicate experiments; values with different superscripts in each column are significantly different according to Tukey's test ($P < 0.05$); NA – no activity.

The isopropanolic extracts of *C. japonica* fruits exerted more significant bacteriostatic effects on the tested Gram-positive bacterial strains, especially *Micrococcus lysodeikticus*, *Staphylococcus aureus* B904, and *S. epidermidis* ATCC149; at the same time, bacterial strains *S. aureus* B209 and *S. epidermidis* B919 were relatively more resistant. As with the Gram-negative bacteria, the extract of *C. japonica* fruits demonstrated lower activity, except a significant effect on *Escherichia coli* B906 strain. Activity of the extracts from *C. speciosa* fruits was the strongest against Gram+ bacteria, such as all tested *S. aureus* and *S. epidermidis* strains, especially against *S. epidermidis* B919. Among Gram-negative bacterial strains, *Erwinia dissolvens* and *Klebsiella pneumoniae* strains were the most susceptible to the action of the *C. speciosa* fruit extracts. The extracts from *C. × superba* fruits exhibited the strongest activity against Gram+ bacteria *Micrococcus lysodeikticus* and both *S. epidermidis* tested strains, namely *S. epidermidis* ATCC149 and *S. epidermidis* B919. As for the Gram-negative bacteria, the extract of *C. × superba* fruits showed significant activity against all the tested strains, especially against *Escherichia coli* and *Pseudomonas aeruginosa*. With regard to the fungal strain *Candida albicans*, the fruit extracts of all plant species demonstrated notable effects with the highest activity of *C. × superba* and *C. speciosa* extracts.

In general, isopropanolic extracts of the tested *Chaenomeles* fruits demonstrated higher activity against the strains of Gram-positive bacteria than Gram-negative strains. Isopropanolic extracts of *C. japonica* fruits showed the greatest activity against *E. coli* (inhibition zone diameter 18.5 mm) and *S. aureus* B904 (17.0 mm). Extracts of *C. speciosa* fruits were most active against *E. dissolvens* (diameter 18.0 mm), *K. pneumoniae* (16.2 mm), *S. aureus* B209 (18.8 mm) and *S. epidermidis* ATCC149 (19.0 mm). Extracts of *C. × superba* fruits demonstrated the highest activity against *P. aeruginosa* (inhibition zone diameter 17.7 mm), *M. lysodeikticus* (21.5 mm), *S. epidermidis* B919 (33.3 mm), and *C. albicans* (19.9 mm).

Discussion

The constituents of soluble fruit cuticular waxes of introduced *Chaenomeles* species, namely *C. japonica*, *C. speciosa*, and interspecific hybrid *C. × superba* were classified into six chemical classes ; fatty acids, aldehydes, alcohols, alkanes, esters, and ethers. Similarly, fatty acids and alkanes were identified as the most abundant components in mandarin fruit epicuticular waxes (Ding et al., 2020). The fruit cuticular waxes of the studied *Chaenomeles* species demonstrated the notable differences in compound composition. Waxes of the *C. japonica* fruits were observed to have the highest amounts of individual components and long-chain fatty acids, as well as the most abundant fractions of alcohols (in the range of

C_{16} – C_{28}) and esters (C_{18} – C_{25}) as compared to two other species. In the fruit waxes of *C. speciosa*, the highest content of alkanes (C_{27} – C_{54}) was found, while aldehydes fraction (C_{16} – C_{60}) was the largest in the waxes of *C.* × *superba* fruits. Similar differences in the compositions of fruit waxes were found between 35 pear cultivars belonging to five different species and hybrid interspecies (Wu et al., 2018), as well as between the melting and non-melting peach varieties (Belge et al., 2014). In general, the obtained results are consistent with the known data (Trivedi et al., 2019) that the chemical compositions of fruit cuticular waxes vary greatly between fruit species and are modified by the developmental and environmental cues affecting the protective properties of the wax. *Prunus* cultivars are dominated by n-alkanes with even and odd numbers of carbons in the range from C_{27} to C_{60} (Lykholat et al., 2021). Many studies have shown that alkanes are predominant in epicuticular wax on many fruits (Rios et al., 2015; Wu et al., 2018), and accumulation of very long chain alkanes on the surface of fruit peel may be induced by low temperature (Hao et al., 2017) or water deficit (Kosma et al., 2009). Prevalence of n-alkanes in the cuticular waxes can be explained by the assumption of Fernández et al. (2016) that the non-polar compounds with low solubility, such as alkanes, can migrate from the cell wall to the epicuticular wax layer, while the polar compounds (i.e. alcohols, acids, esters) will be held in the waxes' inner layer. However, this pattern was not seen in the cuticular waxes of the *Chaenomeles* fruits, and alkanes (2-methylhexacosane C_{27} and tetrapentacontane C_{54}) accounted for the greatest share in the fruit waxes of *C. japonica* only, while the aldehydes' fraction was the largest in both *C. speciosa* and *C.* × *superba* fruit waxes.

The peculiarities of the *Chaenomeles* fruit waxes' composition correlate with the reported regularities and can be explained on the basis of the processes of biosynthesis of wax components. It is known that cuticular wax is produced from the biosynthetic pathway that has two main branches, including acyl reduction and decarbonylation of C_{16} and C_{18} fatty acids (Xue et al., 2017). In the acyl reduction branch, primary alcohols are produced by reduction of very long chain fatty acid precursors. These fatty alcohols can be further combined with fatty acid to produce wax esters. In the other branch, aldehydes are produced from very long chain fatty acid precursors, followed by aldehyde decarbonylation to produce alkanes. Since aldehydes are the direct precursors of alkanes and determine their formation, it is likely that the dominance of alkanes in the fruit waxes can be found in a certain period during *Chaenomeles* fruit ripening. Taking into account the differences in the rates of fruit ripening of the introduced *Chaenomeles* species, it can be assumed that the cuticular waxes of the *C. japonica* fruits were formed faster than *C. speciosa* and *C.* × *superba* fruit waxes. However, despite the differences in the maturation rate, the biosynthesis of *Chaenomeles* fruit waxes apparently occurs along the same path. The total amount of fatty aldehydes and alkanes exceeds the sum of primary alcohols and fatty esters (45.3% compared with 29.0%) in the waxes of *C. japonica* fruits, indicating predominance of the decarbonylation pathway. More significant overweight of total amount of aldehydes and alkanes over the total amount of primary alcohols and ethers was found in *C. speciosa* fruit cuticular waxes (67.0% against 24.2%) and *C.* × *superba* fruit waxes (61.8% against 21.2%). The findings confirm the well-known opinion (Martin & Rose, 2014; Wang et al., 2014) that the composition, structure, and architecture of fruit cuticles show considerable variability among varieties, species, and also throughout the development. The structure and biosynthesis pathway of fruit waxes do not necessarily coincide with the waxes of other plant parts, including leaf waxes, in which acyl reduction may be the main branch for wax biosynthesis (Tomasi et al., 2018).

In our study, isopropanolic fruit extracts of *C. japonica*, *C.* × *superba* and *C. speciosa* inhibited growth of all tested strains, confirming antimicrobial activity of *Chaenomeles* species. In particular, a broad spectrum of antibacterial activity was shown by the essential oil extracted from the dried *C. speciosa* fruits (Xianfei et al., 2007). All extracts of *Chaenomeles* fruit were more active against Gram-positive than Gram-negative bacteria. The most resistant bacterial strains were: *Erwinia dissolvens* against the extracts of *C. japonica* fruits, *Pseudomonas aeruginosa* against the *C. speciosa* extracts, and *Klebsiella pneumonia* against the fruit extracts of *C.* × *superba*. At the same time, these strains were susceptible to the action of the fruit extracts from another species: *Erwinia dissolvens* was inhibited by extracts of *C.* × *superba* and *C. speciosa*, *Pseudomonas aeruginosa* – by *C.* × *superba* extracts, and *Klebsiella pneumonia* – by *C. speciosa* extracts. Similar differences in the antimicrobial activity were found between the fruit extracts of three *C. japonica* cultivars, which also were more active against Gram+ bacteria, as reported by Urbanaviciute et al. (2020). The authors attributed the differences found only to the polyphenols content in fruit extracts. Without questioning the important role of phenolic compounds in the antimicrobial activity of fruits, we also paid attention to the soluble cuticular waxes components which must be present in whole fruit extracts. For example, cinnamaldehyde was represented as a new antibacterial agent that has been shown to have substantial antimicrobial activity, as well as an array of other medicinal properties (Ashakirin et al., 2017; Doyle & Stephens, 2019). Linoleic and oleic acids are well-known as bioactive compounds with versatile health benefits, including antibacterial activity (Dilika et al., 2000), antifungal activities against plant pathogenic fungi (Walters et al., 2004), and cancer prevention (Diab et al., 2021). Moreover, Zheng et al. (2005) demonstrated that antibacterial activities of long-chain unsaturated fatty acids, such as linoleic acid, palmitoleic acid, oleic acid, linolenic acid, and arachidonic acid are mediated by the inhibition of bacterial fatty acid synthesis. Hexadecanoic acid, hexadecanoic acid ethyl ester, (E)-9-octadecenoic acid ethyl ester, and octadecanoic acid ethyl ester, which were identified in the hexanic extract from *Manilkara subsericea* fruits, exhibited antimicrobial activity against *S. aureus* ATCC25923 (Fernandes et al., 2013). Diisooctyl phthalate found in the cuticular wax extract of the *Chaenomeles* fruit is suspected to have bioactivity similar to dibutyl phthalate and mono(2-ethylhexyl) phthalate in fungal filtrates, which exhibited a wide spectrum of antibacterial activity (Bhimba et al., 2012), or di(2-ethylhexyl) phthalate, which exhibited activity against *Candida albicans* fungus and the Gram-positive bacteria *Sarcina lutea* (Lotfy et al., 2018). As for the *Candida albicans*, all isopropanolic extracts of the *Chaenomeles* fruits had notable fungistatic activity, contrasting with the data of Urbanaviciute et al. (2020) where none of the extracts of three *C. japonica* cultivars exerted antifungal activity against *Candida albicans* yeast.

Conclusion

Fruits of all three introduced *Chaenomeles* species (*C. japonica*, *C. speciosa*, and *C.* × *superba*) have a rich component composition of the soluble cuticular waxes, and showed the substantial antimicrobial activity against all the selected strains. The detectable amounts of linoleic acid, oleic acid, hexadecanoic acid, hexadecanoic acid ethyl ester and cinnamaldehyde, well-known as bioactive compounds with versatile health benefits, were found in the soluble waxes of the fruits. Bacteriostatic and fungistatic effects of the fruit extracts of all *Chaenomeles* species against four Gram-negative and five Gram-positive bacteria and one fungal strain varied both between different concentrations of extracts and microbial strains. The findings not only confirmed the well-known versatile health benefits of *Chaenomeles* fruits, but also indicated the implementation of these useful properties by the introduced plants in unfavourable climatic conditions. The studied *Chaenomeles* species can be recommended for broad distribution in the steppe zone and introduction into agricultural practice. The plants can be used both for functional nutrition and for obtaining unique bioactive compounds.

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The authors declare that they have no competing interests.

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