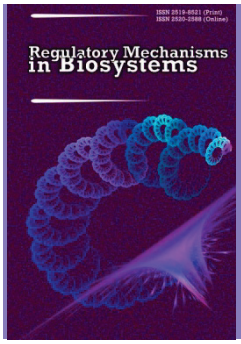

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GC-MS analysis of cuticular waxes and evaluation of antioxidant and antimicrobial activity of *Chaenomeles cathayensis* and *Ch.* × *californica* fruits

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Fruit extracts of the *Chaenomeles* species are a rich source of compounds having health-promoting properties, while their distribution between the species and cultivars varies significantly depending on both genotype and environmental threats. This study aimed at discovering antioxidant and antimicrobial potential of the secondary metabolites of fruit and waxes of fruit cuticular of introduced *Ch. cathayensis* and *Ch.* × *californica* plants. The sum of detected polyphenols in the isopropanolic fruit extracts varied slightly between the species, while significant excesses in indices were seen for both species peel extracts as compared to pulp extracts. Antimicrobial assays carried out by disc diffusion method showed notable activity of the fruit peel and pulp extracts of both species against all tested Gram-negative and Gram-positive bacterial strains, and two *Candida* strains as well. *Pseudomonas aeruginosa* strain was the most resistant to the action of both fruit extracts, especially peel extracts of *Ch. cathayensis* fruits. As identified by gas chromatography-mass spectrometry (GC-MS) assays, chloroformic extracts from the fruits of cuticular waxes of *Ch. cathayensis* and *Ch.* × *californica* contained six prevailing fractions: aldehydes, alkanes, alcohols, esters, fatty acids and various terpenoids. The predominant compounds were tetrapentacontane (21.8% of total amount) and heptacosanal (23.1% of total), respectively in the cuticular waxes of *Ch. cathayensis* and *Ch.* × *californica*. Cinnamaldehyde, cis-9-hexadecenal, hexadecanoic acid, oleic acid, olean-12-ene-3,28-diol (3. beta), lupeol, diisooctyl phthalate, 9-octadecenoic acid, 1,2,3-propanetriyl ester, 1,3,12-nonadecatriene-5,14-diol and some other identified compounds are well-known for their bioactivity, indicating the feasibility of studying the antimicrobial potential of plant fruits.

Keywords: *Chaenomeles* fruits; phenolic compounds; cuticular waxes; antimicrobial activity.

Introduction

Species *Chaenomeles cathayensis* (Hemsl.) Schneider (Chinese quince) is a native plant in China and may be found in the wild, while *Ch.* × *californica* Clarke ex Weber is a tri-species hybrid [*Ch. cathayensis* × (*Ch.* × *superba*), *Californica* group] as Yeung (2000) reported. Plants of all species belonging to the genus *Chaenomeles* Lindley (Rosaceae family) are well known as a rich source of bioactive compounds that can provide the impressive spectrum of useful properties (Miao et al., 2016). The preparations from the fruits, leaves and twigs of various *Chaenomeles* plants have been used in traditional medicine for treating pneumonia, bronchitis (Han et al., 2016), as well as vitamin C deficiency syndrome, rheumatism, cholera, dysentery, and beriberi (Zhang et al., 2014). During the recent years, numerous investigations confirmed the high prophylactic and therapeutic effects of plant extracts from the commonest *Chaenomeles* species. Significant anti-carcinogenic action against colon cancer (Gorlach et al., 2011) and breast cancer (Lewandowska et al., 2013) was exhibited by the extracts from *Ch. japonica* fruits, and for extracts from leaves of *Ch. japonica* – against glioblastoma cells (Zvikas et al., 2021). Immunoregulatory and antiparkinsonian properties were demonstrated by the extracts from *Ch. speciosa* fruits (Zhang et al., 2014). Hepatoprotective activity was shown by the fruit extracts of *Ch. thibetica* (Ma et al., 2016) and *Ch. japonica* (Baranowska-Bosiacka et al., 2017). Strong anti-inflammatory effect along with cytotoxic activities against cultured human tumour cell lines were exhibited by the extracts of *Ch. speciosa* twigs (Suh et al., 2017), while antioxidant and anti-inflammatory effects were shown by the *Ch. sinensis* leaf extracts (Han et al., 2016). Hypoglycemic effect of *Ch. japonica* fruit polyphenols may have preventive anti-diabetic action (Zakłos-Szyda & Pawlik, 2018). In general, most of the studies we mentioned have indicated the association between bioactivities of *Chaenomeles* plant extracts and the polyphenols content in the fruits and other plant organs.

In the recent decades, the remarkable properties of *Chaenomeles* plants have gained wide popularity, and some species were successfully introduced to a number of European countries (Rumpunen, 2002), and to Ukraine as well (Khromykh et al., 2018; Lykholat et al., 2019). However, the ability of introduced plants to accumulate bioactive compounds and retain beneficial properties in a new environment is not fully clear. The survival of plants in a new habitat involves all plant adaptive mechanisms, including the multiple functions of the cuticle and cuticular waxes. The protection of plants from external environments and biotic or abiotic stresses is partly provided by the cuticular waxes as the first defensive barrier of plant organisms. The major components of cuticular wax are very long chain fatty acids and different derivatives (Trivedi et al., 2019) contents of which vary significantly depending on plant species and the growth conditions (Lykholat et al., 2018). However, the chemical composition and defensive properties of *Chaenomeles* plant cuticular waxes are poorly studied. The objective of this study was comparing the introduced natural and hybrid *Chaenomeles* species regarding accumulation of polyphenols, cuticular waxes composition, and antimicrobial activity of plant fruits.

Materials and methods

Fruits of *Ch. cathayensis* and *Ch.* × *californica* were taken from the Botanical Garden of the 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 the *Chaenomeles* plants were collected in the first half of September 2021, packed in plastic containers and immediately delivered to the laboratory.

Plant extracts for polyphenols content determination and bioassays were prepared using 80% isopropanol. Briefly, 2.0 g weighed portion of fresh fruit (peel and flesh as the separated samples) was triturated with 20 ml of isopropanol and kept for 24 hours at the room temperature in dark with occasional shaking. Then, the extracts were filtered through the paper filters, and the total volume was divided into two parts intended for different studies. Total polyphenols content (TPC), total flavonoids content (TFC), free phenolic acids content (PAC), total antioxidant capacity (TAC), and reducing power (RP) were determined in the crude extracts obtained. For the antimicrobial assays, crude extracts were dried at 45 °C using a rotary evaporator IKA® RV 10 (Germany), and a corresponding amount of solid residue was dissolved in isopropanol.

Total polyphenols content (TPC) in the fruit peel and pulp extracts was determined using spectrophotometric method with Folin-Ciocalteu reagent (Singleton et al., 1999); the absorbance was measured at 726 nm, and the results were then calculated using a calibration graph prepared on the solutions of Gallic acid (GA) and expressed as mg GA equivalents per 100 g of wet weight (mg GA/100 g WW). Total flavonoids content (TFC) in fruit peel and pulp was determined by aluminum chloride spectrophotometric method (Pękal & Pyrzynska, 2014); the absorbance was measured at 425 nm; results were calculated using a calibration graph prepared on the rutin (Ru) solutions and expressed as rutin equivalents per 100 g (mg Ru/100 g WW). Free phenolic acids content (FAC) was determined using a spectrophotometric method described by Gawron-Gzella et al. (2012) with Arnov's reagent (10.0 g of sodium molybdate, 10.0 g sodium nitrite in 100.0 mL of water); absorbance at 490 nm was measured; caffeic acid (CA) was used as the standard, and the results were expressed in caffeic acid equivalents (mg CA/100 g WW). Reducing power (RP) of the plant fruit extracts was evaluated using potassium ferricyanide method (Pulido et al., 2000); the absorbance was measured at 700 nm, and the results were expressed in mg Ascorbic acid (AA) equivalents (mg AA/100 g WW). Evaluation of total antioxidant capacity (TAC) of fruit peel and pulp was carried out in accordance with Prieto et al. (1999) using the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate); after the incubation in 95 °C for 90 min, the absorbance at 695 nm was measured against blank; the results were calculated using a calibration graph prepared on the solutions of ascorbic acid, and expressed as mg AA equivalents (mg AA/100 g WW).

Chloroform was used for extraction from *Ch. cathayensis* and *Ch.* × *californica* fruit cuticular waxes (Buschhaus et al., 2007) through immersing fruits in a solvent for 60 seconds, followed by solvent evaporation at 40 ºC using a rotary evaporator (IKA® RV 10, Germany). Obtained solid fraction was stored at 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 equaled 50 °C, with 5 min initial hold, and then programmed temperature gradient increased to 300 °C at the rate of 15 °C per min, and kept constant at 300 °C for 10.5 min. The carrier gas helium was 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 in the fruit cuticular waxes was estimated through the corresponding peak area and expressed as a percentage of the total.

Antimicrobial activity of the isopropanolic extracts from *Ch. cathayensis* and *Ch.* × *californica* fruit peel and pulp were tested using the disc diffusion method described by Bhimba et al. (2012). The test strains of microorganisms were taken from the culture collection of Microbiology, Virology and Biotechnology Department of Oles Honchar University. Four Gram-negative bacteria, namely *Erwinia dissolvens* (strain 170), *Escherichia coli* (strain B 906), *Pseudomonas aeruginosa* (strain B 907), *Klebsiella pneumonia* (strain B 920), and five Gram-positive bacteria, including *Micrococcus lysodeikticus* (strain 2665), *Staphylococcus aureus* (strain B 904), *Staphylococcus aureus* (strain B 209), *Staphylococcus epidermidis* (strain ATCC 149), *Staphylococcus epidermidis* (strain 919), and two fungal strains (*Candida albicans* (clinical strain) and *Candida lipolytica* (strain B 504) were tested. Petri plates containing meat-peptone agar (MPA) medium were inoculated with 10^9 CFU (colony forming units) of 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 of plates at 37 °C for 24 h. Ofloxacin (5.0 μg per disc) was used as the positive control for the bacterial strains; itraconazole 10.0 μg was used as the positive control for the fungal strains. Antimicrobial activity of the fruit peel and pulp extracts was expressed as the diameter of the inhibition zone (mm) around the discs along with disc diameter.

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

Results

The contents of phenolic compounds in *Ch. cathayensis* and *Ch.* × *californica* fruits revealed the domination of polyphenols in fruit peel, and the similarity in the ratio of the peel and pulp indicators of both plant species (Table 1).

Table 1
Phenolic compounds content and antioxidant activity of *Chaenomeles* fruits (x ± SD, n = 5)

Index	Indicator unit	*Ch. cathayensis*		*Ch.* × *californica*	
		peel	pulp	peel	pulp
Total polyphenol content	mg GA/100 g FW	1441.2 ± 5.6^a	533.0 ± 4.1^b	1240.9 ± 5.6^c	534.2 ± 5.6^b
Total flavonoid content	mg Ru/100 g FW	79.45 ± 4.39^a	31.59 ± 2.87^b	45.11 ± 1.77^c	36.60 ± 1.88^b
Free phenolic acids content	mg CA/100 g FW	95.36 ± 0.74^a	36.07 ± 0.42^b	79.42 ± 1.05^c	36.20 ± 0.06^b
Reducing power	mg AA/100 g FW	1871.9 ± 17.4^a	518.6 ± 15.1^b	1598.2 ± 7.6^c	542.2 ± 7.7^d
Total antioxidant capacity	mg AA/100 g FW	3273.0 ± 4.6^a	1703.1 ± 5.4^b	3277.8 ± 31.4^a	1578.7 ± 98.3^b

Note: different letters indicate the values significantly differing one from another within a line of the Table according to the results of comparison using the Tukey test ($P < 0.05$).

The total content of phenolic compounds, total flavonoids and free phenolic acids content in *Ch. cathayensis* fruit peel exceeded the corresponding indicators in the pulp by 2.7, 2.5 and 2.6 times respectively. In the fruits of *Ch.* × *californica*, the above-mentioned excesses were smaller, amounting to 2.3, 1.2 and 2.2 times respectively. Antioxidant activity of the peel extracts of *Ch. cathayensis* was higher compared with the pulp ones: by 3.6 and 1.9 times, respectively for reducing power and total antioxidant property. Similarly, the differences between the antioxidant activity indices of *Ch.* × *californica* fruit peel and pulp were 3.0 and 2.1 times respectively. In general, total contents of phenolic compounds, total flavonoids and free phenolic acids content as well as the antioxidant potential of *Ch. cathayensis* fruits prevailed over the corresponding levels of *Ch.* ×

californica fruits (by 1.1–1.4 times). However, high correlation was seen between the total polyphenols content and total antioxidant capacity of the whole fruits of both species (r = 0.94 and r = 0.90, respectively for *Ch. cathayensis* and *Ch.* × *californica*). Antimicrobial assays carried out by the disc diffusion method showed bioactivity of the isopropanolic extracts from peel and pulp of both *Ch. cathayensis* and *Ch.* × *californica* fruits against all tested strains (Table 2). The isopropanolic extracts of *C. cathayensis* fruit peel showed more notable growth-inhibiting effects on the Gram-negative bacteria, except *P. aeruginosa* strain, while *M. lysodeikticus* and all *Staphylococcus strains* were more resistant, especially *S. aureus* B904 and *S. epidermidis* ATCC149. At the same time, Gram-positive bacteria, especially *M. lysodeikticus* and *S. aureus* B209 were more sensitive to the action of extracts from *Ch. cathayensis* fruit pulp. Both peel and pulp extracts from *Ch. cathayensis* fruits exhibited high activity against *E. coli* B906 and *K. pneumoniae* strains, and the lowest activity against *P. aeruginosa*. With regard to the fungal strains, *C. albicans* was more inhibited by *Ch. cathayensis* fruit peel extract, when *C. lipolytica* was more sensitive to the action of fruit pulp extracts.

Table 2

Diameter of inhibition zones (mm) caused by the *Chaenomeles* fruits isopropanolic extracts (x ± SD, n = 5)

Test-culture	*Ch. cathayensis* extracts		*Ch.* × *californica* extracts		Positive control[1]
	peel, 42.5 μg/μL	pulp, 45.5 μg/μL	peel, 42.5 μg/μL	pulp, 45.5 μg/μL	
Erwinia dissolvens 170	13.33 ± 0.26^{a}	10.88 ± 0.31^{b}	11.38 ± 0.17^{b}	8.53 ± 0.17^{c}	22.78 ± 0.24^{d}
Escherichia coli B906	12.88 ± 0.22^{a}	12.70 ± 0.29^{a}	11.48 ± 0.35^{b}	13.23 ± 0.31^{a}	26.80 ± 0.42^{e}
Pseudomonas aeruginosa B 907	7.70 ± 0.18^{a}	8.93 ± 0.17^{b}	8.90 ± 0.22^{b}	8.58 ± 0.25^{b}	14.68 ± 0.48^{c}
Micrococcus lysodeikticus 2665	8.73 ± 0.17^{a}	12.68 ± 0.33^{b}	9.43 ± 0.17^{c}	8.43 ± 0.22^{a}	12.23 ± 0.15^{b}
Klebsiella pneumonia B 920	11.20 ± 0.32^{a}	11.58 ± 0.28^{a}	11.08 ± 0.25^{a}	9.20 ± 0.18^{b}	24.10 ± 0.34^{c}
Staphylococcus aureus B904	8.13 ± 0.17^{a}	10.60 ± 0.14^{b}	9.63 ± 0.17^{c}	11.38 ± 0.15^{d}	34.23 ± 0.54^{e}
Staphylococcus aureus B209	8.98 ± 0.21^{ab}	11.65 ± 0.37^{c}	9.50 ± 0.16^{a}	8.60 ± 0.28^{b}	32.55 ± 0.44^{d}
Staphylococcus epidermidis ATCC149	8.15 ± 0.21^{a}	10.03 ± 0.29^{b}	10.05 ± 0.21^{b}	12.05 ± 0.17^{c}	26.80 ± 0.73^{d}
Staphylococcus epidermidis B919	9.35 ± 0.26^{a}	11.18 ± 0.22^{b}	9.95 ± 0.26^{ac}	10.15 ± 0.19^{c}	26.60 ± 0.57^{d}
Candida albicans (clinical strain)	11.23 ± 0.19^{a}	10.43 ± 0.33^{b}	10.53 ± 0.25^{b}	10.88 ± 0.43^{ab}	12.62 ± 0.25^{c} – itraconazole
Candida lipolytica B 504	11.18 ± 0.21^{a}	11.88 ± 0.22^{ab}	11.48 ± 0.17^{a}	12.20 ± 0.26^{b}	13.29 ± 0.25^{c} – itraconazole

Notes: [1] – for bacteria, discs with 5.0 μg ofloxacin were used as positive control, and for *C. albicans*, *C. lipolytica* – discs with 10.0 μg itraconazole; the diameter of the inhibition zones (mm), including the disc diameter (6 mm), are given as x ± SD; different letters indicate the values significantly differing one from another within a line of the Table based on the results of comparison using the Tukey test ($P < 0.05$).

The extracts of *Ch.* × *californica* fruit peel caused higher inhibition of Gram-negative bacteria, except *P. aeruginosa*, whereas growth inhibition of *M. lysodeikticus*, *S. aureus* B209 and *S. aureus* B904 was lower. The effect of *Ch.* × *californica* fruit pulp extracts on the strains *E. dissolovens*, *P. aeruginosa*, *M. lysodeikticus* and *S. aureus* B209 was the lowest of all. At the same time, the activity of *Ch.* × *californica* fruit pulp against *E. coli* B906, *S. aureus* B904 and *S. epidermidis* ATCC149 strains was the highest of all. Both peel and pulp extracts from *Ch.* × *californica* fruits caused high inhibition of *E. coli* B906 and *S. epidermidis* ATCC149 strains, while the lowest inhibition was recorded for *P. aeruginosa*, *M. lysodeikticus* and *S. aureus* B209 strains. As for the fungal strains, both *C. albicans* and *C. lipolytica* were more sensitive to the action of *Ch.* × *californica* fruit pulp extracts.

The GC-MS analysis of *Ch. cathayensis* fruit cuticular waxes identified 21 individual compounds in the chloroformic extract. The sum of all identified components was 97.0% of the total amount (Table 3).

Table 3

Chemical constituents of chloroformic extracts of the *Ch. cathayensis* fruit cuticular waxes

Compound name	Formula	RT, min	Peak area, %	Classification	Known bioactivity (References)
2,4-Dimethylhexane	C_8H_{18}	1.961	0.11	alkane	not found
2-Tridecenal	$C_{13}H_{24}O$	4.558	0.35	aldehyde	not found
7-Hexadecenal	$C_{16}H_{30}O$	7.604	1.39	aldehyde	not found
Octadec-9-enoic acid (syn. Oleic acid)	$C_{18}H_{34}O_2$	8.145	0.18	fatty acid	antifungal (Walters et al., 2004); anti-inflammatory, cancer preventive (Diab et al., 2021)
Hexadecanoic acid (syn. Palmitic acid)	$C_{16}H_{32}O_2$	8.175	0.19	fatty acid	antifungal (Pourakbar et al., 2021)
Cis-9-Hexadecenal	$C_{16}H_{30}O$	8.240	1.28	aldehyde	antimicrobial (Hoda et al., 2020)
Heptacosanal	$C_{27}H_{54}O$	8.581	14.52	aldehyde	not found
9-Octadecenoic acid, 1,2,3-propanetriyl ester	$C_{57}H_{104}O_8$	9.045	7.12	fatty acid ester	antibacterial (Ololade et al., 2015)
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	9.289	2.61	alcohol	antifungal (Parveen et al., 2017).
Olean-12-ene-3,28-diol (3.beta)	$C_{30}H_{50}O_2$	9.563	3.07	triterpenoid	antimicrobial (Catteau et al., 2018)
1-Heptatriacontanol	$C_{37}H_{76}O$	9.871	0.78	alcohol	not found
Diisooctyl phthalate	$C_{24}H_{38}O_4$	10.890	14.15	fatty acid ester	antimicrobial (Shanab et al., 2010)
17-Pentatriacontene	$C_{35}H_{70}$	12.152	1.44	alkene	antioxidant (Begum et al., 2016)
Lup-20(29)-en-3-ol (3. beta) (syn. Lupeol)	$C_{30}H_{50}O$	12.508	1.95	pentacyclic triterpenoid	antimicrobial (Catteau et al., 2018)
Tetrapentacontane	$C_{54}H_{110}$	12.945	21.75	alkane	not found
Z,Z-3,13-Octadecedien-1-ol	$C_{18}H_{34}O$	13.327	0.15	alcohol	not found
Oleic acid, 3-(octadecyloxy)propyl ester	$C_{39}H_{76}O_3$	13.798	0.69	fatty acid ester	not found
Tricosanal	$C_{23}H_{46}O$	14.310	3.27	aldehyde	not found
Urs-12-en-28-al	$C_{30}H_{48}O$	14.512	2.07	aldehyde	antibacterial, antioxidant (Do Nascimento et al., 2014)
7,8-Epoxylanostan-11-ol-3-acetoxy-	$C_{32}H_{54}O_4$	14.807	1.21	steroid triterpenoid	antibacterial (Zubair et al., 2017)
1-Hentetracontanol	$C_{41}H_{84}O$	15.036	18.67	alcohol	not found

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

Fatty acids fraction in the *Ch. cathayensis* fruit cuticular waxes consisted of two long-chain compounds with an even carbon number (C_{16} and C_{18}), amounting only to 0.37% of the total. The alcohols fraction in *Ch. cathayensis* cuticular waxes mostly consisted of primary alcohols (three compounds in the range $C_{18} - C_{41}$), reaching 19.6% of the total amount. The fraction of fatty acid esters was represented by three compounds with an even and odd number of carbons in the range of $C_{24} - C_{57}$, accounting for 22.0% of total; of these, diisooctyl phthalate was the most abundant. Aldehydes in the cuticular waxes of *Ch. cathayensis* fruits were represented by six compounds with an even and odd numbers of carbons ($C_{13} - C_{30}$), which amounted to 22.9% of total; heptacosanal and tricosanal were the main compounds among the aldehydes. Fraction of the alkanes and alkenes in the *Ch. cathayensis* fruit cuticular waxes consisted of three compounds ($C_8 - C_{54}$) dominated by tetrapentacontane and reached

23.3% of the total. Other compounds in the *Ch. cathayensis* fruit cuticular waxes included different triterpenoids and accounted for 6.2% of the total. Primary alcohols and esters accounted for 41.6%, while the content of aldehydes and alkanes in total was 46.2% in the cuticular waxes of *Ch. cathayensis* fruits. The phytochemicals study of *Ch.* × *californica* fruit cuticular waxes carried out by GC-MS analysis identified 23 individual compounds in the chloroformic extract. The sum of all identified components was 97.4% of the total amount (Table 4).

Table 4
Chemical constituents of chloroformic extracts of the *Ch.* × *californica* fruit cuticular waxes

Compound name	Formula	RT, min	Peak area, %	Classification	Bioactivity (References)
Nonanal	$C_9H_{18}O$	3.516	0.40	aldehyde	not found
9-Oxononanoic acid	$C_9H_{16}O_3$	4.566	0.41	fatty acid	not found
Cinnamaldehyde	C_9H_8O	5.493	0.12	aldehyde	antimicrobial (Ashakirin et al., 2017)
3,7,11-Trimethyl-1,3,6,10-dodecatetraene (syn. Alpha-Farnezene)	$C_{15}H_{24}$	6.042	0.45	sesquiterpene	antimicrobial (Kuroda et al., 2007)
Tetradecenal (syn. Myristaldehyde)	$C_{14}H_{28}O$	7.612	0.23	aldehyde	antimicrobial (Xianfei et al., 2007)
7-Hexadecenal	$C_{16}H_{30}O$	7.632	1.38	aldehyde	not found
Hexadecanoic acid (syn. Palmitic acid)	$C_{16}H_{32}O_2$	8.188	1.86	fatty acid	antifungal (Pourakbar et al., 2021)
Cis-9-Hexadecenal	$C_{16}H_{30}O$	8.259	9.76	aldehyde	antimicrobial (Hoda et al., 2020)
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	9.285	1.21	alcohol	antifungal (Parveen et al., 2017).
Olean-12-ene-3,28-diol (3.beta)	$C_{30}H_{50}O_2$	9.545	5.37	triterpenoid	antimicrobial (Catteau et al., 2018)
Undec-10-ynoic acid, tetradecyl ester	$C_{25}H_{48}O_2$	9.960	1.82	fatty acid ester	not found
Eicosanal	$C_{20}H_{40}O$	10.225	0.79	aldehyde	not found
Undec-10-ynoic acid, hexadecyl ester	$C_{27}H_{50}O_2$	10.787	0.86	fatty acid ester	not found
Diisooctyl phthalate	$C_{24}H_{38}O_4$	10.884	10.18	fatty acid ester	antimicrobial (Shanab et al., 2010)
Henicosanal	$C_{21}H_{42}O$	11.233	2.44	aldehyde	not found
2-Methyl-hexacosane	$C_{27}H_{56}$	11.549	1.68	alkane	not found
1-Hentetracontanol	$C_{41}H_{84}O$	12.176	6.65	alcohol	not found
Tricosanal	$C_{23}H_{46}O$	12.534	3.83	aldehyde	not found
Lup-20(29)-en-3-ol (3. beta) (syn. Lupeol)	$C_{30}H_{50}O$	12.601	1.60	pentacyclic triterpenoid	antimicrobial (Catteau et al., 2018)
Octacosanol	$C_{28}H_{58}O$	12.801	1.10	alcohol	anti-parkinsonism (Wang et al., 2010)
Tetrapentacontane	$C_{54}H_{110}$	13.012	21.32	alkane	not found
Hexadecyl nonyl ether	$C_{25}H_{52}O$	13.825	0.81	ether	not found
Heptacosanal	$C_{27}H_{54}O$	14.364	23.14	aldehyde	not found

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

Fatty acids in the *Ch.* × *californica* fruit cuticular waxes were represented by two compounds (C_9 and C_{16}), amounting to 2.3% of total. The alcohol fraction in *Ch.* × *californica* fruit cuticular waxes mostly consisted of primary alcohols (two compounds, C_{25} and C_{41}), reaching 7.8% of the total amount. The fatty acid esters were represented by three compounds with an even and odd carbons number ($C_{24} – C_{27}$), equaling 12.9% of the total; among them, diisooctyl phthalate content was the highest. Aldehyde fraction in the fruit cuticular waxes of *Ch.* × *californica* was represented by nine compounds with an even and odd carbon numbers ($C_9 – C_{27}$), which amounted to 42.1% of total; heptacosanal and cis-9-hexadecenal were the main compounds among the aldehydes. Alkane fraction in the *Ch.* × *californica* fruit cuticular waxes consisted of two compounds ($C_{27} – C_{54}$) dominated by tetrapentacontane and reached 23.0% of the total. Other compounds in the *Ch.* × *californica* fruit cuticular waxes included different triterpenoids and accounted for 7.4% of the total. Content of primary alcohols and esters was 20.6%, while aldehydes and alkanes accounted for 65.1% in the cuticular waxes of *Ch.* × *californica* fruits. Some chemical compounds with the well-known biological activity were identified in the fruit cuticular waxes of both *Ch. cathayensis* and *Ch.* × *californica* plants (Fig. 1).

Discussion

Fruit extracts of the *Chaenomeles* species are the rich source of phenolic compounds, including flavonoids and phenolic acids. However, distribution of these groups between the species and cultivars varies significantly depends on both genotype and environmental treats. The total content of polyphenols in the fruits of *Ch. cathayensis* slightly exceeded such in *Ch.* × *californica* fruits, while the accumulation of flavonoids and free phenolic acids prevailed more significantly (1.4 and 1.2 times respectively). Similar variability both of total polyphenols and the main bioactive compounds content was revealed in the fruits of five wild *Chaenomeles* species, namely *Ch. japonica*, *Ch. sinensis*, *Ch. speciosa*, *Ch. cathayensis* and *Ch. thibetica* (Du et al., 2013), and in *Ch. speciosa* fruits from four production areas in China (Zheng et al., 2018). Total contents of polyphenols determined in the fresh fruits of *Ch. cathayensis* and *Ch.* × *californica* were comparable to such found in the dried fruits of *Ch. speciosa* (Zheng et al., 2018) and *Ch. japonica* cultivars (Urbanaviciute et al., 2020). Reducing power capacity and total antioxidant activity also did not differ significantly in the fruits of *Ch. cathayensis* and *Ch.* × *californica*. The obtained results indicate the main dependence of the fruit antioxidant potential on the total polyphenols content, which was also confirmed by high correlation coefficients. In general, particularly the amount of polyphenols in plant extracts is associated with the antioxidant properties and different biological activities (Zakłos-Szyda & Pawlik, 2018). Study of distribution of the phenolic compounds in peel and pulp of both *Ch. cathayensis* and *Ch.* × *californica* fruits revealed significant dominane of fruit peel in the total contents of polyphenols, flavonoids and free phenolic acids. Findings are consistent with the data of Miao et al. (2018) that fruit peel of different *Chaenomeles* varieties contain more polyphenols, flavonoids and triterpenes, and show better antioxidant activity than flesh.

In the present study, we have found that the isopropanolic extracts from peel and pulp of both *Ch. cathayensis* and *Ch.* × *californica* fruits were active against all tested Gram-positive and Gram-negative bacteria, as well as against both *Candida* strains. These results confirm the well-known antimicrobial activity of *Chaenomeles* species, including a broad spectrum of antibacterial activity exhibited by the essential oil from the fruits of *Ch. speciosa* (Xianfei et al., 2007) and *Ch. japonica* (Urbanaviciute et al., 2020). Most of the tested bacterial strains were more sensitive to the inhibitory effect of the extracts from *Ch. cathayensis* fruits, whereas *P. aeruginosa*, *S. aureus* B904 and *S. epidermidis* ATCC149 strains were more effected by *Ch.* × *californica* fruit extracts. *P. aeruginosa* strain was the most resistant to the action of both fruit extracts, especially peel extracts of *Ch. cathayensis* fruits. Overall, compared to the peel extracts, the pulp extracts from the *Ch. cathayensis* fruits caused more notable growth inhibition of most bacterial strains except *E. dissolvens* and *E. coli* B906. However, no regularities were determined for the inhibitory effect of extracts from *Ch.* × *californica* fruit peel and pulp. Similar differences in the antimicrobial activity between the fruit extracts of three *Ch. japonica* cultivars were reported by Urbanaviciute et al. (2020), while none of the extracts showed antifungal activity against *C. albicans* yeast. In our study, both *Ch. cathayensis* and *Ch.* × *californica* peel and pulp fruit extracts exhibited equally high activities against *C. albicans* and *C. lipolytica*, indicating the feasibility of studying the antimicrobial potential of *Chaenomeles* fruits.

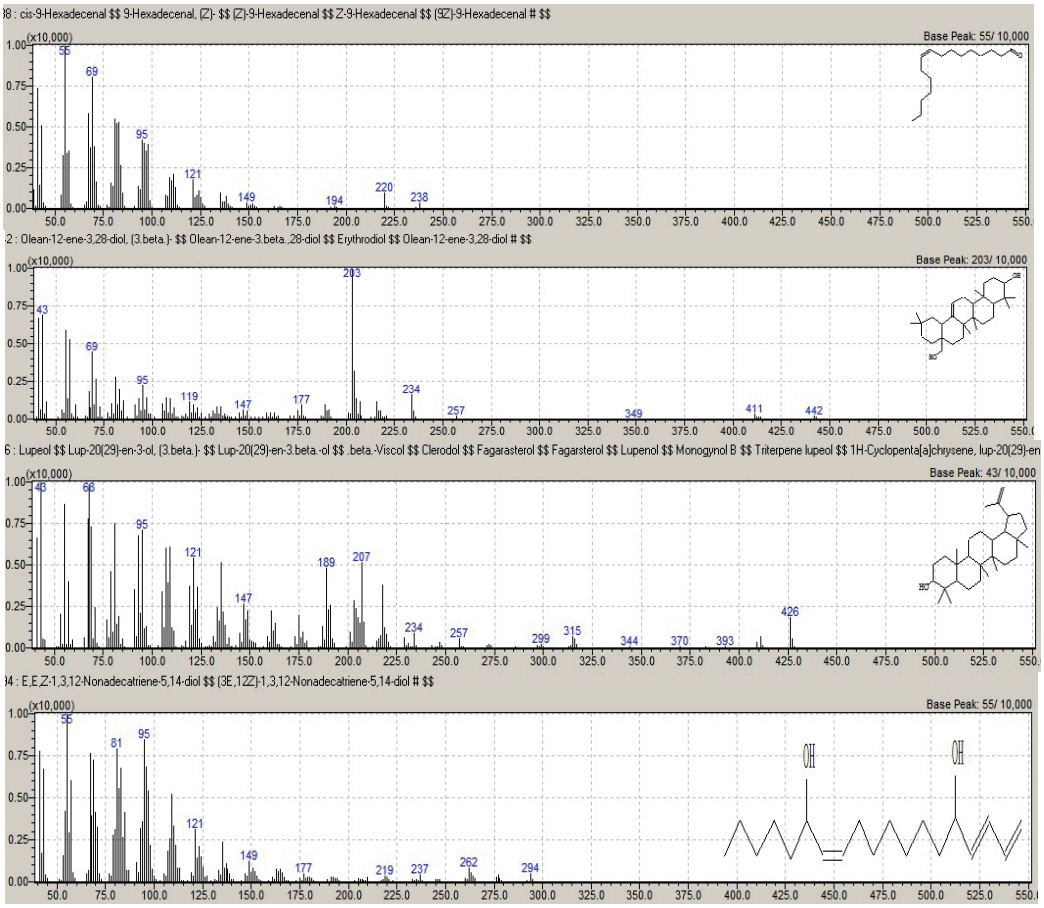

Fig. 1. *Ch. cathayensis* and *Ch. × californica* fruit cuticular wax compounds having the known bioactivity: *a* – cis-9-hexadecenal, *b* – olean-12-ene-3,28-diol (3. Beta), *c* – lupeol, *d* – 1,3,12-nonadecatriene-5,14-diol

Results of GC-MS assays showed the presence of the fatty acids, aldehydes, alkanes, esters, alcohols and different terpenoids in compositions of cuticular waxes of both *Ch. cathayensis* and *Ch. × californica* fruits. However, the amount of these compounds varies significantly, confirming the known data (Trivedi et al., 2019; Lykholat et al., 2021) that the fruit cuticular wax chemical composition varies greatly between fruit species and is modified by developmental and environmental cues affecting the wax protective properties. Identification of the fruit cuticular waxes component composition of *Ch. cathayensis* and *Ch. × californica* revealed the chemical compounds that have known biological activities. Cinnamaldehyde identified in *Ch. × californica* cuticular wax was represented (Ashakirin et al., 2017) as a new antibacterial agent which exerted substantial antimicrobial activity. Oleic acid found in *Ch. cathayensis* fruit cuticular waxes is known as bioactive compounds having antifungal activities against plant pathogenic fungi (Walters et al., 2004), and cancer prevention (Diab et al., 2021). Moreover, Zheng et al. (2005) showed that antibacterial activity of long-chain unsaturated fatty acids, such as oleic acid, is mediated by the inhibition of bacterial fatty acid synthesis. Diisooctyl phthalate that was found in great amount in the cuticular waxes of both *Ch. cathayensis* and *Ch. × californica* fruits was reported by Shanab et al. (2010) as an antimicrobial agent along with many other phthalates. Triterpenoids olean-12-ene-3,28-diol (3.beta) and lupeol identified in the waxes of both species are known (Catteau et al., 2018) for their antimicrobial activity. The presence of the mentioned bioactive compounds in the fruit cuticular waxes suggests their involvement in the bioactivity of extracts from *Ch. cathayensis* and *Ch. × californica* fruit. Further research is needed to detect the bioactivity of fruit extracts and identify compounds responsible for certain effects.

Conclusion

Peel and pulp of *Ch. cathayensis* and *Ch. × californica* fruits can accumulate high contents of polyphenols, flavonoids and free phenolic acids. They showed substantial antimicrobial activities against all selected bacterial and fungal strains. The detectable amounts of cinnamaldehyde, oleic acid, hexadecanoic acid, and some triterpenes, which are well-known as bioactive compounds, were found in the fruit cuticular waxes. Antimicrobial effects of the fruit extracts against four Gram-negative, five Gram-positive bacteria and two fungal strains varied between peel and pulp fruit extracts of both plant species. The findings confirmed the health-promoting abilities of the fruits of *Ch. cathayensis* and *Ch. × californica* plants, and also indicated the implementation of these useful properties by the introduced plants in unfavorable climatic conditions.

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