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 Chaenomeles 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 Chaenomeles cathayensis fruits. As identified by gas chromatography-mass spectrometry (GC-MS) assays, chloroformic extracts from the fruits of cuticular waxes of Chaenomeles cathayensis and Ch. × californica contained six prevailing fractions: aldehydes, alkanes, alcohols, esters, fatty acids and various terpenoids. The predominant compounds were tetratetrapentacontane (21.8% of total amount) and heptacosan (23.1% of total), respectively in the cuticular waxes of Chaenomeles 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-propa-ne1,2,3-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 (Hemsel) 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 [Chaenomeles × (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, choler, 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 (Lewan-tka-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 (Rampanen, 2002), and to Ukraine as well (Khromykh et al., 2018; Lykhollat 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 (Lykhollat 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 *Chamaemelum* 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 *Chamaemelum* 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-Ciocalteau 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 expressed in mg Gallic acid (GA) equivalents (mg GA/100 g WW). 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.

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 °C using a rotary evaporator (IKA® RV 10, Germany). Obtained solid fraction was stored at 4 °C; 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 equalled 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 Bhimha 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 10⁵ 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>
<thead>
<tr>
<th>Table 1</th>
<th>Phenolic compounds content and antioxidant activity of <em>Chamaemelum</em> fruits (x ± SD, n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index</td>
<td>Indicator unit</td>
</tr>
<tr>
<td></td>
<td>Ch. cathayensis</td>
</tr>
<tr>
<td></td>
<td>Ch. × californica</td>
</tr>
<tr>
<td></td>
<td>peel</td>
</tr>
<tr>
<td></td>
<td>pulp</td>
</tr>
<tr>
<td></td>
<td>peel</td>
</tr>
<tr>
<td></td>
<td>pulp</td>
</tr>
<tr>
<td>Total polyphenol content</td>
<td>mg GA/100 g FW</td>
</tr>
<tr>
<td>Total flavonoid content</td>
<td>mg Ru/100 g FW</td>
</tr>
<tr>
<td>Free phenolic acids content</td>
<td>mg CA/100 g FW</td>
</tr>
<tr>
<td>Reducing power</td>
<td>mg AA/100 g FW</td>
</tr>
<tr>
<td>Total antioxidant capacity</td>
<td>mg AA/100 g FW</td>
</tr>
</tbody>
</table>

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*.
on the Gram-negative bacteria, except *C. cathayensis*.

### Table 2

<table>
<thead>
<tr>
<th>Test-culture</th>
<th><em>Ch. cathayensis</em> extracts</th>
<th><em>Ch. × californica</em> extracts</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>peel, 42.5 μg/μL</td>
<td>pulp, 45.5 μg/μL</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>11.38 ± 0.17</td>
<td>8.53 ± 0.17</td>
<td>22.78 ± 0.24</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>11.68 ± 0.18</td>
<td>13.25 ± 0.31</td>
<td>26.80 ± 0.42</td>
</tr>
<tr>
<td><em>M. lysodeikticus</em></td>
<td>9.63 ± 0.17</td>
<td>9.75 ± 0.23</td>
<td>34.23 ± 0.54</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>9.00 ± 0.20</td>
<td>9.00 ± 0.20</td>
<td>32.55 ± 0.44</td>
</tr>
<tr>
<td><em>M. lysodeikticus</em></td>
<td>10.65 ± 0.20</td>
<td>12.05 ± 0.17</td>
<td>26.80 ± 0.42</td>
</tr>
<tr>
<td><em>C. × californica</em></td>
<td>11.08 ± 0.25</td>
<td>9.50 ± 0.16</td>
<td>26.60 ± 0.57</td>
</tr>
</tbody>
</table>

Notes: 1 – for bacteria, discs with 5.0 μg oxolinic acid 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* B204 was lower. The effect of *Ch. × californica* fruit pulp extracts on the strains *E. dissolvens*, *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* B206, *S. aureus* B204, and *S. epidermidis* ATCC149 strains was the highest of all. Both peel and pulp extracts from *Ch. cathayensis* fruits caused high inhibition of *E. coli* B206 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

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Formula</th>
<th>RT, min</th>
<th>Peak area, %</th>
<th>Classification</th>
<th>Known bioactivity (References)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-Dimethylthiophene</td>
<td>C₅H₁₀</td>
<td>3.58</td>
<td>0.11</td>
<td>alkane</td>
<td>not found</td>
</tr>
<tr>
<td>2,4-Di-tert-butylthiophene</td>
<td>C₅H₁₀</td>
<td>4.58</td>
<td>0.35</td>
<td>alkyldehyde</td>
<td>not found</td>
</tr>
<tr>
<td>Octadecenoic acid (syn. Oleic acid)</td>
<td>C₁₈H₃₃O₂</td>
<td>8.145</td>
<td>0.18</td>
<td>fatty acid</td>
<td>antifungal (Walter et al., 2004); anti-inflammatory, cancer preventive (Dibb et al., 2021)</td>
</tr>
<tr>
<td>Hexadecanoic acid (syn. Palmitic acid)</td>
<td>C₁₈H₃₄O₂</td>
<td>8.175</td>
<td>0.19</td>
<td>fatty acid</td>
<td>antifungal (Pourkabir et al., 2021)</td>
</tr>
<tr>
<td>cis-9-Hexadecenoic acid</td>
<td>C₁₈H₃₄O₂</td>
<td>8.240</td>
<td>1.28</td>
<td>alkyldehyde</td>
<td>antifungal (Hoda et al., 2020)</td>
</tr>
<tr>
<td>Hydroxyeicosenoic acid</td>
<td>C₂₀H₄₀O₂</td>
<td>8.581</td>
<td>14.52</td>
<td>aldehyde</td>
<td>not found</td>
</tr>
<tr>
<td>9-Octadecenoic acid, 1,2,3-propenyl) ester</td>
<td>C₁₈H₃₂O₂</td>
<td>9.045</td>
<td>7.12</td>
<td>fatty acid ester</td>
<td>antibacterial (Ookubo et al., 2015)</td>
</tr>
<tr>
<td>E,E,Z-13,12-Nonadecatetra-5,14-diole</td>
<td>C₂₀H₄₀O₂</td>
<td>9.289</td>
<td>2.61</td>
<td>alcohol</td>
<td>antifungal (Parveen et al., 2017)</td>
</tr>
<tr>
<td>Oleic-12-ene-3,8-diol (3beta)</td>
<td>C₁₈H₃₄O₃</td>
<td>9.563</td>
<td>3.07</td>
<td>triterpenoid</td>
<td>antifungal (Cai et al., 2018)</td>
</tr>
<tr>
<td>1-Hexatriacontanol</td>
<td>C₃₃H₆₆O</td>
<td>9.871</td>
<td>0.78</td>
<td>alcohol</td>
<td>not found</td>
</tr>
<tr>
<td>Dicocotyl phthalate</td>
<td>C₁₈H₂₈O₃</td>
<td>10.800</td>
<td>14.15</td>
<td>fatty acid ester</td>
<td>antifungal (Shan et al., 2010)</td>
</tr>
<tr>
<td>17-Pentacyclooctylcyclo</td>
<td>C₁₈H₂₀O₂</td>
<td>12.152</td>
<td>1.44</td>
<td>alkane</td>
<td>antioxidant (Begum et al., 2016)</td>
</tr>
<tr>
<td>Lap[20(S)-en-3-ol (3beta) (syn. Lupol)]</td>
<td>C₂₀H₃₂O₃</td>
<td>12.508</td>
<td>1.59</td>
<td>pentacyclooctylcyclo</td>
<td>antifungal (Cai et al., 2018)</td>
</tr>
<tr>
<td>Tetrapentacontanol</td>
<td>C₃₄H₆₈O</td>
<td>12.945</td>
<td>21.75</td>
<td>alkane</td>
<td>not found</td>
</tr>
<tr>
<td>ZZ,ZZ-Octadecadi-1-ol</td>
<td>C₁₈H₃₄O₂</td>
<td>13.327</td>
<td>0.15</td>
<td>alcohol</td>
<td>not found</td>
</tr>
<tr>
<td>Oleic acid, 3-(octadecyloxy)propyl ester</td>
<td>C₂₈H₄₈O₄</td>
<td>13.796</td>
<td>0.69</td>
<td>fatty acid ester</td>
<td>not found</td>
</tr>
<tr>
<td>Tricosanol</td>
<td>C₂₀H₄₀O₂</td>
<td>14.310</td>
<td>3.27</td>
<td>alcohol</td>
<td>not found</td>
</tr>
<tr>
<td>Uro-12-28-sil</td>
<td>C₂₀H₄₀O₂</td>
<td>14.512</td>
<td>2.07</td>
<td>aldehyde</td>
<td>antibacterial, antioxidant (Do Nascimento et al., 2014)</td>
</tr>
<tr>
<td>7,8-Epoxyoctadecan-11-ol-3-acetoxy</td>
<td>C₂₀H₳₄O₂</td>
<td>14.807</td>
<td>1.21</td>
<td>steroid triketone</td>
<td>antibacterial (Zahari et al., 2017)</td>
</tr>
<tr>
<td>1-Hexatriacontanol</td>
<td>C₃₃H₆₆O</td>
<td>15.036</td>
<td>18.67</td>
<td>alcohol</td>
<td>not found</td>
</tr>
</tbody>
</table>

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 (C₁₆ and C₁₈), accounting for 22.0% of total; of these, dioscycol 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 (C₁₅ – C₃₅), which amounted to 22.9% of total; heptacosanal and tricosanal were the main compounds among the aldehydes. Fraction of the alkanes and alkynes in the *Ch. cathayensis* fruit cuticular waxes consisted of three compounds (C₁₅ – C₃₅) dominated by tetrapentacontane and reached accounting for 22.0% of total; of these, dioscycol 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 (C₁₅ – C₃₅), which amounted to 22.9% of total; heptacosanal and tricosanal were the main compounds among the aldehydes. Fraction of the alkanes and alkynes in the *Ch. cathayensis* fruit cuticular waxes consisted of three compounds (C₁₅ – C₃₅) dominated by tetrapentacontane and reached accounting for 22.0% of total; of these, dioscycol 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 (C₁₅ – C₃₅), which amounted to 22.9% of total; heptacosanal and tricosanal were the main compounds among the aldehydes. Fraction of the alkanes and alkynes in the *Ch. cathayensis* fruit cuticular waxes consisted of three compounds (C₁₅ – C₃₅) dominated by tetrapentacontane and reached accounting for 22.0% of total; of these, dioscycol 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 (C₁₅ – C₃₅), which amounted to 22.9% of total; heptacosanal and tricosanal were the main compounds among the aldehydes. Fraction of the alkanes and alkynes in the *Ch. cathayensis* fruit cuticular waxes consisted of three compounds (C₁₅ – C₃₅) dominated by tetrapentacontane and reached accounting for 22.0% of total; of these, dioscycol 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 (C₁₅ – C₃₅), which amounted to 22.9% of total; heptacosanal and tricosanal were the main compounds among the aldehydes. Fraction of the alkanes and alkynes in the *Ch. cathayensis* fruit cuticular waxes consisted of three compounds (C₁₅ – C₃₅) dominated by tetrapentacontane and reached.
23.3% of the total. Other compounds in the Ch. cathayensis fruit cuticular waxes included different terpenoids 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

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Formula</th>
<th>RT, min</th>
<th>Peak area, %</th>
<th>Classification</th>
<th>Bioactivity (References)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonaonal</td>
<td>C₉H₁₈O</td>
<td>0.40</td>
<td>5.07</td>
<td>fatty acid</td>
<td>not found</td>
</tr>
<tr>
<td>9-Octanoic acid</td>
<td>C₁₀H₁₈O₂</td>
<td>0.40</td>
<td>5.07</td>
<td>fatty acid</td>
<td>not found</td>
</tr>
<tr>
<td>Cinamaldehyde</td>
<td>C₉H₆O</td>
<td>0.012</td>
<td>5.07</td>
<td>fatty acid</td>
<td>not found</td>
</tr>
<tr>
<td>3,7,11-Trimethyl-1,3,6,10-tetradecatetraene (syn. Alph-Farnozone)</td>
<td>C₁₅H₂₆O₈</td>
<td>0.45</td>
<td>0.07</td>
<td>sesquiterpene</td>
<td>antimicrobial (Kuroda et al., 2007)</td>
</tr>
<tr>
<td>Tetradecal (syn. Myristaldehyde)</td>
<td>C₁₄H₂₆O₂</td>
<td>0.23</td>
<td>0.07</td>
<td>aldehyde</td>
<td>antimicrobial (Xiaolun et al., 2007)</td>
</tr>
<tr>
<td>7-Hexadecenal</td>
<td>C₁₆H₃₂O</td>
<td>0.138</td>
<td>0.07</td>
<td>aldehyde</td>
<td>not found</td>
</tr>
<tr>
<td>Hexadecanoic acid (syn. Palmitic acid)</td>
<td>C₁₆H₃₂O₂</td>
<td>0.958</td>
<td>0.07</td>
<td>fatty acid</td>
<td>not found</td>
</tr>
<tr>
<td>Cis-9-Hexadecenal</td>
<td>C₁₆H₃₂O</td>
<td>0.958</td>
<td>0.07</td>
<td>aldehyde</td>
<td>antimicrobial (Chauveau et al., 2018)</td>
</tr>
<tr>
<td>E,E-Z,13,12-Nonadecadiene-5,14-diol</td>
<td>C₁₈H₃₂O</td>
<td>0.121</td>
<td>0.07</td>
<td>alcohol</td>
<td>antimicrobial (Pourakbar et al., 2020)</td>
</tr>
<tr>
<td>Undec-10-ynoic acid, trans-decalyl ester</td>
<td>C₁₁H₁₄O₂</td>
<td>0.82</td>
<td>0.07</td>
<td>aldehyde</td>
<td>not found</td>
</tr>
<tr>
<td>Eicosanal</td>
<td>C₂₀H₄₀</td>
<td>0.079</td>
<td>0.07</td>
<td>aldehyde</td>
<td>not found</td>
</tr>
<tr>
<td>Undec-10-ynoic acid, hexadecyl ester</td>
<td>C₁₁H₁₄O₂</td>
<td>0.079</td>
<td>0.07</td>
<td>aldehyde</td>
<td>not found</td>
</tr>
<tr>
<td>Dodecyl phthalate</td>
<td>C₁₀H₂₀O₄</td>
<td>0.108</td>
<td>0.07</td>
<td>fatty acid ester</td>
<td>not found</td>
</tr>
<tr>
<td>Henicosal</td>
<td>C₁₀H₂₀O</td>
<td>0.079</td>
<td>0.07</td>
<td>aldehyde</td>
<td>not found</td>
</tr>
<tr>
<td>2-Methylhexacosanoic acid</td>
<td>C₂₂H₄₄O</td>
<td>0.168</td>
<td>0.07</td>
<td>alkane</td>
<td>not found</td>
</tr>
<tr>
<td>1-Hentriacontanol</td>
<td>C₂₁H₄₂</td>
<td>0.665</td>
<td>0.07</td>
<td>alcohol</td>
<td>not found</td>
</tr>
<tr>
<td>Triocosanol</td>
<td>C₂₃H₄₆O</td>
<td>0.383</td>
<td>0.07</td>
<td>aldehyde</td>
<td>not found</td>
</tr>
<tr>
<td>Lap-2,6,10-dodecatriene-3-ol (3.beta) (syn. Lupeol)</td>
<td>C₂₃H₄₆O₂</td>
<td>0.660</td>
<td>0.07</td>
<td>pentacetylated terpenoid</td>
<td>antimicrobial (Chauveau et al., 2018)</td>
</tr>
<tr>
<td>Octacosanol</td>
<td>C₂₄H₄₈O</td>
<td>0.110</td>
<td>0.07</td>
<td>alcohol</td>
<td>anti-parkinsonism (Wang et al., 2010)</td>
</tr>
<tr>
<td>Tetrapentacontane</td>
<td>C₃₄H₆₄</td>
<td>0.212</td>
<td>0.07</td>
<td>alkane</td>
<td>not found</td>
</tr>
<tr>
<td>Hexadecyl 3-nonyl ether</td>
<td>C₂₆H₅₀O</td>
<td>0.081</td>
<td>0.07</td>
<td>ether</td>
<td>not found</td>
</tr>
<tr>
<td>Heptacosanol</td>
<td>C₂₇H₅₂O</td>
<td>0.234</td>
<td>0.07</td>
<td>aldehyde</td>
<td>not found</td>
</tr>
</tbody>
</table>

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 (C₁₀ and C₁₂), amounting to 2.3% of the total. The alcohol fraction in Ch. × californica fruit cuticular waxes mostly consisted of primary alcohols (two compounds, C₁₀ and C₁₂), reaching 7.8% of the total amount. The fatty acid esters were represented by three compounds with an even and odd carbon number (C₁₂ – C₂₇), equaling 12.9% of the total; among them, dodecyl 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 (C₆ – C₁₄), accounting for 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 (C₁₂ – C₁₄) dominated by tetrapentacontane and reached 23.0% of the total. Other compounds in the Ch. × californica fruit cuticular waxes included different terpenoids 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, the distribution of these groups between the species and cultivars varies significantly depending 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 compound contents was revealed in the fruits of five wild Chaenomeses species, namely Ch. japonica, Ch. sieversii, 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 (Zaklos-Szyda & Pawlik, 2018). Study of distribution of the phenolic compounds in peel and pulp of both Ch. cathayensis and Ch. × californica fruits revealed significant dominance 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 terpenoids, and show better antioxidant activity than flesh.

In the present study, we have found that the isopropionic 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 (Xiaolun 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. aeruginosain 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. 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 (Xiaolun 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. aeruginosain 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 extracted showed antifungal activity 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 wax 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 terpenes, 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.

References


