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CUTICULAR WAX PROFILING OF *CHAENOMELES* LINDL. LEAVES UNDER DIFFERENT AGROCLIMATIC CONDITIONS

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SUMMARY

This study comprised a comprehensive GC-MS (gas chromatography-mass spectrometry) fingerprinting of leaf cuticular waxes in four *Chaenomeles* Lindl. species—*C. japonica*, *C. speciosa*, *C. × superba*, and *C. cathayensis*. The growth of said species has prevailed growth under diverse agroclimatic conditions of Moscow (temperate) and Crimea (Yalta) (subtropical), Russia. The identified metabolites totaled 54, including terpenoids (13), fatty acids (FA) (9), glycerides (7), alcohols (6), alkanes (5), organic acids (4), phytosterols (4), polyphenols (4), vitamin E, and sucrose. The waxes displayed characteristics of the highest content of long-chain compounds (C28–C33), with C30 triterpenes being the dominant fraction overall. Southern ecotypes exhibited a prolonged profile of terpenoids, alkanes, and sugar alcohols, revealing an adaptive enhancement of the leaf hydrophobic barrier against heat and UV stress conditions. Remarkably, the phytosterol stigmast-5-ene reached its identification for the first time in *Chaenomeles* leaf waxes, exhibiting a considerable accretion in Yalta samples compared with trace amounts in Moscow samples. The results enunciated that non-targeted GC-MS profiling provides a dependable basis for phytochemical recognition and appraising the ecological plasticity. Among these four species, *C. cathayensis* showed the most partial metabolite set, confirming a general trend toward reduced chemical diversity in the Moscow temperate region.

Keywords: *Chaenomeles*, leaves, cuticular wax, GC-MS fingerprinting, agroclimatic adaptation, ecological plasticity

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Key findings: Assimilation of 54 metabolites into group-specific profiles revealed a dual nature of *Chaenomeles* cuticular waxes with species specificity and environmental plasticity, enabling vigorous identification of phytochemicals.

INTRODUCTION

Cuticular wax forms a physico-chemical wall against the mechanical damage, pathogens, and pests (Skrzydeł et al., 2021). Comprising long-chain aliphatics, triterpenoids, and sterols, it confines the non-stomatal transpiration to uphold the water homeostasis (Zeisler-Diehl et al., 2020). High triterpenoid concentrations prevent wax softening at the decisive temperature, ensuring structural reliability under heat stress (He et al., 2020).

Accordingly, the dominance of C₃₀-triterpenoids in *Chaenomeles* is a key adaptive mechanism for thermal resistance in southern ecotopes (Borisova et al., 2020), whereas the temperate climate supports a simplified metabolic profile (Busta and Jetter, 2018). In wax composition, the variations reflect both genetic specificity and ecological plasticity (Borisova et al., 2020). The GC-MS analysis of sterols (stigmast-5-ene) and triterpenes provides the consistent chemotaxonomic markers for species recognition. These profiles emerged as crucial for evaluating the adaptive potential in their introduction to breeding programs (Rumpunen, 2002; Jetter and Kunst, 2008).

This chemical 'fingerprinting' facilitates the selection of climate-resilient genotypes for diverse agroclimatic zones. The genus *Chaenomeles* Lindl. received a global recognition for the highest ascorbic acid and polyphenol content in its fruits (Rumpunen, 2002; Zhao et al., 2022). However, the traditional research focused on breeding and ornamental values (Krasnov et al., 2021), and the recent attention has moved toward in-depth metabolomic analysis of vegetative organs containing exclusive bioactive triterpenoids (Zhang et al., 2023). Although the composition of *Chaenomeles* leaf epicuticular waxes remains only fragmentarily understood.

With their high resilience, these species serve as an ideal model for studying ecological

plasticity (Li et al., 2020). Non-targeted GC-MS metabolomic profiling allows for chemosystematic analysis based on inimitable chemical fingerprints. This approach further facilitates both the clarification of phylogenetic relationships and the discovery of adaptive mechanisms during introduction into contrasting agroclimatic zones (Zhang et al., 2023; Wang et al., 2024). Applying GC-MS to compare the ecotypes revealed specific metabolic shifts in response to heat and UV stress conditions and provided a basis for selecting resilient genotypes (He et al., 2020; Wang et al., 2024).

The cuticular wax layer plays a crucial role in plant adaptation to abiotic stress conditions, yet the chemical diversity and plasticity in *Chaenomeles* species across various agroclimatic zones in Russia remain meagerly studied and understood. Therefore, the concerned study aimed to elucidate the species-specific and environmentally conditioned differences in the composition of cuticular wax in leaves of four *Chaenomeles* species (*C. japonica*, *C. speciosa*, *C. × superba*, and *C. cathayensis*) grown in different agroclimatic conditions of Moscow (continental) and Yalta (subtropical), Russia.

MATERIALS AND METHODS

Preparation of plant materials

Leaves of *Chaenomeles japonica* (Thunb.) Lindl. Ex Spach, *C. speciosa* (Sweet) Nakai, and *C. × superba* (Frahm.) Rehder came from plants in the collection of the Nikita Botanical Gardens, Russian Academy of Sciences (Yalta, Crimea, 44° 30'34"N, 34° 13'58"E). The collection of species *C. cathayensis* (Hemsl.) C.K. Schneid and *C. japonica* leaf samples came from the Main Botanical Garden, Russian Academy of Sciences (Moscow, 55° 50'36.58"N, 37° 35'42.97"E). At least 15 intact leaves collected from the middle third of

shoots along the bush perimeter ensured the representative sampling from at least five individual plants per species during the fruit formation stage. All leaf samples underwent lyophilization before extraction.

Extraction and derivatization

Extraction of leaf surface waxes continued by brief immersion of intact leaves in chloroform (analytical grade) at 25 °C for 20 min. The resulting extract underwent evaporation to a dry residue (approx. 0.0020–0.0025 g). For GC-MS analysis, the residue incurred dissolving in 50 µl of anhydrous pyridine and silylation with 100 µl of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). Derivatization took place at 80 °C for 2 h to ensure the complete conversion of polar metabolites into their trimethylsilyl (TMS) derivatives.

GC-MS conditions

The GC-MS analysis, as performed, used a Chromatec Crystal 5000.2 gas chromatograph equipped with a quadrupole mass-selective detector (Chromatec, Russia). Achieving the separation was successful on an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The helium used served as the carrier gas at a constant flow rate of 0.7 ml/min.

Instrument settings

Injector: 320 °C; injection volume: 3 µl; split ratio: 1:20.

Oven program: Initial 100 °C (2 min), ramp 5 °C/min to 315 °C (held for 15 min). Total run time: 60 min.

MSD: Ion source 300 °C; transfer line 315 °C; and solvent delay 5 min.

Total analysis time: 60 min.

Data processing and fingerprinting

The metabolites' identification resulted from comparing their retention parameters and mass spectra with the National Institute of Standards and Technology (NIST-05) library and literature data. The relative content of each compound had the expression as a

percentage of the total area of all identified peaks. Carrying out the data comparison had the absolute peak areas scaled by a factor of 10^{-7} . The analysis proceeded according to established recommendations (Roessner *et al.*, 2000; Lebedev, 2015).

The conduct of this study served as an exploratory screening of leaf surface profiles. Given the unique nature of the specimens and limited reagent volumes, the analysis focused on establishing the qualitative composition (fingerprinting) and identifying key metabolite classes. The acquired profiles became useful as primary chemotaxonomic markers for species discrimination. Peak areas expression was in relative units (multiplied by 10^{-7}). All identifications ensued using the gas chromatography-mass spectrometry (GC-MS) following MSTFA derivatization.

The comparison of chemical profiles of the studied leaf samples succeeded in the construction of a heat map. The study applied a row-by-row normalization to enhance the precision of the distribution: a color gradient sustained calculation individually for each component, where intense red corresponds to the maximum value in the row, and green corresponds to the minimum or zero content.

Chemicals

All reagents, solvents, and standards used were of analytical grade (minimum purity ≥ 99%), as received from commercial suppliers. The chloroform purchase was from EKOTEK (Russia), the N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) came from Sisco Research Laboratories (SRL, India), and the use of highly pure helium was the carrier gas for the GC-MS analysis.

RESULTS

Analysis by gas chromatography-mass spectrometry

In cuticular wax extracts using chloroform, the identified organic compounds totaled 54. These organic components further reached classification into nine different categories, i.e.,



Figure 1. Comparative metabologram of the studied samples: 1 — *C. japonica* (Yalta); 2 — *C. japonica* (Moscow); 3 — *C. speciosa* (Yalta); 4 — *C. × superba* (Yalta); and 5 — *C. cathayensis* (Moscow). Note: The data presented has a two-panel heat map. The color scale (legend) represents the relative metabolite content, ranging from minimum (green) to maximum (red). Row-wise normalization enables the visualization of qualitative differences in chemical composition independent of absolute compound concentrations.

terpenoids, fatty acids (FA), glycerides, alcohols, alkanes, organic acids, polyphenols, phytosterols, and miscellaneous compounds. A proportional analysis of the identified metabolites succeeded in visualization through a heat map (Figure 1). The color scale ranges

from green (minimum abundance) to red (maximum abundance), providing an obvious spatial demarcation of chemical profiles across the *Chaenomeles* species and collection locations.

The heat map revealed a distinctive clustering of highly concentrated metabolites in the Yalta (subtropical climate) ecotypes, particularly in regions matching to triterpenoids, phytosterols (stigmast-5-ene), and sugar alcohols (D-mannitol). In contrast, the Moscow (temperate climate) samples showed a shift toward green in several metabolic blocks, enunciating a considerable reduction in the total content of the long-chain FA and alkanes. In comparison with *C. japonica* and *C. cathayensis*, this was obviously visible, exhibiting a unique chemical 'fingerprint' dominated by low-concentration zones (green). Meanwhile, the species *C. × superba* and *C. speciosa* showed intermediate patterns, with the glycerides and organic acids localized in fractions (red-orange). These visual results also confirmed that environmental conditions stimulate and enhance the activity of leaf hydrophobic barrier components in the southern region.

1. Terpenoids: 14 compounds of terpenoids prevailed in *Chaenomeles* Lindl. leaf extracts: loliolide, squalene, lanost-8-ene-3 β ,7 α -diol, 3-acetate, lupeol, erythrodil, uvaol, hederagenin, oleanolic, betulinic, ursolic, crategolic, corosolic, and pomolic acids (Table 1). The precision retention time analysis identified two isomer groups: C₃₀H₄₈O₃ (oleanolic, betulinic, and ursolic acids) and C₃₀H₄₈O₄ (hederagenin, crategolic, corosolic, and pomolic acids). The ursolic acid was the major component, and its contents in the species *C. japonica* (Yalta/Moscow), *C. speciosa*, *C. × superba*, and *C. cathayensis* were 137.24, 272.29, 277.31, 196.08, and 12.27 relative units, respectively. The species *C. speciosa* revealed the highest diversity (for all 14 compounds), while the species *C. cathayensis* showed the lowest. The organic compounds loliolide, squalene, and corosolic acid were elite to Crimea (Yalta) samples. Notably, the content of lanost-8-en-3 β ,7 α -diol in *C. japonica* found in Yalta was fivefold higher than in Moscow samples, while the peak areas for lupeol, uvaol, and major triterpene acids were twice as high.

2. Fatty acids (FA): In the studied *Chaenomeles* Lindl. species, the FA profile of the leaf cuticular wax comprised six saturated

FAs (palmitic, heptadecanoic, decanedioic, stearic, arachidic, and behenic) and three unsaturated FAs, viz., α -linolenic (Omega-3), 9,12-octadecadienoic (Omega-6), and oleic (Omega-9). The palmitic, stearic, and α -linolenic acids were the dominant components across all the *Chaenomeles* species. The highest concentrations of palmitic acids (45.06 relative units) were evident in the species *C. japonica*, in the Yalta (Figure 2). In contrast, in the species *C. japonica* (Moscow), the wax showed a two-fold lower accumulation of palmitic, stearic, and behenic acids. Furthermore, the organic compounds heptadecanoic, decanedioic, and α -linolenic acids did not occur in the Moscow samples, while oleic acid was apparent (and was not emergent in the Yalta samples). The heptadecanoic, arachidic, behenic, and oleic acids appeared as minor components, and the peak areas were not exceeding 2 relative units. Overall, the greatest diversity of FA (8) resulted in the species *C. speciosa*, whereas *C. cathayensis* exhibited the lowest diversity (3: palmitic, heptadecanoic, and oleic), which was considerably an excellent example of species-specific fingerprinting.

3. Glycerides: The leaf cuticular wax composition provided four monoglycerides (2-palmitoylglycerol, 1-monopalmitin, 1-linolenoylglycerol, and glycerol monostearate) and three diglycerides: 1,2-dilaurin, 1,2-dimyristin, and 1-lauroyl-3-myristoyl-glycerol (Figure 3). All these organic compounds were notably in the leaf wax of the species *C. × superba*, with the peak areas of 1-monopalmitin and 1,2-dilaurin, which were 2–7 times larger than those of other glycerides. In the species *C. speciosa* wax, 1,2-dimyristin surfaced with a peak area comparable to that in the species *C. × superba*. Additionally, all the four monoglycerides were evident, with the 1-linolenoylglycerol peak area exceeding the others by more than fourfold. In the species *C. japonica* leaf wax, only two monoglycerides were noticeable (1-monopalmitin and 1-linolenoylglycerol), with the 1-monopalmitin being predominant (2.35 and 1.21 rel. u. for Yalta and Moscow, respectively). Similarly, the leaf wax of the species *C. cathayensis* contained two glycerides, i.e., 1-

Table 1. Characteristics of identified terpenes, phytosterols, and alkanes in *Chaenomeles* leaf waxes.

No	RT	Component	Formula	MW	<i>C. japonica</i> (Moscow)	<i>C. japonica</i> (Yalta)	<i>C. speciosa</i> (Yalta)	<i>C. superba</i> (Yalta)	× <i>C. cathayensis</i> (Moscow)
					Peak area, relative units				
Terpenes									
1	19.09	loliolide	C ₁₁ H ₁₆ O ₃	196.24	-	1.58	0.97	1.41	-
2	36.39	squalene	C ₃₀ H ₅₀	410.73	-	-	2.92	0.76	-
3	40,45	lanost-8-ene-3β,7α-diol	C ₃₀ H ₅₂ O	444,73	0.71	3.76	2,21	1,92	-
4	43.7	lupeol	C ₃₃ H ₅₀ O	426.72	0.24	0.58	0.30	0.36	-
5	44.94	erythrodiol	C ₁₈ H ₂₄ O ₂	384.51	0.11	0.11	0.31	0.41	0.55
6	45.33	uvaol	C ₃₀ H ₅₀ O ₂	442.71	1.53	3.8	4.33	3.18	-
7	45.74	oleanolic acid	C ₃₀ H ₄₈ O ₃	456.72	30.98	59.6	60.52	51.22	2.66
8	45.92	betulinic acid	C ₃₀ H ₄₈ O ₃	456.71	2.93	5.46	4.82	1.52	0.67
9	46.36	ursolic acid	C ₃₀ H ₄₈ O ₃	456.71	137.24	272.29	277.31	196.08	12.27
10	47.56	hederagenin	C ₃₀ H ₄₈ O ₄	472.72	0.32	0.23	1.01	0.38	-
11	47.92	crategolic acid	C ₃₀ H ₄₈ O ₄	472.70	2.06	1.87	7.89	3.11	0.12
12	48.4	corosolic acid	C ₃₀ H ₄₈ O ₄	472.71	-	0.28	1.31	-	-
13	49.18	pomolic acid	C ₃₀ H ₄₈ O ₄	472.71	22.32	21.9	35.01	1.11	1.34
Phytosterols									
1	42.0	campesterol	C ₂₈ H ₄₈ O	400.7	0.32	0.36	0.62	0.29	-
2	42.37	stigmasterol	C ₂₉ H ₄₈ O	412.7	0.82	0.78	1.44	0.56	-
3	43.06	stigmast-5-ene	C ₂₉ H ₅₀	398.7	5.94	20.33	18.78	30.33	0.21
4	43.25	isofucosterol	C ₂₉ H ₄₈ O	412.7	1.71	1.87	0.31	1.87	-
Alkanes (saturated hydrocarbons)									
1	34.36	2-methyl dodecane	C ₁₃ H ₂₈	184.36	-	-	-	-	0.1
2	35.93	2-methyl-nonadecane	C ₂₀ H ₄₂	282.55	0.26	0.59	0.27	-	-
3	37.29	eicosane	C ₂₀ H ₄₂	282.55	3.03	3.98	1.73	0.28	-
4	39.68	2-methyl octadecane	C ₁₉ H ₄₀	268.52	-	-	0.11	1.35	0.74
5	39.87	pentacosane	C ₂₅ H ₅₂	352.68	5.04	11.82	8.58	9.97	-

Note: RT = retention time; MW = molecular weight; all values are expressed in relative peak areas scaled by 10⁻⁷; - Not detected.

linolenoylglycerol (0.15 rel. u.) and 1,2-dilaurin (0.1 rel. u.).

4. Alcohols: The wax alcohols had representations of six compounds: glycerol (a trihydric alcohol), D-mannitol (a sugar alcohol), phytol (an unsaturated diterpene alcohol), and three fatty alcohols—1-hexacosanol, 1-octacosanol, and 1-triacontanol (Table 2). The largest peak areas for mannitol (41.85 rel. units) and phytol (7.12 rel. units) were evident in the species *C. japonica* samples collected from Yalta. In the wax of the species *C. japonica* (Moscow), *C. speciosa*, and *C. × superba*, the mannitol peak areas ranged from 2.42 to 2.91 rel. u. However, the recorded lowest phytol content (0.26 rel. units) occurred in the leaf cuticular wax of *C. japonica*

(Moscow). The differences in glycerol and 1-hexacosanol content between the species *C. speciosa* and *C. × superba* were nonsignificant. Higher levels of mannitol, phytol, and 1-hexacosanol were notable in the leaf cuticular wax of the species *C. japonica* (Yalta) compared with the Moscow samples. Notably, the 1-triacontanol was existent in the wax of the species *C. speciosa*, *C. × superba*, and *C. cathayensis*, while 1-octacosanol was the only identified alcohol in the leaf cuticular wax of the species *C. cathayensis*.

5. Alkanes (saturated hydrocarbons): Five alkanes were available in the leaf cuticular wax of *Chaenomeles* species, namely, 2-methyldodecane, 2-methyloctadecane, pentacosane, and the

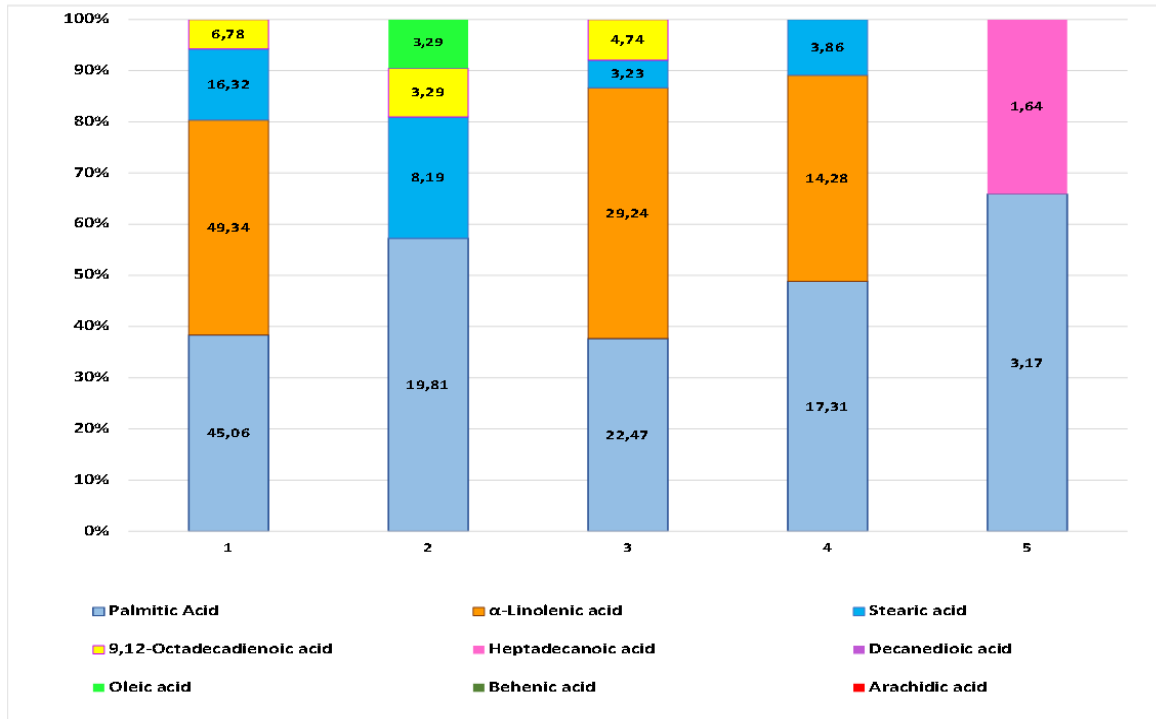


Figure 2. Relative content of FA in leaf cuticular waxes of *Chaenomeles* species (100% stacked bar chart). Samples: 1 – *C. japonica* (Yalta); 2 – *C. japonica* (Moscow); 3 – *C. speciosa* (Yalta); 4 – *C. × superba* (Yalta); and 5 – *C. cathayensis* (Moscow). Minor components (< 2%) are not labeled: heptadecanoic, decanedioic, oleic, behenic, and arachidic acids.

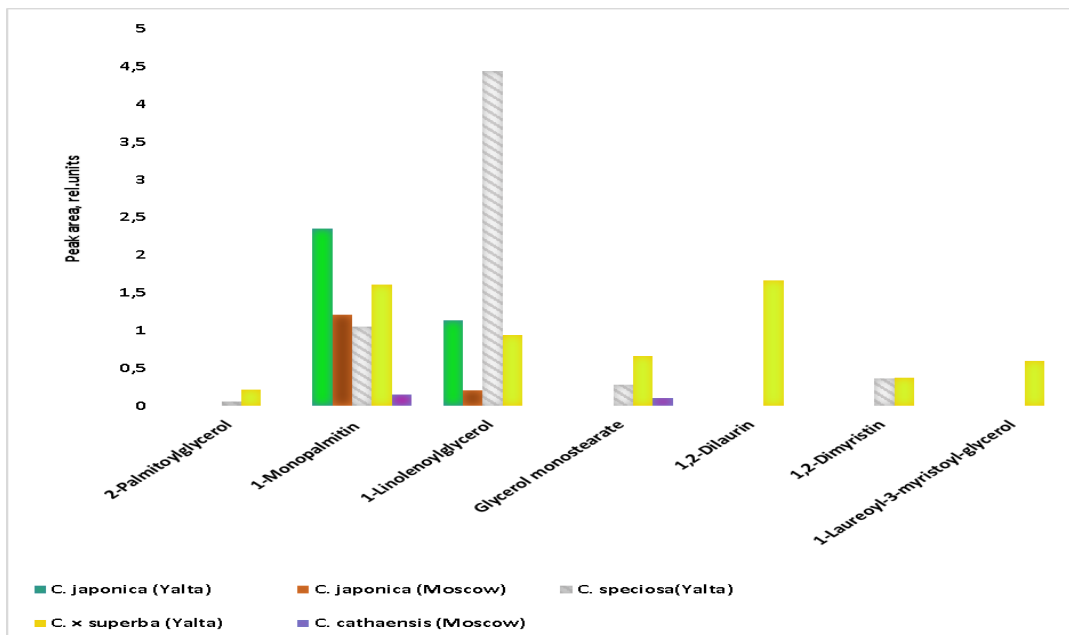


Figure 3. Peak area comparison of glycerides in *Chaenomeles* Lindl. cuticular wax.

Table 2. Alcohols, organic acid, polyphenols, and other components identified by GC-MS.

No.	RT	Component	Formula	MW	C.	C.	C.	C.	×	C.
					japonica (Moscow)	japonica (Yalta)	speciosa (Yalta)	superba (Yalta)		cathayensis (Moscow)
Peak area. relative units										
Alcohols										
1	7.86	glycerol	C ₃ H ₈ O ₃	92.09	1.91	0.63	0.59	0.47		-
2	22.71	d-mannitol	C ₃₂ H ₅₄ O ₃	182.17	2.42	41.85	2.61	2.91		-
3	26.4	phytol	C ₃₃ H ₅₀ O	296.54	0.26	7.12	2.48	2.91		-
4	37.95	1-hexacosanol	C ₂₆ H ₅₄ O	382.71	0.57	1.34	0.76	0.86		-
5	40.26	1-octacosanol	C ₂₈ H ₅₈ O	410.76	-	-	-	-		0.46
6	42.62	1-triacontanol	C ₃₀ H ₆₂ O	438.81	0.60	-	2.29	2,22		0.11
Organic acid										
1	7.13	benzoic acid	C ₇ H ₆ O ₂	122.12	1.36	1.87	-	-		0.21
2	8.61	butanedioic acid	C ₄ H ₆ O ₄	118.09	0.13	0.28	0.16	0.21		0.21
3	19.34	azelaic acid	C ₉ H ₁₆ O ₄	188.22	0.05	0.03	0.13	-		-
4	21.17	quinic acid	C ₇ H ₁₂ O ₆	192.17	7.09	11.91	1.05	4.81		1.24
Polyphenol										
1	22.12	4-coumaric acid	C ₉ H ₈ O ₃	164.16	-	0.18	0.06	0.27		-
2	25.88	caffeic acid	C ₉ H ₈ O ₄	180.16	0.67	6.48	1.45	1.86		-
3	39.00	5-O-coumaroylquinic acid	C ₁₆ H ₁₈ O ₈	338.31	0.27	-	0.06	0.27		-
4	4.87	chlorogenic acid	C ₁₆ H ₁₈ O ₉	354.31	13.84	15.48	3.81	3.36		-
Other connections										
1	34.71	sucrose	C ₁₂ H ₂₂ O ₄	342.30	1,0	15,38	4.12	27.96		0.19
2	40.59	α-tocopherol	C ₂₉ H ₅₀ O ₂	430.71	0.21	1.13	0.78	1.41		-

Note: RT = retention time; MW = molecular weight; all values are expressed in relative peak areas scaled by 10⁻⁷; - Not detected.

structural isomers eicosane and 2-methylnonadecane. These organic compounds revealed distinct physical and chemical properties due to their different molecular structures (Table 1). The alkanes pentacosane and eicosane were predominant in the leaf cuticular wax of the species *C. japonica*, *C. speciosa*, and *C. × superba*. The isomer 2-methylnonadecane manifested in the species *C. japonica* and *C. speciosa*, while 2-methyloctadecane was evident in the species *C. speciosa*, *C. × superba*, and *C. cathayensis*. Particularly, 2-methyldodecane appeared exclusively in the leaf wax of the species *C. cathayensis*. In *C. japonica* leaf samples collected from Yalta, the peak areas of the identified alkanes were considerably higher than those collected from Moscow. Thus, the alkane composition of leaf cuticular wax emerged to be species-specific in *Chaenomeles*, and the environmental

conditions significantly influenced the qualitative and quantitative profiles of these metabolites.

6. The organic acid: The organic acid fraction in the epicuticular wax of *Chaenomeles* Lindl. leaves comprised four organic compounds, viz., benzoic, butanedioic, azelaic, and quinic acids (Table 2). The quinic acid was the dominant organic acid across all the studied species, revealing its maximum concentration in the species *C. japonica* (11.91 rel. u.) and its minimum value in the species *C. speciosa* (1.05 rel. u.). Benzoic acid was entirely evident in the species *C. japonica* and *C. cathayensis*. Overall, the concentration of all organic acids was lower in the species *C. japonica* samples collected from Moscow than in said species samples from Yalta. The levels of butanedioic and azelaic acids were negligible in all species, and the peak areas were not exceeding 0.3 rel. u.

7. Phytosterols: Four phytosterols, such as campesterol, stigmasterol, stigmast-5-ene, and isofucosterol, were notable in *Chaenomeles* Lindl. leaf wax (Table 1). Stigmast-5-ene was predominant, with relative peak areas in the species *C. japonica* (Yalta/Moscow), *C. speciosa*, and *C. × superba* at 5.94, 20.33, 18.78, and 30.33, respectively, and it was the sole phytosterol in the species *C. cathayensis*. Other sterols did not exceed 2 rel. u. Overall, phytosterol levels were higher in the species *C. japonica* samples collected from Yalta than in samples from Moscow.

8. Polyphenols: The leaf cuticular wax of *Chaenomeles* leaves contains four polyphenols, viz., 4-coumaric acid, caffeic acid, 5-O-coumaroyl-D-quinic acid, and chlorogenic acid (Table 2). The predominant polyphenol was the chlorogenic acid, with the highest concentrations observed in the species *C. japonica* (Yalta) at 15.48 rel. u. and *C. japonica* (Moscow) at 13.84 rel. u. In the leaf wax of species *C. speciosa* and *C. × superba*, the chlorogenic acid content was four times lower. However, the peak areas for 4-coumaric and 5-O-coumaroyl-D-quinic acids seemed negligible, not exceeding 0.3 rel. u. The maximum peak area for caffeic acid resulted in the species *C. japonica* (Yalta) at 6.48 rel. u., while the species *C. × superba* and *C. speciosa* showed 1.45 and 1.86 rel. u., respectively. The species *C. japonica* samples (Moscow) showed a slight decrease in chlorogenic acid and a tenfold decrease in caffeic acid compared with the species *C. japonica* (Yalta). No detection of polyphenols materialized in the species *C. cathayensis*.

9. Other connections: The vitamin E (α -tocopherol) and the disaccharide sucrose were also existent in the leaf cuticular wax of *Chaenomeles* (Table 2). The relative peak areas of sucrose and α -tocopherol were species-specific, with the highest values recorded in samples from the agroclimatic zone of Crimea (Yalta). For the species *C. japonica*, *C. speciosa*, and *C. × superba*, the values were 15.38, 4.12, and 27.96 rel. u., respectively.

Overall, the integration of 54 identified metabolites into group-specific profiles confirmed that the leaf cuticular wax composition in *Chaenomeles* was both species-

specific and environmentally influenced, providing a reliable basis for phytochemical fingerprinting. The samples from Yalta exhibited a more diverse chemical profile (up to 48 compounds) than those from Moscow, revealing a pronounced adaptive response to the region's higher insolation and thermal conditions.

DISCUSSION

Triterpenoids were the primary components of cuticular waxes in four *Chaenomeles* species from different ecogeographic zones of Russia. These include two groups: ursolic, oleanolic, and betulinic acids, alongside hederagenin, crategolic, corosolic, and pomolic acids. Presented results align with recent past studies (Zhou *et al.*, 2020; Lykholat *et al.*, 2021; Xu *et al.*, 2023), which reported these triterpenes as key bioactive compounds. Notably, betulinic acid concentrations in the species *C. speciosa* and *C. japonica* were 4–6 times higher than in *C. × superba* and *C. cathayensis*. Combined with ursolic and oleanolic acids, the said trait served as a chemotaxonomic marker (Tao *et al.*, 2023). Significant variations were evident in acid accumulation, depending on environmental conditions (Moscow vs. Yalta). In Yalta's subtropical climate, characterized by high insolation and moisture deficit conditions, the triterpene acid concentrations were twice as high as in temperate Moscow. These results were greatly analogous to the observations made in past studies (Chen *et al.*, 2020; Diarte *et al.*, 2021; Guo *et al.*, 2021; Moneva *et al.*, 2023; Yan *et al.*, 2024; Trivedi *et al.*, 2025).

The FA profile of *Chaenomeles* leaf cuticular wax comprises a complex mixture of saturated and unsaturated components. The supremacy of palmitic (C16:0), stearic (C18:0), and linolenic (C18:3) acids aligns with recent data (Chojnacka *et al.*, 2020). Notably, the species *C. japonica* from the subtropical climate of Yalta exhibited a twofold increase in dominant FAs compared with samples collected in Moscow, revealing an adaptive response to higher temperature and UV-B radiation. Heat stress often stimulates FA synthesis to maintain the thermal stability of the wax layer

(Zeisler-Diehl et al., 2020; He et al., 2020). The presence of unsaturated linolenic (omega-3) and oleic (omega-9) acids was in association with modulating surface conductivity and mitigating oxidative stress (Oliveira et al., 2020). The absence of linolenic acid in Moscow samples versus its presence in Yalta emphasized the specific biochemical adaptation to high insolation. Furthermore, FA diversity ranged from eight compounds in *C. speciosa* to three in *C. cathayensis*, which highlighted the highest species-specific identification. Such ecological plasticity was consistent with other Rosaceae members (Wang et al., 2024; Yan et al., 2024; Trivedi et al., 2025).

Although glycerides often become recognized as minor leaf cuticle components compared to alkanes and fatty alcohols. However, recent studies highlighted their viable role as functional modifiers of the hydrophobic barrier (Zeisler-Diehl et al., 2020; Diarte et al., 2021; Yan et al., 2024; Trivedi et al., 2025) and for the identification and selection of resistant genotypes for breeding programs (Wang et al., 2024). In this study, the exclusive presence of all seven identified glycerides in the species *C. × superba*, contrasted with a minimal profile in the species *C. cathayensis*, suggested as specialized adaptive traits. Furthermore, the significant accumulation of 1-monopalmitin and 1-linolenoylglycerol in the leaf cuticle wax of the species *C. × superba* and *C. speciosa* highlighted their distinct chemical signatures.

The leaf cuticular wax of *Chaenomeles* species contains a diverse alcohol fraction, including D-mannitol, phytol, and very-long-chain fatty acids (VLCFAs) (C₂₆–C₃₀), reflecting a multi-layered defense strategy. A significant finding was the high accumulation of D-mannitol in the species *C. japonica* from subtropical Yalta (41.85 rel. units) versus Moscow (2.42 rel. units), authenticating its primary role as a critical osmolyte and antioxidant (Gudoityte et al., 2021). Increased 1-hexacosanol in Yalta samples suggested that subtropical environments stimulate the carbon chain elongation to enhance surface hydrophobicity (He et al., 2020). Furthermore, the exclusive presence of 1-octacosanol in the species *C. cathayensis* and 1-triacontanol in

other species enunciated the highest chemotaxonomic specificity. These VLCFAs provide a vital physical barrier against water loss and pathogens (Diarte et al., 2021; Yan et al., 2024).

The alkane fraction of *Chaenomeles* leaf cuticle wax features a specific blend of n-alkanes and iso-alkanes. Dominant pentacosane (C₂₅) and eicosane (C₂₀) in the species *C. japonica*, *C. speciosa*, and *C. × superba* provided the critical protection against non-stomatal water loss (Chen et al., 2023; Wang et al., 2024). Considerably higher alkane levels in Yalta samples versus Moscow confirmed that the highest temperature and insolation stimulate the decarbonylation pathway. Recent studies suggested that this accumulation prevents tissue overheating (He et al., 2020; Trivedi et al., 2025). Furthermore, the exclusive presence of 2-methyldodecane in the species *C. cathayensis* and species-specific isomer distribution serve as distinct chemical fingerprints, highlighting the chemotaxonomic specificity of the genus.

The significant accumulation of quinic acid in the species *C. japonica* from Yalta (11.91 rel. units) compared with Moscow accessions highlighted its primary role in osmoprotection and UV defense. The quinic acid derivatives perform as precursors for complex polyphenols that protect the photosynthetic apparatus from high solar radiation in subtropical ecotopes (Tao et al., 2023; Wang et al., 2024). Furthermore, the elite presence of benzoic acid in the species *C. japonica* and *C. cathayensis* underscored the highest genetic specificity and potentially enhanced the antimicrobial surface properties (Zhang et al., 2023). Negligible levels of azelaic and butanedioic acids suggested these were the minor metabolic intermediates (Mathur et al., 2025). The overall decrease in organic acids in Moscow samples confirmed that metabolic flux entailed determination largely by environmental conditions (Moneva et al., 2023; Xu et al., 2023; Yan et al., 2024).

The clear dominance of stigmasterol across all studied species, with particularly high concentrations in the species *C. × superba* (30.33 rel. u.) and *C. speciosa* (18.78 rel. u.), suggested its primary function as a

structural stabilizer (Diarte *et al.*, 2021; Mathur *et al.*, 2025; Trivedi *et al.*, 2025). The significant increase in phytosterol content in the Yalta samples (subtropical) compared with the Moscow samples (temperate) for the species *C. japonica* points toward an environment-driven adaptive shift. The current metabolomic research confirmed that plants upregulate sterol biosynthesis in response to high temperature and intense UV radiation (Farzan *et al.*, 2024; Socaciu *et al.*, 2025).

A remarkable increase in phytosterol content in the species *C. japonica* samples from Yalta (subtropical climate) versus samples from Moscow (temperate climate) disclosed an adaptive shift driven by environmental conditions. Recent studies enunciated that phytosterols maintained the mechanical flexibility of the leaf cuticle, which was vital for preventing microcracks under environmental stress conditions (Diarte *et al.*, 2021; Farzan *et al.*, 2024; Mathur *et al.*, 2025; Socaciu *et al.*, 2025; Trivedi *et al.*, 2025).

The species-specific levels of α -tocopherol and sucrose, peaking in Yalta samples (up to 27.96 rel. u.), highlighted the environmental plasticity of the *Chaenomeles* species. A more diverse chemical profile in Crimea (48 compounds), including loliolide and squalene, confirmed an adaptive strengthening of the hydrophobic barrier against the highest insolation, providing a reliable foundation for phytochemical fingerprinting.

The leaf cuticular wax of the studied *Chaenomeles* species revealed the highest degree of chemical complexity, representing a multi-layered defense system and the ability to acclimate to the agroclimatic zones of Moscow (temperate) and Crimea (Yalta) (subtropical), Russia.

CONCLUSIONS

This study found the highest species-specificity pattern in the leaf epicuticular wax composition of *Chaenomeles*, supporting the use of alkane, fatty alcohol, and glyceride profiles as reliable chemotaxonomic markers. The quantitative dominance of palmitic acid, pentacosane, and D-mannitol in subtropical samples (Yalta)

compared with temperate samples (Moscow) confirmed a robust adaptive mechanism aimed at enhancing thermal resistance and osmoprotection. The observed metabolic plasticity of the hydrophobic barrier, evidenced by the accumulation of phytosterols and triterpenoids, ensured the cuticular structural integrity under high-temperature and UV stress. These metabolic signatures served as valuable selection criteria for breeding resilient genotypes adapted to diverse agroclimatic zones.

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