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GC–MS-Based Compositional Profiling of Cosmetic Plant Waxes Using Solvent-Dependent Extraction

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Abstract

The utilisation of cosmetic plant waxes as sustainable functional ingredients in personal care formulations is on the rise. This is due to their emollient, structuring and film-forming properties. Nevertheless, the intricate lipid composition of these substances engenders considerable analytical difficulties, impeding the reliable characterisation and quality assessment thereof. In this study, an optimised gas chromatography–mass spectrometry (GC–MS) workflow was developed and evaluated for the compositional profiling of waxes derived from *R. succedanea* and *R. verniciflua*, two East Asian botanical species that have historically been utilised in cosmetic and lacquer-related applications. Wax samples originating from China, Vietnam, Korea, and Japan were extracted using solvents of different polarity, including petroleum ether, dichloromethane (DCM), ethyl acetate, and acetone. Following the process of BSTFA derivatisation, the extracts were subjected to analysis by GC–MS to evaluate the efficiency of the extraction process and the chemical composition of the extracts. DCM was found to provide the highest extraction yields and the most representative compositional profiles. Across all samples, palmitic acid was identified as the predominant constituent (80–90%), followed by lower amounts of oleic acid, stearic acid, and minor monoacylglycerols. The proposed analytical workflow exhibited satisfactory reproducibility and effective discrimination of lipid constituents, thereby substantiating its application in comparative compositional evaluation, potential support for future quality assessment studies, and formulation development of cosmetic plant waxes.

Keywords: GC–MS; cosmetic wax; composition analysis; derivatization; botanical wax; fatty acid profiling; method development

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1. Introduction

Cosmetic waxes represent a pivotal functional ingredient in a broad spectrum of personal care and decorative products, encompassing lipsticks, balms, mascaras, and emulsions. It has been demonstrated that these elements contribute significantly to product texture, consistency, gloss, melting behaviour, and overall stability. Consequently, they influence both product performance and consumer perception [1–4]. Chemically speaking, cosmetic waxes are complex mixtures composed primarily of hydrocarbons, fatty acids, long-chain alcohols, and wax esters. The relative abundance of these components determines the physicochemical and sensory properties of the waxes [5,6].

The growing consumer demand for transparency, in conjunction with the increasingly stringent regulatory requirements regarding ingredient traceability and product safety,

has intensified the need for reliable methods to verify the authenticity and composition of cosmetic raw materials [7–9]. This trend has also promoted the use of renewable and plant-derived waxes as sustainable alternatives to conventional ingredients. Of particular interest are waxes obtained from *Rhus succedanea* and *Rhus verniciflua*, which have garnered attention due to their favourable functional properties and their contribution to the natural and sustainable profile of cosmetic formulations [10–12]. Accurate compositional characterisation is therefore essential for quality control, formulation verification, and the detection of potential adulteration.

Notwithstanding their industrial pertinence, the analytical characterisation of cosmetic waxes remains challenging. Their intricate composition, extensive molecular-weight distribution, and the coexistence of structurally related compounds frequently impede both chromatographic separation and compound identification [4–6]. Gas chromatography–mass spectrometry (GC–MS) is a technique that has gained widespread recognition for its efficacy in the analysis of lipid-rich and wax-based matrices. This is because it combines high separation efficiency with structural information derived from mass spectra [1–3,13–15]. Nonetheless, the extraction step is of critical importance in determining the range and abundance of compounds detected, particularly in the case of plant waxes, where constituents exhibit markedly different polarities and solubilities.

Despite the extensive application of gas chromatography–mass spectrometry (GC–MS) in the characterisation of natural and synthetic waxes, including beeswax, carnauba wax, candelilla wax, and related materials [1–3,14], there is a paucity of research specifically addressing the influence of extraction solvents on the compositional profiling of cosmetic plant waxes. Moreover, there is an absence of a standardised workflow for the reproducible GC-MS characterisation of these materials at present [3,6,13].

The objective of this study was to investigate the impact of solvent-dependent extraction on the GC–MS compositional profiling of cosmetic plant waxes. A workflow analysis was developed and applied to a selection of botanical waxes. This workflow was based on simplified sample preparation, optimised chromatographic conditions, and compound identification through mass spectral libraries and Similarity Index (SI) matching. The proposed approach aims to identify the most suitable extraction system, thereby facilitating the acquisition of detailed and comprehensive information on the wax composition through a rapid protocol. In the event of comparisons, the approach will evaluate the composition, and assess its authenticity.

2. Materials and Methods

2.1. Samples and Reagents

The raw plant-based waxes utilised in this study were supplied by ROELMI HPC (Via Celeste Milani 22, 21040 Origgio, VA, Italy). In collaboration with the company, a selection of waxes from different geographical origins of *R. succedanea* and *R. verniciflua* was subjected to a comparative investigation. Three samples were obtained from *R. succedanea* fruits (RS1, RS2, and RS3), and three from *R. verniciflua* berries (RV1, RV2, and RV3). RS samples were obtained from China, Vietnam and Japan, respectively, whereas RV samples were obtained from China, Korea and Japan, respectively.

The following reagents were procured from Merck KGaA (Darmstadt, Germany): Petroleum ether (ETP), dichloromethane (DCM), ethyl acetate (EtOAc), acetone, analytical-grade hexane, and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Solvents and reagents were utilised without undergoing additional purification.

2.2. Sample Preparation

Approximately 20 g of each wax sample was ground using a mechanical grinder (MRC Ltd., Harlow, CM19 5QF, UK). To preserve the powdered state of the substance and thereby enable subsequent weighing, dry ice was incorporated during the grinding process. Following the grinding and removal of residual dry ice, 1 g of the resulting powder was suspended in 20 mL of solvent (ETP, DCM, EtOAc or Acetone). The suspension was then stirred at room temperature (RT) for 24 h in a closed flask covered with aluminium foil. This procedure was adopted to prevent possible photolabile substances from degrading during the 24 h extraction period [16].

The solutions were subjected to vacuum filtration over Celite, after which the extracts were permitted to evaporate at room temperature. The residues were weighed to determine the quantity that was recovered. To assess the repeatability of the method, the extraction process was performed in triplicate.

For derivatization, 5 mg of each extract were transferred into a vial and treated with 150 µL of BSTFA. Samples were subjected to an incubation process for a period of 24 h at room temperature (RT). This was performed to ensure derivatization, a process which is imperative in highly heterogeneous mixtures. In such mixtures, functional groups of differing accessibility and reactivity may necessitate extended reaction times. Subsequently, excess BSTFA was evaporated under a gentle stream of nitrogen, and the residues were re-dissolved in 1 mL of analytical-grade hexane prior to GC–MS analysis.

2.3. GC–MS Analysis

GC-MS analyses were performed using a Thermo Fisher Scientific (Waltham, MA, USA) system consisting of a TRACE™ 1600 gas chromatograph coupled to a TSQ™ 9610 triple quadrupole mass spectrometer. A TG-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was used with helium as the carrier gas at a flow rate of 1 mL/min.

Samples (1 µL) were injected in fast injection mode using a splitless injector maintained at 230 °C. The split flow was set to 50 mL/min, and the splitless time was 0.80 min. Electron impact ionization was performed at 70 eV and 200 °C. The oven temperature was initially held at 85 °C for 1 min and then ramped at 10 °C/min to 305 °C, which was maintained for 5 min. The mass filter was maintained at 250 °C, and the mass spectrometer was operated in scan mode with m/z ranges of 50–500.

2.4. Data and Statistical Analysis

The identification of the compounds was achieved through a comparison of the acquired mass spectra with those contained within the NIST Mass Spectral Library. The SI provided by the library search was used as an additional criterion to support compound assignment. The Chromatographic resolution between adjacent peaks was calculated in order to evaluate the quality of peak separation.

The semi-quantification of the compounds was achieved by employing the normalized peak area percentages obtained from the total ion chromatogram (TIC), a method that did not necessitate the use of calibration curves or internal standards. Consequently, the results are reported as relative abundances rather than absolute concentrations.

A one-way analysis of variance (ANOVA) was conducted, followed by Tukey's post hoc test. The statistical software used was Statistica 7 v.13 (TIBCO Software, Palo Alto, CA, USA).

3. Results and Discussion

It is evident that Species belonging to the Anacardiaceae family, including *R. succedanea* and *R. verniciflua*, are significant botanical sources of natural waxes, resins, and lacquer-

like exudates. These substances have a long history of traditional use in East Asian manufacturing, cosmetics, and protective coatings [17]. *R. verniciflua* is native to East and Southeast Asia, and is principally distributed in China, Korea, and Japan, whereas *R. succedanea* is widespread in Taiwan, Vietnam, and other subtropical Asian regions [18,19]. These species have historically been cultivated for the extraction of sap and fruit-derived waxes used in varnishes, candles, pharmaceuticals, personal care products, and traditional lacquerware due to their remarkable film-forming properties, hydrophobicity, gloss, and oxidative stability [20].

The chemical composition of these plant-derived materials is characterised predominantly by long-chain catechol derivatives, fatty acids, esters, hydrocarbons, and phenolic lipids [19,20]. In particular, *R. verniciflua* contains urushiol-type compounds, while *R. succedanea* is mainly associated with laccol derivatives. Both consist of substituted catechols bearing unsaturated alkyl side chains, which are responsible for their characteristic polymerisation behaviour and bioactive properties [19]. It is evident that these constituents contribute not only to the traditional use of oriental lacquers as durable protective coatings, but also to the increasing interest in these natural products as renewable raw materials for cosmetic and analytical applications [20].

In recent years, the growing demand for sustainable and plant-based cosmetic ingredients has renewed scientific interest in the compositional characterisation of vegetable waxes and lacquer-related plant extracts [4]. It is evident that advanced chromatographic and mass spectrometric techniques, most notably GC/MS, have been demonstrated to be effective in the detailed profiling of complex lipidic matrices. This has enabled the identification of characteristic molecular markers and the evaluation of solvent-dependent extraction behaviours [19–21].

3.1. Comparative Evaluation of Solvent Extraction Efficiency

In order to verify the reliability of the extraction method, the extraction process was repeated on each sample and each solvent on three occasions. The extraction solvents employed—namely, ETP, DCM, EtOAc, and acetone—were meticulously selected to constitute an ascending polarity sequence of organic solvents, progressing from non-polar to moderately polar media. Alcohols were deliberately excluded from the study because of their strong hydrogen bonding capacity and high polarity. It is well established that alcohols tend to solubilise unwanted polar impurities and disrupt wax matrices, rendering them unsuitable for extraction protocols targeting waxes.

The data presented in Table S1 demonstrate minimal variability between triplicates of each species, thereby substantiating the efficacy of the sequential extraction method in ensuring reproducibility and consistency in solvent-dependent extraction profiles across replicates. This consistency confirms that the method is both robust and suitable for the purpose of comparative chemical analyses of plant waxes.

As demonstrated in Figure 1, it is evident that samples of the same species, yet originating from disparate locations, exhibit divergent extraction quantities. This discrepancy is particularly pronounced in the case of *R. succedanea*, especially during extraction with EtOAc. The discrepancy is less pronounced in the three samples of *R. verniciflua*, apart from RV1 in conjunction with ETP. The mean DCM yield was found to be higher in *R. succedanea* (~951 mg; yield: 95.10%) than in *R. verniciflua* (~874 mg; yield: 87.4%). A similar trend was observed for EtOAc, where *R. succedanea* produced the highest yields among all samples. In contrast, extraction with ETP exhibited an inverse trend, with *R. verniciflua* demonstrating the highest mean yield (626 mg; yield: 62.60%). Extraction with acetone yielded comparable values in both species.

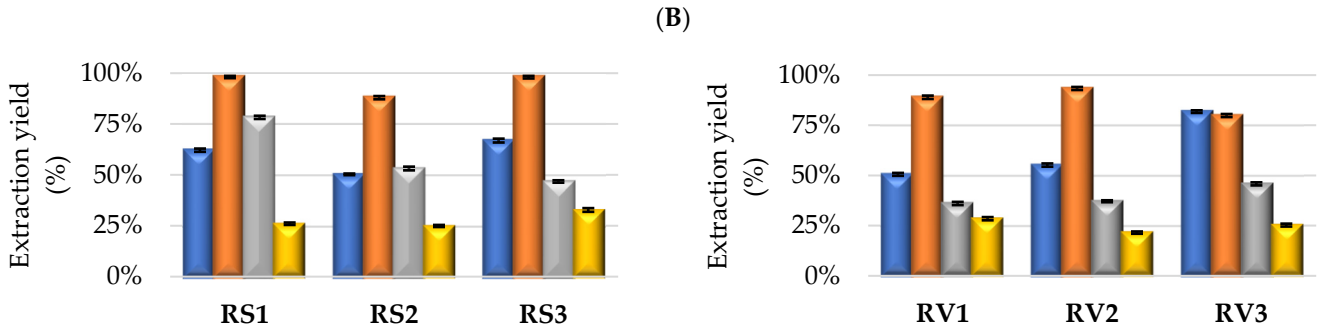


Figure 1. Individual extraction yields for *R. succedanea* (**A**) and *R. verniciflua* (**B**) across ETP (Blue), DCM (Orange), EtOAc (Gray), and Acetone (Yellow). Extraction yields are expressed as percentage recovery (% *w*/*w*) relative to the initial sample mass (1.00 g). The figure highlights the influence of solvent polarity on extraction efficiency and provides a comparative assessment of solvent performance for the recovery of wax constituents from the two Rhus species. Mean + SD of three independent extraction.

In consideration of the collective impact of solvents within the extraction process (cf. Figure 2), DCM was identified as the optimal solvent, with ETP ranking second. As anticipated, acetone exhibited the lowest extraction capacity for both species (216–328 mg; yield: 21.60–32.80%), thereby emphasising the limited compatibility between this solvent and the prevalent chemical classes present in the resins.

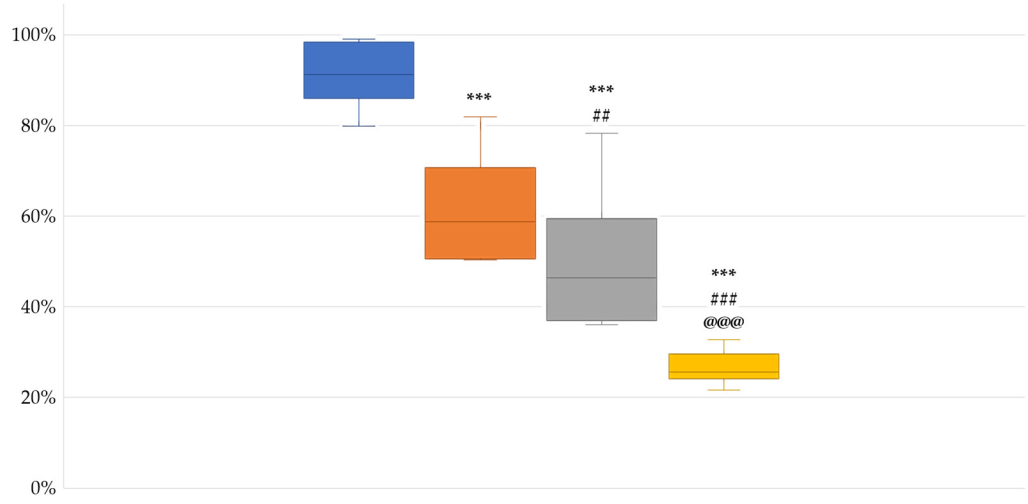


Figure 2. Extraction yields obtained from *R. succedanea* (RS1–RS3) and *R. verniciflua* (RV1–RV3) wax samples using DCM (Blue), ETP (Orange), EtOAc (Gray), and Acetone (Yellow) as extraction solvents. Values represent mean ± SD of three independent extractions. The figure highlights the influence of solvent polarity on wax recovery, with DCM generally providing the highest extraction yields across the investigated samples. Statistical significance among extraction solvents was evaluated by one-way ANOVA followed by Tukey's post hoc test. *** $p < 0.001$ vs. DCM; ### $p < 0.001$; ## $p < 0.01$ vs. ETP; @@@ $p < 0.001$ vs. EtOAc.

This finding suggests that the Rhus genus contains a greater abundance of non-polar constituents that are efficiently solubilised by DCM. It has been demonstrated that analogous findings were reported for other biomass-derived wax resins. For instance, in a study on Musa acuminata biomass, non-polar solvents such as ETP and toluene were shown to recover the wax fraction more effectively than polar solvents [22].

The impact of solvent polarity on the lipid extract composition has been thoroughly documented in studies conducted on Saccharum officinarum (sugarcane) straw and bagasse. In these studies, the solvent significantly influenced the recovery of non-polar lipids

(e.g., hydrocarbons, fatty alcohols, free fatty acids) or more polar lipids (e.g., glycolipids, phospholipids) [23].

3.2. GC–MS Analysis

As a preliminary evaluation of the GC–MS analyses, the extracts obtained using different solvents were compared to assess variations in their chemical profiles. The focus of this study was directed towards a selection of samples (RS1 and RV1) in order to ascertain the existence of any potential disparities in extraction patterns.

It is acknowledged that the composition of the waxes can vary between the two plants and the various origins. to facilitate the identification of the compounds, the extracts were derivatised with BSTFA. This process has been shown to improve the volatility of the hydroxylated and carboxylated constituents, increase the thermal stability of thermolabile compounds that could be present in the waxes, and improve the resolution of the peaks. This is of particular importance when dealing with complex samples in which many molecules could be present and therefore where high resolution becomes important [24–26]. The identification of the compounds was achieved by comparing the retention times and mass spectra with those of reference compounds available in spectral libraries.

3.2.1. RS1 Wax Extracts

GC-MS analysis of the RS1 extracts revealed a composition dominated by saturated and unsaturated fatty acids and their derivatised forms (TMS and TBDMS). Across all solvent systems, palmitic acid (C16:0) and oleic acid (C18:1) derivatives were consistently the principal constituents, thus confirming the lipid-rich nature of the wax matrix.

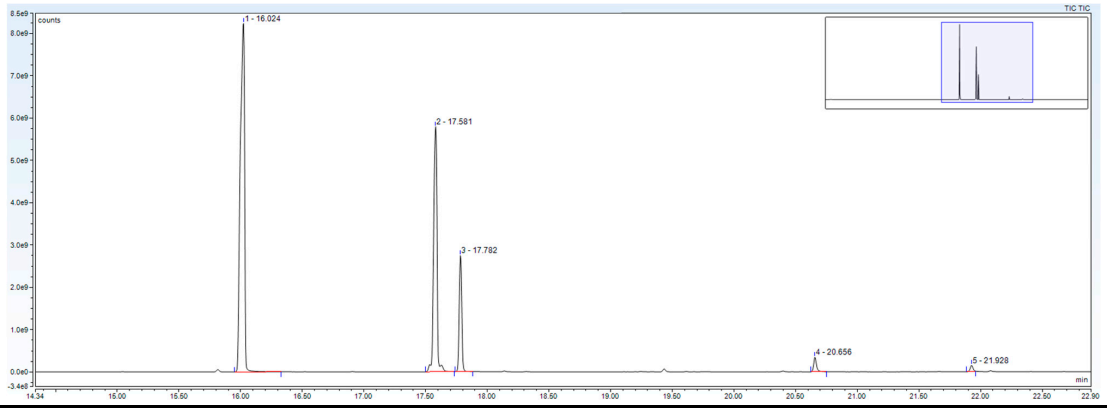
The ETP extract (Figures 3A and S1, Table 1) exhibited a profile exclusively characterised by the presence of fatty acids, with palmitic acid (TMS) accounting for 55.70%, oleic acid (TMS) contributing 31.16%, and stearic acid (C18:0) making up 11.01%. A low abundance of monoacylglycerols was observed, with a percentage of less than 2%. This could be due to their low presence in the sample or poor extraction due to the greater polarity of the compounds. Despite employing divergent extraction conditions, Nayebpour's report indicated that the ETP extract of a different plant species from the Rhus genus exhibited a comparable percentage of oleic acid, approximately 40%. In contrast, *Rhus coriaria* demonstrated a pronounced predominance of unsaturated fatty acids, approximately 70%, with only 30% saturated fatty acids, predominantly palmitic acid. These differences serve to confirm a certain difference in metabolic profiles within the Rhus genus [27].

Table 1. Relative area percentage of the major lipid constituents identified by GC–MS in *R. succedanea* RS1 wax extracts obtained using ETP, DCM, EtOAc, and acetone after BSTFA derivatization. Values are expressed as relative chromatographic peak areas (%).

	ETP	DCM	EtOAc	Acetone
Palmitic Acid [a]	55.70	86.58	68.67	80.44
Oleic Acid [b]	31.16	8.28	23.59	13.35
Stearic acid (TMS derivative)	11.01	<LOQ [c]	4.43	<LOQ
1-Monopalmitin (2TMS derivative)	1.51	3.13	2.05	4.11
1-Monooleoylglycerol (2TMS derivative)	0.63	2.01	1.26	2.10

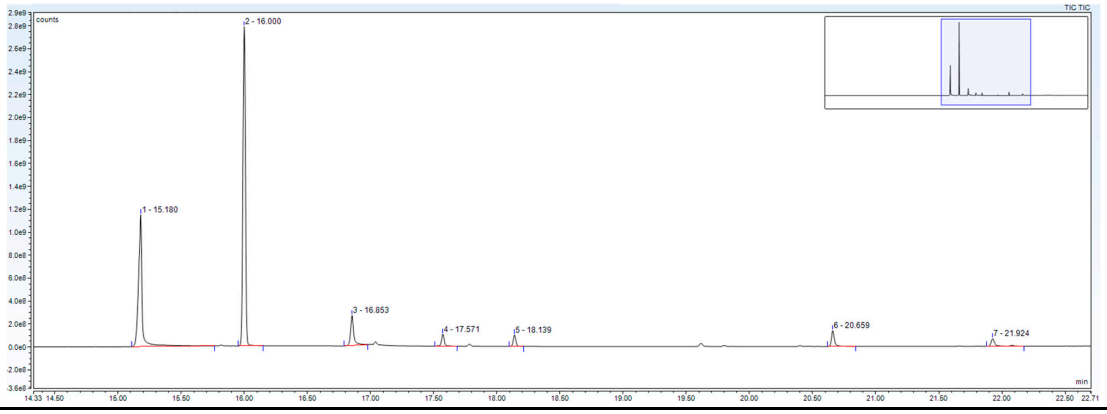
[a] Include free, TMS, 2TMS, and TBDMS form of Palmitic Acid. [b] Include free and TMS form of Oleic Acid. [c] Below the limit of quantification (<LOQ).

(**A**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	16.024	Palmitic Acid TMS derivative	3.34×10^8	8.23×10^9	55.70	923	25.34
2	17.581	Oleic Acid, (Z)- TMS derivative	1.87×10^8	5.79×10^9	31.16	893	4.45
3	17.782	Stearic acid TMS derivative	6.60×10^7	2.74×10^9	11.01	927	73.60
4	20.656	1-Monopalmitin 2TMS derivative	9.03×10^6	3.44×10^8	1.51	858	32.64
5	21.928	1-Monooleoylglycerol 2TMS derivative	3.75×10^6	1.48×10^8	0.63	865	

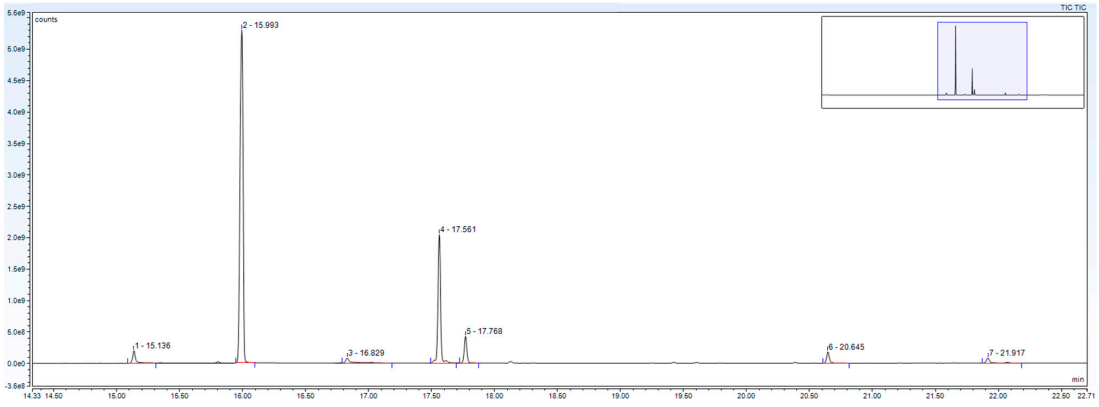
(**B**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	15.180	Palmitic Acid	3.85×10^7	1.15×10^9	31.25	870	19.71
2	16.000	Palmitic Acid TMS derivative	6.56×10^7	2.78×10^9	53.34	915	22.42
3	16.853	Oleic Acid	7.51×10^6	2.64×10^8	6.11	885	19.33
4	17.571	Oleic Acid, (Z)- TMS derivative	2.67×10^6	1.05×10^8	2.17	865	15.36
5	18.139	Palmitic Acid TBDMS derivative	2.46×10^6	1.02×10^8	2.00	868	66.64
6	20.659	1-Monopalmitin 2TMS derivative	3.85×10^6	1.39×10^8	3.13	805	30.82
7	21.924	1-Monooleoylglycerol 2TMS derivative	2.47×10^6	6.83×10^7	2.01	848	

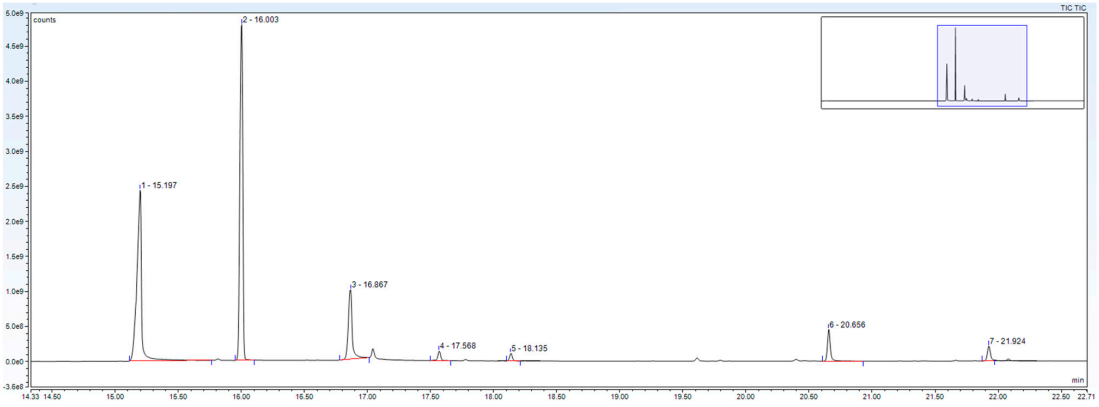
Figure 3. *Cont.*

(**C**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	15.136	Palmitic Acid	5.87×10^6	1.97×10^8	2.60	876	20.75
2	15.993	Palmitic Acid TMS derivative	1.49×10^8	5.30×10^9	66.07	916	18.32
3	16.829	Oleic Acid	3.90×10^6	7.84×10^7	1.72	876	18.41
4	17.561	Oleic Acid, (E)- TMS derivative	4.95×10^7	2.05×10^9	21.87	933	5.81
5	17.768	Stearic acid TMS derivative	1.00×10^7	4.26×10^8	4.43	909	78.72
6	20.645	1-Monopalmitin 2TMS derivative	4.64×10^6	1.82×10^8	2.05	787	31.71
7	21.917	1-Monooleoylglycerol, 2TMS derivative	2.86×10^6	8.00×10^7	1.26	853	

(**D**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	15.197	Palmitic Acid	1.04×10^8	2.44×10^9	35.38	841	15.40
2	16.003	Palmitic Acid TMS derivative	1.30×10^8	4.79×10^9	44.10	914	18.23
3	16.867	Oleic Acid	3.55×10^7	9.89×10^8	12.10	894	15.83
4	17.568	Oleic Acid, (Z)- TMS derivative	3.67×10^6	1.36×10^8	1.25	871	14.68
5	18.135	Palmitic Acid TBDMS derivative	2.81×10^6	1.11×10^8	0.96	873	65.39
6	20.656	1-Monopalmitin 2TMS derivative	1.21×10^7	4.52×10^8	4.11	862	31.34
7	21.924	1-Monooleoylglycerol 2TMS derivative	6.16×10^6	2.13×10^8	2.10	866	

Figure 3. Enlarged GC–MS chromatograms of *R. succedanea* RS1 wax extracts obtained using ETP (**A**), DCM (**B**), EtOAc (**C**), and Acetone (**D**) following BSTFA derivatization. The expanded chromatographic

regions highlight the major constituents detected in the wax matrix, including fatty acids, monoacylglycerol derivatives, and minor hydrocarbon components. Peak numbers shown in the chromatograms correspond to those reported in the associated peak assignment table, which includes retention time (min), compound name, peak area (Counts·min), peak height (Counts), relative area (%), similarity index (SI), and chromatographic resolution (Rs). The chromatograms illustrate solvent-dependent differences in the relative abundance of individual components while maintaining a generally consistent compositional profile across extracts, thereby facilitating comparison between chromatographic features and tentatively assigned compounds.

In the DCM extract (Figures 3B and S2, Table 1), palmitic acid (TMS derivative) was the predominant compound (53.34% relative area), followed by the same palmitic acid but in free form (31.25%), which was absent in the ETP extract. The presence of Oleic acid (6.11%) and its derivatized form (2.17%) was detected in moderate amounts, along with minor mono-acylglycerols such as 1-monopalmitin (3.13%) and 1-monooleoylglycerol (2.01%). It is a matter of some surprise that no traces of stearic acid were detected. This distribution suggests that the DCM extract contains both free fatty acids and partially esterified lipid species, the latter being present in higher percentages than ETP, consistent with its greater polarity. As demonstrated in seminal works by Tsujimoto and subsequently by Wang, who employed chromatographic techniques, a similar composition for Rhus succedanea had already been identified in very early works without the use of GC-MS [28,29].

In the EtOAc extract (Figures 3C and S3, Table 1), palmitic acid derivatives attained the highest percentage (66.07%), accompanied by a substantial fraction of oleic acid TMS derivative (21.87%). The presence of stearic acid (4.43%) and minor glycerides provides support for the hypothesis of a chemically diverse lipid fraction.

The acetone extract (Figures 3D and S4, Table 1) exhibited a more balanced composition, with free and silylated palmitic acid predominating (79.5%), while oleic acid reached its highest relative abundance (12.10%) among all extracts, with monoglycerides identified as trace components.

3.2.2. RV1 Wax Extracts

GC-MS analysis of RV1 wax extracts obtained using different solvents revealed a mixture dominated by long-chain fatty acids, their silylated derivatives, and small amounts of monoacylglycerols. the chromatographic profiles were found to be characterised by consistent retention times, with the main compounds eluting between 15 and 18 min. This finding serves to confirm the predominance of C16-C18 lipid species [30–32].

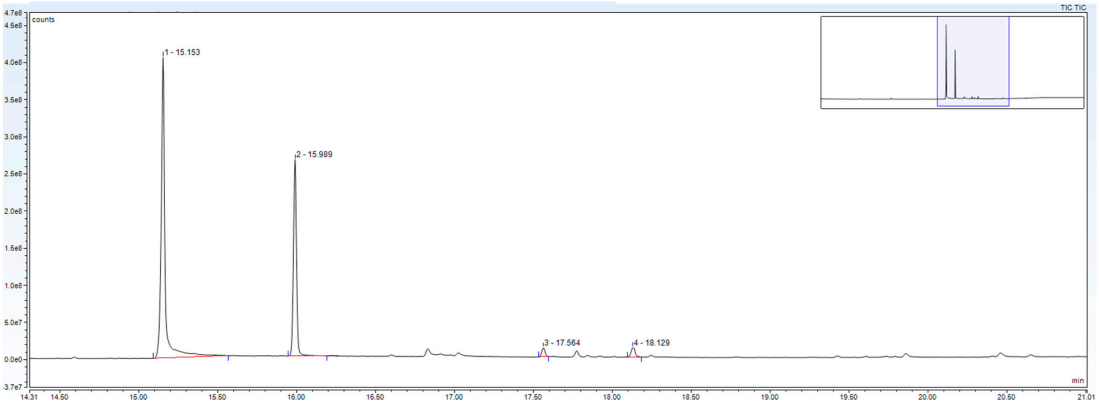
In the ETP extract (Figures 4A and S5, Table 2), the only compound detected was palmitic acid, both in free form (tr = 15.15 min) and in silylated form (tr = 15.99 min). The presence of other components was also noted, albeit in trace amounts. These included oleic acid, which constituted 1.58% of the total sample. As illustrated in Figures 4B and S6 and Table 2, the DCM extract demonstrates that free and silylated palmitic acid constitutes approximately 90% of the total, with oleic acid (6%) and stearic acid (1.6%) identified as additional components of the mixture. Trace amounts of 1-monopalmitin (1.3%) were identified. The ETP extract exclusively favours palmitic acid, while the DCM also allows the extraction of both saturated and unsaturated 18-carbon fatty acids [33].

In the EtOAc extract (Figures 4C and S7, Table 2), palmitic acid is still present, but in smaller percentages than the other less polar solvents (81%). Furthermore, Oleic acid (3%) and trace amounts of stearic acid (1%) were identified. The extract was found to contain 1-monopalmitin, which constituted 11.3% of the total.

In the acetone extract (Figures 4D and S8, Table 2), palmitic acid (free and silylated) accounted for approximately 87%, while oleic acid accounted for approximately 4%. Glyc-

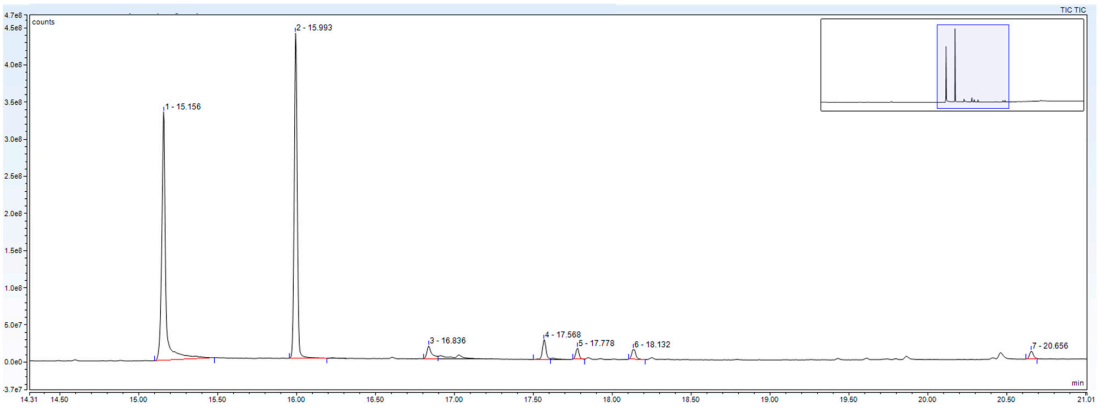
eride derivatives of palmitic acid accounted for almost 7%, with 1-monopalmitin being the dominant species (5.6%) [34].

(**A**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	15.153	Palmitic Acid	1.15×10^7	4.05×10^8	63.26	865	24.28
2	15.989	Palmitic Acid TMS derivative	6.02×10^6	2.65×10^8	33.11	904	43.52
3	17.564	Oleic Acid, (Z)- TMS derivative	2.88×10^5	1.21×10^7	1.58	774	13.60
4	18.129	Palmitic Acid TBDMS derivative	3.72×10^5	1.26×10^7	2.04	769	

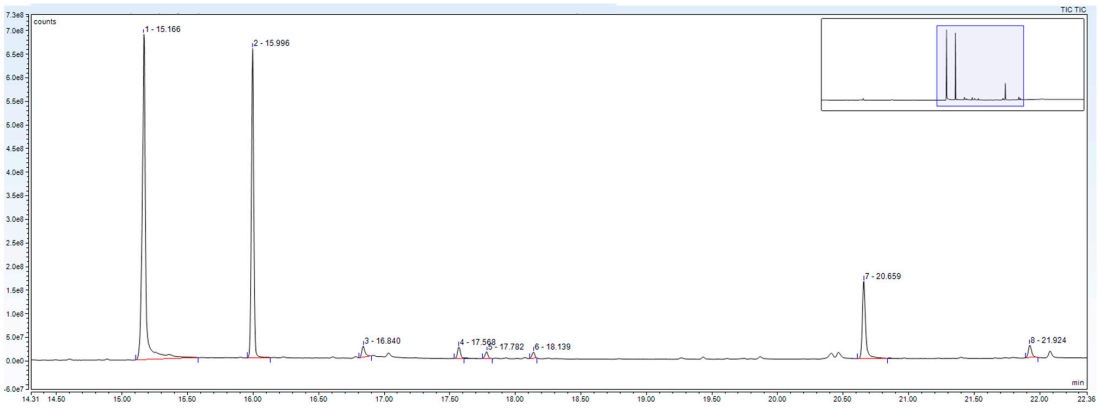
(**B**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	15.156	Palmitic Acid	9.74×10^6	3.35×10^8	44.46	861	23.83
2	15.993	Palmitic Acid TMS derivative	9.80×10^6	4.38×10^8	44.75	919	21.20
3	16.836	Oleic Acid	6.22×10^5	1.73×10^7	2.84	825	16.89
4	17.568	Oleic Acid, (Z)- TMS derivative	7.15×10^5	2.67×10^7	3.26	829	5.38
5	17.778	Stearic acid TMS derivative	3.53×10^5	1.49×10^7	1.61	748	8.58
6	18.132	Palmitic Acid TBDMS derivative	3.92×10^5	1.33×10^7	1.79	765	57.03
7	20.656	1-Monopalmitin 2TMS derivative	2.80×10^5	1.03×10^7	1.28	632	

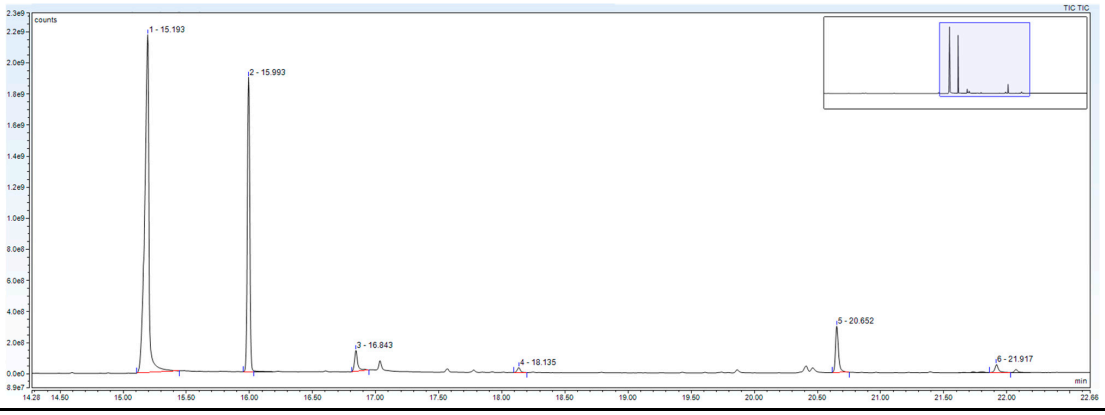
Figure 4. *Cont.*

(**C**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	15.166	Palmitic Acid	2.11×10^{7}	6.90×10^{8}	49.69	866	23.50
2	15.996	Palmitic Acid TMS derivative	1.38×10^{7}	6.56×10^{8}	32.48	918	23.04
3	16.840	Oleic Acid	6.74×10^{5}	2.38×10^{7}	1.59	849	17.91
4	17.568	Oleic Acid, (Z)- TMS derivative	6.36×10^{5}	2.40×10^{7}	1.50	825	5.46
5	17.782	Stearic acid TMS derivative	3.60×10^{5}	1.44×10^{7}	0.85	756	9.30
6	18.139	Palmitic Acid TBDMS derivative	3.10×10^{5}	1.31×10^{7}	0.73	732	63.89
7	20.659	1-Monopalmitin 2TMS derivative	4.81×10^{6}	1.64×10^{8}	11.34	800	30.55
8	21.924	1-Monooleoylglycerol 2TMS derivative	7.71×10^{5}	2.66×10^{7}	1.82	788	

(**D**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	15.193	Palmitic Acid	8.52×10^{7}	2.17×10^{9}	59.71	836	17.55
2	15.993	Palmitic Acid TMS derivative	4.26×10^{7}	1.90×10^{9}	29.901	924	22.51
3	16.843	Oleic Acid	4.00×10^{6}	1.35×10^{8}	2.80	875	5.13
4	18.135	Palmitic Acid TBDMS derivative	8.84×10^{5}	3.18×10^{7}	0.62	799	62.22
5	20.652	1-Monopalmitin 2TMS derivative	8.11×10^{6}	2.98×10^{8}	5.69	821	29.70
6	21.917	1-Monooleoylglycerol 2TMS derivative	1.84×10^{6}	5.31×10^{7}	1.29	831	

Figure 4. Enlarged GC–MS chromatograms of *R. verniciflua* RV1 wax extracts obtained using ETP (**A**), DCM (**B**), EtOAc (**C**), and Acetone (**D**) following BSTFA derivatization. The expanded chromatographic

regions highlight the major constituents detected in the wax matrix, including fatty acids, monoacylglycerol derivatives, and minor hydrocarbon components. Peak numbers shown in the chromatograms correspond to those reported in the associated peak assignment table, which includes retention time (min), compound name, peak area (Counts·min), peak height (Counts), relative area (%), similarity index (SI), and chromatographic resolution (Rs). The chromatograms illustrate solvent-dependent differences in the relative abundance of individual components while maintaining a generally consistent compositional profile across extracts, thereby facilitating comparison between chromatographic features and tentatively assigned compounds.

Table 2. Relative area percentage of the principal compounds identified by GC–MS in *R. verniciflua* RV1 wax extracts obtained using different extraction solvents (ETP, DCM, EtOAc, and acetone) following BSTFA derivatization. Data are reported as relative chromatographic peak areas (%).

	ETP	DCM	EtOAc	Acetone
Palmitic Acid [a]	98.42	91.00	82.90	89.37
Oleic Acid [b]	1.58	6.11	3.09	3.72
Stearic acid (TMS derivative)	<LOQ [c]	1.61	0.85	<LOQ
1-Monopalmitin (2TMS derivative)	<LOQ	1.28	11.34	5.63
1-Monooleoylglycerol (2TMS derivative)	<LOQ	-	1.82	1.27

[a] Include free, TMS, and TBDMS form of Palmitic Acid. [b] Include free and TMS form of Oleic Acid. [c] Below the limit of quantification (<LOQ).

3.2.3. Influence of Solvent Polarity, Functional Implications, and Comparative Interpretation

The GC-MS profiles of wax extracts RS1 and RV1 demonstrate notable conservation across a range of four extraction solvents. In RS, palmitic acid is the most abundant acid, with percentage variations of up to 28% observed across the four extraction solvents, ranging from 56% in ETP to 84% in DCM. Given that DCM is also the solvent with the highest extraction percentage, these data suggest that palmitic acid is by far the most abundant component in the sample. It is noteworthy that the silylated component of palmitic acid is the most abundant in the various solvents, while free palmitic acid is only partially observed in DCM and acetone. The other fatty acid with a satisfactory degree of abundance is oleic acid (C18), the sole unsaturated fatty acid present in the sample. The presence of the substance was not detected in EtOAc, while its highest percentage was observed in the ETP extract. This suggests that the non-polarity of ETP favours its extraction. The other fatty acid identified is stearic acid (C18), which was observed especially in the ETP extract. This finding indicates that the two fatty acids possessing longer carbon chains are more readily extractable with ETP than with alternative solvents. The trace components include the monoglycerides of palmitic acid (in greater quantities) and oleic acid.

In the case of RV1, the content of the extracts is found to be almost identical, with minor variations. Palmitic acid is present in all extracts, with concentrations ranging from 96% in ETP to 83% in EtOAc. The unsaturated component, provided by oleic acid, never exceeds the 6% identified in DCM. The analysis revealed the presence of Stearic acid, albeit in negligible quantities. Conversely, RV1 exhibited a higher concentration of palmitic acid monoglyceride, with a maximum of 11% observed in EtOAc, the solvent that was identified as the optimal medium for extraction.

A comparison of the two samples, both of which were sourced from China and belonged to two different plant species, revealed a clear preponderance of palmitic acid in both instances. However, while oleic acid was found to be the predominant fatty acid in RV1, RS1 also exhibited significant percentages of oleic acid, as well as stearic acid. With

regard to monoglycerides, the palmitic acid derivative was manifestly more prevalent, albeit only at significant levels were attained in RV1.

The predominance of palmitic acid, as well as the presence, albeit in a minority, of oleic acids across all extracts, aligns with the reported compositions of plant waxes and lipid-based cosmetic ingredients [35], thus confirming their central role in such matrices.

From a functional perspective, the high abundance of palmitic acid contributes to emollient and occlusive properties, enhancing skin barrier integrity, while oleic acid is known to improve skin permeability and facilitate transdermal delivery of active compounds [36]. The presence of stearic acid, particularly in sample RS1, enhances its suitability as a texturising and stabilising agent in formulations, owing to its well-documented thickening and emulsifying properties. In addition, it has been demonstrated that the substance under discussion contributes to enhanced structural integrity and rheological behaviour. It functions effectively as a thickener and stabiliser in cosmetic applications [37]. Furthermore, minor components such as monoacylglycerols (e.g., 1-monopalmitin and 1-monooleoylglycerol) have been demonstrated to contribute to emulsification and formulation stability, despite their relatively low abundance [38].

From an analytical perspective, the presence of TMS and TBDMS derivatives is indicative of the derivatisation strategy employed to enhance the volatility and chromatographic performance of fatty acids in GC–MS analysis. The prevalence of TMS derivatives indicates enhanced derivatisation efficiency and compatibility for GC–MS analysis under the prevailing conditions. This finding is in accordance with standard lipid analysis protocols, wherein TMS derivatisation is extensively utilised to enhance the volatility and detectability of analytes [39,40]. The reproducibility of retention times and high-resolution values across all extracts is indicative of effective peak discrimination and evaluates the robustness of the analytical method [41].

It can be posited that the various solvents yield extraction patterns that are analogous, albeit not congruent. The disparities observed can be ascribed exclusively to the varying concentrations of the constituents present in the extracts. Consequently, in order to carry out a comprehensive analysis of all six samples, it was decided to continue analysing only the DCM extract.

3.3. Comparative GC–MS Analysis of DCM Extracts

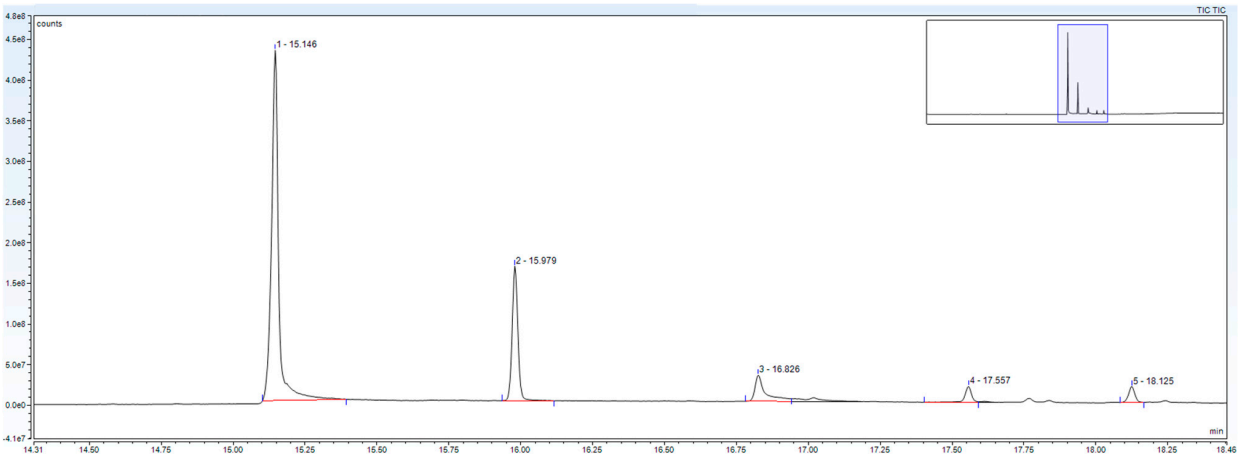
In view of the findings from analysing the two samples (RS and RV) from China with different extraction solvents, it was decided to compare only the DCM extracts for all available samples. DCM exhibited the highest extraction capacity, suggesting a more realistic composition of the various components for the different waxes. DCM combines moderate polarity with strong solvation ability toward long-chain lipids and fatty acids, allowing extraction of both non-polar hydrocarbons and slightly polar lipid species, which likely contributes to its superior extraction performance.

When comparing the three *R. succedanea* samples, it is observed that the sample from China (RS1) (Figure 3B, Table 1) and the sample from Vietnam (RS2) (Figures 5A and S9–S12, Table 3) have very similar percentages. Indeed, the addition of the values of palmitic acid and the derivative 1-monopalmitin, as well as oleic acid and 1-monooleoylglycerol, yields a composition of saturated fatty acid (palmitic acid) and monounsaturated component (oleic acid) that is almost identical. The outcome of this study is not unexpected, given that the regions of China and Vietnam are politically distinct yet geographically proximate. The findings from the gas chromatographic analysis substantiate the shared provenance of the two plants from which the wax is derived. In consideration of the wax derived from Japanese *R. succedanea* (RS3) (see Figures 5B and S13–S16, Table 3), it is evident that a higher concentration of monounsaturated fatty acids is present, in conjunction with the

presence of 2-methyleicosane, a branched saturated hydrocarbon composed of 21 carbon atoms. It has been established that analogous compounds are frequently associated with plant epicuticular waxes, thereby contributing to the protection of the plant surface. Its presence is both characteristic and of interest; indeed, in a recent study by Rabeh et al., it was observed that molecules from this family were found to be metabolic biomarkers of drought tolerance [42]. The increase in the presence of this molecule in Japanese fruit may be attributed to the country's geographical and meteorological isolation, a factor that has been demonstrated to influence the synthesis of the fruit's hydrophobic barrier, which plays a role in protecting the fruit from water loss during the ripening process [19,43,44].

A comparison of the three samples of *R. verniciflua* reveals a marked predominance of palmitic acid, accompanied by a variable percentage of monounsaturated acid (oleic acid). The three samples demonstrate a higher degree of differentiation. The palmitic acid and oleic acid composition of the samples derived from the Chinese (RV1) (Figure 4B, Table 2) and Korean (RV2) plants (Figures 5C and S17–S20, Table 3) bracket that of the sample derived from Japan (RV3) (Figures 5D and S21–S24, Table 3), which exhibits an intermediate composition between the two. The presence of stearic acid, a saturated fatty acid with a longer chain than palmitic acid (C18 vs. C16), was observed in both continental samples. In this case, it is more challenging to identify an origin with greater intraspecific variability, as evidenced by the Japanese sample's lack of stearic acid in comparison to the Chinese and Korean samples. However, when considering the composition of palmitic acid and oleic acid, the Korean sample is the most unique. In this case, it should be noted that, although Korea is geographically adjacent to China, it is also geographically unique.

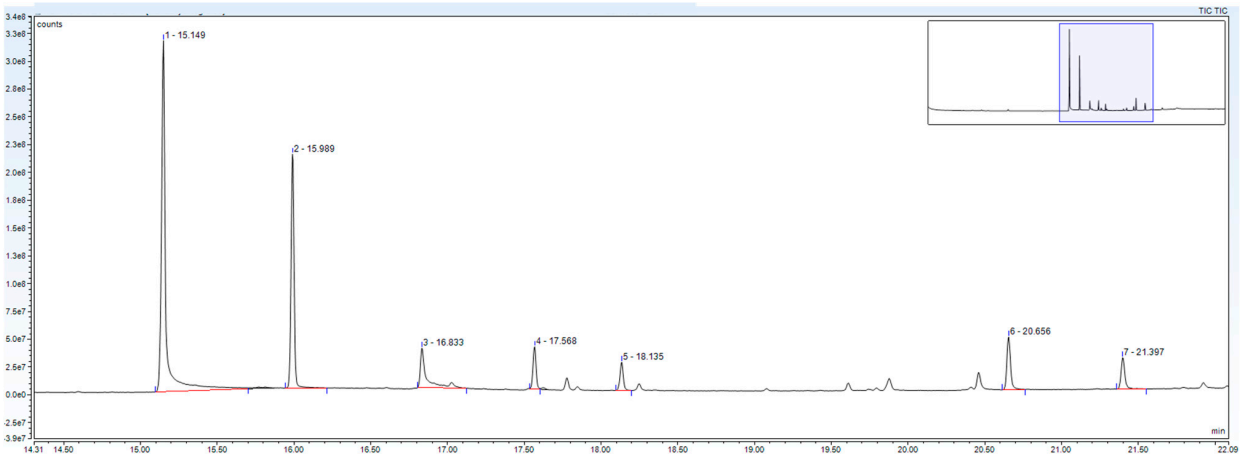
(**A**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	15.146	Palmitic Acid	1.23×10^7	4.31×10^8	66.36	871	22.96
2	15.979	Palmitic Acid TMS derivative	3.84×10^6	1.66×10^8	20.72	876	21.02
3	16.826	Oleic Acid	1.37×10^6	3.22×10^7	7.40	862	17.67
4	17.557	Oleic Acid, (Z)- TMS derivative	5.29×10^5	1.96×10^7	2.85	802	14.93
5	18.125	Palmitic Acid TBDMS derivative	4.92×10^5	1.95×10^7	2.65	784	

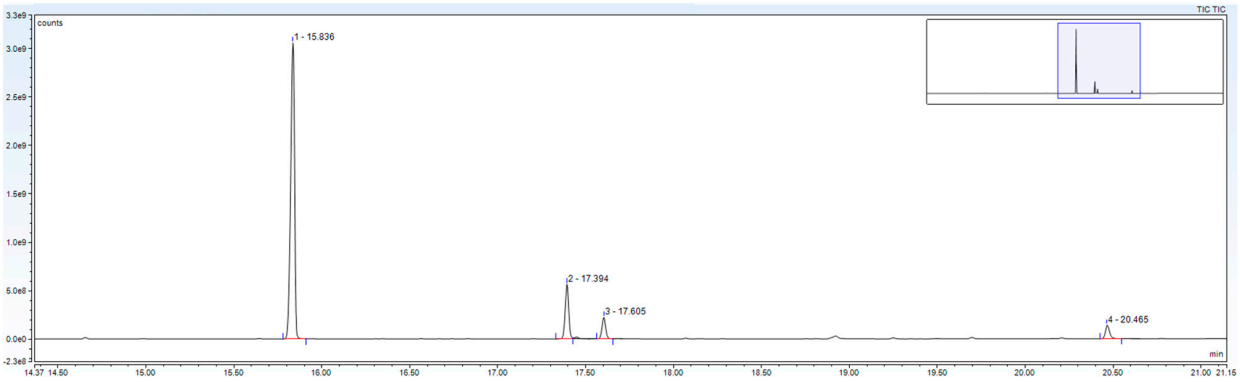
Figure 5. *Cont.*

(**B**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	15.149	Palmitic Acid	1.04×10^{7}	3.16×10^{8}	50.36	869	23.12
2	15.989	Palmitic Acid TMS derivative	4.85×10^{6}	2.10×10^{8}	23.51	893	21.45
3	16.833	Oleic Acid	1.70×10^{6}	3.55×10^{7}	8.22	867	18.37
4	17.568	Oleic Acid, (Z)- TMS derivative	8.89×10^{5}	3.81×10^{7}	4.31	849	15.39
5	18.135	Palmitic Acid TBDMS derivative	6.25×10^{5}	2.54×10^{7}	3.03	787	63.00
6	20.656	1-Monopalmitin 2TMS derivative	1.34×10^{6}	4.76×10^{7}	6.51	718	17.43
7	21.397	Eicosane, 2-methyl-	8.35×10^{5}	2.83×10^{7}	4.05	793	

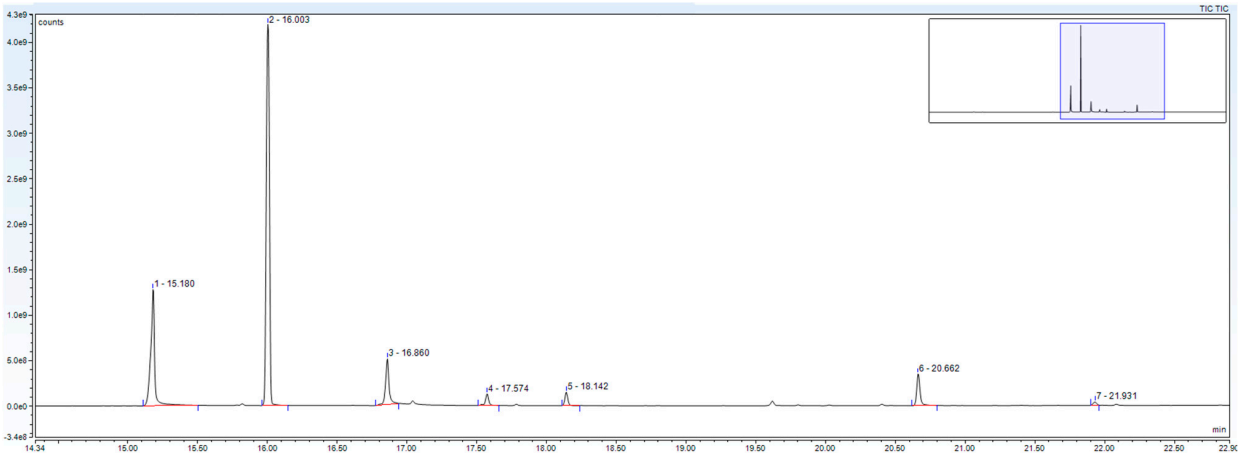
(**C**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	15.836	Palmitic Acid TMS derivative	7.87×10^{7}	3.06×10^{9}	77.93	772	40.65
2	17.394	Oleic Acid, (Z)- TMS derivative	1.31×10^{7}	5.67×10^{8}	12.97	820	5.75
3	17.605	Stearic acid TMS derivative	5.28×10^{6}	2.22×10^{8}	5.23	776	73.82
4	20.465	1-Monopalmitin 2TMS derivative	3.92×10^{6}	1.43×10^{8}	3.88	593	

Figure 5. *Cont.*

(**D**)



	Ret. Time (min)	Peak Name	Area (Counts × min)	Height (Counts)	Rel. Area (%)	SI	Resolution
1	15.180	Palmitic Acid	4.17×10^7	1.28×10^9	22.62	865	19.06
2	16.003	Palmitic Acid TMS derivative	1.11×10^8	4.19×10^9	60.03	908	20.79
3	16.860	Oleic Acid	1.45×10^7	5.03×10^8	7.87	898	18.84
4	17.574	Oleic Acid, (Z)- TMS derivative	3.46×10^6	1.28×10^8	1.88	872	15.28
5	18.142	Palmitic Acid TBDMS derivative	3.48×10^6	1.48×10^8	1.89	888	65.15
6	20.662	1-Monopalmitin 2TMS derivative	9.51×10^6	3.49×10^8	5.16	858	29.37
7	21.931	1-Monooleoylglycerol 2TMS derivative	1.01×10^6	3.74×10^7	0.55	806	

Figure 5. Enlarged GC–MS chromatograms of DCM extracts obtained from *R. succedanea* RS2 (**A**) and RS3 (**B**), and *R. verniciflua* RV2 (**C**) and RV3 (**D**) wax samples following BSTFA derivatization. The expanded chromatographic regions highlight the major constituents detected in the wax matrix, including fatty acids, monoacylglycerol derivatives, and minor hydrocarbon components. Peak numbers shown in the chromatograms correspond to those reported in the associated peak assignment table, which includes retention time (min), compound name, peak area (Counts·min), peak height (Counts), relative area (%), similarity index (SI), and chromatographic resolution (Rs). The chromatograms illustrate solvent-dependent differences in the relative abundance of individual components while maintaining a generally consistent compositional profile across extracts, thereby facilitating comparison between chromatographic features and tentatively assigned compounds.

A comparison of the two species, *R. succedanea* and *R. verniciflua*, reveals no significant differences in chemical composition, despite the different origins of the waxes (from the fruits of *R. succedanea* and from the berries of *R. verniciflua*). In all cases, the most abundant compound is palmitic acid, which varies in percentages from 80 to 90%, followed by oleic acid, which provides greater fluidity to the wax. The presence of stearic acid has been observed in the *R. verniciflua* samples from China and Korea; conversely, the *R. succedanea* sample from Japan has been found to contain 2-methyleicosane.

It is evident, therefore, that the compositional profiles observed in all samples indicate that the plant waxes examined are significantly rich in saturated fatty acids, particularly palmitic acid, which is known to confer structural rigidity, high melting points, and oxidative stability [31]. These characteristics are advantageous for cosmetic formulations, including sticks, balms, and creams [45]. This observation is consistent with the prevailing composition of plant waxes.

Nevertheless, the presence of oleic acid has been demonstrated to enhance the emollient properties, skin absorption and spreadability of the waxes when utilised for cosmetic applications.

Table 3. Comparative relative area percentages of the major lipid constituents identified in DCM extracts of *R. succedanea* (RS1–RS3) and *R. verniciflua* (RV1–RV3) samples from different geographical origins, determined by GC–MS after BSTFA derivatization. Values are expressed as relative chromatographic peak areas (%).

	RS1	RS2	RS3	RV1	RV2	RV3
Palmitic Acid [a]	86.58	89.74	76.91	91.00	77.93	84.54
Oleic Acid [b]	8.28	10.26	12.53	6.11	12.97	9.75
1-Monopalmitin (2TMS derivative)	3.13	<LOQ [c]	6.51	1.28	3.88	5.16
1-Monooleoylglycerol (2TMS derivative)	2.01	<LOQ	<LOQ	<LOQ	<LOQ	0.55
Eicosane, 2-methyl-	<LOQ	<LOQ	4.05	<LOQ	<LOQ	<LOQ
Stearic acid (TMS derivative)	<LOQ	<LOQ	<LOQ	1.61	5.23	<LOQ

[a] Include free, TMS, and TBDMS form of Palmitic Acid. [b] Include free and TMS form of Oleic Acid. [c] Below the limit of quantification (<LOQ).

4. Conclusions

The present study successfully developed and evaluated a reproducible GC-MS-based analytical procedure for the compositional profiling of cosmetic plant waxes derived from *R. succedanea* and *R. verniciflua*. The combination of solvent extraction, BSTFA derivatisation, and GC–MS analysis yielded robust and consistent chromatographic profiles. This facilitated tentative compound assignment and semi-quantitative assessment of the major lipid constituents. The low variability observed among extraction replicates, in conjunction with the effective chromatographic discrimination of lipid species, serves to demonstrate the suitability of the proposed workflow for routine analytical applications.

The findings indicated that DCM was the most effective solvent for optimising the extraction process. Furthermore, GC-MS analyses demonstrated that all samples were dominated by palmitic acid, constituting approximately 80–90% of the total lipid fraction. It is important to note that the proposed workflow demonstrated sufficient sensitivity and reproducibility to detect compositional differences. Even though the overall lipid composition remained consistent across the various samples, discernible variations were identified among waxes originating from China, Vietnam, Korea, and Japan. These variations encompassed differences in the distribution of fatty acids and the presence of minor marker compounds. The findings indicate that compositional differences were observed among samples obtained from different sources and regions. Further studies including larger sample sets would be required to determine the specific influence of geographical origin.

The developed methodology is a valuable instrument for comparative compositional evaluation and traceability of cosmetic plant waxes. The capacity to generate reproducible compositional fingerprints lends support to the notion of its potential application in the domains of raw-material verification, supplier qualification, and the detection of adulteration in cosmetic supply chains.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/analytica7030044/s1, Table S1. Extraction yield (mean ± SD) of samples from *Rhus succedanea* (RS1–RS3) and *Rhus verniciflua* (RV1–RV3) obtained using different solvents: ETP, DCM, EtOAc, and Acetone. GC–MS characterization of the BSTFA-derivatized wax extract. The figure comprises (A) the full GC–MS total ion chromatogram (TIC), and (B) the

corresponding peak assignment table. For each peak, retention time (min), compound name, peak area (Counts·min), peak height (Counts), relative area (%), similarity index (SI), and chromatographic resolution (Rs) are reported. Peak numbers shown in the chromatograms correspond to those listed in the identification table, enabling straightforward comparison between chromatographic features and assigned compounds. Figures S1–S4. GC–MS chromatograms of *R. succedanea* RS1 wax extracts. Figures S5–S8. GC–MS chromatograms of *R. verniciflua* RV1 wax extracts. Figures S9–S12. GC–MS chromatograms of *R. succedanea* RS2 wax extracts. Figures S13–S16. GC–MS chromatograms of *R. succedanea* RS3 wax extracts. Figures S17–S20. GC–MS chromatograms of *R. verniciflua* RV2 wax extracts. Figures S21–S24. GC–MS chromatograms of *R. verniciflua* RV3 wax extracts.

Author Contributions: Conceptualization, S.C. and E.C.; methodology, M.L. and M.C.M.; writing—original draft preparation, E.C. and M.C.M.; writing—review and editing, S.C.; All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in the article.

Conflicts of Interest: Authors Manuela Loiacono and Simone Conti were employed by the company Roelmi HPC. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

<LOQ	Below the limit of quantification
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
DCM	Dichloromethane
EtOAc	Ethyl acetate
ETP	Petroleum ether
GC-MS	Gas chromatography coupled with mass spectrometry
RT	Room temperature
SI	Similarity Index
TBDMS	tert-butyldimethylsilyl
TMS	Trimethylsilyl

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