

Article

UPLC-QTOF-ESI-MS/MS-Based Comparative Study of Phytochemicals in *Sapindus mukorossi*

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Abstract: This study examined the phytochemical compositions of ten *Sapindus mukorossi* samples from two regions in Korea: Suwon and Daegu. The Folin–Ciocalteu method was used to calculate the total polyphenol content (TPC). Among all extracts tested, leaf samples from Suwon and Daegu (SLE and DLE) exhibited the highest TPC at 2.70 and 2.90 mg tannic acid equivalent/g extract. Similarly, a modified aluminum chloride colorimetric test was used to determine the total flavonoid content (TFC). Similar results were obtained, with SLE and DLE having TFC values of 40.71 and 41.07 mg quercetin equivalent/g extract, respectively. Liquid chromatography with tandem mass spectrometry was used to detect 13 compounds, whereas high-performance liquid chromatography was used to quantify the prominent compounds: rutin, nicotiflorin, and narcissin. Among these, rutin was the most abundant, especially in SLE and DLE (54.37 and 70.21 mg/g, respectively). Furthermore, rutin significantly contributed to the total content of these samples at 78.31 and 85.44 mg/g, respectively. There were significant variations in the distribution of these compounds across different parts of the plant. These findings highlight the importance of *S. mukorossi* as a source of natural bioactive chemicals and pave the way for further research into its potential applications in healthcare products.



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Keywords: flavonoid glycosides; saponin; UPLC-QTOF-ESI-MS/MS; rutin; phytochemicals

1. Introduction

The soap nut tree, also known as *Sapindus mukorossi* is a deciduous tree belonging to the Sapindaceae family [1]. This species is native to South-Central and Southeast China and has been introduced to various regions, including India, Japan, Korea, and Nepal [2]. In recent years, there has been increasing emphasis on exploring plant-derived bioactive compounds as natural alternatives to synthetic antioxidants and therapeutic agents [3]. This global trend aligns with the growing consumer preference for sustainable and eco-friendly products in pharmaceuticals, nutraceuticals, and cosmetics. *S. mukorossi*, with its diverse phytochemical profile, represents an untapped resource to address these demands. It has been a staple in Traditional Chinese Medicine for centuries [4], highlighting its potential as a source of bioactive compounds with therapeutic value. Recent studies have shown that *S. mukorossi* possesses antipyretic, anti-inflammatory, antioxidant, and analgesic properties [5].

Beyond these medicinal applications, *S. mukorossi* has been traditionally used in the aforementioned countries for centuries mainly due to its abstergent properties [6]. This is

attributed to the abundance of saponins in the pericarps of its fruit. Saponins are the primary phytochemicals found in soap nuts [7]. These medicinally important phytochemicals play a key role in plant defense against bacteria [8]. Furthermore, saponins are used in industries that manufacture detergents and shampoos due to their surfactant properties [9].

Although the saponins of *S. mukorossi* are well-documented, less attention has been paid to its other bioactive constituents, particularly flavonoids. These secondary metabolites exhibit antioxidant, anti-inflammatory, and therapeutic properties [10]. Their relevance to health and industry underscores the importance of further exploration into the phytochemical composition of *S. mukorossi*. Despite the extensive traditional and industrial use of this plant, detailed studies on the flavonoid glycosides present in various parts of the plant are lacking [11]. Previous research has primarily focused on saponin content, leaving significant gaps in the profiling and quantification of other bioactive compounds [12]. Additionally, variations in these phytochemicals across different plant parts (e.g., leaves, fruits, and seeds) have not been comprehensively examined. Addressing these gaps is essential to fully elucidate the bioactive potential of *S. mukorossi*.

Flavonoid glycosides, such as rutin, nicotiflorin, and narcissin, are bioactive compounds with potent antioxidant properties [13–15]. They play crucial roles in reducing oxidative stress and have potential applications in pharmaceuticals, nutraceuticals, and cosmetics. Investigating these specific compounds allows for a deeper understanding of the therapeutic potential of *S. mukorossi* and paves the way for its future applications in various industries.

This study aimed to address these gaps by extracting and analyzing the compounds present in various parts of *S. mukorossi* using colorimetric assays and ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-ESI-MS/MS), quantifying the identified flavonoid glycosides using high-performance liquid chromatography (HPLC), and comparing the phytochemical content across different plant parts to identify patterns and potential applications.

2. Materials and Methods

2.1. Plant Materials

The *S. mukorossi* samples were acquired from the Korea National Arboretum, Pocheon, Republic of Korea. The samples were named according to the plant part used, and their geographical locations were the National Institute of Forest Science, Suwon, and Kyungpook National University, Daegu, Republic of Korea (Figure 1). The samples from Suwon, Republic of Korea, are referred to as leaf (SLE), stem (SST), fruit receptacle (SFR), flesh (SPF), and seed (SSE), whereas the samples from Daegu, Republic of Korea, are referred to as leaf (DLE), stem (DST), fruit receptacle (DFR), flesh (DPF), and seed (DSE).

2.2. Instruments and Reagents

Chromatographic analysis was performed using a Waters Alliance e2695 separation module (Milford, MA, USA) equipped with a Waters 2998 photodiode array (PDA) detector, quaternary pump, and autosampler. The HPLC solvents (water and acetonitrile (ACN)) were purchased from Honeywell (Burdick and Jackson, Muskegon, MI, USA), and trifluoroacetic acid (TFA) was purchased from J. T. Baker (Phillipsburg, PA, USA). Standard compounds rutin (1), nicotiflorin (2), and narcissin (3) were procured from the Natural Product Institute of Science and Technology (www.nist.re.kr; accessed on 12 January 2025), Anseong, Republic of Korea (Figure 2).

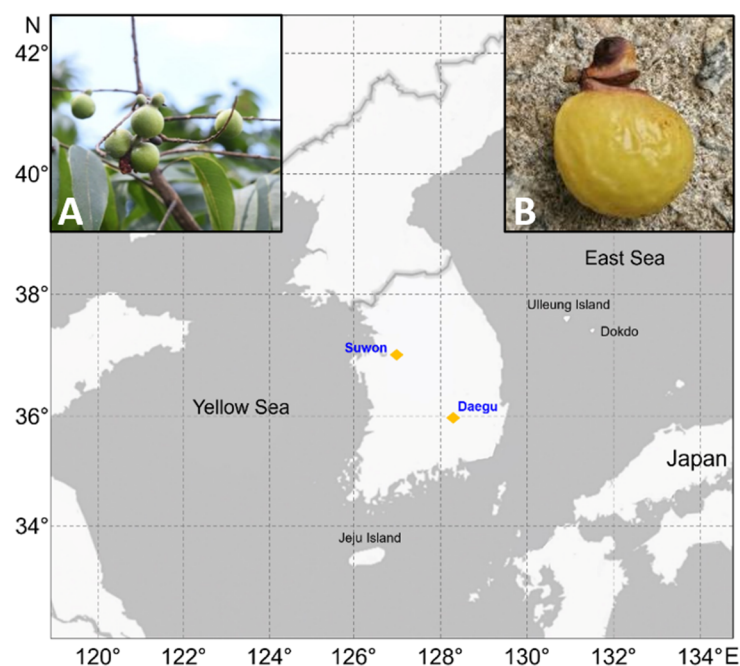


Figure 1. The collection sites of the whole *S. mukorossi* plant (A) and its fruit (B) that were used in the study.

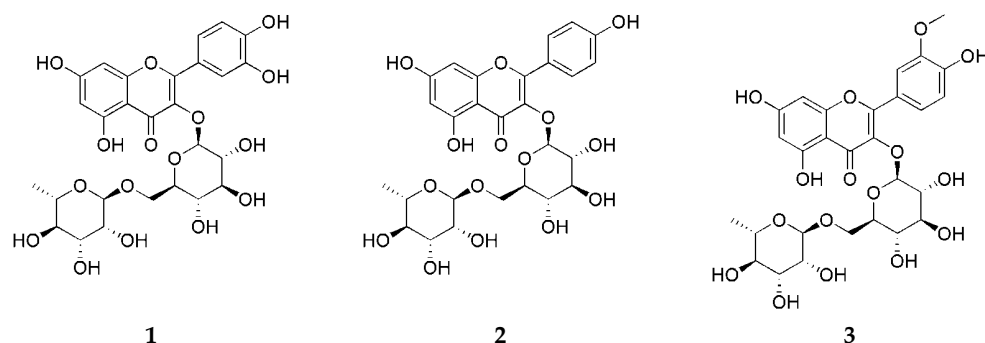


Figure 2. Chemical structures of the standard compounds rutin (1), nicotiflorin (2), and narcissin (3).

2.3. Crude Extraction

Ten grams of homogenized air-dried samples were combined with 200 mL of 95% ethanol. The mixture was then refluxed for 5 h at 80 °C three times. The extracts were filtered and subsequently concentrated in a vacuum with a rotary evaporator (Eyela, Tokyo, Japan) at 55 °C to obtain concentrated EtOH extracts. The extracts were set aside until further use.

2.4. Total Polyphenol Content Assay

The TPC of the ten *S. mukorossi* extracts was determined using a previously described method with minimal changes [16]. First, a stock solution (2.5 mg/mL) of each sample was serially diluted to achieve three concentrations (2.5–0.63 mg/mL). Next, 60 µL of each sample was placed in the 96-well microtiter plate. Then, 40 µL of Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) was added to wells containing the samples. After adding 100 µL of 7.5% sodium carbonate (Na_2CO_3), the samples were left to react for 30 min at 37 °C in the dark. Subsequently, the samples' absorbance was measured at 760 nm using a microplate reader (Epoch; BioTek, Winooski, VT, USA). The TPC was then constructed using a standard curve built with various tannic acid concentrations (200–12.5 µg/mL).

2.5. Total Flavonoid Content Assay

The TFC of the extracts was determined using a modified method adapted from a previous study [16]. First, a stock solution (2.5 mg/mL) of each sample was serially diluted to achieve three concentrations (2.5–0.63 mg/mL). Subsequently, 100 µL of each diluted sample was transferred into the wells of a 96-well microtiter plate. An equal volume (100 µL) of 2% aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) solution was added to the wells. The plate was incubated at room temperature for 10 min and the absorbance was recorded at 430 nm using a microplate reader (Epoch; BioTek, Winooski, VT, USA). The TFC was then calculated using a standard curve generated using various quercetin concentrations (200–12.5 µg/mL).

2.6. Phytochemical Detection by Ultra-Performance Liquid Chromatography (UPLC), Quadrupole Time-of-Flight Mass Spectrometry (QTOF-MS), and Electrospray Ionization (ESI) (UPLC-QTOF-ESI-MS/MS)

The analysis was conducted utilizing an LC system with Thermo Vanquish UHPLC equipment (Thermo Fisher Scientific, Waltham, MA, USA) fitted with a Waters Cortex T3 column (150 mm × 2.1 mm, particle size 1.6 µm). The column temperature was maintained at 45 °C. The mobile phase consisted of acetonitrile (eluent B: 0.1% HCOOH) and water (eluent A: 0.1% HCOOH). A flow rate of 0.25 mL/min and a gradient elution program (97% A from 0 to 0.5 min, 85% A at 15 min, 0% A from 50 to 55 min, and 3% B from 55.1 to 60 min) were employed. For spectrometric analysis, a Triple TOF 5600+ System (AB SCIEX, Marlborough, MA, USA) equipped with a heated electrospray ion source was used. The mass spectrometer was run in both positive and negative ion modes to produce survey full-scan MS spectra (m/z 100–1500) using a quadrupole system at a resolution of 70,000. The spray voltage was set to 3.5 kV in positive ion mode. The 13 most energetic precursor ions were chosen for MS2 fragmentation at a resolution of 17,500. The MS specifications also included a capillary temperature of 320 °C, a sheath gas of 50 AU, a sweep gas of 0 AU, and an auxiliary gas of 10 AU.

2.7. Quantification of Flavonoid Glycosides by HPLC

The HPLC analysis was conducted using a Waters Alliance e2695 separation module (Milford, MA, USA) coupled with a Waters 2998 PDA detector, following a previously described method with modifications [17]. The separation was performed on an INNO C18 column (25 cm × 4.6 mm, 5 µm). The mobile phase of the gradient elution system consisted of 0.1% TFA in water (A) and ACN (B), with the following gradient elution system: 95% A from 0 to 10 min, 0% A at 40 min, and 95% B from 50 to 60 min. The sample injection volume was 10 µL, with a flow rate of 1.0 mL/min. Detection was performed at 254 nm.

2.8. Calibration Curves

Standard stock solutions were prepared by dissolving the reference compounds in methanol (MeOH) to generate a concentration of 1 mg/mL. Working solutions used to construct the calibration curve were prepared by serially diluting the selected stock solutions to the desired concentrations (0.500–0.03125 mg/mL). The samples were dissolved in the same solvent and each extract had a final concentration of 15 mg/mL. The standard and sample solutions were filtered using a 0.45 µm polyvinylidene fluoride (PVDF) filter before use. Calibration functions for the standard compounds were calculated based on peak areas (Y), concentrations (X, µg/10 µL), and mean values ± standard deviation (SD) ($n = 3$).

2.9. Statistical Analysis

The results are expressed as mean $\pm$ SD, and all analyses were conducted in triplicate. Data were normalized using one-way analysis of variance (ANOVA) and Tukey's post hoc test. All statistical tests were performed using GraphPad Prism 8.0.2 software (GraphPad Software, Boston, MA, USA). p -values < 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Total Polyphenol and Total Flavonoid (TPC and TFC) Content

The TPC of *S. mukorossi* extracts SLE, SST, SFR, SPF, SSE, DLE, DST, DFR, DPF, and DSE were evaluated. The results varied significantly (Figure 3). Generally, SLE and DLE exhibited the highest TPC among all the extracts with values of 2.70 mg tannic acid equivalent (TAE)/g extract and 2.90 mg TAE/g extract, respectively. This was followed by SST (2.38 mg TAE/g extract), DFR (2.11 mg TAE/g extract), and DST (1.66 mg TAE/g extract). In comparison, the remaining samples exhibited significantly lower TPCs with DPE, SSE, SPE, DSE, and SFR showing values of 0.43 mg TAE/g extract, 0.35 mg TAE/g extract, 0.30 mg TAE/g extract, 0.06 mg TAE/g extract, and 0.04 mg TAE/g extract, respectively, indicating minimal polyphenolic contributions to the overall phytochemical profile.

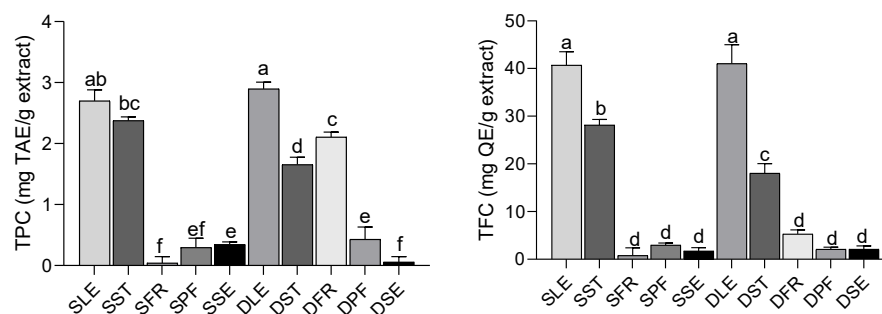


Figure 3. Total polyphenol and total flavonoid content (TPC and TFC) of the different *S. mukorossi* extracts. TAE, tannic acid equivalent; QE, quercetin equivalent. The results are expressed as mean $\pm$ SD. Different letters in each column indicate significant differences at $p < 0.05$.

Similarly, the total flavonoid content (TFC) of the tested extracts followed a similar trend to TPC with SLE and DLE exhibiting the highest TFC values at 40.71 mg quercetin equivalent (QE)/g extract and 41.07 mg QE/g extract, respectively. This was followed by SST (28.14 mg QE/g extract), DST (18.08 mg QE/g extract), DFR (5.33 mg QE/g extract), SPE (2.99 mg QE/g extract), DPE (2.10 mg QE/g extract), DSE (2.09 mg QE/g extract), SSE (1.74 mg QE/g extract), and SFR (0.80 mg QE/g extract). A previous study by Singh and Kumari (2015) [18] examined the TPC and TFC of *S. mukorossi* leaves and fruits extracted with different solvents (ethanol, methanol, and acetone). The results showed that the TPC of the leaves was highest when extracted with water compared to methanol and ethanol, whereas the TFC of the leaves would be highest if extracted with ethanol. For the fruits, TPC was highest when extracted with methanol, whereas TFC was highest in the ethanolic extracts. Conversely, a study by the same authors that analyzed the callus of the plant reported that ethanol extraction yielded high TPC, whereas methanol extraction gave the highest TFC compared to ethanol and water. Another study evaluated the TPC and TFC of *S. mukorossi* partitioned using solvents of varying polarities [19]. Compared to the results of the present study, their TPC values were comparable to those of TFC. Differences in methods and samples may explain the variation in results between these studies and the present work. Furthermore, the present study used only ethanol as the extraction solvent, which is widely used and generally recognized as safe (GRAS) [20].

3.2. UPLC-QTOF-ESI-MS/MS Profiling

To characterize the flavonoid glycosides in *S. mukorossi* extracts, a representative sample (SLE) was subjected to ultra-performance liquid chromatography-quadrupole time-of-flight-electrospray ionization-mass spectrometry/mass spectrometry (UPLC-QTOF-ESI-MS/MS), resulting in the emergence of three prominent peaks. The base peak chromatogram and the MS² spectra of these peaks in SLE are shown in Figure 4.

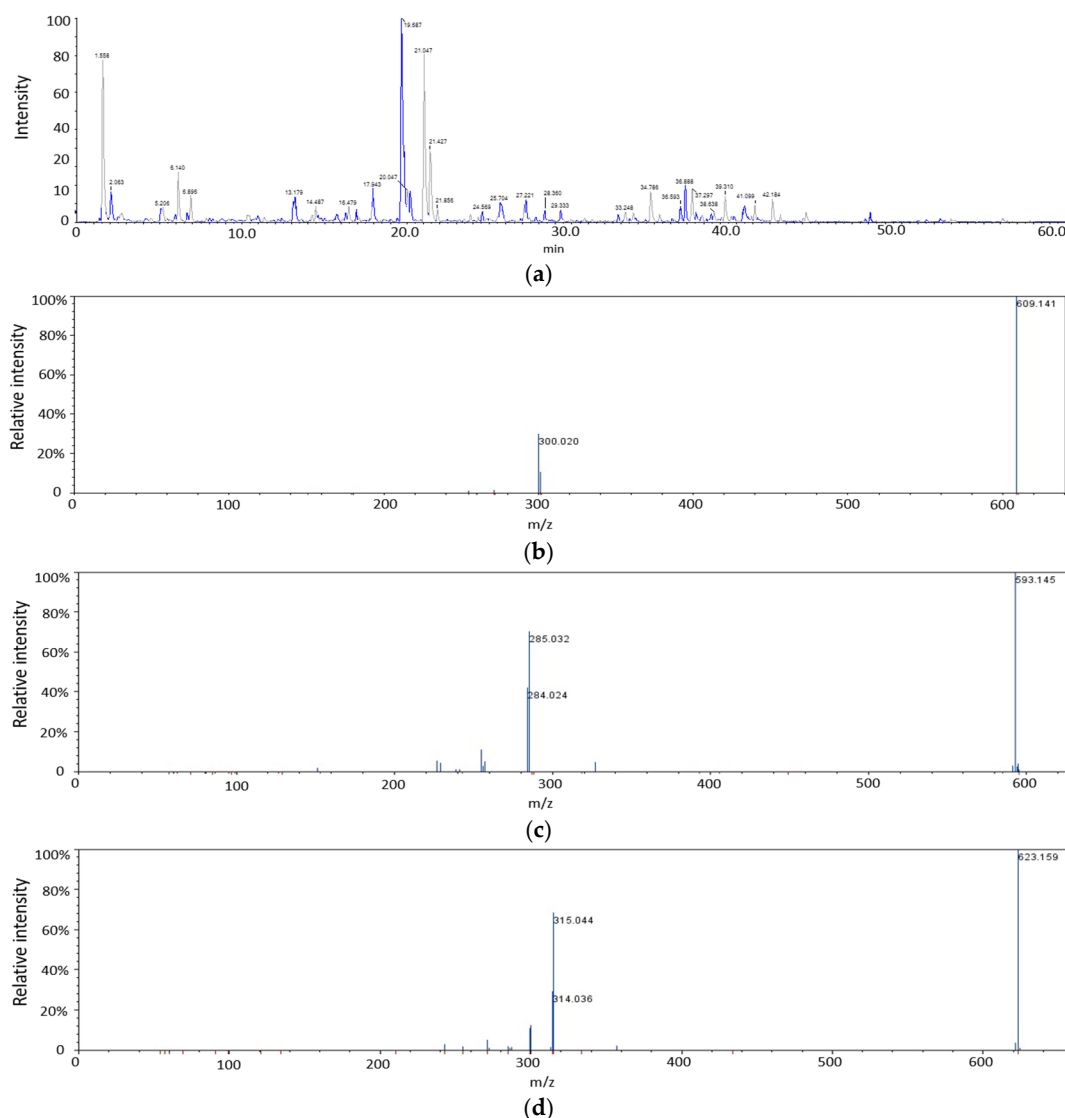


Figure 4. Base peak chromatogram (a) of SLE analyzed in negative ionization mode and the MS² spectra of rutin (b), nicotiflorin (c), and narcissin (d).

A UPLC-QTOF-ESI-MS/MS system was employed for these studies. This versatile and highly sensitive technique enabled the detection of the 13 most energetic peaks, which were identified by comparing their molecular weights and fragmentation patterns with spectral libraries. All phytochemicals were flavonoid glycosides (Table 1).

Peak 1 (molecular weight = 772.2) eluted at 13.60 min and was identified as quercetin 3-rutinoside-7-glucoside. The fragmentation pattern exhibited classic losses of rhamnose and glucose, indicating the presence of two sugar moieties linked to the quercetin backbone. Similarly, Peak 2, with a retention time of 15.71 min and a molecular weight of 594.2, was identified as vicianin-2, a C-glycosyl flavonoid [21]. Its MS² spectrum supported its identity as a di-C-glycosyl apigenin derivative by exhibiting neutral losses of glucose [22]. Peak 3

was identified as schaftoside based on its molecular weight of 564.1 and fragment ions at m/z 443.1 and m/z 353.1, suggesting consecutive sugar moiety losses, consistent with its glycosylated structure [23].

Table 1. Proposed structures by UPLC-QTOF-ESI-MS/MS analysis (negative mode).

^a t_R	Molecular Formula	Molecular Weight	Tentative Identification
13.60	C ₃₃ H ₄₀ O ₂₁	772.2	Quercetin 3-rutinoside-7-glucoside
15.71	C ₂₇ H ₃₀ O ₁₅	594.2	Vicenin-2
18.00	C ₂₆ H ₂₈ O ₁₄	564.1	Schaftoside
19.67	C ₂₇ H ₃₀ O ₁₆	610.2	Rutin
20.06	C ₂₁ H ₂₀ O ₁₂	464.1	Hyperoside
21.04	C ₂₇ H ₃₀ O ₁₅	594.2	Nicotiflorin
21.40	C ₂₈ H ₃₂ O ₁₆	624.2	Narcissin

^a Retention time.

Peak 4 with a molecular weight of 610.2 eluted at 19.67 min and was identified as rutin (quercetin-3-rutinoside) [24]. Its fragmentation pattern showed distinct losses of rhamnose and glucose, supporting its identity. Peak 5, observed at 20.06 min with a molecular weight of 464.1, was identified as hyperoside (quercetin-3-galactoside) based on the loss of galactose in its fragmentation spectrum [25]. Peak 6, with a molecular weight of 594.2 was identified as nicotiflorin (kaempferol-3-rutinoside) and eluted at 21.04 min [26]. Its MS² spectrum displayed fragment ions, indicating the loss of rutinose. Peak 7 with a molecular weight of 624.2, was tentatively identified as narcissin (isorhamnetin-3-rutinoside) due to the loss of rhamnose during fragmentation, further confirming its structure [27].

A previous study used LC-MS/MS to rapidly identify the phytochemical constituents of the fruits of *S. mukorossi* [28], detecting 31 compounds, of which 16 were acyclic sesquiterpene oligoglycosides and 15 were triterpenoid saponins. These results were not unexpected, as the fruits of this plant are the ones most utilized because of their rich saponin content [29].

3.3. HPLC Quantitative Analysis

To quantify the prominent compounds in the extracts, high-performance liquid chromatography was employed. The analysis quantified three major flavonoid glycosides, rutin (1), nicotiflorin (2), and narcissin (3) from the extracts of *S. mukorossi* collected from the Suwon and Daegu regions. With the methods employed, the standard compounds demonstrated good retention times and linearity (Figure 5, Table 2).

The results revealed variations in their contents across different plant parts (Figure 6). In Suwon samples, the highest rutin (1) content (54.37 mg/g) was observed in the leaf extract (SLE), contributing to a total content of 78.31 mg/g (Table 3). Nicotiflorin (2) and narcissin (3) were also abundant in SLE at 14.25 mg/g and 5.19 mg/g, respectively.

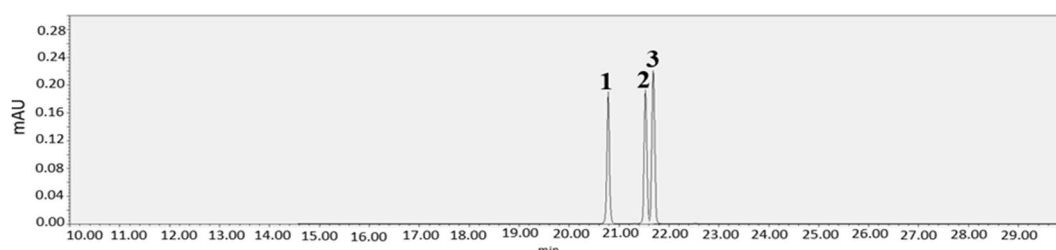
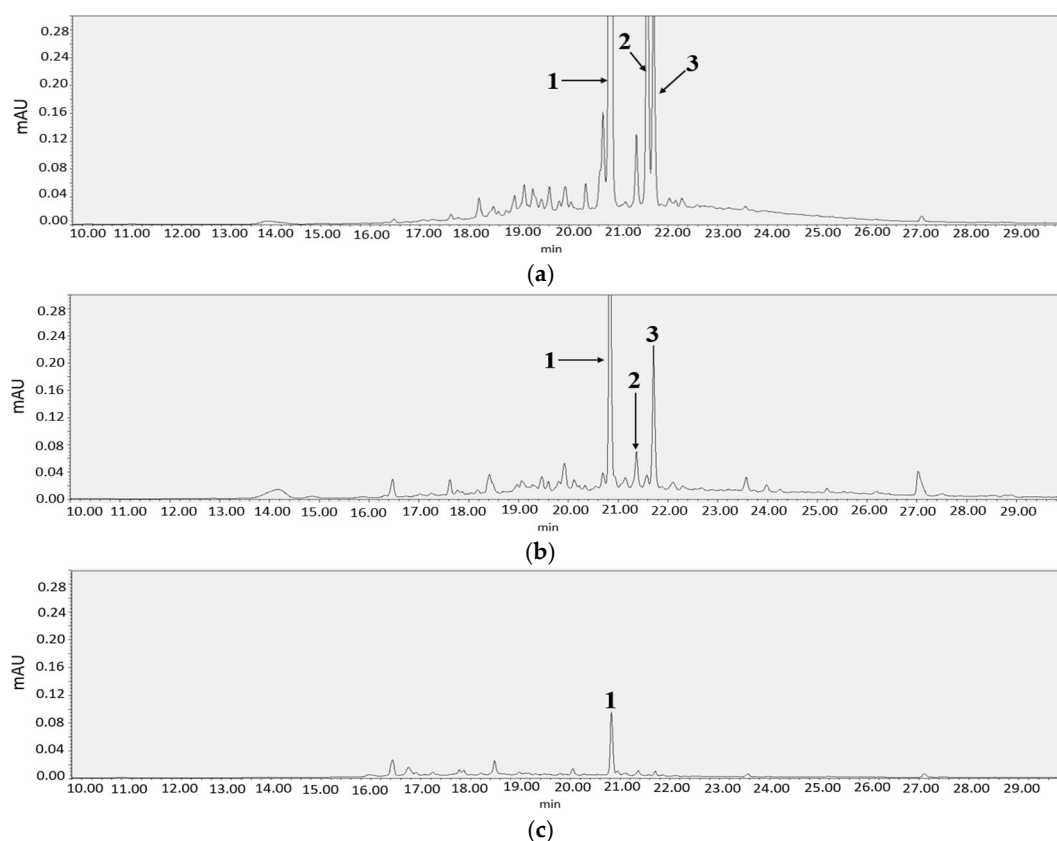


Figure 5. High-performance liquid chromatography (HPLC) chromatogram of the standard compounds: rutin (1), nicotiflorin (2), and narcissin (3).

Table 2. Calibration data for rutin (1), nicotiflorin (2), and narcissin (3).

Compound	^a t _R	Regression Equation	^b R ²
1	20.77	y = 9846.2x + 95,280	0.9991
2	21.51	y = 10,370x + 72,185	0.9997
3	21.63	y = 16,616x + 323,207	0.9983

^a Retention time. ^b Coefficient of determination.

**Figure 6.** HPLC chromatograms of SLE (a), SST (b), and SPF (c) showing peaks corresponding to rutin (1), nicotiflorin (2), and narcissin (3).**Table 3.** HPLC quantification of rutin (1), nicotiflorin (2), and narcissin (3) in each *S. mukorossi* extract.

Sample	Content (mg/g)			Total
	1	2	3	
SLE	54.37 ± 0.63 ^b	14.25 ± 0.38 ^a	5.19 ± 0.25 ^a	78.31
SST	11.59 ± 0.10 ^c	0.92 ± 0.04 ^c	2.78 ± 0.04 ^c	15.29
SFR	¹ ND	ND	ND	ND
SPF	1.89 ± 0.07 ^f	tr	tr	1.89
SSE	² tr	tr	tr	tr
DLE	70.21 ± 0.23 ^a	11.21 ± 0.11 ^b	4.02 ± 0.05 ^b	85.44
DST	5.89 ± 0.65 ^d	0.41 ± 0.04 ^d	0.76 ± 0.08 ^d	7.06
DFR	4.71 ± 0.20 ^e	0.02 ± 0.12 ^d	0.62 ± 0.17 ^d	5.35
DPF	tr	tr	tr	tr
DSE	ND	ND	ND	ND

¹ Not detected. ² Trace. Different lower-case letters in each column indicate statistically significant differences at $p < 0.05$.

Conversely, SST exhibited significantly lower levels of all three compounds, with a total content of 15.29 mg/g. In other plant parts, including the SFR, SPF, and SSE, most

compounds were either not detected (ND) or present in trace amounts (tr), with the total content in SPF being only 1.89 mg/g. These results highlight that the leaves were the richest source of flavonoid glycosides among the Suwon samples.

Similarly, Daegu samples exhibited a similar pattern, where DLE contained the highest rutin (1) content (70.21 mg/g), significantly surpassing other plant parts and contributing to a total content of 85.44 mg/g (Figure 7).

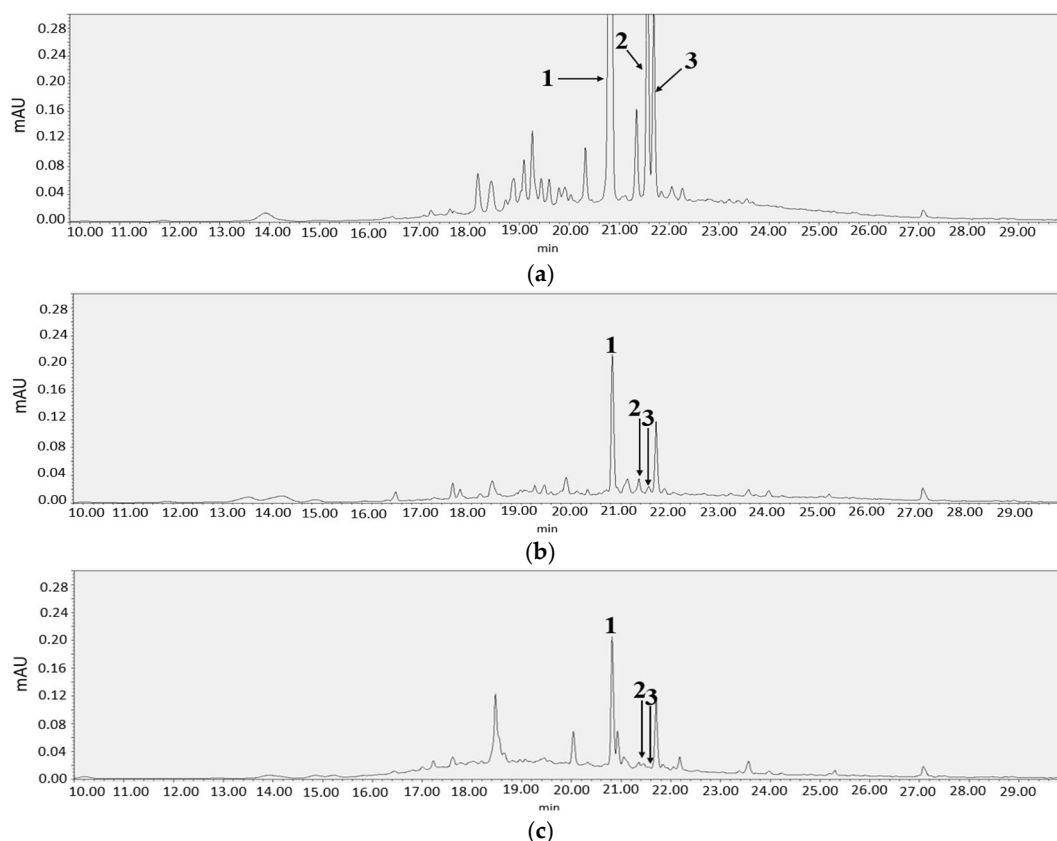


Figure 7. HPLC chromatograms of DLE (a), DST (b), and DFR (c) showing peaks corresponding to rutin (1), nicotiflorin (2), and narcissin (3).

Nicotiflorin (2) and narcissin (3) were also prominent in DLE at 11.21 mg/g and 4.02 mg/g, respectively. The stem (DST) and fruit receptacle (DFR) had lower flavonoid glycoside contents, with total contents of 7.06 mg/g and 5.35 mg/g, respectively.

As in Suwon, most compounds were not detected or were present in trace amounts in the flesh (DPF) and seeds (DSE) from the Daegu samples. The significantly higher rutin (1) content in Daegu leaf extracts than that in Suwon leaf extracts (70.21 mg/g vs. 54.37 mg/g) highlights potential regional differences in secondary metabolite biosynthesis, possibly influenced by climatic and soil conditions [30]. The variations in flavonoid glycoside content among different plant parts and regions suggest that environmental factors and plant morphology significantly influence phytochemical accumulation [31]. Leaves consistently exhibit the highest flavonoid glycoside content, making them a promising source of bioactive compounds. Generally, leaves contain a higher flavonoid glycoside content compared to other plant tissues because they are more directly exposed to harmful UV rays [32]. On the other hand, the rest of the plant parts that are not rich in flavonoid glycosides may be abundant in other phytochemicals; for example, the fruit pericarp is known to have the most saponins [33]. These results underscore the importance of selecting the appropriate plant parts to maximize the yield of bioactive compounds for pharmaceutical and nutraceutical applications.

This study is the first to report on the flavonoid glycosides present in the leaves of *S. mukorossi* using the UPLC-QTOF-ESI-MS/MS method and the quantification of their prominent peaks by HPLC. Successful quantification of rutin (1), nicotiflorin (2), and narcissin (3) provides valuable insights into the phytochemical profile of *S. mukorossi*, emphasizing its potential as a source of health-beneficial flavonoid glycosides. Specifically, these compounds were abundant in the leaves of both the Suwon and Daegu samples, indicating that they have the potential to be a source of anticancer [34], anti-inflammatory [35], and insecticide [36] agents.

The findings of this study have the potential to advance the utilization of *S. mukorossi* beyond its traditional applications and contribute to the development of sustainable bio-based industries. By identifying and quantifying the key flavonoid glycosides, this research not only provides a foundation for future pharmacological studies but also opens avenues for eco-friendly product innovations in health and personal care.

4. Conclusions

This study elucidates the phytochemical composition of *S. mukorossi* from two distinct locations in Korea, highlighting its diverse bioactive component profiles. The high total polyphenol and flavonoid contents of the leaf extracts from Suwon and Daegu suggest the potential of these plant parts as sources of bioactive compounds, particularly rutin (1), the most prevalent flavonoid glycoside. The presence of this phytochemical, along with nicotiflorin (2) and narcissin (3), underscores the therapeutic potential of *S. mukorossi*, particularly its antioxidant and anti-inflammatory properties. The successful identification of flavonoid glycosides and saponins using UPLC-QTOF-ESI-MS/MS and HPLC reinforces the potential of *S. mukorossi* as a source of compounds with pharmacological, nutraceutical, and cosmetic applications. The diverse phytochemical distribution among the plant parts warrants further research to identify the most suitable parts for extracting these chemicals. The findings of this study open avenues for further exploration of the health benefits of *S. mukorossi*, particularly in the development of sustainable plant-based products for medicinal and industrial purposes. These results address the gaps in the literature regarding the flavonoid profile of *S. mukorossi* and contribute to its already well-established medicinal and industrial uses.

Author Contributions: HPLC/PDA, TPC, and TCC analyses, N.P.U.; UPLC-QTOF-ESI-MS/MS analysis, H.-D.L.; Resources, data curation, and funding, J.K. and K.C.; Supervision and editing: S.L. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data are available upon request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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