pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 41, No. 2, pp. 71~88 (2026)
https://doi.org/10.13103/JFHS.2026.41.2.71

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Development and Validation of an LC-MS/MS Method for Determination of α-Solanine, Solamargine, and Solasonine in Dried *Solanum* Products

Bo Reum Park‡, Sun Hee Lee‡, Hyun Kyoung Lee, Hyung Soo Kim, Hyun-Kyung Kim*

Advanced Analysis Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Cheongju, Korea

(Received January 16, 2026/Revised March 20, 2026/Accepted March 28, 2026)

ABSTRACT - *Solanum nigrum* L. is widely used as both a food and medicinal plant; however, its berries may contain toxic steroidal glycoalkaloids that pose potential health risks. In this study, a liquid chromatography-tandem mass spectrometry (LC–MS/MS) method was developed and validated to simultaneously quantify α-solanine, solamargine, and solasonine in dried commercial *Solanum* products. The optimized sample preparation involved ultrasonic extraction with 50% aqueous methanol, followed by dispersive solid-phase extraction. Ammonium bicarbonate (5 mM) was added to the mobile phase, and a divert valve was employed to exclude highly abundant solamargine and solasonine during the α-solanine acquisition window, thereby improving the detection of trace-level α-solanine. The method showed satisfactory linearity, accuracy, precision, and recovery in a solid matrix. Molecular characterization using five chloroplast loci (*accD, rpoC1*, *ndhA, rpl16*, and *rpl32*), supplemented by *ITS2* and *matK* analyses for selected representative samples, indicated that commercial products marketed as *S. nigrum* in Korea, including materials showing high sequence similarity to multiple closely related taxa within the *S. nigrum* complex, were not taxonomically uniform. Solamargine and solasonine were detected in all 26 samples analyzed, whereas α-solanine was not detected in 22 samples and was detected only below the limit of quantification in the remaining 4 samples. Glycoalkaloid concentrations were markedly higher in berries than in leaves and stems and berries were present in 23 of the 26 samples. These findings support the utility of the developed LC–MS/MS method for the safety assessment of commercial *Solanum* products and highlight the need for continued monitoring of berry-containing products due to potential glycoalkaloid exposure.

Keywords: *Solanum nigrum* L., α-Solanine, Solamargine, Solasonine, Herbal safety assessment

Solanum nigrum L., an annual herb belonging to the family Solanaceae, is widely distributed in the temperate regions of Asia, Europe, and the Americas. This species has been traditionally utilized worldwide for both culinary and medicinal purposes, and numerous studies have examined its pharmacological properties, including its anti-inflammatory, analgesic, anti-allergic, hepatoprotective, and anticonvulsant activities[1]. Additionally, a diverse array of phytochemicals, such as alkaloids, flavonoids, tannins, saponins, glycosides, proteins, carbohydrates, coumarins, and phytosterols, have been detected in *S. nigrum*[2].

Among these phytochemicals, the glycoalkaloids have received considerable attention because of their potential toxicities. More specifically, glycoalkaloids are nitrogen-containing steroidal glycosides that function as secondary metabolites and aid plants in resisting environmental stressors. These compounds are widely distributed throughout the Solanaceae family[3], with representative glycoalkaloids including α-solanine and α-chaconine in potatoes, α-tomatine and dehydrotomatine in tomatoes, and solasonine and solamargine in eggplants[4]. Although low levels of glycoalkaloids are generally not considered hazardous, high levels of glycoalkaloids can result in a potent anticholinesterase activity, and excessive consumption can lead to gastrointestinal and nervous system disorders. In severe cases, glycoalkaloids may cause a rapid decline in the central nervous system function, potentially resulting in fatal outcomes[5]. Consequently, several countries, including the United States and Canada, have regulated the glycoalkaloid contents of food products by establishing appropriate safety limits[6].

‡These authors contributed equally to this work
*Correspondence to: Hyun-Kyung Kim, Advanced Analysis Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Cheongju 28159, Korea
Tel: +82-43-719-5300, Fax: +82-43-719-5300
E-mail: hyunkkim@korea.kr

Copyright © The Korean Society of Food Hygiene and Safety. All rights reserved. The Journal of Food Hygiene and Safety is an Open-Access journal distributed under the terms of the Creative Commons Attribution Non-Commercial License(http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

The aerial parts of *S. nigrum* are known to contain various glycoalkaloids, including α-solanine, solanine, and solamargine[7]. Guo et al.[8] isolated and identified solamargine, solasonine, uttroside A, and uttroside B by high-performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy following the ethanolic extraction of a whole plant of *S. nigrum*. Later, Chester et al.[9] extracted the leaves and berries of *S. nigrum* using ethanol, which allowed the quantification of solasonine, solamargine, and solasodine using high-performance thin-layer chromatography (HPTLC) and ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS). In another study, Singh et al.[10] quantified solasodine in the berries of eight *Solanum* species using HPLC. Additionally, Zhu et al.[11] identified and quantified solamargine, solasonine, and various polysaccharides in unripe berries and the whole plant of *S. nigrum* using both ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) and HPLC with ultraviolet detection. Based on these previous studies, the main steroidal alkaloids in *S. nigrum* were identified as solanine, solasonine, and solamargine. Although these glycoalkaloids share the same steroidal aglycone core, i.e., solasodine, their carbohydrate side chains are different[12].

The *S. nigrum* plant is known to grow naturally in Korea and is commonly referred to as “Yong-gyu” in traditional medicine and “Kka-ma-jung” when used as a food ingredient[13]. However, since unripe green berries of *S. nigrum* contain high levels of the toxic compound α-solanine[2,7,14-17], only the leaves and stems are permitted for use as food ingredients or herbal medicines in Korea[18,19].

In this study, an analytical method was established for the quantification of glycoalkaloids to support the safety assessment of commercial *Solanum* products sold for food or medicinal use. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS), α-solanine, solamargine, and solasonine were quantified in commercial products marketed as “Kka-ma-jung” or “Yong-gyu” in Korea. In addition, molecular marker analysis was performed as a supportive characterization of the purchased materials to examine whether the marketed products were taxonomically homogeneous.

Materials and Methods

Reagents and standards

The α-solanine standard (>99%) was purchased from Sigma-Aldrich (99%, Saint Louis, MO, USA), while the solasonine and solamargine standards (>98%) were purchased from ChemFaces (Wuhan, China). HPLC grade methanol, ethanol, acetonitrile, ammonium acetate, and ammonium formate, as well as liquid chromatography-mass spectrometry (LC-MS) grade ammonium bicarbonate, were purchased from Sigma-Aldrich. LC-MS grade formic acid was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Ultrapure water (18.2 MΩ·cm at 25°C) was obtained using a Milli-Q system (Millipore, Burlington, MA, USA). The dispersive solid-phase extraction (d-SPE) cleanup tubes were purchased from Waters (Milford, MA, USA). The PTFE filter (0.2 μm) was purchased from Whatman (Maidstone, UK).

Plant materials

Commercial herbal products marketed under the names “Kka-ma-jung” or “Yong-gyu” were purchased between February and March 2022 from herbal medicine markets in Seoul and from online retailers. Most samples consisted of dried plant materials and were labeled as products of domestic origin. All samples were finely ground using a blender (Tube Mill 100 control, IKA, Staufen, Germany) at 25,000 rpm for at least 3 minutes prior to analysis.

DNA isolation, PCR amplification, and sequence analysis

DNA was extracted from the samples using the DNeasy Plant Pro Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s protocol. Homogenized samples (25-30 mg) were used for DNA extraction. For selected samples, the ITS2 and matK barcode regions were amplified using the primer pairs nrITS2-S2F/nrITS2-S3R and matK-3F/matK-1R, respectively, and the corresponding PCR cycling conditions followed the DNA Barcoding 101 protocol/manual[20]. In addition, based on comparative inspection of *Solanum* sequences available in GenBank/NCBI, primers were designed in this study for five chloroplast loci (*accD, rpoC1, ndhA, rpl16* and *rpl32*) containing candidate informative nucleotide differences within the available reference dataset. The primer sequences for these five chloroplast loci were as follows: accD-F (5′-CAATGGGCAGTCTTGGTCCT-3′), accD-R (5′-CTCTCCGACTACGGATCCCA-3′), rpoC1-F (5′-TGGGCTTTTTAGTTATGGGCCT-3′), rpoC1-R (5′-AGACTTTAAGGGCGTGGTGG-3′), ndhA-F (5′-CATCACTGTGCCCATCGCTA-3′), ndhA-R (5′-TCCACAACGGGAGGTTTTTGA-3′), rpl16-F (5′-TCATCCAGCTCCTCGCAAAT-3′), rpl16-R (5′-AAAAGCCCAGCCCACATAGT-3′), rpl32-F (5′-AGCGAAATCTCTTTCTACCGGG-3′) and rpl32-R (5′-AGGAATTTGAGGTTGAAGGTTCATT-3′). PCR amplification of the five chloroplast loci was carried out in 20 μL reaction mixtures containing 10 μL of AccuPower® PCR Master Mix (Bioneer, Korea), 1 μL of each primer (10 pmol), 1 μL of DNA extract, and nuclease-free water to a final volume of 20 μL. Amplification was

performed on a Veriti 96-Well Fast Thermal Cycler (Applied Biosystems, Waltham, MA, USA) under the following conditions: initial denaturation at 95°C for 5 min; 30 cycles of 95°C for 20 s, annealing at the primer-specific temperature for 1 min, and extension at 72°C for 30 s; and a final extension at 72°C for 5 min. The annealing temperatures were 65°C for *accD* and *rpoC1*, 60°C for *rpl16* and *rpl32*, and 55°C for *ndhA*. The PCR products were purified using the AccuPrep® PCR/Gel Purification Kit (Bioneer, Daejeon, Korea) and sequenced at Macrogen (Daejeon, Korea). The resulting sequences were compared with publicly available reference sequences using the Basic Local Alignment Search Tool (BLAST) in NCBI to identify the closest matches in the current database. All newly generated high-quality sequences were submitted to GenBank.

Preparation of the standard solutions

Stock solutions of α-solanine, solasonine, and solamargine were prepared at a concentration of 1 mg/mL by separately dissolving the corresponding compounds in methanol under ultrasonication (POWERSONI 620, Hwashin Technology, Seoul, Korea). Aliquots of these stock solutions were subsequently diluted using 50% aqueous methanol to obtain a series of concentrations for validation.

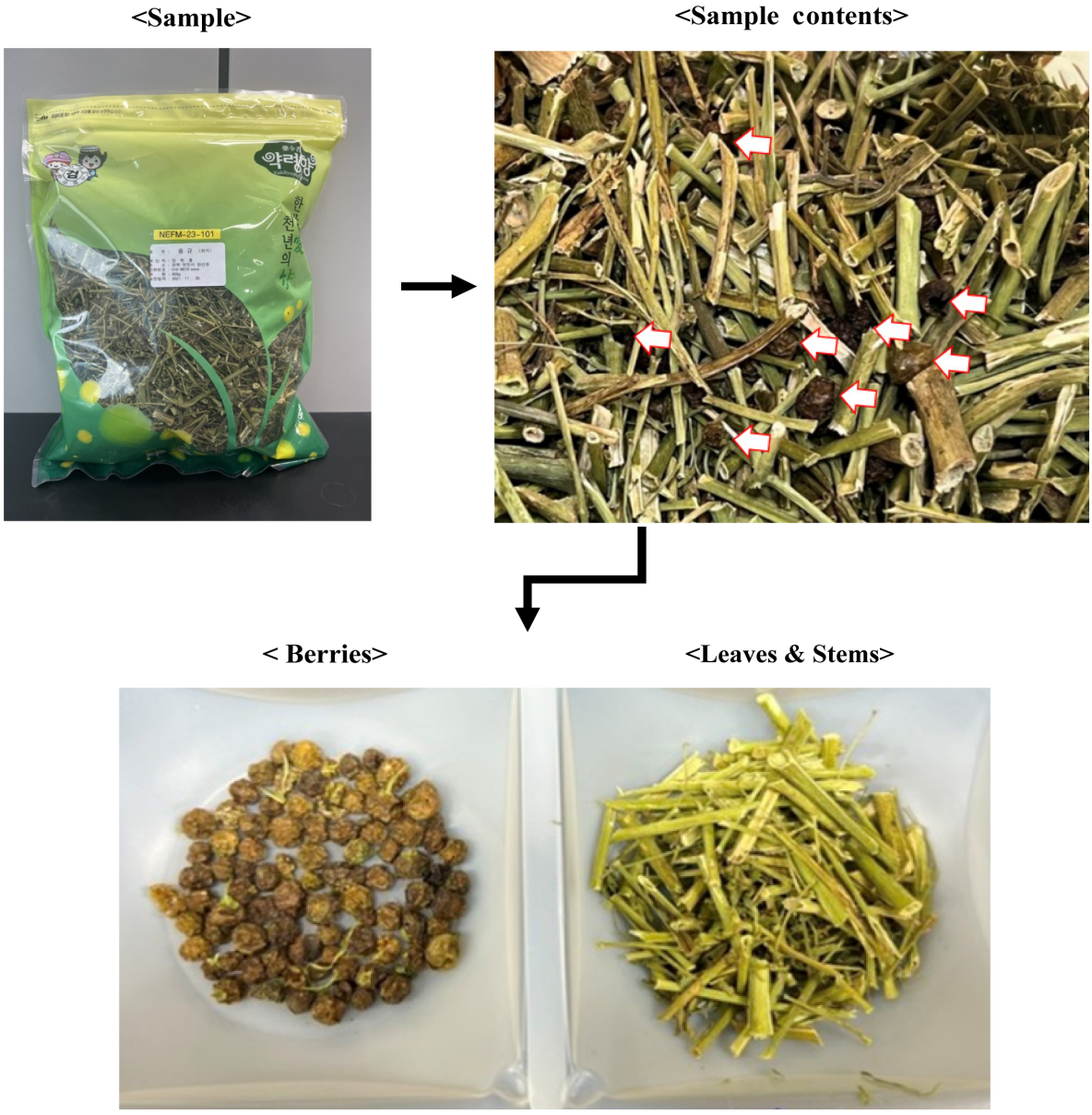

Fig. 1. Separated into two fractions: (1) Berries and (2) Leaves & Stems.

Sample preparation

To analyze the contents of the three target compounds in the different parts of the *Solanum spp.* plant, 26 samples were divided into two groups: (1) Berries and (2) Leaves&Stems (Fig. 1). Each sample was finely ground into a powder and used for analysis. More specifically, the homogenized sample (1.0 g) was placed in a 50 mL conical tube and extracted using a mixture of methanol and water (50 mL, 50:50, v/v). The resulting mixture was subjected to ultrasonic treatment for 30 min, followed by centrifugation at 3,220 ×g for 5 min. After this time, an aliquot (5 mL) of the supernatant was transferred to a 15 mL d-SPE cleanup tube containing $MgSO_4$ (900 mg) and primary secondary amine (PSA, 150 mg), and the mixture was vigorously shaken for 1 min. After subsequent centrifugation at 3,220 ×g for 5 min, the supernatant was filtered using a 0.2 μm Whatman syringe filter, and analyzed by LC-MS/MS.

LC-MS/MS analysis

The LC instrument used in this study was a Nanospace NASCA HPLC system (Shiseido, Tokyo, Japan), consisting of a vacuum degasser, binary pump, autosampler, and column oven. The column used for analysis was a CAPCELL PAK C18 MGIII-H column (3 μm, 2.0 mm×100 mm, Osaka Soda, Japan) with column a temperature of 40°C. Mobile phase A consisted of 5 mM ammonium bicarbonate in water, while mobile phase B consisted of acetonitrile and water (80:20, v/v). The flow rate was set as 0.40 mL/min and a 2 μL aliquot of the sample was injected for analysis. The HPLC elution conditions were as follows: 10% B (0-2 min), 10-50% B (2-3 min), 50-65% B (3-10 min), 65-100% B (10-11 min), and 100% B (11-13 min), followed by an immediate reduction to 10% B at 13.5-16 min. The temperature of the auto-sampler was maintained at 10°C throughout this process. The MS connected to the LC instrument was a SCIEX Triple Quad 5500^+ system (AB SCIEX, Marlborough, MA, USA) equipped with an electrospray ionization (ESI) source. The ionization source conditions were as follows: curtain gas pressure=35 psi, collision gas pressure=7 psi, ion spray voltage=5500 V, source temperature=450°C, ion source gas 1 pressure=60 psi, and ion source gas 2 pressure=45 psi. To avoid contamination of the MS instrument by dirty matrices, analysis was performed only between 5.2 and 8 min using a divert valve, and for the quantitative analysis of α-solanine, injection into the mass spectrometer was performed only between 7 and 8 min.

Method validation

To establish a suitable LC-MS/MS quantification method, the developed method was validated using a blank solid sample (cabbage tablet). Specifically, validation was performed based on the linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, and recovery according to the International Council for Harmonization and the US Food and Drug Administration guidelines[21]. The LOD and LOQ were evaluated based on the standard deviation (σ) of the response and the slope (S) of the calibration curve. The values of σ and S were estimated by analyzing seven replicates of blank samples spiked with the lowest concentration of each compound that was expected to be close to the detection limit. The spike levels (1-40 ng/mL) were selected to ensure that they were close to the expected detection limit of the method, and included the lowest concentration employed for the estimation of σ. The LOD and LOQ were calculated as follows: $LOD=3.3\times\sigma/S$; $LOQ=3\times LOD$. The linearity was evaluated using matrix-matched calibration curves to minimize the influence of matrices. Considering the LOQ value obtained for each analyte, calibration curves were obtained by plotting the peak areas against six individual concentrations (LOQ, 2.5 LOQ, 5 LOQ, 10 LOQ, 20 LOQ, and 40 LOQ). It was confirmed that all components exhibited good linearity with a correlation coefficient (R^2) of ≥0.99. The accuracy and precision were determined by analyzing solid blank samples spiked with low (LOQ), medium (10 LOQ), and high (40 LOQ) concentrations on the same day (intra-day) and over three separate days (inter-day). The recovery was defined as the ratio of the peak areas of the spiked blank samples to those of the standard solutions. Solid blank samples spiked with low, medium, and high concentrations were examined in triplicate.

Results and Discussion

Molecular characterization of commercial *Solanum* products

To molecularly characterize the commercial *Solanum* products, five chloroplast loci (*accD, rpoC1, ndhA, rpl16* and *rpl32*) were analyzed, and the resulting sequences were compared with reference records in NCBI using BLAST. Based on the current database, samples 1-3 showed the highest sequence similarity to reference sequences annotated as *S. nigrum*, samples 11-24 to those annotated as *S. emulans*, and samples 25-26 to those annotated as *S. scabrum* (Table 1 and Table S1). These results indicate that the commercial products marketed as *S. nigrum* are not taxonomically uniform and include materials showing the closest similarity to multiple closely related taxa within the *S. nigrum* complex. Additional *ITS2* and *matK* analyses showed concordant sequence-similarity patterns with the chloroplast-based grouping, providing supplementary support for the grouping pattern observed across the chloroplast loci (Table S1). In contrast to the clean chromatograms observed in samples with

Table 1. Summary of commercial Solanum products analyzed in this study and their descriptive molecular grouping based on multilocus sequence-similarity patterns.

Sample no.	Marketed name	Labeled origin	Plant part (s) included	Purchase source	Descriptive molecular grouping
1	Yong-gyu	Domestic (Yeongcheon, Korea)	leaf, stem and berries	online store	*S. nigrum*-like group
2	Yong-gyu	Domestic	leaf, stem and berries	online store	*S. nigrum*-like group
3	Kka-ma-jung	Domestic (Cheongju, Korea)	leaf, stem and berries	online store	*S. nigrum*-like group
4	Yong-gyu	Domestic	leaf, stem and berries	Seoul Yaknyeongsi Market	molecularly ambiguous group
5	Yong-gyu (Kka-ma-jung)	Domestic (Hoengseong, Korea)	leaf, stem and berries	Seoul Yaknyeongsi Market	molecularly ambiguous group
6	Kka-ma-jung	Domestic	leaf, stem and berries	online store	molecularly ambiguous group
7	Kka-ma-jung	Domestic	leaf, stem and berries	online store	molecularly ambiguous group
8	Kka-ma-jung	Domestic	leaf and stem	online store	molecularly ambiguous group
9	Yong-gyu (Kka-ma-jung)	Domestic (Yeongcheon, Korea)	leaf, stem and berries	online store	molecularly ambiguous group
10	Kka-ma-jung	Domestic (Yeosu, Korea)	leaf, stem and berries	online store	molecularly ambiguous group
11	Yong-gyu	Domestic	leaf, stem and berries	Seoul Yaknyeongsi Market	*S. emulans*-like group
12	Yong-gyu (Kka-ma-jung)	Domestic (Yeongwol, Korea)	leaf, stem and berries	Seoul Yaknyeongsi Market	*S. emulans*-like group
13	Yong-gyu (Kka-ma-jung)	Domestic (Yeongwol, Korea)	leaf, stem and berries	Seoul Yaknyeongsi Market	*S. emulans*-like group
14	Yong-gyu (Kka-ma-jung)	Domestic (Hoengseong, Korea)	berries only	Seoul Yaknyeongsi Market	*S. emulans*-like group
15	Kka-ma-jung	Domestic	leaf, stem and berries	Seoul Yaknyeongsi Market	*S. emulans*-like group
16	Kka-ma-jung	Domestic (Jecheon, Korea)	leaf, stem and berries	online store	*S. emulans*-like group
17	Kka-ma-jung	Domestic (Jeolla-do, Korea)	leaf, stem and berries	online store	*S. emulans*-like group
18	Yong-gyu	Domestic	leaf, stem and berries	online store	*S. emulans*-like group
19	Kka-ma-jung	Domestic (Jecheon, Korea)	leaf, stem and berries	online store	*S. emulans*-like group
20	Kka-ma-jung	Domestic	leaf and stem	online store	*S. emulans*-like group
21	Kka-ma-jung (Yong-gyu)	Domestic	leaf, stem and berries	online store	*S. emulans*-like group
22	Yong-gyu (Kka-ma-jung)	Domestic (Yeongwol, Korea)	leaf, stem and berries	online store	*S. emulans*-like group
23	Kka-ma-jung	Domestic (Cheorwon, Korea)	leaf, stem and berries	online store	*S. emulans*-like group
24	Kka-ma-jung (Yong-gyu leaf)	Domestic	leaf, stem and berries	online store	*S. emulans*-like group
25	Wang-kka-ma-jung	Domestic (Buyeo, Korea)	stem only	online store	*S. scabrum*-like group
26	Wang-kka-ma-jung	Domestic	leaf, stem and berries	online store	*S. scabrum*-like group

Grouping was based on multilocus sequence-similarity patterns obtained from five chloroplast loci (*accD, rpoC1, ndhA, rpl16* and *rpl32*), with supplementary *ITS2* and *matK* data. "Descriptive molecular grouping" indicates sequence-similarity-based grouping and does not represent definitive species identification. "Labeled origin" refers to the origin stated on the product label. Detailed locus-by-locus sequence-similarity results and GenBank accession numbers are provided in Table S1.

unambiguous single-sequence profiles (sample 1-3 and 11-26), samples 4-10 showed recurrent dual peaks at specific nucleotide positions across multiple loci. These consistent patterns suggest the possibility of mixed *Solanum* materials; however, due to potential alternative causes such as non-specific amplification or sequencing artifacts, these samples were conservatively treated as molecularly ambiguous.

These taxa are morphologically similar and can be difficult to distinguish by visual inspection, particularly in dried commercial materials[22]. According to the National Institute of Biological Resources (NIBR), *S. nigrum* is a native species of Korea, whereas *S. emulans* and *S. scabrum* are not listed in the database (National Institute of Biological Resources)[23]. The present results suggest that products marketed as *S. nigrum* in Korea are not taxonomically uniform and that some products may include *Solanum* taxa other than *S. nigrum*. Given that all analyzed products were labeled as being of domestic origin, these findings raise the possibility that *Solanum* taxa other than *S. nigrum* may also be present in Korea. However, because this study was conducted on only a subset of commercially distributed products, the present data do not allow conclusions regarding the natural distribution or wild occurrence of these taxa in Korea. Imported raw materials, labeling inaccuracies, and post-harvest mixing cannot be excluded, and further investigation with broader sampling will be required.

Optimization of the MS instrument

To optimize the analytical conditions for glycoalkaloid analysis, standard solutions (1.0 mg/L) were individually injected into the MS instrument and the ionization conditions were evaluated in both the positive and negative ESI modes. All three glycoalkaloids exhibited optimal sensitivities in the positive mode, consistent with the findings of previous studies[24-29]. After MS acquisition in the full-scan mode, the precursor ions were selected for multiple reaction monitoring (MRM) quantification, and the product ions and corresponding optimal MS parameters were evaluated. The Q1 full-scan mass spectra of α-solanine, solamargine, and solasonine showed peaks corresponding to predominant protonated precursor ions (i.e., $[M+H]^+$) at an *m/z* value of 868.5 for α-solanine and solamargine, and at *m/z* 884.5 for solasonine. The optimized MS conditions, including the MRM transitions, are summarized in Table 2. The most sensitive product ion was selected as the quantifier ion and the remaining ions were used as qualifier ions.

Table 2. MRM transitions and optimized MS conditions for the three glycoalkaloids

Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	Dp[a] (V)	Ce[b] (eV)	CXP[c] (V)
α-solanine	868.5	398.5*	266	97	16
		706.4		95	32
		722.6		95	24
solamargine	868.5	850.4*	181	69	32
		157.2		77	14
		253.2		63	18
solasonine	884.5	866.7*	201	67	28
		85.1		117	10
		70.1		145	36

[a]Declustering potential.
[b]Collision energy.
[c]Collision cell exit potential.
*Quantitative ion.

Development of the HPLC method

Optimization of the chromatographic conditions

During optimization of the sample preparation method, remarkably high concentrations of solamargine and solasonine were detected in the *S. nigrum* extract. For the purpose of LC-MS/MS analysis, the extract was diluted 100-10,000-fold in 50% aqueous methanol to achieve the accurate quantification of solasonine and solamargine, while also ensuring safe operation of the analytical instrument. However, this dilution process has the drawback of diluting already low concentrations of α-solanine, potentially hindering its detection. To address the issue of these large concentration differences between the target components, a divert valve was used during the separation process, as described below. Initially, peak separation of three glycoalkaloids was confirmed using acceptable additives available for LC-MS/MS, including formic acid, ammonium formate, ammonium acetate, and ammonium bicarbonate (Fig. S2)[30]. Although an aqueous 5 mM ammonium acetate solution yielded the highest peak intensities, it failed to achieve sufficient separation of the α-solanine component. In contrast, although an aqueous 5 mM ammonium bicarbonate solution resulted in slightly lower peak intensities compared to those achieved using ammonium acetate, it provided an improved resolution, with α-solanine eluting last. Based on these results, an aqueous 5 mM ammonium bicarbonate solution was selected as mobile phase A, while 80% acetonitrile in water was employed as mobile phase B. Under these mobile phase conditions, the retention times of the three glycoalkaloids were 5.7 min for solasonine, 6.0 min for solamargine, and 7.6 min for α-solanine. The retention time gap between solamargine and α-solanine exceeded 1 min. Therefore, a divert valve was employed to ensure that only α-solanine was introduced into

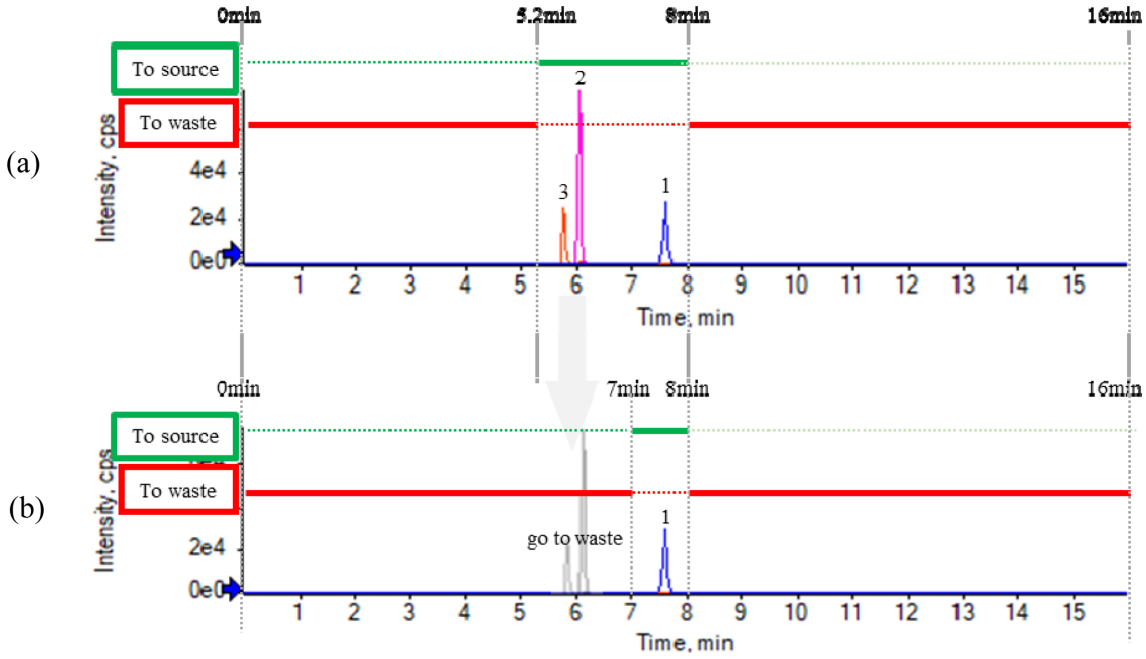

Fig. 2. Selective detection of glycoalkaloids using divert valve control.
(a) The divert valve was configured to direct the eluent to the MS between 5.2 and 8 min, allowing simultaneous detection of all three glycoalkaloids. (b) The divert valve was operated to transfer only the 7-8 min elution window to the MS, during which α-solanine eluted, while earlier the eluting compounds solasonine and solamargine were diverted to the waste. Peak numbering: 1=α-solanine, 2=solamargine, and 3 = solasonine. The concentration of each standard solution was 50 ng/mL.

the MS instrument, thereby excluding the high concentrations of solasonine and solamargine (Fig. 2).

Optimization of sample preparation

The sample preparation process, including the extraction and purification steps, was optimized based on the obtained recovery rates. A blank sample was prepared using cabbage extract tablets devoid of the three target glycoalkaloids. Additionally, a sample of cabbage powder (1.0 g) was spiked with glycoalkaloids at a known concentration, followed by sample preparation and LC-MS/MS analysis. The recovery was calculated based on the peak area of the standard solution at a known concentration. To extract the three glycoalkaloids from the samples, simple and rapid ultrasonic methods were employed due to their ability to provide a high extraction efficiency when paired with reflux conditions[31]. Various extraction solvents (methanol, 50% aqueous methanol, ethanol, and 50% aqueous ethanol) were tested to evaluate their corresponding extraction efficiencies. During extraction of the spiked blank sample, the recovery approached 100% for the majority of investigated solvents, with the exception of water (Table S2). Upon the extraction of *S. nigrum* using these solvents, the highest peak area for the glycoalkaloid components was observed using 50% aqueous methanol (Fig. S1), wherein the extraction was performed by mixing 1.0 g of *S. nigrum* with 50% aqueous methanol (50 mL), followed by ultrasonication for 30 min. To select an appropriate purification method for removing any interfering substances present in the sample, two d-SPE methods were compared, namely Method 1 (150 mg PSA, 900 mg $MgSO_4$) and Method 2 (150 mg PSA, 900 mg $MgSO_4$, 150 mg C18). As detailed in Table S3 (supplementary information), d-SPE Method 1 was selected for further experiments as it gave a recovery close to 100%. Following d-SPE and subsequent syringe filtration of the *S. nigrum* methanol extract, the peak shape became sharper and the sensitivity improved.

Method validation

The LC-MS/MS method was validated with respect to its specificity, LOD, LOQ, linearity, recovery, accuracy, and precision according to the International Conference of Harmonization (ICH) Q2 (R1) guidelines. All experiments were repeated in triplicate, and the results are presented in Table 3. The calibration curves for α-solanine, solamargine, and solasonine were constructed by plotting the peak areas versus the corresponding concentrations for the three compounds. The developed method exhibited a good linearity over the concentration ranges of 3.72-148.72 ng/mL for α-solanine, 0.82-32.64 ng/mL for solamargine, and 2.86-114.25 ng/mL for solasonine, with correlation coefficients (R^2) of 0.999 being obtained for all analytes.

Table 3. Validation results of the glycoalkaloids in the solid sample, as obtained using LC-MS/MS (n=9)

Compound	LOD[a] (ng/mL)	LOQ[b] (ng/mL)	Conc. (ng/mL)	Recovery		Accuracy (%)		Precision (RSD%)		R^2
				Mean (%)	RSD (%)	Intra-day	Inter-day	Intra-day	Inter-day	
α-Solanine	1.23	3.72	3.72	105	3.24	103	101	11.4	4.68	0.999
			37.2	106	0.784	103	101	5.69	1.72	
			148	100	1.72	101	99.0	2.62	3.59	
Solamargine	0.272	0.816	0.816	99.2	3.42	104	106	9.39	4.70	0.999
			8.16	101	3.38	102	101	1.92	2.67	
			32.6	100	1.98	108	103	6.21	4.63	
Solasonine	0.957	2.86	2.86	100	3.28	108	108	4.45	10.0	0.999
			28.6	104	4.35	102	101	3.92	2.10	
			114	101	2.48	99.8	99.7	4.88	1.04	

[a] Limits of detection.
[b] Limits of quantification.

The intra-day precision and accuracy were evaluated for each glycoalkaloid by performing measurements in triplicate at three different concentrations within a single day. To assess the inter-day precision and accuracy, the measurements were repeated over three days. The intra- and inter-day accuracies ranged from 99.81 to 102.34%, and the precision of the assay was determined to be <11.40%. These results demonstrate that this method is reproducible and sufficiently reliable for the analysis of solid samples.

Glycoalkaloid analysis of dried commercial *Solanum* products

The 26 samples were analyzed using the optimized sample preparation method combined with the developed LC-MS/MS approach (Table 4). For descriptive comparison, the samples were grouped according to their multilocus sequence-similarity patterns as follows: the *S. nigrum*-like group (samples 1-3), the molecularly ambiguous group (samples 4-10), the *S. emulans*-like group (samples 11-24), and the *S. scabrum*-like group (samples 25-26). Solamargine and solasonine were detected in all 26 samples, whereas α-solanine was undetectable or present only below the LOQ in the four samples (Table 4). In the *S. nigrum*-like group (samples 1-3), solamargine was quantified at levels of 9.29-10.1 mg/g in the berries and 0.51-1.27 mg/g in the leaves and stems, while solasonine was detected at levels of 7.38-8.38 mg/g in the berries and 0.244-0.868 mg/g in the leaves and stems. In the molecularly ambiguous group (samples 4-10), the solamargine levels ranged from 5.70 to 8.13 mg/g in the berries and from 0.0339 to 0.393 mg/g in the leaves and stems, while the solasonine concentrations varied from 5.08 to 9.62 mg/g in the berries and from 0.0522 to 1.58 mg/g in the leaves and stems. Among the *S. emulans*-like group (samples 11-24), the solamargine concentrations ranged from 3.25 to 12.1 mg/g in the berries and from 0.0480 to 0.685 mg/g in the leaves and stems, while the solasonine levels were measured at levels of 3.72-12.5 mg/g in the berries and 0.0473-2.79 mg/g in the leaves and stems. In *S. scabrum*-like group (samples 25-26), solamargine was detected at a concentration of 0.172 mg/g in the berries and 0.026-0.0941 mg/g in leaves and stems, whereas solasonine was present at a concentration of 0.158 mg/g in the berries and 0.026-0.0739 mg/g in the leaves and stems.

Numerous studies have reported that the unripe berries of *S. nigrum* contain α-solanine, thereby posing a potential health risk[2,7,14-17]. In the present study, α-solanine was not detected in the *S. nigrum*-like or *S. scabrum*-like groups. It was detected only below the LOQ in two samples of the molecularly ambiguous group and in two samples of the *S. emulans*-like group. The absence of quantifiable α-solanine should not be interpreted as evidence of safety, because substantial levels of solamargine and solasonine were observed across the analyzed groups, particularly in berry-containing samples. Specifically, in the present study, substantial levels of solamargine and solasonine were observed across the analyzed groups, especially in the berries. Indeed, the solamargine levels in the berries were between 6.54- to 192-fold higher than those in the leaves and stems, and the solasonine levels were 1.33- to 153-fold higher. Among the 26 samples analyzed, only three did not contain berries. Therefore, consumption of berry-containing commercial *Solanum* products may pose a potential risk of excessive glycoalkaloid intake.

Previously, Chester et al.[9] quantified solasonine, solamargine, and solasodine in leaf- and berry-derived preparations of authenticated *S. nigrum* using validated UPLC-ESI-MS/MS and HPTLC methods. Their results showed that berry-derived preparations generally contained comparable or higher levels of

Table 4. Quantification of the three glycoalkaloids in 26 samples

Descriptive molecular grouping	Sample no	Plant part	α-Solanine (mg/g)	Solamargine (mg/g)	Solasonine (mg/g)
S. nigrum-like group	1	berries	N.D. [a]	9.55	7.38
		leaf&stem	N.D.	1.27	0.868
	2	berries	N.D.	10.1	7.78
		leaf&stem	N.D.	0.510	0.335
	3	berries	N.D.	9.29	8.38
		leaf&stem	N.D.	0.961	0.244
Molecularly ambiguous group	4	berries	N.D.	6.66	8.00
		leaf&stem	Trace[b]	0.0480	0.0522
	5	berries	N.D.	8.13	7.13
		leaf&stem	N.D.	0.385	1.58
	6	berries	N.D.	5.70	5.08
		leaf&stem	N.D.	0.393	0.305
	7	berries	N.D.	8.08	8.30
		leaf&stem	Trace	0.289	0.214
	8	leaf&stem	N.D.	0.111	0.120
	9	berries	N.D.	7.82	9.62
		leaf&stem	N.D.	0.121	0.141
	10	berries	N.D.	6.52	7.30
		leaf&stem	N.D.	0.0339	0.0489
S. emulans-like group	11	berries	N.D.	7.29	7.63
		leaf&stem	N.D.	0.685	0.790
	12	berries	N.D.	8.69	7.63
		leaf&stem	N.D.	0.209	0.226
	13	berries	N.D.	4.57	3.72
		leaf&stem	N.D.	0.663	2.79
	14	berries	Trace	3.28	5.93
	15	berries	N.D.	6.01	6.68
		leaf&stem	N.D.	0.156	0.659
	16	berries	N.D.	5.07	7.36
		leaf&stem	N.D.	0.180	0.189
	17	berries	N.D.	7.67	7.46
		leaf&stem	N.D.	0.264	0.213
	18	berries	N.D.	6.52	6.59
		leaf&stem	Trace	0.530	0.470
	19	berries	N.D.	0.184	0.212
		leaf&stem	N.D.	4.14	5.50
	20	leaf&stem	N.D.	0.0480	0.0473
	21	berries	N.D.	3.25	4.77
		leaf&stem	N.D.	0.152	0.106
	22	berries	N.D.	6.26	9.51
		leaf&stem	N.D.	0.0951	0.131
	23	berries	N.D.	12.1	12.5
		leaf&stem	N.D.	0.316	0.360
	24	berries	N.D.	9.34	9.11
		leaf&stem	N.D.	0.640	0.619
S. scabrum-like group	25	leaf&stem	N.D.	0.0941	0.0739
	26	berries	N.D.	0.172	0.158
		leaf&stem	N.D.	0.0259	0.0260

[a] Not detected.
[b] Detection below LOQ.

these steroidal alkaloids than the corresponding leaf-derived preparations, which is consistent with our finding that berry-containing samples exhibited markedly higher solamargine and solasonine contents than leaves and stems. In the same study, solasodine was also quantified together with solasonine and solamargine, suggesting that commercial *Solanum* products may contain a broader steroidal alkaloid profile than the two glycoalkaloids measured in the present work. Although solasodine was not analyzed in this study, additional steroidal alkaloids may also contribute to the overall alkaloid burden of dried commercial *Solanum* products. Although these samples are commonly consumed after steeping or boiling in water, the present extraction study showed that water was less efficient than methanol for glycoalkaloid extraction (Fig. S1). Therefore, further studies are needed to determine the total steroidal alkaloid burden of these materials, evaluate their potential human health risks, and more comprehensively assess their suitability and safety for food use.

Conclusion

Solanum nigrum L. has long been used as both a food and a medicinal plant, although its berries have raised safety concerns because of the reported presence of α-solanine. In the present study, a reliable LC-MS/MS method was developed and validated for the simultaneous determination of α-solanine, solamargine, and solasonine in commercial “Yong-gyu” and “Kka-ma-jung” products sold in Korea, and the method proved useful for the analysis of market samples. Molecular characterization further indicated that products marketed as S. nigrum in Korea were not taxonomically uniform and included materials showing sequence similarity to multiple closely related *Solanum* taxa. Among the 26 samples analyzed, α-solanine was detected only below the LOQ in four samples, whereas solamargine and solasonine were detected at markedly higher levels, particularly in berries compared with leaves and stems. These findings suggest that safety evaluation of commercial *Solanum* products should not rely solely on α-solanine, but should also consider the broader steroidal alkaloid burden of berry-containing materials. Further studies involving a larger number of commercial products, as well as voucher-authenticated *Solanum* materials collected in Korea, will be needed for a more comprehensive assessment of their taxonomic identity, glycoalkaloid composition, and suitability for food use.

Acknowledgments

This study was financially supported by the Ministry of Food and Drug Safety of the Korea [grant number 23211MFDS241].

국문요약

까마중(*Solanum nigrum* L.)은 식용 및 약용으로 널리 사용되는 식물이지만, 그 열매에는 잠재적인 건강 위험을 초래할 수 있는 스테로이드성 글리코알칼로이드가 함유된 것으로 알려져 있다. 본 연구에서는 건조된 시판 까마중 제품 중 α-solanine, solamargine, solasonine을 동시에 정량하기 위한 LC–MS/MS 분석법을 개발하고 검증하였다. 시료 전처리는 50% 메탄올 수용액을 이용한 초음파 추출과 분산형 고체상 추출법(d-SPE)을 적용하였다. 이동상에는 5 mM 탄산수소암모늄을 첨가하고, divert 밸브를 이용하여 고농도의 solamargine과 solasonine을 제외함으로써 미량 α-solanine의 검출 감도를 향상시켰다. 개발된 분석법은 고체 매트릭스에서 우수한 직선성, 정확성, 정밀도 및 회수율을 나타내었다. 엽록체 유전자 5개 부위(*accD, rpoC1, ndhA, rpl16, rpl32*)를 이용한 분자생물학적 특성 분석 결과, 국내에서 까마중으로 유통되는 제품은 *S. nigrum*을 포함한 근연종과 높은 서열 유사성을 보였으며, 시판 제품은 단일 종으로 구성된 경우도 있었으나 여러 종이 혼재된 것으로 추정되는 시료도 확인되었다. 확립된 LC–MS/MS 분석법을 통해 분석된 26개 시료 모두에서 고농도의 solamargine과 solasonine이 검출된 반면, α-solanine은 22개 시료에서 검출되지 않았고 나머지 4개 시료에서도 정량한계 이하 수준으로만 검출되었다. 글리코알칼로이드 함량은 잎과 줄기보다 열매에서 유의하게 높았으며, 26개 시료 중 23개에서 열매가 포함되어 있었다. 이러한 결과는 본 연구에서 개발된 LC–MS/MS 분석법이 시판 까마중 제품의 안전성 평가에 유용함을 시사하며, 글리코알칼로이드 노출 가능성에 대비하여 열매가 포함된 제품에 대한 지속적인 모니터링의 필요성을 강조하였다.

Conflict of interests

The authors declare no potential conflict of interest.

ORCID

Bo Reum Park https://orcid.org/0000-0003-4082-8956
Sun Hee Lee https://orcid.org/0000-0002-8431-0289
Hyun Kyoung Lee https://orcid.org/0009-0001-4533-9775
Hyung Soo Kim https://orcid.org/0000-0001-5995-4579
Hyun-Kyung Kim https://orcid.org/0009-0006-9241-2986

References

1. Mandal, S., Vishvakarma, P., Verma, M., Alam, M.S., Agrawal, A., Mishra, A., *Solanum nigrum* Linn: an analysis of the medicinal properties of the plant. *J. Pharm. Negative Results*, **14**, 1595-1600 (2023).
2. Saleem, T.S.M., Chetty, C.M., Ramkanth, S., Alagusunda-

ram, M., Gnanaprakash, K., Thiruvengada Rajan, V.S., Angalaparameswari, S., *Solanum nigrum* Linn: a review. *Pharmacogn. Rev.*, **3**, 342-345 (2009).
3. Gürbüz, N., Uluişik, S., Frary, A., Doğanlar, S., Health benefits and bioactive compounds of eggplant. *Food Chem*., **268**, 602-610 (2018).
4. Friedman, M., Chemistry and anticarcinogenic mechanisms of glycoalkaloids produced by eggplants, potatoes, and tomatoes. *J. Agric*. *Food Chem*., **63**, 3323-3337 (2015).
5. Sohn, H.B., Kim, S.J., Lee, Y.Y., Park, H.M., Mekapogu, M., Chang, D.C., Hong, S.Y., Nam, J.H., Suh, J.T., Kweon, K.B., Jeong, J.C., Kim, Y.H., Distribution of glycoalkaloid content in tubers of 24 potato varieties in Korea. *Korean J. Breed. Sci*., **47**, 357-365 (2015).
6. EFSA Panel on Contaminants in the Food Chain (CONTAM), Scientific opinion on the risks for public health related to the presence of glycoalkaloids in food and feed. *EFSA J*., **18**, 6222 (2020).
7. Atanu, F.O., Ebiloma, U.G., Ajayi, E.I., A review of the pharmacological aspects of *Solanum nigrum* Linn. *Biotechnol. Mol. Biol. Rev*., **6**, 1-7 (2011).
8. Guo, S.B., Tian, Y., Jian, L.Y., Optimization of ethanol extraction process of *Solanum nigrum* Linn. and structural confirmation of its compounds. *Asian J. Chem*., **26**, 4615-4618 (2014).
9. Chester, K., Paliwal, S., Khan, W., Ahmad, S., UPLC-ESI-MS/MS and HPTLC method for quantitative estimation of cytotoxic glycosides and aglycone in bioactivity guided fractions of *Solanum nigrum* L. *Front. Pharmacol*., **8**, 434 (2017).
10. Singh, P., Singh, P., Singh, M.P., Quantitative estimation of steroidal drug solasodine in wild species of *Solanum* by reversed-phase HPLC method. *J. Pharmacogn. Phytochem*., **8**, 920-926 (2019).
11. Zhu, L., Lu, Y., Sun, Z., Han, J., Tan, Z., The application of an aqueous two-phase system combined with ultrasonic cell disruption extraction and HPLC in the simultaneous separation and analysis of solanine and *Solanum nigrum* polysaccharide from *Solanum nigrum* unripe fruit. *Food Chem*., **304**, 125383 (2020).
12. Blankemeyer, J.T., McWilliams, M., Rayburn, J.R., Weissenberg, M., Friedman, M., Developmental toxicology of solamargine and solasonine glycoalkaloids in frog embryos. *Food Chem. Toxicol*., **36**, 383-389 (1998).
13. Yun, H.J., Jung, J.H., Hyun, S.K., Kim, B.W., Kwon, H.J., Isolation and identification of a novel anticancer compound from *Solanum nigrum*. *J. Life Sci*., **24**, 234-241 (2014).
14. Jain, R., Sharma, A., Gupta, S., Sarethy, I.P., Gabrani, R., *Solanum nigrum*: current perspectives on therapeutic properties. *Altern. Med. Rev*., **16**, 78-85 (2011).
15. Anzoom, S., Tahsin, M.R., Kabir, S., Amran, M.S., A comprehensive review on black nightshade (*Solanum nigrum*): chemical constituents, pharmacological activities, and its role in COVID-19 treatment. *J. Asiatic Soc. Bangladesh Sci*., **49**, 237-263 (2023).
16. Roelen, C., Mulder-Spijkerboer, H.N., Gee, E.S., Kolukirik, P., Biesta-Peters, E.G., Royen, H., Public health risk due to contamination of Solanum nigrum in frozen green beans - collaboration effort between a poison centre, a hospital and health authorities. *Clini. Toxicol*., **62**, 126-128 (2024).
17. Rani, Y.S., Reddy, V.J., Basha, S.J., Koshma, M., Hanumanthu, G., Swaroopa, P., A review on *Solanum nigrum*. *World J. Pharm. Pharm. Sci.*, **6**, 293-303 (2017).
18. Korea Ministry of Food and Drug Safety (MFDS), (2025, February 25). Annex 2: raw materials that can be used in food with restrictions. In Food standards and specifications. Retrieved from https://www.law.go.kr
19. Korea National Institute for Food and Drug Safety Evaluation (NIFDS), (2025, February 25). Herbal organoleptic test manual, Retrieved from https://www.mfds.go.kr/brd/m_218/view.do?seq=33456
20. DNA Learning Center, (2025 February 25). Using DNA barcodes to identify and classify living things. Retrieved from https://dnabarcoding101.org/files/using-dna-barcodes.pdf
21. U.S. Food and Drug Administration (FDA), (2025 February 25). Bioanalytical method validation guidance for industry. Retrieved from https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf
22. Knapp, S., Barboza, G.E., Bohs, L., Särkinen, T., A revision of the Morelloid clade of Solanum L. (Solanaceae) in North and Central America and the Caribbean. *PhytoKeys*, **123**, 1-144 (2019).
23. National Institute of Biological Resources (NIBR), (2025, February 10). Species detailed information. Retrieved from https://species.nibr.go.kr/home/mainHome.do?cont_link=009&subMenu=009002&contCd=009002&pageMode=view&ktsn=120000062904
24. Paul, A.T., Vir, S., Bhutani, K.K., Liquid chromatography-mass spectrometry-based quantification of steroidal glycoalkaloids from Solanum xanthocarpum and effect of different extraction methods on their content. *J. Chromatogr. A*, **1208**, 141-146 (2008).
25. Zheng, X., Xu, L., Liang, Y., Xiao, W., Xie, L., Zhang, Y., Zhang, L., Zhao, L., Chao, L., Chen, J., Wang, G., Quantitative determination and pharmacokinetic study of solamargine in rat plasma by liquid chromatography-mass spectrometry. *J. Pharm. Biomed. Anal*., **55**, 1157-1162 (2011).
26. Chen, Y., Zhang, S., Chen, D., Zhou, M., Zheng, J., Xiang, Z., An UPLC-MS/MS method for determination of solasonine in rat plasma and its application to a pharmacokinetic and bioavailability study. *J. Chromatogr. B*, **985**, 1-5 (2015).
27. Nielsen, S.D., Schmidt, J.M., Kristiansen, G.H., Dalsgaard, T.K., Larsen, L.B., Liquid chromatography mass spectrometry quantification of α-solanine, α-chaconine, and solanidine in potato protein isolates. *Foods*, **9**, 416 (2020).
28. Liu, W., Zhang, N., Li, B., Fan, S., Zhao, R., Li, L.P., Wu, H.H., Zhao, Y., Determination of α-chaconine and α-solanine in commercial potato crisps by QuEChERS extraction and UPLC-MS/MS. *Chem. Pap.*, **68**, 1498-1504 (2014).
29. Li, T.C., Chen, N.J., Chen, Y.Y., He, B.J., Zhou, Z.F., Sola-

sonine induces apoptosis of the SGC-7901 human gastric cancer cell line in vitro via the mitochondria-mediated pathway. *J. Cell. Mol. Med.*, **26**, 3387-3395 (2022).
30. Hodson, G., (2020, January 23). Mobile phase preparation - steps to take for ideal analysis. Phenomenex Blog. Retrieved from https://www.phenomenex.com
31. Pietsch, J., Günther, J., Henle, T., Dreßler, J., Simultaneous determination of thirteen plant alkaloids in a human specimen by SPE and HPLC. *J. Sep. Sci.*, **31**, 2410-2416 (2008).

Supplementary Information

Table S1. Locus-by-locus sequence-similarity results for commercial *Solanum* samples based on BLAST comparison with NCBI reference sequences

Sample no.	Locus	GenBank accession no. (this study)	Closest reference taxon	Closest reference accession no.	% Identity	Query coverage (%)	Interpretation
1	*5.8S~ITS2~28S*	OQ978663	*S. nigrum*	KX166238.1	99.01	100%	Multilocus results support *S. nigrum*-like affinity
	accD	OR046452	*S. nigrum*	MT621037.1	100%	100%	
	rpoC1	OR046392	*S. nigrum*	OZ176049.1	100%	100%	
	ndhA	OR046432	*S. nigrum*	ON641346.1	100%	100%	
	rpl16	OR046412	*S. nigrum*	NC_028070.2	100%	100%	
	rpl32	OR046471	*S. nigrum*	NC_028070.2	100%	100%	
2	*5.8S~ITS2~28S*	OQ978665	*S. nigrum*	MG730621.1	99.00%	100%	Multilocus results support *S. nigrum*-like affinity
	accD	OR046454	*S. nigrum*	MT621037.1	100%	100%	
	rpoC1	OR046394	*S. nigrum*	OZ176049.1	100%	100%	
	ndhA	OR046434	*S. nigrum*	ON641346.1	100%	100%	
	rpl16	OR046414	*S. nigrum*	PZ094981.1	100%	100%	
	rpl32	OR046473	*S. nigrum*	NC_028070.2	100%	100%	
3	*accD*	OR046461	*S. nigrum*	NC_028070.2	100%	100%	Multilocus results support *S. nigrum*-like affinity
	rpoC1	OR046401	*S. nigrum*	MZ221865.1	100%	100%	
	ndhA	OR046441	*S. nigrum*	MT621037.1	100%	100%	
	rpl16	OR046421	*S. nigrum*	OZ176049.1	100%	100%	
	rpl32	OR046480	*S. nigrum*	NC_028070.2	100%	100%	
11	*5.8S~ITS2~28S*	OQ978654	*S. nigrum*	MG730621.1	100%	100%	Overall pattern most consistent with reference *S. emulans.*
	matK	OR046371	*S. emulans*	NC_062499.1	100%	100%	
			S. ptychanthum	HQ235335.1	100%	100%	
	accD	OR046443	*S. emulans*	NC_062499.1	100%	100%	
	rpoC1	OR046383	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	
	ndhA	OR046423	*S. emulans*	NC_062499.1	100%	100%	
	rpl16	OR046403	*S. emulans*	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046462	*S. emulans*	NC_062499.1	99.74%	100%	
12	*5.8S~ITS2~28S*	OQ978655	*S. nigrum*	MG730621.1	100%	100%	Overall pattern most consistent with reference *S. emulans.*
	matK	OR046372	*S. emulans*	NC_062499.1	100%	100%	
			S. ptychanthum	HQ235335.1	100%	100%	
	accD	OR046444	*S. emulans*	NC_062499.1	100%	100%	
	rpoC1	OR046384	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	

Table S1. Locus-by-locus sequence-similarity results for commercial *Solanum* samples based on BLAST comparison with NCBI reference sequences

Sample no.	Locus	GenBank accession no. (this study)	Closest reference taxon	Closest reference accession no.	% Identity	Query coverage (%)	Interpretation
12	*ndhA*	OR046424	*S. emulans*	NC_062499.1	99.87%	100%	Overall pattern most consistent with reference S. emulans.
	rpl16	OR046404	*S. emulans*	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046463	*S. emulans*	NC_062499.1	99.87%	100%	
13	*5.8S~ITS2~28S*	OQ978656	*S. nigrum*	MG730621.1	100%	100%	Overall pattern most consistent with reference *S. emulans.*
	matK	OR046373	*S. emulans*	NC_062499.1	100%	100%	
			S. nigrum	FN668839.1	100%	100%	
			S. ptychanthum	HQ235335.1	100%	100%	
	accD	OR046445	*S. emulans*	NC_062499.1	100%	100%	
	rpoC1	OR046385	*S. nigrescens*	NC_062429.1		100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100	100%	
			S. scabrum	MT621038.1		100%	
	ndhA	OR046425	*S. emulans*	NC_062499.1	99.86%	100%	
	rpl16	OR046405	*S. emulans*	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046464	*S. emulans*	NC_062499.1	99.87%	100%	
14	*5.8S~ITS2~28S*	OQ978657	*S. nigrum*	MG730621.1	100%	100%	Overall pattern most consistent with reference *S. emulans.*
	accD	OR046446	*S. emulans*	NC_062499.1	100%	100%	
	rpoC1	OR046386	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	
	ndhA	OR046426	*S. emulans*	NC_062499.1	100%	100%	
	rpl16	OR046406	*S. emulans*	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046465	*S. emulans*	NC_062499.1	99.87%	100%	
15	*5.8S~ITS2~28S*	OQ978660	*S. nigrum*	MG730621.1	100%	100%	Overall pattern most consistent with reference *S. emulans.*
	accD	OR046449	*S. emulans*	NC_062499.1	100%	100%	
	rpoC1	OR046389	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	
	ndhA	OR046429	*S. emulans*	NC_062499.1	100%	100%	
	rpl16	OR046409	*S. emulans*	NC_062499.1	100%	100%	
			S.ptychanthum	AY727389.1	100%	100%	
			S. alpinum	NC_062515.1	100%	100%	
			S. nigrum	MW285079.1	100%	100%	
	rpl32	OR046468	*S. emulans*	NC_062499.1	99.74%	100%	

Table S1. Locus-by-locus sequence-similarity results for commercial *Solanum* samples based on BLAST comparison with NCBI reference sequences

Sample no.	Locus	GenBank accession no. (this study)	Closest reference taxon	Closest reference accession no.	% Identity	Query coverage (%)	Interpretation
16	*5.8S~ITS2~28S*	OQ978661	*S. nigrum*	MG730621.1	100%	100%	Overall pattern most consistent with reference *S. emulans*
	accD	OR046450	*S. emulans*	NC_062499.1	100%	100%	
	rpoC1	OR046390	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	
	ndhA	OR046430	*S. emulans*	NC_062499.1	100%	100%	
	rpl16	OR046410	*S. emulans*	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046469	*S. emulans*	NC_062499.1	99.87%	100%	
17	*5.8S~ITS2~28S*	OQ978662	*S. nigrum*	MG730621.1	100%	100%	Overall pattern most consistent with reference *S. emulans*
	accD	OR046451	*S. emulans*	NC_062499.1	100%	100%	
	rpoC1	OR046391	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	
	ndhA	OR046431	*S. emulans*	NC_062499.1	100%	100%	
	rpl16	OR046411	*S. emulans*	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046470	*S. emulans*	NC_062499.1	99.87%	100%	
18	*5.8S~ITS2~28S*	OQ978664	*S. nigrum*	MG730621.1	100%	100%	Overall pattern most consistent with reference *S. emulans*
	accD	OR046453	*S. emulans*	NC_062499.1	100%	100%	
	rpoC1	OR046393	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	
	ndhA	OR046433	*S. emulans*	NC_062499.1	100%	100%	
	rpl16	OR046413	*S. emulans*	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046472	*S. emulans*	NC_062499.1	99.88%	100%	
19	*5.8S~ITS2~28S*	OQ978666	*S. nigrum*	MG730621.1	100%	100%	Overall pattern most consistent with reference *S. emulans*
	accD	OR046455	*S. emulans*	NC_062499.1	100%	100%	
	rpoC1	OR046395	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	

Table S1. Locus-by-locus sequence-similarity results for commercial *Solanum* samples based on BLAST comparison with NCBI reference sequences

Sample no.	Locus	GenBank accession no. (this study)	Closest reference taxon	Closest reference accession no.	% Identity	Query coverage (%)	Interpretation
19	*ndhA*	OR046435	*S. emulans*	NC_062499.1	100%	100%	Overall pattern most consistent with reference *S. emulans*
	rpl16	OR046415	*S. emulans*	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046474	*S. emulans*	NC_062499.1	99.87%	100%	
20	*5.8S~ITS2~28S*	OQ978667	*S. nigrum*	MG730621.1	100%	100%	Overall pattern most consistent with reference *S. emulans*
	accD	OR046456	*S. emulans*	NC_062499.1	100%	100%	
	rpoC1	OR046396	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	
	ndhA	OR046436	*S. emulans*	NC_062499.1	100%	100%	
	rpl16	OR046416	*S. emulans*	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046475	*S. emulans*	NC_062499.1	99.87%	100%	
21	*5.8S~ITS2~28S*	OQ978668	*S. nigrum*	MG730621.1	100%	100%	Overall pattern most consistent with reference *S. emulans*
	accD	OR046457	*S. emulans*	NC_062499.1	100%	100%	
	rpoC1	OR046397	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	
	ndhA	OR046437	*S. emulans*	NC_062499.1	100%	100%	
	rpl16	OR046417	*S. ptychanthum*	AY727389.1	100%	100%	
			S. alpinum	NC_062515.1	100%	100%	
			S. nigrum	MW285079.1	100%	100%	
	rpl32	OR046476	*S. emulans*	NC_062499.1	99.72	100%	
22	*accD*	OR046458	*S. emulans*	NC_062499.1	100%	100%	Overall pattern most consistent with reference *S. emulans*
	rpoC1	OR046398	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	
	ndhA	OR046438	*S. emulans*	NC_062499.1	100%	100%	
	rpl16	OR046418	*S. emulans*	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046477	*S. emulans*	NC_062499.1	99.87%	100%	
23	*accD*	OR046459	*S. emulans*	NC_062499.1	100%	100%	Overall pattern most consistent with reference *S. emulans*

Table S1. Locus-by-locus sequence-similarity results for commercial *Solanum* samples based on BLAST comparison with NCBI reference sequences

Sample no.	Locus	GenBank accession no. (this study)	Closest reference taxon	Closest reference accession no.	% Identity	Query coverage (%)	Interpretation
23	*rpoC1*	OR046399	*S. nigrescens*	NC_062429.1	100%	100%	Overall pattern most consistent with reference *S. emulans*
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	
	ndhA	OR046439	*S. emulans*	NC_062499.1	100%	100%	
	rpl16	OR046419	*S. emulans*	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046478	*S. emulans*	NC_062499.1	99.87%	100%	
24	*accD*	OR046460	*S. emulans*	NC_062499.1	100%	100%	Overall pattern most consistent with reference *S. emulans*
	rpoC1	OR046400	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	MT621038.1	100%	100%	
	ndhA	OR046440	*S. emulans*	NC_062499.1	100%	100%	
	rpl16	OR046420	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. americanum	AY727387.1	100%	100%	
	rpl32	OR046479	*S. emulans*	NC_062499.1	99.87%	100%	
25	*5.8S~ITS2~28S*	OQ978658	*S. villosum*	PZ012403.1	99.75%	100%	Overall pattern most consistent with reference *S. scabrum*
	accD	OR046447	*S. scabrum*	NC_062483.1	100%	100%	
	rpoC1	OR046387	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	NC_062483.1	100%	100%	
	ndhA	OR046427	*S. scabrum*	MT621038.1	100%	100%	
	rpl16	OR046407	*S. scabrum*	NC_062483.1	100%	100%	
	rpl32	OR046466	*S. scabrum*	NC_062483.1	100%	100%	
26	*5.8S~ITS2~28S*	OQ978659	*S. villosum*	PZ012403.1	99.75%	100%	Overall pattern most consistent with reference *S. scabrum*
	accD	OR046448	*S. scabrum*	NC_062483.1	100%	100%	
	rpoC1	OR046388	*S. nigrescens*	NC_062429.1	100%	100%	
			S. emulans	NC_062499.1	100%	100%	
			S. opacum	NC_062487.1	100%	100%	
			S. annuum	NC_062873.1	100%	100%	
			S. villosum	MT621039.1	100%	100%	
			S. umalilaense	NC_062472.1	100%	100%	
			S. scabrum	NC_062483.1	100%	100%	
	ndhA	OR046428	*S. scabrum*	MT621038.1	100%	100%	
	rpl16	OR046408	*S. scabrum*	NC_062483.1	100%	100%	
	rpl32	OR046467	*S. scabrum*	NC_062483.1	100%	100%	

Samples 4-10 are not included in this table because recurrent dual peaks across multiple loci precluded unambiguous sequence interpretation and GenBank deposition.

Table S2. Recovery of the three compounds from S. nigrum using different extraction solvents

No.	Compound	Mean ±RSD (%)			
		Methanol	50% Methanol	Ethanol	50% Ethanol
1	α-Solanine	96.5±0.92	110±0.73	103±5.0	102±7.5
2	Solamargine	96.9±0.83	106±1.1	99.5±0.88	98.3±4.5
3	Solasonine	101±3.8	104±2.72	96.1±1.3	92.9±3.2

Table S3. Recovery of the three compounds from S. nigrum using the QuEChERS extraction kit

No.	Compound	Mean±RSD (%)	
		Method 1[a)]	Method 2[b)]
1	α-Solanine	95.7±8.9	24.6±20
2	Solamargine	110±12	59.0±19
3	Solasonine	106±13	58.0±32

[a)] DisQuE™ 900 mg $MgSO_4$+ 150 mg PSA.
[b)] DisQuE™ 900 mg $MgSO_4$+ 150 mg PSA+150 mg C18.

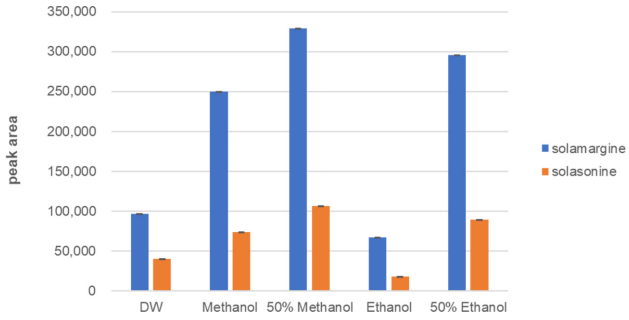

Fig. S1. Comparison of the peak areas of solamargine (blue) and solasonine (orange) in *S. nigrum* berry extracts obtained using the four different solvents (n=3). The extracts were diluted 1:100 with the respective solvents prior to LC-MS/MS analysis. α-Solanine was not detected and therefore was excluded from the data.

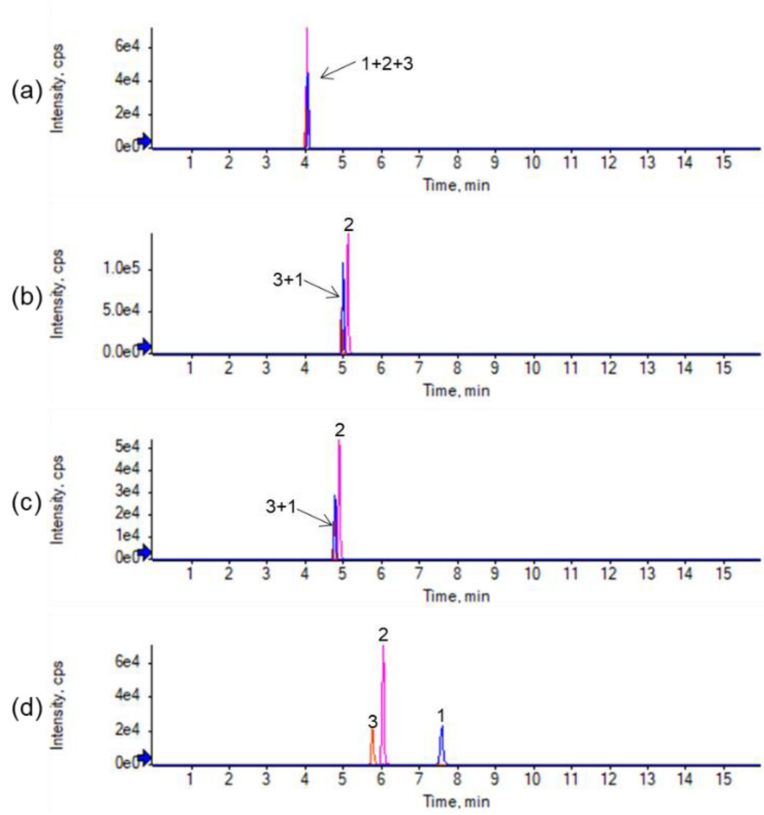

Fig. S2. Effect of the mobile phase A composition on the chromatographic separation of the three glycoalkaloids. Chromatograms were obtained using the following aqueous mobile phases: (a) 0.1% formic acid in water, (b) 5 mM ammonium acetate in water (c) 5 mM ammonium formate in water and (d) 5 mM ammonium bicarbonate in water. Peak numbering: 1=α-solanine, 2=solamargine, and 3=solasonine. Mobile phase B consisted of 80% acetonitrile in water, and the concentration of each standard solution was 50 ng/mL.