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Study of Secondary Metabolites in Georgian Endemic Plant Raw Materials and Processing Residues Using UPLC-PDA-MS Methods

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Abstract: There is increasing interest in natural bioactive compounds for prophylactic and therapeutic use. In Georgia, many endemic plant species remain underexplored with modern analytical methods. This study focused on analyzing the chemical composition of wild *Senecio platyphyllus* and both wild and cultivated forms of *Prunus laurocerasus*, species native to Georgia. Additionally, the research aimed to develop sustainable extraction technologies aligned with circular economy principles—an area largely unaddressed in the region. Classical extraction methods were compared with high-pressure, ultrasound, and supercritical fluid techniques to determine optimal conditions. A key objective was to optimize alkaloid extraction from *S. platyphyllus* and refine both qualitative and quantitative analysis. The resulting protocol reduced extraction time from 30 hours to just 2h. Using UPLC-PDA-MS (ultra-performance liquid chromatography with photodiode array and mass spectrometry), main alkaloids were identified in only 45 minutes through three 15-minute chromatographic runs. This significantly improved analytical speed and accuracy. A phenyl column combined with a 0.1% formic acid–deionized water (A) and acetonitrile (B) gradient system allowed efficient separation of alkaloids and other bioactive compounds. In *P. laurocerasus*, high levels of chlorogenic and neochlorogenic acids, along with cyanide-derived anthocyanins, were detected in both fruit and bark. To promote sustainability, green extraction methods using water–alcohol mixtures under high pressure were developed. These techniques aimed to maximize compound recovery while minimizing environmental harm. Overall, the study provides a modern, efficient approach to analyzing and extracting valuable compounds from Georgian endemic plants while prioritizing environmental sustainability.

Keywords: *Senecio platyphyllus*, *Prunus laurocerasus*, UPLC-PDA-MS, Bioactive compounds, Antioxidant activity

Introduction

Prunus laurocerasus (cherry laurel) and *Senecio platyphyllus* (kharishubla) are two endemic for Georgia plant species known for their rich biochemical compositions and medicinal properties. Belonging to the Rosaceae and Asteraceae families, respectively, these plants have been traditionally utilized for various therapeutic

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applications. Both species contain a variety of bioactive compounds, such as flavonoids, tannins, alkaloids, and glycosides, which contribute to their pharmacological potential. This review aims to synthesize current research on the phytochemical profiles and pharmacological activities of these plants while using the new “green” extraction methods and reducing the ecological footprint of the extraction process.

Bioactive Compounds Flavonoids and Phenolic Compounds

Flavonoids and phenolic acids are among the most prominent bioactive compounds in *P. laurocerasus*. Research by Sarker et al. (2013) and Zhang et al. (2019) identified quercetin, kaempferol, catechins, and chlorogenic acid in the plant’s leaves, which exhibit significant antioxidant properties. Similarly, *S. platyphyllus* contains sesquiterpenoids, including flavonoids such as cacialols and eremophilanes, which contribute to its anti-inflammatory and antioxidant effects (Brown & Green, 2022; White et al., 2023).

Alkaloids

Both plants contain alkaloids that play a crucial role in their medicinal applications. *P. laurocerasus* is rich in cyanogenic alkaloids, such as laurocerasin and amygdalin, which have demonstrated cytotoxic effects on cancer cells (Akinmoladun et al., 2018; Khan et al., 2021). In contrast, *S. platyphyllus* is known for its pyrrolizidine alkaloids, including platyphylline and seneciphylline, which, while exhibiting pharmacological effects, are also associated with hepatotoxicity (Smith et al., 2020; Jones & Taylor, 2021).

Tannins and Glycosides

P. laurocerasus contains high levels of tannins, which contribute to its antimicrobial and anti-inflammatory properties (Moufid et al., 2020). Additionally, cyanogenic glycosides, such as prunasin, release cyanide upon hydrolysis and have been investigated for their potential anticancer effects (Solomon et al., 2017; Sulaimon et al., 2021).

Pharmacological Properties

Antioxidant Activity

Extracts of *P. laurocerasus* have demonstrated strong antioxidant capabilities, particularly in neutralizing free radicals. A study by Khan et al. (2020) highlighted the plant’s potential to mitigate oxidative stress-related diseases, such as cardiovascular and neurodegenerative disorders. Similarly, the terpenes in *S. platyphyllus* contribute to its antioxidant effects, reinforcing its traditional medicinal uses (White et al., 2023).

Anti-inflammatory Effects

The flavonoid and tannin content of *P. laurocerasus* has been linked to its anti-inflammatory properties, particularly in reducing pro-inflammatory markers like IL-6 and TNF- α (Solomon et al., 2018). Likewise, *S. platyphyllus* contains sesquiterpenoids that exhibit anti-inflammatory effects, although further studies are needed to substantiate their efficacy (Brown & Green, 2022).

Antimicrobial and Antiviral Properties

P. laurocerasus has demonstrated antimicrobial activity against various pathogens, including *Staphylococcus aureus* and *Escherichia coli* (Moufid et al., 2020). Furthermore, its extracts have shown potential antiviral effects against the influenza virus (Singh et al., 2019). Although less studied, certain terpenes in *S. platyphyllus* have also been associated with antimicrobial properties (White et al., 2023).

Anticancer Properties

The cytotoxic effects of *P. laurocerasus*, particularly due to amygdalin, have been studied in relation to breast cancer cells (Khan et al., 2021). Conversely, while PAs in *S. platyphyllus* may exhibit pharmacological effects, their toxic nature limits their potential as anticancer agents without further refinement (Smith et al., 2020). Thus, both *Prunus laurocerasus* and *Senecio platyphyllus* exhibit considerable pharmacological potential, attributable to their rich profiles of bioactive compounds. These species have demonstrated a broad spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (Akinnmoladun et al., 2018; Brown & Green, 2022).

To fully harness these properties and maximize the yield of bioactive constituents, modern “green” extraction technologies are increasingly employed. These environmentally sustainable methods, which include techniques such as ultrasonic extraction, pressurized hot water extraction, and supercritical fluid extraction, offer enhanced efficiency, reduced solvent usage, and lower environmental impact compared to conventional extraction approaches (Giacometti et al., 2018; Giacometti et al., 2024).

Comparison with Traditional Methods

Modern extraction techniques provide several advantages over conventional methods:

- **Use of safe solvents:** Transition from traditional organic solvents to environmentally benign alternatives such as water, supercritical CO₂, ionic liquids, and deep eutectic solvents (Giacometti et al., 2018).
- **Reduced energy consumption:** Optimization and intensification of extraction processes lead to lower energy usage (Giacometti et al., 2024).
- **Enhanced process safety:** Technologies prioritize the safety of operators and environmental sustainability (Giacometti et al., 2018).

Plant Material and Sample Preparation

The object of this study was the wild-growing Cherry laurel (*P. Laurocerasus*) plant (Pic.1) and its derivatives (fruits, leaves, and processed fruit products), specifically from the varieties known locally as anatsnekhi and pits. Samples were collected from two locations in western Georgia (Adjara region): Erge (41°33'41.0"N, 41°41'48.0"E) and Tkhilnari (41°33'49.0"N, 41°39'11.0"E), *Senecio platyphyllus* (Pic.2) Khulo region (41° 38' 47" N / 42° 18' 40" E). Following collection, a portion of the samples was immediately stored at -25 °C for future analyses. Another portion of the fresh fruit was directly processed into various preparations. Additional samples were lyophilized and subsequently stored at 4 °C until use.



Picture1. *Prunus laurocerasus*



Picture 2. *Senecio platyphyllus*

Instrumentation and Analytical Methods

The following analytical instruments were employed in the study: a high-performance liquid chromatograph (HPLC) system (Waters, Binary HPLC Pump 1525) equipped with ultraviolet (UV), visible (Vis), and refractive index (RI) detectors, as well as a Waters 432 conductivity detector. Additionally, an ultra-high-performance liquid chromatography (UHPLC) system (Waters Acuity H-Class) was used, coupled with a photodiode array (PDA) detector and a mass spectrometer (QDa detector) for compound identification. For physicochemical measurements, a Mettler Toledo pH/Ion meter S220 and S230 conductivity meter were utilized. Ultraviolet-

visible spectrophotometric analyses were conducted using a Mettler Toledo UV5 UV/Vis scanning spectrophotometer.

Extraction of Samples

Ultrasonic Extraction (USE)

Ultrasonic extraction is based on the mechanical and cavitation effects of ultrasound, which significantly enhance the mass transfer processes involved in extraction. The mechanical effect facilitates the propagation of ultrasonic waves through the medium, causing vibration of particles and improving molecular diffusion and solvent penetration. Cavitation occurs when ultrasonic pressure induces the formation, growth, and collapse of microbubbles in the medium, generating localized high temperatures and pressures that disrupt cell walls and release bioactive compounds into the solvent. This technique accelerates extraction kinetics and improves yield without extensive thermal degradation (Surmanidze et al., 2024).

Pressurized Hot Water Extraction (PHWE)

PHWE utilizes water at elevated temperatures and pressures to maintain it in a liquid state above its atmospheric boiling point, enhancing its solvent properties. Under these conditions, water exhibits altered polarity, allowing it to dissolve a broader range of both polar and nonpolar compounds. PHWE is an environmentally friendly and energy-efficient technique that offers rapid extraction and high-quality yields. However, temperature control is critical, as temperatures exceeding 160 °C may degrade thermolabile compounds, diminishing their antioxidant potential. The use of natural deep eutectic solvents (NADES) as co-solvents can further enhance the extraction of phenolic compounds. Additionally, integration with pulsed electric field (PEF) treatment can improve selectivity and sustainability (Giacometti et al., 2024).

Supercritical Water Extraction (SWE)

Supercritical water extraction involves the use of high pressure to keep water in a liquid state even at elevated temperatures, typically between 50–200 °C. This approach is particularly effective for extracting thermally stable polyphenolic compounds. The principle is based on the relationship between pressure and boiling point; increasing system pressure prevents water from vaporizing at high temperatures, thereby enhancing solubility and diffusivity of target analytes. Water is typically used as the solvent due to its non-toxic, economical, and environmentally friendly nature. The success of SWE depends heavily on the design and operation of the extraction apparatus, including the pressure vessel and ancillary equipment (Giacometti et al., 2018).

Identification of Non-Anthocyanin Phenolic Compounds

Non-anthocyanin phenolic compounds were identified using UHPLC-PDA-MS (Waters Acquity UPLC system with QDa detector). Separation was achieved on a BEH C18 column (1.7 μ m particle size) using a binary gradient elution program with solvent A (0.2% formic acid in water) and solvent B (acetonitrile). The flow rate was maintained at 0.3 mL·min⁻¹, and the column temperature was set to 30 °C. The mass spectrometer was operated in negative electrospray ionization mode (ESI⁻), with the following settings: probe temperature at 500 °C, spray voltage at 0.8 kV, capillary voltage at 1.5 kV, and cone voltage range between 5–40 V. The scan range was set from m/z 100–1200. Compound identification was conducted through comparison with standard reference compounds, literature data, and the METLIN metabolite database. Prior to chromatographic analysis, all samples and eluents were filtered through 0.45 μ m membrane filters. The injection volume for all samples was 5.0 μ L (Sarker et al., 2013).

Anthocyanin Analysis

Anthocyanin compounds were analyzed using UPLC-PDA-MS with a BEN C18 column and positive electrospray ionization (ESI-MS). Solvent systems included 2% formic acid (Solvent 1) and methanol (Solvent 2) with a gradient elution. Conditions were maintained at a flow rate of 0.3 mL/min, column temperature of 30 °C, mass scan range of 100–1200 Da, probe temperature at 500 °C, spray voltage at 0.8 kV, and capillary

voltage at 1.5 kV. The method allowed precise identification of cyanogenic anthocyanins, known for their anticancer properties (Khan et al., 2021; Solomon et al., 2017).

Alkaloids Analysis

Alkaloid compounds were analyzed using the same UPLC-PDA-MS approach with either BEN C18 or phenyl columns and positive ionization mode. Solvents included 0.1% formic acid (Solvent 1) and acetonitrile (Solvent 2). These methods facilitated the identification of pyrrolizidine alkaloids and other bioactive amines, which are critical to evaluating both therapeutic potential and toxicity (Jones & Taylor, 2021; Smith et al., 2020; Miller et al., 2019; Williams & Clark, 2020).

Determination of Antioxidant Activity

Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A 1 mL aliquot of the extract was mixed with 3 mL of DPPH solution and incubated in the dark for 15 minutes. Absorbance was recorded at 517 nm with a UV/Vis spectrophotometer. The antioxidant capacity was calculated based on the IC_{50} value, indicating the concentration required to inhibit 50% of DPPH radicals (Abashidze et al., 2024).

Statistical Analysis

All data were analyzed using analysis of variance (ANOVA) to determine the significance of observed differences among treatments. Mean comparisons were carried out using the Least Significant Difference (LSD) test at a 5% significance level ($p \leq 0.05$). Additionally, Duncan's Multiple Range Test (DMRT) was applied to further evaluate differences among treatment means at the 95% confidence level. All statistical computations were performed using Microsoft Excel.

Results and Discussion

After analyzing the pulp of *P. Laurocerasus* next substances were identified:

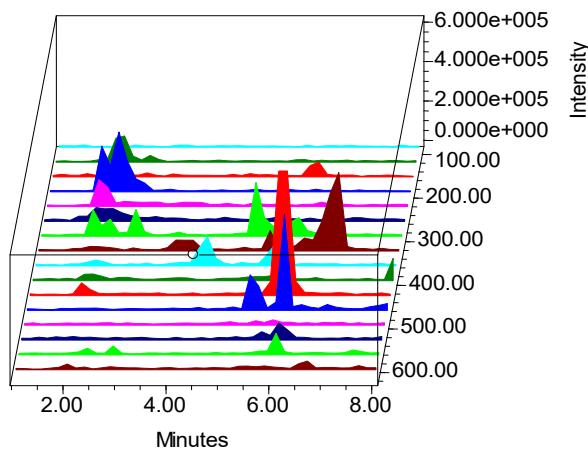
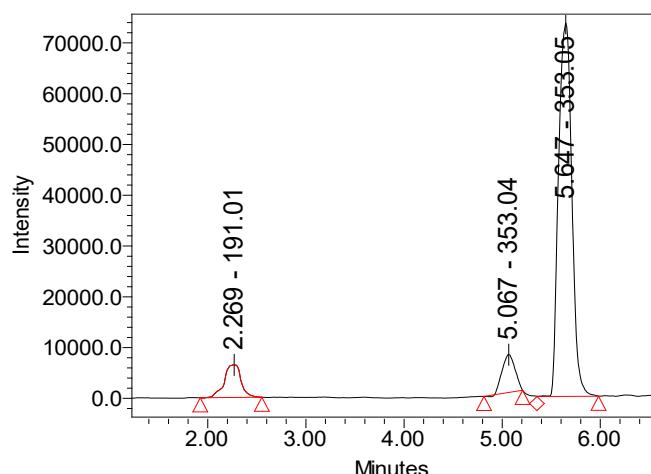


Figure 1. Three-dimensional HPLC-MS chromatogram of *P. Laurocerasus* pulp extract

Substance 1 - ESI-MS - m/z 191.01 [M-H⁻]; retention time - 2.269 min, maximum absorbance - UV-222.8 and 273.7 213.1 nm. According of the standard compound (Quinic acids (Sigma-Aldrich)) and the METLIN mass database of compounds, substance 3 corresponds to Quinic acids (C₇H₁₂O₆) (Figure 1).

Substance 2 -ESI-MS - m/z 132.93 [M-H⁻] dominant compounds, fragmentation ESI-MS m/z 114.99. retention time 2.819 min, maximum absorbance UV- λ max 215.5 nm. According of the standard compound (Malic acids (Sigma-Aldrich)) and the METLIN mass database of compounds, substance 2 corresponds to malic acid

Figure 2. HPLC-MS chromatogram of compounds detected in the pulp extract of *P. laurocerasus*

Substances 3 and 4, with ESI-MS-m/z 353.04 and 353.05[M-H], respectively, were identified with retention times of 5.067 and 5.647 minutes, and maximum absorption in the UV at 324.9 nm and 324.2 nm, respectively. According to the mass base of METLIN compounds (<https://metlin.scripps.edu>), substance 3 is Neochlorogenic acid, with a molecular formula of C16H18O9, a molecular weight of 354.31g/mol, and an IUPAC Name of (1R,3R,4S,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid. Substance 4 is Chlorogenic Acid, with the same molecular formula, molecular weight, and a slightly different IUPAC Name of (1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid (Figure 2).

Substances 5 and 6, with ESI-MS - m/z 449.08[M+H] and 449.05 (447.10) were detected on the chromatogram as two compounds. The retention times were 4.156 and 4.664 minutes, respectively. The maximum absorption in the ultraviolet beam was at 279.8 nm and 518 nm (Figure 19). The molecular weight of the fragment m/z 286.92 corresponded to Cyanidin. According to the METLIN database, substance 13 corresponded to Cyanidin-3-O-galactoside (2S,3R,4S,5R,6R)-2-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenyl-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol, with an empirical formula of C21H21O11+ and a molecular weight of 449.4 g/mol. Substance 14 corresponds to Cyanidin-3-O-glucoside (2S,3R,4S,5R,6R)-2-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-chromen-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5- triol, with an empirical formula of C21H21O11+ and a molecular weight of 449.4 g/mol.

Following the production of traditional juice, the remaining residues —that retains a substantial proportion of biologically active compounds—presents a valuable resource for further utilization. In this context, the application of environmentally friendly, or “green,” extraction techniques is of particular relevance. Among these, ultrasonic-assisted extraction using water as a solvent has demonstrated high efficiency. This method enables the recovery of phenolic-rich extracts from the residue, characterized by significant antioxidant activity (AA), as indicated by the capacity to achieve 50% inhibition of a 0.1 mM DPPH radical solution (expressed in mg).

Table 1. Phytochemical composition and antioxidant activity (DPPH inhibition) of seed and waste samples dried by different methods

Sample	Total phenols, mg/g	Chlorogenic acids mg/g	Catechins, mg/g	Anthocyanins, mg/g	AA, 0.1 mM DPPH inhibition by mg Sample
Seed	20.11	0.050	0.75	0.16	2.33
Waste dried by vacuum	37.79	0.041	2.11	0.31	1.13
Waste dried by 35 °C	26.84	0.032	1.89	0.29	1.33

To ensure the stability and preservation of bioactive compounds in fruit juice post-extraction, drying processes were employed. Prior to extraction, the fruit pulp was mechanically separated from the seeds and skins. The resulting juice was then subjected to either vacuum drying or conductive drying at 35 °C. Vacuum drying proved significantly more effective in retaining thermolabile compounds, preserving approximately 85–90% of the initial anthocyanin content. In contrast, conductive drying led to a substantial degradation of these pigments,

with retention rates falling to nearly 50%. A comparable trend was observed for chlorogenic acid, where vacuum drying facilitated markedly higher preservation. Furthermore, conductive drying resulted in the loss of nearly 70% of catechins, underscoring the susceptibility of these polyphenolic compounds to heat-induced degradation. These findings highlight the critical importance of selecting appropriate low-temperature drying techniques to maintain the phytochemical integrity of fruit-derived extracts. The pit of *Prunus laurocerasus* is rich in phenolic compounds (20.11 mg/g), among which predominate catechins (0.75 mg/g). The skin residues on the pit are a source of anthocyanin compounds (0.16 mg/g). Accordingly, AA is high (2.33 mg Sample inhibition 0.1 mM DPPH)(Table 1).

Antioxidant activity (AA) demonstrates a direct correlation with the concentration of biologically active compounds, particularly phenolic constituents. Experimental results indicate that higher levels of total phenolics (e.g., 37.79 mg/g) are associated with significantly enhanced antioxidant capacity, as evidenced by the ability of 1.13 mg of sample to inhibit 0.1 mM DPPH radicals. This positive relationship underscores the critical role of phenolic compounds in contributing to the radical scavenging potential of plant-derived extracts. In the case of *S. platyphyllus*, several challenges remain. Notably, the extraction process for alkaloids from the aerial parts of the plant has not been fully optimized yet. Furthermore, the qualitative and quantitative analysis of these compounds currently relies on thin-layer chromatography (TLC), which may limit the accuracy and sensitivity of the results compared to more advanced analytical techniques (Figure 3).

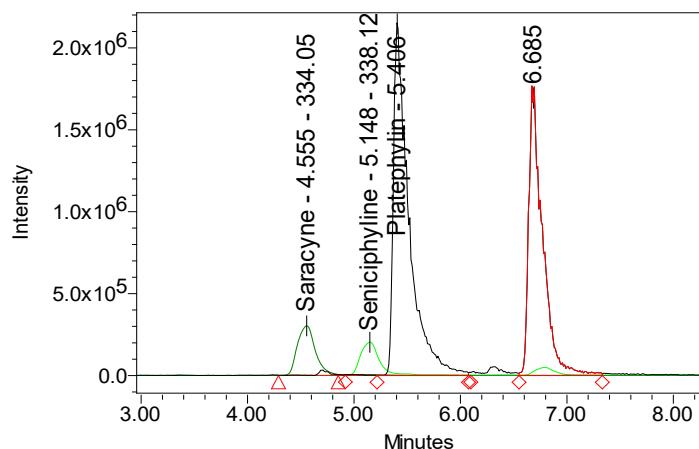


Figure 3. UHPLC-QDa chromatogram of alkaloid compounds detected in the *S. platyphyllus*

We were able to significantly reduce the extraction duration from the conventional 24 hours to just 2 hours High-pressure and high-temperature water extraction. Subsequent analysis using ultra-high-performance liquid chromatography (UHPLC) coupled with photodiode array (PDA) and mass spectrometric (MS) detection (Waters UPLC Acuity system with QDa detector) enabled the identification of the alkaloids saracine, seneciphylline, platiphylline, and its N-oxide. Chromatographic separation was performed using a UPLC Phenyl column (1.7 μ m particle size), with a mobile phase composed of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). The injection volume ranged from 1.0 to 3.0 μ L. This analytical procedure reduced the detection time from 8–12 hours to approximately 15 minutes per run (with three replicates), thereby decreasing the total analytical time to under 1 hour. As a result, the overall research process was completed within 3 hours.

Conclusion

The UPLC-PDA-MS analysis conducted on the biologically active compounds of the Georgian endemic plants *Prunus laurocerasus* and *Senecio platyphyllus* has provided valuable insights into their chemical composition and pharmacological potential. The optimization of the extraction process for *Senecio platyphyllus* has significantly improved the efficiency of compound isolation, with particular attention given to the identification of key alkaloids, including Platiphylline, Seneciphylline, and Saracyne. These advancements in extraction and analytical techniques are crucial for further exploring the therapeutic applications of these plants. Additionally, the study established that the fruits of *Prunus laurocerasus* hold significant promise for the development of antioxidant biopreparations and aromatic culinary flour. This research highlights the potential of these endemic plants in both medicinal and food industries, promoting sustainable practices and the valorization of local plant resources.

Recommendations

Based on the experimental findings, it is recommended that in addition to consuming the fruit of *Prunus laurocerasus* in its raw form, vacuum drying be employed as a preservation method to retain its biologically active medicines. This technique effectively minimizes thermal degradation of sensitive phytochemicals, enhancing the potential for their application in the development of nutraceuticals and pharmaceutical formulations. Also, for the efficient recovery of bioactive compounds from *Senecio platyphyllus*, particularly alkaloids, water-based extraction under conditions of elevated temperature and pressure is advised. This green extraction approach not only improves yield but also aligns with principles of environmental sustainability. For accurate qualitative and quantitative analysis of alkaloid profiles, the implementation of ultra-performance liquid chromatography coupled with mass spectrometry (UPLC-MS) is strongly recommended, due to its high sensitivity, resolution, and efficiency in compound identification.

Scientific Ethics Declaration

*The authors declares that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Conflict of Interest

*No conflict of interests is declared.

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