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Dihydrofuscin Profiling of *Solanum muricatum* using UHPLC-Q-Orbitrap HRMS Metabolomics

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Abstract

Pepino (*Solanum muricatum*) is an Andean-origin fruit that has attracted growing interest as a functional food due to its nutritional and health-promoting properties, yet detailed information on its chemical composition remains limited, particularly for fruit grown under Indonesian conditions. This study aimed to characterize the metabolite composition of pepino fruit extract using ultra-high-performance liquid chromatography coupled with quadrupole-Orbitrap high-resolution mass spectrometry (UHPLC-Q-Orbitrap HRMS). Pepino fruit was processed into powder and extracted by maceration in ethanol, followed by concentration using a rotary evaporator. The resulting extract was analyzed using UHPLC-Q-Orbitrap HRMS operated in positive and negative ionization modes, with compound identification performed through mass spectral library and database matching. Analysis in positive ionization mode revealed a dominant secondary metabolite eluting at a retention time of 5.82 minutes, showing a protonated molecular ion at a mass-to-charge ratio of 279.12341, corresponding to the molecular formula $C_{15}H_{18}O_5$. Based on accurate mass matching and fragmentation pattern analysis, with a diagnostic fragment ion at mass-to-charge ratio 109.02867, this compound was tentatively identified as Dihydrofuscin, an oxygenated polyketide compound with a fused chromone-pyranone ring skeleton. The mass measurement showed high accuracy, with an error below three parts per million, supporting confident identification of the compound's molecular formula. These findings provide preliminary evidence of a polyketide-type secondary metabolite in pepino fruit that has not been widely reported in this species, offering new insight into its phytochemical diversity. Further confirmation using nuclear magnetic resonance analysis or comparison with an authentic reference standard is recommended to establish definitive structural identity.

Keywords: Dihydrofuscin; metabolomics; pepino; *Solanum muricatum*; UHPLC-Q-Orbitrap HRMS.

INTRODUCTION

Solanum muricatum Aiton, commonly known as pepino, is a fruit species native to the Andean highlands of South America and has since been introduced to various tropical and subtropical regions worldwide (Cueva Manchego et al., 2025). The plant belongs to the Solanaceae family, placing it taxonomically close to other economically important crops such as tomato, potato, and eggplant (Gui et al., 2026). Pepino has traditionally been consumed as fresh fruit and processed into juice, salad, and dessert preparations because of its high water content, distinctive aroma, and mild sweet flavour (Öncü & Yeşil, 2024). Besides its sensory appeal, the fruit has been recognized as a promising source of natural bioactive compounds that may contribute to disease prevention and functional food development. The composition and quality of pepino fruit are strongly influenced by both genetic and environmental factors (Yang et al., 2023). Considerable physical variation exists among cultivars in terms of fruit size, shape, and color, which in turn affects their internal chemical composition. Environmental conditions during cultivation, such as temperature and humidity, further influence fruit development and, consequently, the accumulation of internal metabolites (Zhu et al., 2023). Maintaining fruit quality during postharvest handling and storage also remains a major challenge in the pepino supply chain, as metabolite degradation during this period can significantly affect the fruit's nutritional and commercial value. Given these factors, a comprehensive understanding of pepino's metabolite composition is essential not only for quality assurance but also for supporting its development as an export commodity (Jayan et al., 2026).

Despite growing interest in pepino as a functional food candidate, comprehensive metabolomic characterization of the fruit remains limited, particularly for cultivars grown under Indonesian conditions (Ullah et al., 2026). A detailed metabolite profile would provide valuable insight into the compounds responsible for pepino's nutritional properties and would serve as a scientific basis for its further utilization and product development. Recent advances in analytical chemistry have enabled comprehensive metabolomics-based approaches for plant metabolite characterization. Among the available techniques, ultra-high-performance liquid chromatography coupled with quadrupole-Orbitrap high-resolution mass spectrometry (UHPLC-Q-Orbitrap HRMS) has become a powerful tool for metabolite identification due to its high sensitivity, accurate mass determination, and high-resolution MS/MS fragmentation capability (Choudhury et al., 2025). This technique allows reliable separation and identification of a wide range of metabolites, even within complex plant matrices, making it particularly suitable for untargeted metabolomic studies of fruit crops. Although pepino has attracted increasing scientific attention, comprehensive information regarding its metabolomic profile using UHPLC-Q-Orbitrap HRMS remains scarce (Bayram & Ozturkcan, 2022). Therefore, this study aimed to characterize the metabolite composition of pepino fruit (*Solanum muricatum*) using UHPLC-Q-Orbitrap HRMS-based metabolomics. The findings are expected to provide comprehensive information regarding the metabolite profile of pepino and support its future development as a value-added product and valuable natural resource in Indonesia (Xu et al., 2026).

MATERIALS AND METHODS

Tool and Material

The tools used in this study were standard laboratory glass, air bubble washer, rhizome chopper, hybrid dryer warehouse, oven (Memmert), food dehydrator, grinding machine (Grinder) with gasoline motor drive (Honda), machine and sieve (Sieve Shaker) no. mesh 12, 18, 30, 60, 70, 120, 170, and 230, and rotary evaporator (IKA RV 10 digital). The materials used in this study were pepino fruit, 96% alcohol (Merck), and Whatman filter paper no. 42.

Processing Pepino Fruit into Powder

The process begins with pepino fruit washing using an air bubble washer to remove physical contaminants without damaging the fruit tissue. After wet sorting and size reduction, the fruit's moisture content is significantly reduced through a thermal process using a food dehydrator, oven, or hybrid drying building until it reaches a critical dry point safe from microbial growth. The dried fruit then enters the grinding stage using a grinder machine to reduce its size to fine particles. To produce fruit powder with uniform homogeneity and meet quality standards, the product is sieved using a sieve shaker with the appropriate mesh number (ranging from 12 to 230 mesh). The result is pepino fruit powder which is ready for maceration extraction (Ardyansyah et al., 2026; Wijaya et al., 2026).

Maceration of Pepino Fruit Powder

A 250 g sample of pepino fruit powder was added to 2500 mL of ethanol (1:10 w/v) and left to stand for 120 hours. The mixture was filtered using Whatman No. 42 paper. Next, use a rotary evaporator at 60°C and 300 rpm. Continue until there is no solvent left in the pepino fruit extract to achieve the maximum thickness result.

LC-HRMS Preparation and Testing

Sample preparation was carried out by weighing 10 mg of sample and then dissolving it in 1 mL of HPLC-grade methanol (Fisher Chemical). The mixture was homogenized using a digital vortex (Thermo Scientific) for 2 minutes (Fathimah et al., 2026). Once homogenized, the solution was filtered through a 0.2 µm nylon filter before being introduced into the chromatography system. Metabolomics analysis was performed using a Thermo Scientific™ Vanquish™ Horizon ultra-high-performance liquid chromatography (UHPLC) system equipped with a binary pump. Analyte separation was carried out using a Thermo Scientific™ Accucore™ Phenyl Hexyl column with dimensions of 100 mm x 2.1 mm and a particle size of 2.6 µm. The column temperature was set at 40 °C with a sample injection volume of 5 µL. The mobile phase consisted of (A) MS-grade water with 0.1 % formic acid and (B) MS-grade acetonitrile with 0.1% formic acid. The flow rate was set at 0.3 mL/min for a total analysis time of 25 minutes. Gradient elution was set as follows: starting at 5% B, gradually increasing to 90% B in 16 minutes, maintained at 90% B for 4 minutes, and returning to the initial 5% B level until the 25th minutes (Windarsih et al., 2022). Parameters mass detection was performed using a Thermo Scientific™ Orbitrap™ Exploris 240 HRMS. Data acquisition was performed in Full MS/dd-MS2 mode with polarity switching (positive and negative). The spray voltage was set at 3500 V for positive mode and 2500 V for negative mode. The ion source used was Optamax™ NG Heated Electrospray Ionization (H-ESI) with the following gas parameters: sheath gas 35 AU, ux gas 7 AU, and sweep gas 1 AU. The ion transfer tube temperature was set at 300 °C and the vaporizer temperature at 320 °C. Full MS resolution was set at 60,000 FWHM with a scan range of m/z 70-800, while dd-MS2 resolution was set at 22,500 FWHM. Fragmentation was performed using nitrogen gas with variations in Normalized Collision Energy (NCE) of 30, 50, and 70. Data Analysis and Compound Identification Raw data were processed using Thermo Scientific Compound Discoverer 3.3 software. Compound

identification was performed through a search of the mzCloud mass spectra library, which includes endogenous metabolites, natural products, toxins, and vitamins (Azani et al., 2026). In addition, data matching was carried out with various mass databases such as Arita Lab 6549 Flavonoid, Natural Products Atlas, Lipid Maps, as well as various databases on ChemSpider such as KEGG, ChEBI, and PubChem (Cooper & Yang, 2024).

RESULTS AND DISCUSSION

Based on characterization using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) in positive ionization mode (ESI+), one of the main secondary metabolite compounds in the Pepino Fruit extract sample (F1) was successfully identified. The compound eluted at a retention time (RT) of approximately 5.82 minutes, with the main chromatographic peak observed in the range of RT 5.648-5.82 minutes (MS1 data recorded RT = 5.820 minutes and MS2 data at RT = 5.846 minutes), with an abundance intensity reaching ±4.11 × 10^6 counts. In addition to this main peak, the sample chromatogram profile also showed several minor peaks at RT 3.283, 3.907, 4.167, 5.006, and 5.326 minutes, indicating the presence of other metabolite compounds that were co-extracted from the pepino fruit matrix. First-order mass spectrometry (MS1) data showed the formation of a dominant protonated molecular ion $[M+H]^+$ at a mass-to-charge ratio (m/z) of 279.12341. This experimental value corresponds to the molecular formula $C_{15}H_{18}O_5$, with a theoretical calculated molecular weight (Calc. MW) of 278.11614 Da. Based on comparative compound database searching, this chromatographic and mass profile accurately leads to the compound Dihydrofuscin, a secondary metabolite belonging to the oxygenated polyketide group, characterized by a fused chromone/pyranone core skeleton (J. Zhang et al., 2025).

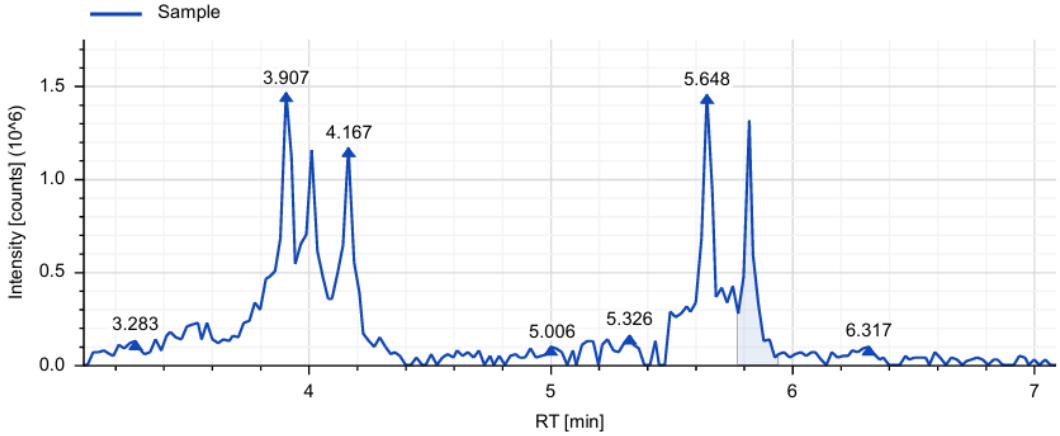


Figure 1. Chromatogram of the results of LC-HRMS analysis of Pepino fruit sample (F1) with an elution peak at RT 5.82 minutes.

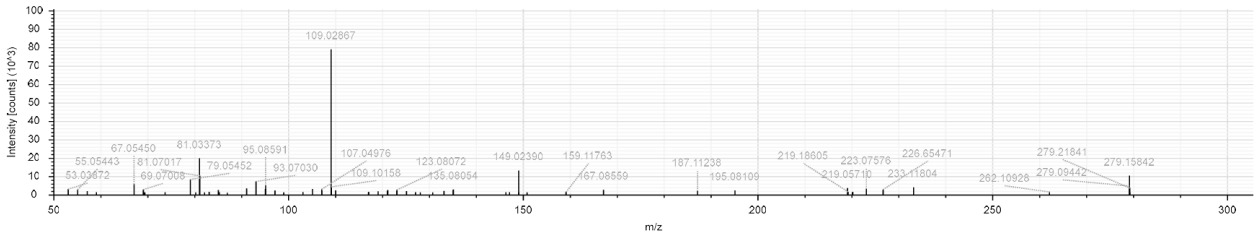


Figure 2. First-order fragmentation (MS1) mass spectrum of the Dihydrofuscin in Pepino fruit samples.

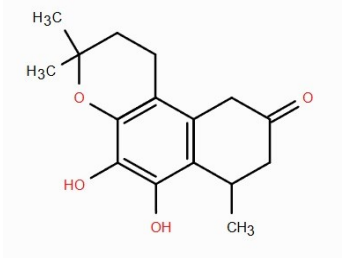


Figure 3. Chemical structure of the Dihydrofuscin

The liquid chromatogram profile showed that Dihydrofuscin eluted relatively late, at around minute 5.82, in contrast to polar glycoside-type compounds that typically elute earlier. This reflects the compound's relatively low-to-moderate polarity (moderately lipophilic character) within the reversed-phase C18 chromatography system. The absence of a sugar (glycone) moiety attached to the core skeleton, together with the presence of a gem-dimethyl substituent on the pyran ring and a methyl group on the lactone ring,

increases the molecule's lipophilicity. As a result, its interaction with the nonpolar C18 stationary phase becomes stronger, causing a longer retention time compared to glycosylated compounds. High-resolution mass spectrometry (HRMS) analysis provided a very high degree of certainty regarding the empirical formula through accurate mass matching. The experimental $[M+H]^+$ ion detected at m/z 279.12341 showed very good agreement with the theoretical mass of the protonated ion $C^{15}H_{19}O_5^+$ (calculated = 279.1227 Da), with a mass error value below 3 ppm. This value demonstrates the reliability of the FTMS instrument system (Orbitrap) in accurately confirming the elemental composition of the target compound (Tenea et al., 2024).

The most convincing structural evidence was obtained from the fragmentation pattern of the MS2 spectrum using the High-energy Collision Dissociation (HCD) method. The fragmentation results showed the formation of a dominant product peak (base peak) at m/z 109.02867, accompanied by a series of minor fragment ions at m/z 149.02390, 123.08072, 107.04976, 95.08591, 81.03373, and 67.05450. The base peak at m/z 109.02867 corresponds well to a dihydroxybenzene aromatic fragment ion ($C_6H_5O_2^+$ calculated mass = 109.0284 Da) formed through cleavage of the pyranone/chromene ring in the core skeleton. This fragment serves as a diagnostic fingerprint confirming the presence of a doubly oxygenated aromatic ring (the dihydroxy groups, shown as HO and OH in the structure), a characteristic feature of the partially hydrogenated fuscin/chromone-type skeleton (W. Zhang et al., 2026).

Structurally, Dihydrofuscin consists of a fused tricyclic polyketide ring system: a chroman ring bearing a gem-dimethyl substituent (2,2-dimethylchroman) fused to an aromatic ring substituted with two hydroxyl groups, along with a lactone (pyranone) ring bearing a methyl substituent at a specific position. The adjacent dihydroxy groups on the aromatic ring play an important role in antioxidant capacity through hydrogen atom donation, while the carbonyl group on the lactone ring provides an extended π-electron conjugation system that contributes to resonance stabilization of the molecule following redox interactions. Chemotaxonomically, the fuscin family of compounds and their derivatives (fuscin, dihydrofuscin, dihydrosecofuscin, and secofuscin) are fundamentally known as polyketide metabolites that have been isolated and characterized using LC-MS/MS and NMR analyses from fungi, such as Oidiodendron griseum.Interestingly, the biological activity reported for this group of compounds includes relatively weak antibacterial activity, mainly demonstrated by the derivatives dihydrosecofuscin and secofuscin against Gram-positive bacteria, whereas Dihydrofuscin itself has not been widely reported to exhibit significant specific bioactivity in that literature. The detection of a fuscin-type compound in the pepino fruit extract (*Solanum muricatum*) is noteworthy and warrants further investigation, given that this class of compounds is typically associated with fungal metabolism — raising the possibility of endophytic microbial involvement within the fruit tissue, or a structural resemblance to other plant metabolites matched by the compound database. This should be confirmed through further analysis, such as NMR or isolation of the pure compound (DiGeronimo & Barr, 2026).

CONCLUSIONS

The results of UHPLC-Q-Orbitrap HRMS characterization of the pepino fruit extract (F1) revealed the presence of a compound whose mass and fragmentation profile most closely matches Dihydrofuscin ($C_{15}H_{18}O_5$), confirmed through the $[M+H]^+$ ion at m/z 279.12341 and the dominant diagnostic fragment at m/z 109.02867. This identification is tentative, based on accurate-mass database matching, and ideally should be supported by NMR data or comparison with an authentic standard for definitive structural confirmation.

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Competing Interests: The authors declare that there are no competing interests.

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