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Phytochemical Screening and LC-HRMS Metabolite Profiling of Red Dragon Fruit and Barlin Banana Pseudostem from Banyuwangi: Exploring Local Natural Resources for Biomaterial Development

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ABSTRACT

Background: Banyuwangi Regency is rich in agricultural resources, including red dragon fruit (*Hylocereus polyrhizus*) and Barlin banana (*Musa balbisiana Colla* cv. Barlin), which have promising potential as renewable biomaterial sources. However, metabolomic investigations of these resources remain limited, particularly regarding comprehensive untargeted profiling of locally specific materials such as Barlin banana pseudostem from Banyuwangi, Indonesia. ***Objective:*** This study aimed to investigate the phytochemical characteristics and metabolite profiles of red dragon fruit and Barlin banana pseudostem using qualitative phytochemical screening and ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS). ***Methods:*** Both plant materials were taxonomically authenticated before extraction. Qualitative phytochemical screening was performed to detect flavonoids, phenolics, alkaloids, and saponins. Metabolite profiling was conducted using UHPLC-HRMS, and compound annotation was performed using the mzCloud™ and ChemSpider™ databases with a mass tolerance of ±5 ppm. ***Results:*** Phytochemical screening showed that the red dragon fruit extract contained flavonoids, alkaloids, and saponins, whereas the Barlin banana pseudostem extract contained only saponins. UHPLC-HRMS detected 185 and 220 chromatographic peaks in the red dragon fruit and Barlin banana pseudostem extracts, respectively. Metabolite classification identified 100 metabolites in the red dragon fruit extract and 117 metabolites in the Barlin banana pseudostem extract, predominantly comprising amino acids and derivatives, organic acids, phenolic compounds, flavonoids, alkaloids, fatty acids, terpenoids, nucleosides, vitamins, and other metabolites. ***Conclusion:*** The integration of qualitative phytochemical screening and UHPLC-HRMS provided complementary chemical information on red dragon fruit and Barlin banana pseudostem from Banyuwangi, Indonesia. The resulting phytochemical and putatively annotated metabolite profiles provide preliminary chemical baseline information that may support subsequent targeted, quantitative, and functional investigations. Further biological and biomaterial-based validation is required to determine the functional relevance and potential applications of the detected metabolites.

Keywords: Barlin banana pseudostem; LC-HRMS; metabolomics; phytochemical screening; red dragon fruit

Introduction

The growing demand for sustainable biomaterials has stimulated increasing interest in renewable plant-derived resources for the development of bio-based materials with functional properties (Thirumavalavan et al., 2024; Chang et al., 2025; Hanifa et al., 2023; Qomariyah et al., 2021). Beyond serving as renewable sources of structural biomass, plants contain diverse secondary metabolites, including phenolic compounds, flavonoids, alkaloids, terpenoids, and organic acids (Chutia et al., 2024; Rahmana Putra et al., 2024), which may provide functional properties relevant to biomaterial development, such as antioxidant, antimicrobial, and anti-inflammatory activities (Guo et al., 2026; Jafari et al., 2026; Yan et al., 2026). Consequently, characterization of plant-derived metabolites is important for identifying bioactive constituents that may contribute to the future development of multifunctional bio-based materials (Andrade et al., 2024; Chauhan et al., 2026; Veer et al., 2026). Comprehensive metabolite profiling can

therefore provide a chemical basis for selecting and valorizing locally available plant resources for subsequent biomaterial engineering and biomedical applications.

Chronic wounds and microbial infections remain important challenges in biomedical care (Afshin et al., 2026; J. Wang et al., 2026), creating a continuing need for sustainable biomaterials with functional properties that may support future wound-management applications (Deeh et al., 2025; Ejegu et al., 2025). Plant-derived resources containing diverse bioactive metabolites are therefore of interest as potential sources for the development of multifunctional biomaterials (Ali et al., 2026; J. Wang et al., 2026). However, the potential relevance of these metabolites to wound-healing applications requires further biological and biomaterial-based evaluation and is beyond the scope of the present metabolomic characterization study.

Indonesia is recognized as one of the world's richest megabiodiversity countries, harboring thousands of medicinal and economically important plant species (Chel-Guerrero et al., 2026; Deb et al., 2026; Rajput et al., 2025). Among its regions, Banyuwangi Regency, located at the eastern tip of Java Island, represents one of Indonesia's major agricultural and horticultural centers. According to regional agricultural statistics, Banyuwangi produced approximately 265,683 tons of dragon fruit and 206,382 tons of bananas in 2023, highlighting the substantial availability of these agricultural resources in the region (Dinas Pertanian dan Pangan Kabupaten Banyuwangi, 2024). These abundant agricultural resources not only contribute significantly to the regional economy but also generate substantial quantities of agricultural biomass that may be further valorized for sustainable bio-based applications.

Among these local commodities, red dragon fruit (*Hylocereus polyrhizus*) has attracted considerable scientific attention because it contains abundant betalains, flavonoids, phenolic acids, vitamins, and various antioxidant compounds exhibiting antimicrobial, anti-inflammatory, cytoprotective, and free radical scavenging activities (Carmen et al., 2025; Deng et al., 2026; Nguyen et al., 2022). Meanwhile, the pseudostem of the local Barlin banana cultivar, which is commonly discarded as agricultural waste after fruit harvesting, represents a renewable lignocellulosic biomass rich in cellulose and various phytochemicals (Boyapati et al., 2023; Rahmana Putra et al., 2024; Veer et al., 2026). The utilization of banana pseudostem not only offers opportunities for value-added agricultural waste management but also supports the development of sustainable biomaterial resources aligned with circular bioeconomy principles.

Previous studies have predominantly focused on the nutritional value of dragon fruit, crude phytochemical screening, antioxidant activity, or the physicochemical characteristics of banana fibers (Chelliah et al., 2025; Gurumayum et al., 2025; Lopez-Nuñez et al., 2025; Rangra & Aggarwal, 2025; Sarma et al., 2026). Other investigations have emphasized cellulose extraction or fiber reinforcement applications (Miranda-Salas et al., 2026; Olivares et al., 2022; Soraisham et al., 2025). However, metabolomic information on these two locally important agricultural resources remains limited, particularly for the pseudostem of the local Barlin banana cultivar collected from Banyuwangi. Existing studies have generally emphasized nutritional, physicochemical, or broad phytochemical characteristics rather than untargeted LC-HRMS-based metabolite profiling of this specific plant material. Therefore, the novelty of the present study lies primarily in providing a preliminary untargeted LC-HRMS metabolite profile of Barlin banana pseudostem from Banyuwangi and characterizing it alongside red dragon fruit from the same region. This approach provides a region-specific chemical baseline for these local agricultural resources, rather than claiming biomaterial efficacy or biological activity.

Recent advances in liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) have enabled comprehensive untargeted metabolomic profiling with exceptional sensitivity and mass accuracy (Baskaran et al., 2025; Muchahary et al., 2024; Rawat et al., 2026). This analytical platform allows rapid identification of hundreds of metabolites simultaneously, facilitating the discovery of valuable phytochemicals that may contribute to future pharmaceutical, nutraceutical, and biomaterial innovations (Hailu et al., 2026; Li et al., 2026; Vupru & Deb, 2026). Integrating conventional phytochemical screening with LC-HRMS therefore

provides a more comprehensive understanding of the chemical composition of locally available biological resources.

From a biomaterial-development perspective, metabolite characterization represents an important preliminary step because the chemical composition of plant-derived resources can provide information for subsequent selection and functional evaluation of biomass for bio-based material applications (Li et al., 2026). Certain classes of plant metabolites, including phenolic compounds, flavonoids, organic acids, and other secondary metabolites, have been associated in previous studies with properties such as antioxidant or antimicrobial activity that may be relevant to multifunctional material systems (Muchahary et al., 2024). However, the presence of these metabolites alone does not establish biomaterial performance. Their functional contribution and compatibility with specific biomaterial matrices require subsequent targeted characterization, material fabrication, and biological evaluation.

Motivated by the enormous agricultural potential of Banyuwangi and the limited metabolomic information available for its indigenous plant resources, this study investigates the phytochemical characteristics and LC-HRMS metabolite profiles of red dragon fruit (*Hylocereus polyrhizus*) and Barlin banana pseudostem collected from Banyuwangi Regency, Indonesia. Unlike previous studies that primarily emphasize nutritional composition or fiber properties, the present work provides a comprehensive metabolite inventory to reveal the diversity of bioactive compounds present in these two important local commodities.

The findings of this study provide a preliminary chemical and metabolomic baseline for the characterization of red dragon fruit and Barlin banana pseudostem from Banyuwangi. The resulting phytochemical and putatively annotated metabolite profiles may support subsequent investigations into the chemical diversity and potential functional relevance of these locally available agricultural resources. However, the present study does not establish biomedical or biomaterial efficacy, and further targeted, quantitative, structural-confirmation, and biological studies are required to determine the functional properties and potential applications of the annotated metabolites.

Methods

Setting

This study was conducted over a one-month period from June to July 2026. Plant extraction, phytochemical screening, and sample preparation were carried out at the Chemistry Laboratory, Universitas Dr. Soekardjo, Banyuwangi, Indonesia. Plant taxonomic identification was performed at the Plant Taxonomy Laboratory, Universitas PGRI Banyuwangi, Indonesia. LC-HRMS analysis was conducted at the Integrated Research Laboratory, Universitas Brawijaya, Malang, Indonesia.

Materials

Fresh red dragon fruit (*Hylocereus polyrhizus*) and fresh Barlin banana (*Musa balbisiana* Colla cv. Barlin) pseudostems were collected from Banyuwangi Regency, East Java, Indonesia. Plant authentication was performed at the Plant Taxonomy Laboratory prior to analysis. The analytical-grade chemicals used in this study included methanol (CH_3OH, LC-MS grade, Merck, Darmstadt, Germany), formic acid (HCOOH, LC-MS grade, Merck, Darmstadt, Germany), acetonitrile (CH_3CN, LC-MS grade, Merck, Darmstadt, Germany), hydrochloric acid (HCl, 37%, Merck, Darmstadt, Germany), ferric chloride ($FeCl_3$, Merck, Darmstadt, Germany), magnesium powder (Mg, Merck, Darmstadt, Germany), Dragendorff reagent (Merck, Darmstadt, Germany), Mayer reagent (Merck, Darmstadt, Germany), distilled water (Aquadest, Ikapharmindo, Indonesia), and Whatman No. 1 filter paper (Cytiva, United Kingdom). Syringe filters (PTFE, 0.22 µm, Whatman, Cytiva, United Kingdom) were used prior to LC-HRMS analysis.

Instruments

The instruments used in this study included Q Exactive™ Hybrid Quadrupole-Orbitrap™ High Resolution Mass Spectrometer coupled with an UltiMate™ 3000 UHPLC System (Thermo

Fisher Scientific, Bremen, Germany), Ultrasonic cleaner (Branson 3510 Ultrasonic Bath, Branson Ultrasonics, USA), Analytical balance (Sartorius Entris II BCE224I-1S, Sartorius AG, Germany), Drying oven (Memmert UN55 Universal Oven, Memmert GmbH, Germany), Hot plate magnetic stirrer (IKA C-MAG HS 7, IKA-Werke GmbH, Germany), High-speed blender (Philips HR2223, Philips, Netherlands), Refrigerator (Sharp SJ Series, Sharp Corporation, Japan). Glassware and laboratory accessories included Volumetric flasks (Pyrex, USA), Beakers (Pyrex, USA), Erlenmeyer flasks (Pyrex, USA), Graduated cylinders (Pyrex, USA), Test tubes (Pyrex, USA), Glass funnels (Pyrex, USA), Micropipettes (Eppendorf Research Plus, Germany), Disposable pipette tips (Axygen, USA), Glass stirring rods, Syringes (Terumo, Japan), PTFE syringe filters (0.22 µm, Whatman, UK)

Procedures

a. Plant Collection and Taxonomic Identification

Fresh ripe red dragon fruit (Hylocereus polyrhizus) and a fresh Barlin banana (Musa balbisiana Colla cv. Barlin) pseudostem were collected from Purwoharjo Village, Purwoharjo District, Banyuwangi Regency, East Java, Indonesia, during the harvesting season. One fresh ripe red dragon fruit and one fresh Barlin banana pseudostem were used as biological specimens for the present study. The samples were collected and processed separately and were not pooled with other biological specimens prior to extraction. The red dragon fruit was selected based on a fresh ripe condition, excluding unripe, damaged, or spoiled fruits. The Barlin banana pseudostem was collected from a healthy plant and selected based on its fresh and good physical condition. Representative specimens of each plant were photographed in situ, and their morphological characteristics were documented during collection. Plant identification and taxonomic authentication were performed at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas PGRI Banyuwangi, Banyuwangi, Indonesia. The Barlin banana pseudostem was taxonomically determined as Musa balbisiana Colla cv. Barlin based on Taxonomic Determination Report No. 004/LHU/LAB-BIO/UNIBA/VI/2026, whereas the red dragon fruit was determined as Hylocereus polyrhizus (F.A.C. Weber) Britton & Rose based on Taxonomic Determination Report No. 005/LHU/LAB-BIO/UNIBA/VI/2026. The official determination reports were retained as supporting documentation for botanical authentication. No formal herbarium voucher accession number was assigned to the specimens used in the present study.

b. Preparation of Red Dragon Fruit Extract

Fresh ripe red dragon fruits (*Hylocereus polyrhizus*) were washed thoroughly under running tap water to remove adhering impurities and then rinsed with distilled water. Approximately 500 g of peeled fruit flesh was cut into small pieces and homogenized using a laboratory blender until a uniform slurry was obtained. The homogenized sample was transferred into a 1000 mL beaker and heated at 70°C for 30 min using a hot plate magnetic stirrer with continuous stirring at 300 rpm. The extraction temperature of 70°C for 30 min was selected to facilitate the extraction of water-soluble constituents from the homogenized fruit matrix within a relatively short extraction period. Nevertheless, this thermal treatment may affect the stability of heat-sensitive metabolites and potentially cause degradation or transformation of susceptible compounds. Therefore, the resulting LC-HRMS profile was interpreted as representing metabolites detectable under the applied extraction conditions rather than the complete metabolite composition of the fresh fruit. After heating, the extract was allowed to cool naturally to room temperature (25 ± 2°C). The cooled extract was filtered through Whatman No. 1 filter paper, and the obtained filtrate was collected in sterile amber bottles. A portion of the filtrate was immediately subjected to LC-HRMS analysis, while the remaining extract was stored at 2–4°C until further analysis.

c. Preparation of Barlin Banana Pseudostem Extract

Fresh pseudostems of Barlin banana (*Musa balbisiana* Colla cv. Barlin) were cleaned with running tap water followed by distilled water to remove soil and surface contaminants. The pseudostems were sliced into thin sections of approximately 2–3 mm thickness and dried in a

laboratory oven at 60°C for 48 h until a constant weight was achieved. The dried material was ground into fine powder using a laboratory grinder and sieved through a 60-mesh sieve. Approximately 100 g of powdered pseudostem was extracted with 1000 mL distilled water (1:10, w/v) under continuous stirring at 70°C for 30 min. The extract was cooled to room temperature and filtered through Whatman No. 1 filter paper. The resulting aqueous filtrate was directly used for phytochemical screening and LC-HRMS analysis without further drying or concentration.

The extraction procedures were adapted to the distinct physical characteristics and moisture content of the two plant matrices. The fresh red dragon fruit flesh was directly homogenized and subjected to aqueous extraction because of its soft and high-moisture matrix, whereas the more fibrous Barlin banana pseudostem was dried, milled, and sieved before extraction to obtain a homogeneous powdered material and facilitate handling. Both extracts were subjected to aqueous extraction at 70°C for 30 min; however, the differences in sample pretreatment and matrix characteristics were recognized as potential sources of variation in metabolite recovery.

d. Phytochemical Screening

Qualitative phytochemical screening was independently performed for both the red dragon fruit extract and Barlin banana pseudostem extract based on previous study (Darge et al., 2026; Novianti et al., 2025; Zhou et al., 2024). Each assay was performed once for each extract as a preliminary qualitative assessment. No dedicated positive or negative controls were included, and the presence of the respective phytochemical classes was assessed based on the characteristic color change or precipitation reaction described in the referenced methods. **Flavonoid Test**: One milliliter of extract was transferred into a test tube, followed by the addition of 1 g magnesium powder and 20 drops of concentrated hydrochloric acid (HCl). The mixture was gently shaken until homogeneous. The formation of orange, yellow, reddish, or dark red coloration indicated the presence of flavonoids. **Saponin Test:** One milliliter of extract was mixed with 10 mL distilled water in a test tube and heated for approximately 15 min. The solution was vigorously shaken for 15 s and allowed to stand for 10 min. The persistence of stable foam measuring at least 1 cm in height after adding 2–3 drops of 2 N HCl indicated the presence of saponins. **Alkaloid Test**: One milliliter of extract was mixed with 2 mL of 2 N hydrochloric acid and shaken thoroughly. The mixture was divided equally into two test tubes. The first tube received one drop of Dragendorff reagent, while the second tube received one drop of Mayer reagent. The appearance of a reddish-orange precipitate after addition of Dragendorff reagent and a creamy yellow precipitate after addition of Mayer reagent confirmed the presence of alkaloids. **Phenolic Test**: One milliliter of extract was mixed with 20 drops of 1% ferric chloride ($FeCl_3$) solution. The development of blue, dark green, purple, reddish, or black coloration indicated the presence of phenolic compounds.

e. LC-HRMS Analysis

LC-HRMS analysis was conducted at the Integrated Research Laboratory, Universitas Brawijaya, Malang, Indonesia. Approximately 10 mg of each dried extract was accurately weighed and dissolved in 1.0 mL LC-MS grade methanol. The solution was sonicated for 30 min using an ultrasonic bath at room temperature to facilitate metabolite extraction. The extracts were subsequently filtered through a 0.22 µm PTFE syringe filter before instrumental analysis. Chromatographic separation was performed using an UltiMate™ 3000 UHPLC system (Thermo Fisher Scientific, Germany) equipped with an Accucore™ Phenyl-Hexyl column (100 mm × 2.1 mm, 2.6 µm particle size). The mobile phase consisted of Solvent A (0.1% formic acid in ultrapure water) and Solvent B (0.1% formic acid in acetonitrile). The flow rate was maintained at 0.30 mL min^{-1}.

The column temperature was maintained at 40°C, and the injection volume was 3 µL. Mass spectrometric detection was performed using a Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer operating under positive electrospray ionization (ESI^+) mode. The operating parameters were Spray voltage: 3.30 kV, Capillary temperature: 320°C, Mass scan range: m/z 66.7–1000, Full MS resolution: 70,000, dd-MS^2 resolution: 17,500, Collision mode: Data-

dependent MS/MS (dd-MS^2), Sheath gas: Nitrogen, Auxiliary gas: Nitrogen, Sweep gas: Nitrogen. Raw data were processed using Compound Discoverer™ software (Thermo Fisher Scientific, version 3.3). Metabolite identification was performed by matching accurate mass spectra with the mzCloud™ and ChemSpider™ databases using a mass tolerance of ±5 ppm.

Data Analysis

Qualitative phytochemical screening results were interpreted based on the characteristic color changes or precipitate formation observed after the addition of specific reagents. These reactions were considered preliminary qualitative indicators of the corresponding phytochemical classes, including flavonoids, alkaloids, saponins, and phenolic compounds, rather than definitive confirmation of individual metabolites. The observed reactions were recorded and tabulated for both plant extracts.

The relative signal contribution of detected metabolites was evaluated based on chromatographic peak area percentages generated by Compound Discoverer™. These peak area percentages were used as semi-quantitative indicators of the detected LC-HRMS signal and were not interpreted as absolute metabolite concentrations because ionization efficiency and detector response may differ among compounds. Peak detection, chromatographic alignment, background subtraction, isotope grouping, and molecular feature extraction were automatically performed using the default workflow. Compound annotation was performed by comparing the experimental mass spectra with reference information in the mzCloud™ and ChemSpider™ databases using a precursor mass tolerance of ±5 ppm. Metabolite features showing consistent database-supported spectral matching were reported as putatively annotated metabolites rather than definitive structural identifications, as authentic analytical standards were not used. Features without sufficient supporting evidence were excluded from the reported metabolite list. The LC-HRMS measurements were performed at the Integrated Research Laboratory, Universitas Brawijaya, under the laboratory's established instrument operating procedures. Study-specific blank samples, pooled quality control samples, and dedicated carry-over assessment were not included in the experimental design of the present study. The annotated metabolites were subsequently classified into their respective phytochemical groups, including flavonoids, phenolic compounds, alkaloids, terpenoids, fatty acids, amino acids, organic acids, and other secondary metabolites based on available chemical databases and published literature. The putatively annotated metabolites were subsequently classified into their respective phytochemical groups, including flavonoids, phenolic compounds, alkaloids, terpenoids, fatty acids, amino acids, organic acids, and other secondary metabolites based on available chemical databases and published literature. The metabolite datasets obtained from LC-HRMS were exported into Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA) for data organization, tabulation, and descriptive analysis. The relative abundance of metabolites was evaluated based on chromatographic peak area percentages generated by Compound Discoverer™. The annotated compounds were ranked according to their chromatographic peak areas to identify metabolites contributing the highest relative analytical signals under the applied LC-HRMS conditions.

Result

Plant Taxonomic Identification

Plant taxonomic identification was successfully performed for both collected specimens at the Plant Taxonomy Laboratory, Universitas PGRI Banyuwangi, Indonesia. The red dragon fruit specimen was identified as *Hylocereus polyrhizus* (F.A.C.Weber) Britton & Rose (Family: Cactaceae), while the banana specimen was identified as *Musa balbisiana* Colla cv. Barlin (Family: Musaceae). The authenticated scientific names were consistently used throughout this study. Representative photographs of both plant materials obtained during the taxonomic identification process are presented in Figure 1, whereas the detailed taxonomic classification is summarized in Table 1.

Table 1. Taxonomic identification of plant materials used in this study.

Plant material	Scientific name	Family	Local name	Identification institution
Red dragon fruit	*Hylocereus polyrhizus* (F.A.C.Weber) Britton & Rose	Cactaceae	Buah naga merah	Plant Taxonomy Laboratory, Universitas PGRI Banyuwangi
Banana pseudostem	*Musa balbisiana* Colla cv. Barlin	Musaceae	Pisang Barlin	Plant Taxonomy Laboratory, Universitas PGRI Banyuwangi

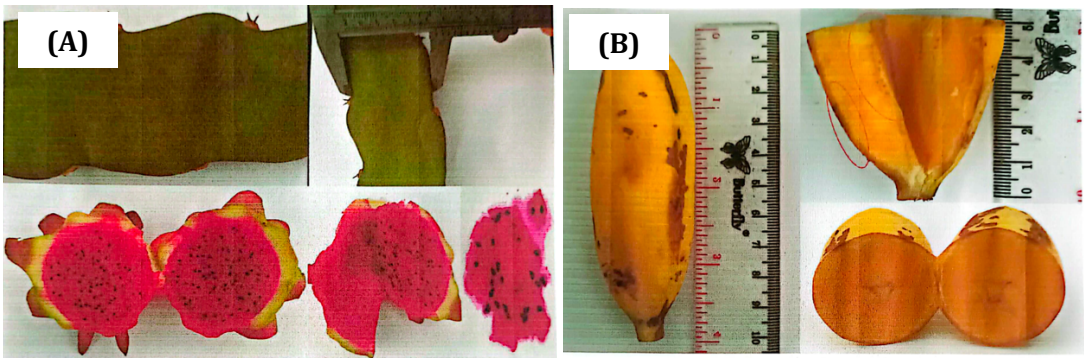

Figure 1. Taxonomic identification of the plant materials used in this study. (A) Morphological characteristics of *Hylocereus polyrhizus* (F.A.C.Weber) Britton & Rose. (B) Morphological characteristics of *Musa balbisiana* Colla cv. Barlin.

Phytochemical Screening

Qualitative phytochemical screening was performed to determine the presence of major secondary metabolites in the red dragon fruit and Barlin banana pseudostem extracts. The results of the phytochemical analysis are summarized in Table 2.

Table 2. Qualitative phytochemical screening of red dragon fruit and Barlin banana pseudostem extracts.

Phytochemical compounds	Observation	Red dragon fruit extract		Barlin banana pseudostem extract	
		Result	Color change	Result	Color change
Flavonoids	Orange/reddish coloration after Mg-HCl reaction	+		-	
Phenolics	Blue-green/black coloration after $FeCl_3$ addition	-		-	
Alkaloids	Reddish-orange precipitate (Dragendorff Method)	+		-	

	Creamy-yellow precipitate (Mayer Method)	+		-	
Saponins	Stable foam after HCl addition	+		+	

Note: (+) Presence of phytochemical compounds; (−) Absence of phytochemical compounds.

LC-HRMS Chromatographic Profile

The metabolite profiles of the red dragon fruit and Barlin banana pseudostem extracts were analyzed using Ultra-High Performance Liquid Chromatography coupled with High Resolution Mass Spectrometry (UHPLC-HRMS). Representative total ion chromatograms (TICs) obtained from both extracts are presented in Figure 2. Distinct chromatographic patterns were observed for each plant extract, indicating differences in their metabolite compositions.

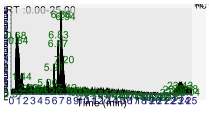

Figure 2. Representative UHPLC-HRMS total ion chromatograms of red dragon fruit (*Hylocereus polyrhizus*) extract

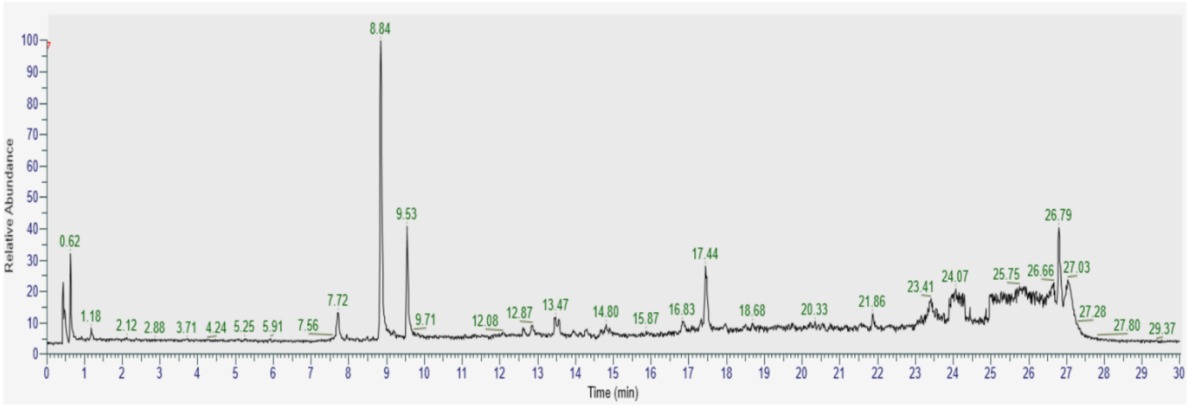

Figure 3. Representative UHPLC-HRMS total ion chromatograms of barlin banana pseudostem (*Musa balbisiana* Colla cv. Barlin) extract.

A total of 185 and 220 LC-HRMS-derived analytical features were detected in the red dragon fruit and Barlin banana pseudostem extracts, respectively, prior to metabolite annotation. These features represent detected analytical signals and should not be interpreted as individual metabolites or compounds prior to database-supported annotation.

Identification of Metabolites by LC-HRMS

LC-HRMS analysis enabled the annotation of metabolites detected in both plant extracts based on accurate mass measurement and spectral matching against the mzCloud™ and ChemSpider™ databases. The representative metabolites presented in the main text were selected based on their relative chromatographic peak areas and the availability of database-supported spectral annotation, with the aim of highlighting prominent analytical signals and representative chemical diversity. These representative compounds do not represent the complete set of metabolites detected in the extracts. The putatively annotated metabolites together with their retention times, molecular formulas, observed *m/z* values, and mass errors are summarized in Tables 3 and 4.

Table 3. Representative metabolites identified in red dragon fruit (*Hylocereus polyrhizus*) extract using UHPLC-HRMS.

No.	Compound	Retention Time (min)	Molecular Formula	Calculated MW	Mass Error (ppm)
1	L-Histidine	0.700	$C_6H_9N_3O_2$	155.0695	−0.11
2	N-Methylethanolamine phosphate	0.790	$C_3H_{10}NO_4P$	155.0347	0.21
3	Choline	0.807	$C_5H_{13}NO$	103.0997	1.00
4	L-(+)-Arginine	0.824	$C_6H_{14}N_4O_2$	174.1117	−0.23
5	1-[(3-Carboxypropyl)amino]-1-deoxy-β-D-fructofuranose	0.832	$C_{10}H_{19}NO_8$	281.1111	0.06
6	L-Histidine	0.832	$C_6H_9N_3O_2$	155.0695	−0.11
7	3,4-Diaminopyridine	0.832	$C_5H_7N_3$	109.0639	0.84
8	D-(+)-Proline	0.838	$C_5H_9NO_2$	115.0633	0.71
9	Betaine	0.843	$C_5H_{11}NO_2$	117.0789	0.15
10	L-Pyroglutamic acid	0.849	$C_5H_7NO_3$	129.0426	−0.74
11	Cytosine	0.856	$C_4H_5N_3O$	111.0433	0.54
12	N-Acetylmuramic acid	0.900	$C_{11}H_{19}NO_8$	293.1111	−1.24
13	Methyl isonicotinate	0.958	$C_7H_7NO_2$	137.0477	−1.51
14	D-(+)-Pipecolinic acid	0.972	$C_6H_{11}NO_2$	129.0789	−1.04
15	4-Guanidinobutyric acid	1.015	$C_5H_{11}N_3O_2$	145.0851	−1.02
16	DL-Stachydrine	1.072	$C_7H_{13}NO_2$	143.0946	−1.01
17	Adenine	1.145	$C_5H_5N_5$	135.0545	0.19
18	Benzocaine	1.236	$C_9H_{11}NO_2$	165.0789	−0.72
19	Pyridoxal	1.364	$C_8H_9NO_3$	167.0582	−0.17

Table 4. Representative metabolites identified in *Musa balbisiana* Colla cv. Barlin pseudostem extract using UHPLC-HRMS (sorted by retention time).

No.	Compound	Retention time (min)	Molecular formula	Observed *m/z*	Mass error (ppm)
1	Proline	0.029	$C_5H_9NO_2$	116.07037	0.029
2	L-Glutamic acid	0.502	$C_5H_9NO_4$	148.06006	0.502
3	L-Pyroglutamic acid	0.504	$C_5H_7NO_3$	147.07603	0.504

4	Chelidonic acid	0.611	$C_7H_4O_6$	185.00771	0.611
5	Leucylproline	0.627	$C_{11}H_{20}N_2O_3$	229.15416	0.627
6	Adenine	0.652	$C_5H_5N_5$	136.06146	0.652
7	Adenosine	0.653	$C_{10}H_{13}N_5O_4$	268.10333	0.653
8	Isoleucine	0.693	$C_6H_{13}NO_2$	132.10162	0.693
9	Acetophenone	0.729	C_8H_8O	121.06467	0.729
10	3-Nitrostyrene	0.842	$C_8H_7NO_2$	150.05463	0.842
11	L(-)-Pipecolinic acid	0.494	$C_6H_{11}NO_2$	130.08611	0.494
12	Cyclohexylamine	0.953	$C_6H_{13}N$	100.11217	0.953
13	Salicylic acid	0.973	$C_7H_6O_3$	139.03865	0.973
14	L-Phenylalanine	1.074	$C_9H_{11}NO_2$	166.08595	1.074
15	Isovanillic acid	1.668	$C_8H_8O_4$	151.03868	1.668
16	Syringic acid	1.979	$C_9H_{10}O_5$	199.05978	1.979
17	trans-3-Indoleacrylic acid	2.307	$C_{11}H_9NO_2$	188.07028	2.307
18	Indole	2.309	C_8H_7N	118.06509	2.309
19	(±)-Abscisic acid	3.743	$C_{15}H_{20}O_4$	265.14279	3.743

Overall, UHPLC-HRMS analysis successfully identified a diverse range of metabolites in both the red dragon fruit and Barlin banana pseudostem extracts. The detected compounds comprised various classes of primary and secondary metabolites with distinct chromatographic characteristics and molecular compositions. Detailed information regarding the putatively annotated metabolites, including their retention times, molecular formulas, and mass errors, is presented in Tables 3 and 4. These results provide a comprehensive metabolite dataset for subsequent interpretation and discussion.

Classification of Putatively annotated metabolites

LC-HRMS analysis revealed that the putatively annotated metabolites from both plant extracts could be classified into several major chemical groups, including amino acids and derivatives, organic acids, phenolic compounds, flavonoids, alkaloids, fatty acids and lipids, terpenoids, nucleosides and nucleobases, vitamins and cofactors, as well as other metabolites. The distribution of metabolite classes varied between the red dragon fruit and Barlin banana pseudostem extracts. A summary of the metabolite classification based on the identified compounds is presented in Table 5. Metabolite classification was performed only for database-supported annotated metabolites included in the final metabolite list. For each extract, the percentage assigned to each phytochemical class was calculated relative to the total number of annotated metabolites in that extract; unannotated LC-HRMS features were not included in the denominator.

Table 5. Classification of putatively annotated metabolites detected in red dragon fruit and Barlin banana pseudostem extracts by UHPLC-HRMS. Percentages were calculated using the total number of database-supported annotated metabolites in each extract as the denominator.

Metabolite class	**Red dragon fruit**	**Barlin banana pseudostem**
Amino acids and derivatives	18	24
Organic acids	12	19
Phenolic compounds	15	11
Flavonoids	9	6
Alkaloids	10	8
Fatty acids and lipids	7	14
Terpenoids	5	4
Nucleosides and nucleobases	6	7
Vitamins and cofactors	4	3

Others	14	21

Discussion

Phytochemical Characteristics and Their Relationship with LC-HRMS Profiling

The qualitative phytochemical screening indicated the presence of flavonoids, alkaloids, and saponins in the red dragon fruit extract, whereas the Barlin banana pseudostem extract showed a positive qualitative reaction only for saponins under the conditions employed. Interestingly, several metabolite groups that were not detected by the conventional phytochemical assays were subsequently identified through UHPLC-HRMS analysis. For example, although the ferric chloride test did not indicate the presence of phenolic compounds, UHPLC-HRMS successfully annotated several phenolic metabolites, including salicylic acid, syringic acid, and isovanillic acid in the Barlin banana pseudostem extract. Likewise, amino acids, nucleosides, vitamins, and numerous nitrogen-containing compounds identified by UHPLC-HRMS were not represented in the preliminary phytochemical screening because these metabolite classes are not specifically targeted by conventional qualitative assays.

The apparent discrepancy between the phytochemical screening and UHPLC-HRMS results can be explained by the fundamental differences in analytical principles. Qualitative phytochemical tests rely on color development or precipitate formation resulting from reactions between specific chemical reagents and functional groups. Consequently, these methods are primarily designed as rapid preliminary screening techniques and generally possess relatively low analytical sensitivity and selectivity. The intensity of the observed color reaction is strongly influenced by metabolite concentration, extraction efficiency, reagent stability, and the presence of interfering compounds within the plant matrix. Therefore, metabolites present at low concentrations may not produce visible color changes, leading to false-negative results despite their actual presence in the extract.

In contrast, UHPLC-HRMS provides high sensitivity, excellent mass accuracy, and superior analytical resolution, enabling the detection of metabolites even at trace concentrations. The combination of chromatographic separation and high-resolution mass spectrometry minimizes matrix interference and facilitates the annotation of numerous individual compounds based on accurate mass measurements and spectral matching against metabolomic databases. Consequently, UHPLC-HRMS revealed a much broader chemical diversity than conventional phytochemical screening, identifying multiple classes of metabolites that could not be distinguished by colorimetric methods alone. Similar observations have been reported in previous metabolomic studies (Al-Mekhlafi et al., 2021; Gao et al., 2026; Y. Wang et al., 2026), where LC-HRMS consistently detected substantially more metabolites than those identified using conventional phytochemical assays.

The complementary nature of both analytical approaches represents one of the strengths of the present study. Qualitative phytochemical screening provides rapid preliminary information regarding the occurrence of major secondary metabolite groups, whereas UHPLC-HRMS offers comprehensive molecular characterization with substantially higher analytical sensitivity. The integration of these two methods therefore enables a more complete understanding of the chemical composition of red dragon fruit and Barlin banana pseudostem, providing a stronger scientific basis for their future utilization as local biomaterial resources.

Comprehensive Metabolite Profiling by UHPLC-HRMS

Compared with qualitative phytochemical screening, UHPLC-HRMS provided a broader characterization of the detectable metabolite features in both plant extracts. A total of 185 LC-HRMS-derived analytical features were detected in the red dragon fruit extract and 220 features in the Barlin banana pseudostem extract under the applied analytical conditions. The higher number of detected features in the Barlin banana pseudostem extract indicates a broader analytical feature profile under these conditions, but does not necessarily imply greater biological activity, bioactivity, or functional potential. High-resolution mass spectrometry enabled putative

metabolite annotation based on accurate mass measurements and database-supported spectral matching, with mass accuracy generally within ±5 ppm.

Differences in sample pretreatment and matrix characteristics should also be considered when comparing the LC-HRMS profiles of the two plant extracts. The red dragon fruit extract was prepared from fresh fruit tissue, whereas the Barlin banana pseudostem was dried and powdered before aqueous extraction. Such differences may influence the recovery and stability of individual metabolites and may consequently affect the number and relative analytical signals of detected LC-HRMS features. Therefore, the differences observed between the two metabolite profiles should be interpreted as matrix- and extraction-condition-dependent chemical characteristics rather than direct evidence of differences in absolute metabolite concentrations or biological superiority.

Several representative metabolites identified in the red dragon fruit extract included amino acids, nucleobases, vitamins, and nitrogen-containing compounds such as L-histidine, arginine, betaine, adenine, pyridoxal, and choline. Meanwhile, the Barlin banana pseudostem extract contained amino acids, organic acids, phenolic acids, nucleosides, and plant-related metabolites including glutamic acid, proline, salicylic acid, syringic acid, isovanillic acid, adenosine, and abscisic acid. These findings are generally consistent with previous metabolomic studies reporting that tropical fruits and banana biomass contain diverse metabolites involved in antioxidant defense, plant metabolism, and cellular regulation (Rao et al., 2025; Rivera-Pérez et al., 2026). The present findings further demonstrate that untargeted LC-HRMS can reveal metabolite diversity that cannot be detected using conventional phytochemical assays alone.

Metabolite Classification and Potential for Biomaterial Development

Classification of the putatively annotated metabolites showed that amino acids and their derivatives represented the largest annotated metabolite group in both plant extracts, followed by organic acids, phenolic compounds, flavonoids, alkaloids, fatty acids, terpenoids, nucleosides, vitamins, and other metabolites. The predominance of amino acids and organic acids suggests that both natural resources possess metabolically active compositions that may contribute to various biological processes.

The phytochemical classes represented among the putatively annotated metabolites, including phenolic compounds, flavonoids, amino acids, fatty acids, and terpenoids, have been associated with diverse biological functions in previous studies (Gao et al., 2026; Miranda-Salas et al., 2026; Olivares et al., 2022). These reported activities include antioxidant and antimicrobial properties for certain phenolic and flavonoid compounds, roles in cellular metabolism for amino acids, and membrane-associated or antimicrobial functions for certain fatty acids and terpenoids. However, these biological activities were not evaluated in the present study, and the detection of these metabolite classes should not be interpreted as evidence of their functional activity or suitability for biomaterial applications. Further studies involving biomaterial fabrication and mechanical, cytotoxicity, antimicrobial, and wound-healing evaluations are required to establish their potential functional relevance.

The phytochemical classes represented among the putatively annotated metabolites, including phenolic compounds, flavonoids, amino acids, fatty acids, and terpenoids, have been associated with diverse biological functions in previous studies (Gao et al., 2026; Miranda-Salas et al., 2026; Olivares et al., 2022). These reported properties include antioxidant and antimicrobial activities for certain phenolic and flavonoid compounds, roles in cellular metabolism for amino acids, and membrane-associated functions for certain fatty acids and terpenoids. However, these biological properties were not experimentally evaluated in the present study and therefore should not be interpreted as confirmed activities of the investigated extracts or their putatively annotated metabolites. Similarly, the detection of these metabolite classes does not establish their suitability or efficacy for biomaterial applications. The metabolomic profiles generated in this study instead provide preliminary chemical information that may support subsequent targeted, quantitative, structural-confirmation, and biological

investigations of these locally available plant resources (Chel-Guerrero et al., 2026; Deb et al., 2026; Huo et al., 2024; Liu et al., 2024; Rajput et al., 2025).

The potential translation of the metabolomic findings into biomaterial applications remains hypothetical and requires systematic experimental validation. Future studies should first investigate the fabrication of plant-derived biomaterial formulations, followed by physicochemical and mechanical characterization to determine material stability and functional properties. Subsequent cytotoxicity and antimicrobial evaluations are required to assess biological safety and antimicrobial performance, while appropriate in vitro and/or in vivo wound-healing models would be necessary to determine therapeutic relevance. Thus, the present metabolomic findings should be regarded as preliminary chemical information that may guide the selection and further investigation of plant-derived resources rather than as evidence of biomaterial efficacy.

Study Novelty, Implications, and Limitations

This study provides one of the first comprehensive metabolomic reports describing both red dragon fruit and the local Barlin banana pseudostem collected from Banyuwangi Regency using UHPLC-HRMS. Unlike previous investigations that primarily focused on nutritional composition, fiber characterization, or qualitative phytochemical screening, the present work offers detailed metabolite annotation together with metabolite classification. The integration of conventional phytochemical screening and untargeted LC-HRMS therefore provides a more comprehensive chemical characterization of two important local agricultural resources.

The findings provide baseline chemical information on the LC-HRMS-detectable metabolites of Banyuwangi's locally important plant resources, particularly red dragon fruit and Barlin banana pseudostem, and may support future research on their sustainable utilization. Nevertheless, several limitations should be acknowledged. Metabolite assignments in the present study were based on accurate mass measurements and database-supported spectral annotation without confirmation using authentic analytical standards. Definitive metabolite identification requires further confirmation through MS/MS spectral comparison with authentic reference standards and, where appropriate, complementary orthogonal analytical approaches. In addition, quantitative metabolite determination and biological activity evaluation were not performed. Future studies should therefore include targeted metabolomics, quantitative validation, authentic-standard-based MS/MS confirmation, and appropriate biological assays to verify the identity and functional relevance of the annotated metabolites.

An important limitation of the present study is the use of a single biological specimen for each plant material. Therefore, the observed LC-HRMS profiles represent the chemical characteristics of the sampled specimens under the applied extraction and analytical conditions and cannot be used to estimate biological variation within the species or cultivar. Future studies should incorporate multiple independent biological replicates to assess biological variability and improve the generalizability of the metabolomic findings.

The present study has several analytical limitations. Study-specific solvent blanks, pooled QC samples, and independent analytical replicates were not included in the LC-HRMS workflow. Consequently, analytical repeatability, potential background contamination, carry-over, and batch-related variation could not be quantitatively assessed within the present dataset. In addition, the precursor mass tolerance used for database-supported annotation should not be interpreted as an independent validation of instrument mass accuracy. Future metabolomics studies should incorporate appropriate blank samples, pooled QC samples, analytical replicates, documented instrument calibration and system-suitability procedures, and mass-accuracy validation to strengthen analytical quality assurance and metabolite annotation confidence.

Conclusion

This study provides qualitative phytochemical screening and broad untargeted UHPLC-HRMS metabolite profiling of red dragon fruit (Hylocereus polyrhizus) and Barlin banana pseudostem (Musa balbisiana Colla cv. Barlin) from Banyuwangi, Indonesia. The integrated

approach provided complementary information on the major phytochemical classes and database-supported metabolite annotations detected under the applied analytical conditions. These findings provide baseline chemical information for further investigation of these locally available agricultural resources. However, the putatively annotated metabolites should not be interpreted as definitively identified compounds or as evidence of biological or biomaterial efficacy, as authentic-standard confirmation and biological evaluation were not performed in the present study. Further targeted, quantitative, structural-confirmation, and biological studies are required to establish the identity, abundance, and functional relevance of the annotated metabolites and to evaluate their potential in future biomedical or biomaterial applications.

SUGGESTIONS

The present findings provide a valuable foundation for further studies on the utilization of red dragon fruit and Barlin banana pseudostem as local biomaterial resources. Future research should include quantitative metabolomic analysis, biological activity validation, and structural characterization to confirm the functional roles of the putatively annotated metabolites. In addition, these plant resources are recommended for further investigation in the development of green-synthesized nanomaterials, natural wound dressing materials, and other sustainable biomedical applications.

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DECLARATION OF INTEREST

The authors declare that there is no conflict of interest

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