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Quercetin-3-β-D-Glucoside Profiling of *Stelechocarpus burahol* using UHPLC-Q-Orbitrap HRMS Metabolomics

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Abstract

Stelechocarpus burahol (*kepel*) is a native Indonesian plant that is traditionally used to treat various health problems, including as a uric acid lowering agent (anti-gout). This study aimed to identify and profile the main active secondary metabolite compounds from kepel fruit extract using a Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) based metabolomics method. *Kepel* fruit was extracted through a maceration method using 96% ethanol solvent. Metabolomics analysis was carried out using a UHPLC system equipped with an Accucore Phenyl Hexyl column and an Orbitrap Exploris 240 HRMS mass spectrometer in positive ionization mode (ESI+). The characterization results successfully identified a flavonoid compound of the flavonol glycoside type, namely Quercetin-3-β-D-glucoside (Isoquercitrin). This compound eluted at a retention time (RT) of 4.187 minutes with an abundance intensity reaching $13x10^6$. First-order mass spectrometry (MS1) data showed the formation of a very dominant protonated molecular ion $[M+H]^+$ at m/z 465.10245, corresponding to the molecular formula $C_{21}H_{20}O_{12}$. Second order fragmentation (MS2) using the HCD method produced a main peak (base peak) at m/z 303.04987 due to the loss of the neutral anhydroglucose fragment of 162.05258 Da. The presence of Quercetin-3-β-D-glucoside provides a strong scientific basis for the traditional use of kepel fruit as an anti-gout agent through inhibition of xanthine oxidase enzyme activity, as well as an antioxidant and anti-inflammatory. This study confirms that the LC-HRMS method is very valid and efficient in the process of dereplication of active compounds in local medicinal plants.

Keywords: Anti-gout; Isoquercitrin; LC-HRMS; Metabolomics; *Stelechocarpus burahol*.

INTRODUCTION

Indonesia is one of the world's megabiodiverse countries, possessing an exceptionally high wealth of biological resources. Its tropical geographical location, combined with an archipelagic condition comprising thousands of islands, gives the country a high level of floral diversity, including many endemic species with potential as sources of bioactive compounds (Eugelio et al., 2026). This biodiversity positions Indonesia as an important center for natural product exploration, with potential applications as pharmaceutical raw materials, functional foods, cosmetics, and other health products (Ashokkumar et al., 2025). Furthermore, natural products remain a primary source for the discovery of new bioactive compounds due to their diverse chemical structures and broad range of biological activities, making them widely utilized in the development of modern medicine and phytopharmaceuticals (Kuban-Jankowska et al., 2026). Natural product chemistry is a branch of science that studies the isolation, characterization, structural identification, and biological activity evaluation of secondary metabolites produced by organisms, particularly plants. Various secondary metabolites such as flavonoids, alkaloids, phenolics, terpenoids, saponins, and tannins are known to exhibit pharmacological activities including antioxidant, antibacterial, anticancer, anti-inflammatory, and antidiabetic effects (Atalla et al., 2026; Gashtasebi et al., 2026). Therefore, the exploration of local medicinal plants represents an important step in discovering new bioactive compound candidates that can be developed as therapeutic agents (Teibo et al., 2026).

One native Indonesian plant with considerable potential that remains underutilized is *kepel* (*Stelechocarpus burahol* (Blume) Hook. f. & Thomson). *Kepel* is a plant native to the island of Java that is historically known as a royal palace plant, particularly in the Special Region of Yogyakarta. This fruit was traditionally consumed by palace princesses as a natural body fragrance and is believed to offer various health benefits. However, cultivation of *kepel* remains very limited, causing it to become increasingly rare and

necessitating conservation efforts (Qiu et al., 2024). Various studies have shown that nearly all parts of the *kepel* plant contain secondary metabolites with potential as bioactive compounds (Li et al., 2023). The leaves and fruit of kepel have been reported to contain flavonoids, polyphenols, alkaloids, saponins, tannins, and terpenoids, which contribute to various biological activities. *Kepel* extracts have been reported to exhibit antioxidant, antibacterial, antihyperuricemic, anti-inflammatory, anti-implantation, antihalitosis, and potential antiaging activities (Ardyansyah et al., 2026). These biological activities are closely related to the phenolic and flavonoid content, which are capable of scavenging free radicals and inhibiting the growth of various pathogenic microorganisms (Sundari et al., 2023). *Kepel* has a natural contraceptive effect and is traditionally used to reduce unwanted body odors, such as sweat, urine, and breath (Angio & Firdiana, 2021). Flavonoids and tannins found in Burahol fruit flour combine with linoleic acid and methionine to improve the effectiveness of protein metabolism. With quantities of 73.09% and 30.99%, respectively, phenolic and flavonoid content contribute to reducing damage from free radicals (Shafirany et al., 2021). According to other research, the flavonoid content of burahol leaves contributes to the inhibition of bacterial development (Shafirany et al., 2021). Additionally, the tannins in burahol have the ability to absorb excess protein, ensuring that it is available for absorption in the small intestine and preventing initial damage by bacteria in the rumen or gut. Because burahol leaf extract contains flavonoids, it can increase quail ration efficiency by lowering conversion and increasing output. This study focused on quercetin-3-β-D-glucoside profiling of *Stelechocarpus burahol* using UHPLC-Q-Orbitrap HRMS Metabolomics.

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 *kepel* fruit, 96% alcohol (Merck), and Whatman filter paper no. 42.

Processing Kepel Fruit into Powder

The process beg**an** with *kepel* 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~~ was *kepel* fruit powder which is ready for maceration extraction.

Maceration of Kepel Fruit Powder

A 250 g sample of *kepel* 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 *kepel* fruit extract to achieve the maximum thickness result (Wijaya et al., 2026).

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 *Kepel* Fruit extract sample (F1) was successfully identified. The compound eluted at a retention time (RT) of 4.19 minutes, with a sharp chromatogram peak specifically detected at RT 4.187 minutes with an abundance intensity reaching 13 × 10^6 counts (Chromatogram Figure). First-order mass spectrometry (MS1) data showed the formation of a very dominant protonated molecular ion $[M+H]^+$ at a mass to charge ratio (m/z) of 465.10245. This experimental value corresponds to the molecular formula $C_{21}H_{20}O_{12}$ with a theoretical calculated molecular weight (Calc. MW) of 464.09510 Da. Based on comparative compound database search, this chromatographic and mass profile accurately leads to a flavonoid compound of the flavonol glycoside type, namely Quercetin-3-β-D-glucoside (Isoquercitrin). Further structural characteristics were studied through the MS2 spectrum using the High-energy Collision Dissociation (HCD) dissociation method. The fragmentation results showed the formation of a very abundant and sharp main peak (base peak) at m/z 303.04987, which is the main product fragment from the cleavage of its precursor ion.

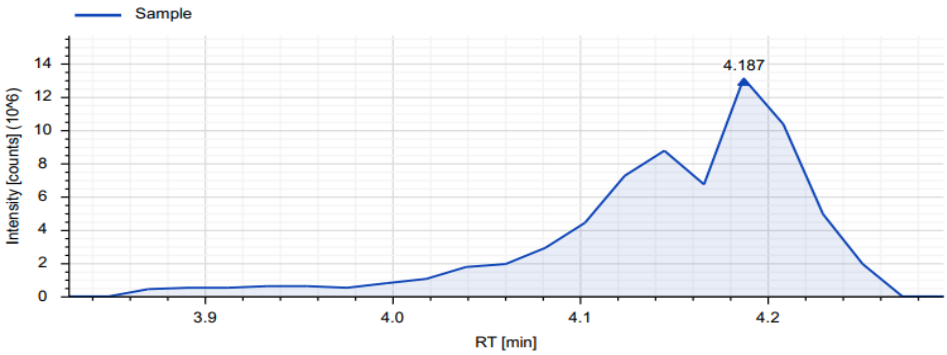

Figure 1. Chromatogram of the results of LC-HRMS analysis of Kepel fruit sample (F1) with an elution peak at RT 4.187 minutes.

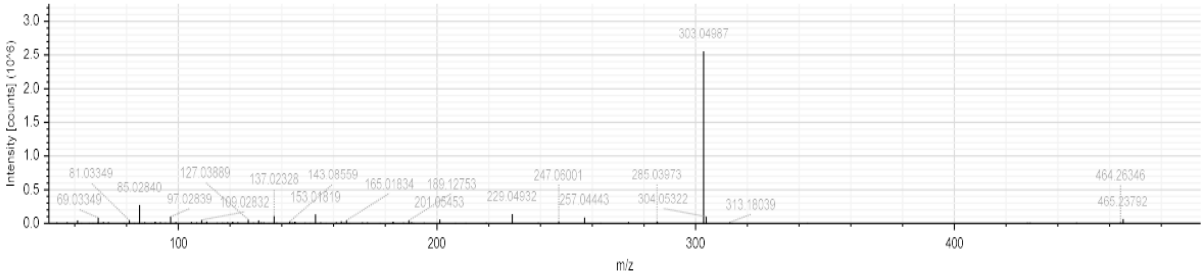

Figure 2. Second-order fragmentation (MS2) mass spectrum of the Quercetin-3-β-D-glucoside compound in Kepel fruit samples.

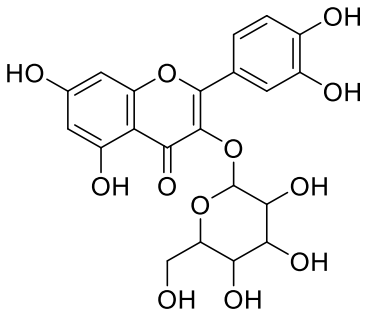

Figure 1. Chemical structure of the isoquercitrin compound Quercetin-3-β-D-glucoside

The liquid chromatogram profile showed that the Quercetin-3-β-D-glucoside compound eluted relatively quickly at 4.187 minutes. This reflects the moderate to high polarity of the molecule in the reversed-phase C18 chromatography system. The presence of a glucose (hexose) functional group attached to the C-3 position of the flavonoid core significantly increases the hydrophilicity of the molecule when compared to its free aglycone form (quercetin). As a result, the interaction of this glycoside compound with the polar mobile phase becomes stronger, causing it to move more quickly

through the non-polar stationary phase. High-resolution mass spectrometry (HRMS) analysis provides a very high certainty of the empirical formula through accurate mass matching. The experimental $[M+H]^+$ ion detected at m/z 465.10245 has a very high agreement with the theoretical mass of the protonated ion of $C_{21}H_{21}O_{12}^+$ with a calculated mass of 465.10238 Da. The mass error value below 1 ppm proves the reliability of the FTMS instrument system (such as Orbitrap) in confirming the elemental composition of the target compound. The most convincing structural evidence is obtained from the fragmentation pattern of the MS2 spectrum. The dominant product peak at m/z 303.04987 is formed due to the loss of a specific neutral fragment with a mass of 162.05258 Da (465.10245 - 303.04987 = 162.05258 Da). The mass loss of 162 Da is a very typical diagnostic fingerprint for the release of radicals or neutral molecules of anhydroglucose (hexose group $C_6H_{10}O_5$) which can result in the cleavage of the O-C glycosidic bond at the C-3 position. The product peak at m/z 303.04987 was identified as the aglycone ion of quercetin ($[Y_0]^+$), which absolutely confirms that the original compound is quercetin bound to one hexose group. Chemotaxonomically and pharmacologically, the presence of Quercetin-3-β-D-glucoside in the extract of Kepel Fruit (Stelechocarpus burahol) provides a strong scientific basis for the medicinal properties of the plant. This flavonol glycoside compound is widely known to have very high bioactivity, including strong free radical scavenging antioxidant activity, anti-inflammatory agents, and plays an important role in inhibiting the activity of the xanthine oxidase enzyme. This enzyme inhibition activity supports the traditional use of kepel fruit in Indonesia as a herbal medicine to reduce uric acid levels (anti-gout). The results of this study confirm that the use of the LC-HRMS method is very valid and efficient in the process of identifying and dereplicating high-value active compounds from local medicinal plants.

The compound shown in the figure is isoquercitrin, also known as Quercetin-3-β-D-glucoside, which belongs to the large group of flavonoids, a subclass of flavonol glycosides. Structurally, this hybrid molecule consists of two main components linked by a glycosidic bond. The first part is the aglycone in the form of a quercetin core that has a heterocyclic basic skeleton with a system of rings A, B, and C. The second part is the glycone which is a single unit of β-D-glucose molecules. The hexose sugar group at the bottom is specifically bonded to carbon atom number 3 (position C-3) of ring C through an oxygen bridge, thus forming an O-glycosidic bond. The chemical properties and biological activities of isoquercitrin are greatly influenced by the presence of several key functional groups in its structure. The presence of a catechol group, namely two hydroxyl groups (-OH) located side by side at positions 3' and 4' in ring B (top right), is the most important part because it plays an active role in donating hydrogen to stabilize free radicals, thus providing strong antioxidant activity. In addition, this compound has a chromone group in rings A and C which is characterized by the presence of a carbonyl group (C=O) at C-4 which conjugates with the double bond between C-2 and C-3. This long π electron conjugation system facilitates electron delocalization throughout the molecule, providing high resonance stability after scavenging free radicals. Additional contributions are also made by free hydroxyl groups at positions C-5 and C-7 in ring A which also affect the polarity and chelating ability towards metals. From the perspective of the relationship between structure and activity, the addition of a glucose group at position C-3 has a significant influence on the physicochemical properties of this compound. This glycosylation significantly increases the solubility of isoquercitrin in water compared to its pure aglycone form (free quercetin). In biological systems, this increased hydrophilicity facilitates the transport of compounds in the aqueous phase such as in plant body fluids or the gastrointestinal tract, although the presence of these sugar groups slightly reduces their direct antioxidant effectiveness in vitro. As a naturally occurring polyphenolic compound abundant in various plants such as tea leaves and medicinal herbs, the unique combination of the electron-rich quercetin core and hydrophilic sugar groups makes isoquercitrin a highly potent active compound as an antioxidant, anti-inflammatory, and cardioprotective agent.

CONCLUSIONS

This study successfully identified the main active compound Quercetin-3-β-D-glucoside (Isoquercitrin) in *kepel* fruit extract (Stelechocarpus burahol) using a metabolomics method based on UHPLC-Q-Orbitrap HRMS. Chromatography and mass spectrometry profiles showed that this flavonol glycoside compound eluted at a retention time of 4.187 minutes, with confirmation of the protonated ion $[M+H]^+$ at m/z 465.10245 and a specific fragment of quercetin aglycone at m/z 303.04987. This discovery provides a strong scientific basis in supporting the traditional use of kepel fruit in Indonesia as a herbal medicine to lower uric acid (anti-gout) through the mechanism of xanthine oxidase enzyme inhibition, as well as an antioxidant and anti-inflammatory agent. Overall, the application of the LC-HRMS method proved to be very valid, reliable, and efficient in accelerating the dereplication process and characterization of high-value active compounds from local medicinal plants.

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

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