Current **A**pplied **S**cience and **T**echnology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. ….., Vol... (No...), e0270013

Research article

Comparative Analysis of Glucosylceramide, Steryl glucoside, and Cytotoxicity in Rice Bran Extracts from Hom Pathum and Riceberry

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Received: 1 December 2025, Revised: 3 April 2026, Accepted: 21 April 2026, Published: 8 September 2026

Abstract

This study evaluated residual methanol levels, lipid class distribution, and the glucosylceramide (GlcCer) and steryl glucoside (SG) contents of rice bran extracts from Hom Pathum (HP) and Riceberry (RB) cultivars and assessed the cytotoxicity of the GlcCer- and SG-enriched extracts using skin-relevant cell models. Rice bran from both cultivars was sequentially extracted with ethyl acetate followed by methanol. Residual methanol in crude extracts was quantified using headspace GC–MS. Total lipids were fractionated by solid-phase extraction into neutral lipids (NL), glycolipids (GL), and phospholipids (PL), and the GL fraction was analyzed by thin-layer chromatography with image-based quantification to determine GlcCer and SG contents. The RB extract, which showed higher glycolipid content, was selected for cytotoxicity testing using a resazurin reduction assay in HaCaT keratinocytes and BJ fibroblasts. HS-GC–MS results confirmed that residual methanol was below 10 ppm in all extracts, meeting international safety guidelines. Lipid profiling revealed clear varietal differences: HP bran was dominated by NL (90.3% w/w), whereas RB contained higher proportions of GL (14.3% w/w) and PL (17.3% w/w). GlcCer (3.08% of GL; 0.027% w/w of bran) and SG (6.34% of GL; 0.055% w/w of bran) were detected exclusively in RB. In vitro assays showed that RB extract was non-cytotoxic to HaCaT and BJ cells at 6.25–50 µg/mL and increased fibroblast viability (113-114%) at 100-200 µg/mL. Overall, the findings indicate that Riceberry bran provides a safe, GlcCer-rich lipid extract with potential dermal benefits. This integrated assessment identifies Riceberry bran as a promising source of functional sphingolipids for nutraceutical, cosmeceutical, and topical applications.

Keywords: Hom Pathum rice bran extract; Riceberry rice bran extract; glucosylceramide; steryl glucoside; residual solvents; cytotoxicity; HaCaT keratinocytes; BJ fibroblasts

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https://doi.org/10.55003/cast.2026.270013

1. Introduction

Rice (*Oryza sativa* L.) is a globally important cereal and a staple food for billions. Rice milling and processing generate rice bran, a nutrient-dense by-product that contains triglycerides, phospholipids, tocopherols and tocotrienols, γ-oryzanol, phenolic compounds, phytosterols, and minor but bioactive lipid species, including plant glycosphingolipids such as glucosylceramides and steryl glucosides (Guazzotti et al., 2023; Liu et al., 2023; Takeda et al., 2023). Because of this compositional complexity, rice bran has attracted increasing interest from the food, nutraceutical, and cosmetic industries as a source of functional ingredients for oral and topical applications.

Glucosylceramides (GlcCer) are glycosphingolipids comprised of a glucose headgroup attached to a ceramide backbone. Structurally, glucosylceramides consist of a ceramide backbone composed of a sphingoid base linked to a fatty acid, with a glucose moiety attached via a β-glycosidic linkage, forming amphiphilic molecules that integrate into lipid bilayers and contribute to membrane stability (Reza et al., 2021; Guo, 2022). Plant-derived GlcCer display substantial molecular heterogeneity—varying in sphingoid-base isomers, N-acyl chain length, and hydroxylation—which governs their physicochemical characteristics and bioactivity. Convergent evidence from in vitro, animal, and human studies indicates that GlcCer enhance epidermal barrier integrity by reinforcing the stratum corneum, reducing transepidermal water loss (TEWL), and improving skin hydration, with structure-dependent effects mediated by differences in fatty-acid chain length and sphingoid-base composition that modulate ceramide metabolism and differentiation pathways (Ohta et al., 2021; Takara et al., 2021; Sapwarobol et al., 2021; Takeda et al., 2023). Growing interest in oral applications is supported by absorption studies showing that ingested GlcCer and their hydrolyzed metabolites can access systemic pools and influence host lipid profiles (Reza et al., 2021; Takeda et al., 2023), while ex vivo and cellular assays corroborate roles in cellular cohesion, barrier restoration, and dermal function (Reza et al., 2021; Takara et al., 2023). Steryl glucosides (SGs)—plant sterols O-glycosylated with moieties such as sitosterol or stigmasterol—are abundant in edible plants and oils, contribute to membrane architecture, and have emerged as multifunctional food and cosmetic bioactives. Structurally, steryl glucosides are formed by glycosylation of plant sterols, contributing to membrane organization and biological function (Rondelli et al., 2021). Dietary SGs and their acylated derivatives have been associated with anti-inflammatory, antidiabetic, and cholesterol-lowering effects (Murai et al., 2020), with fermentation processes increasing acylated SG content and potentiating these metabolic benefits (Murai et al., 2020). In topical formulations, certain SGs (e.g., β-sitosterol β-D-glucoside) have been reported to act as penetration enhancers and emollients (Evtyugin et al., 2023). Together, GlcCer and SGs represent complementary classes of plant-derived glycosides whose structural diversity necessitates rigorous analytical and functional characterization in candidate source materials—such as diverse rice cultivars—before development as nutraceutical or cosmeceutical ingredients.

For translational development, compositional analysis must be paired with rigorous safety assessment. Organic solvents commonly used for lipid extraction (e.g., methanol, ethyl acetate, hexane) may persist as residual solvents in crude or fractionated extracts; because solvent toxicology varies widely, identification and quantification should conform to internationally harmonized guidance such as ICH Q3C impurities: Guideline for residual solvents — ICH Q3C(R8) (ICH Expert Working Group, 2021). Compliance with these guidelines—including solvent classification, permissible daily exposures (PDEs), and concentration limits—should inform extraction method selection and analytical testing

strategies for materials intended for human consumption or dermal application (Opuni et al., 2021; Cravotto et al., 2022; Huang, et al., 2024). Cytotoxicity testing in relevant skin models (e.g., HaCaT keratinocytes and human dermal fibroblasts) is a standard early-phase safety screen to establish non-cytotoxic concentration ranges and to detect potential adverse effects arising from extract components or residual solvents. Recent studies of rice bran extracts and lipid fractions typically report broad safety margins at low to moderate concentrations (commonly 5-50 $\mu g \cdot mL^{-1}$), with occasional observations of pro-survival or pro-metabolic responses at higher, non-toxic doses (Yamashita et al., 2021; Oktavya et al., 2023; Son et al., 2023; Takeda et al., 2023). These data support further mechanistic evaluation of rice-derived lipids, particularly GlcCer, for dermatological and cosmetic applications.

Riceberry, an anthocyanin-rich Thai purple rice cultivar, has been intensively studied for its high antioxidant capacity and favorable bioactivity relative to many non-pigmented varieties (Settapramote et al., 2021; Yamuangmorn & Prom-U-Thai, 2021; Muangchan et al., 2022; Wongwisitchai et al., 2022). In contrast, locally significant cultivars such as Hom Pathum, although widely cultivated in central Thailand, remain comparatively understudied. Because cultivar genetics, geographic origin, agronomic practices, and post-harvest handling can substantially influence the abundance and molecular profile of bioactive lipids (including GlcCer and SG) in rice bran, direct comparisons using standardized extraction and analytical workflows are essential to support ingredient development and health-claim substantiation (Barros Santos et al., 2023; Guazzotti et al., 2023). Accordingly, this study aimed to determine residual methanol levels, compared lipid class distribution, and quantify the GlcCer and SG content in rice bran extracts prepared from Hom Pathum and Riceberry varieties harvested in Pathum Thani Province, Thailand, and to evaluate the in vitro cytotoxicity of the selected Riceberry extract in HaCaT keratinocytes and BJ fibroblasts.

2. Materials and Methods

Rice samples of Hom Pathum (HP) and Riceberry (RB) varieties were collected from Pathum Thani Province, Thailand, in December 2024. The harvested rice was milled to obtain polished rice and bran. The rice bran obtained from both varieties was used as experimental samples for subsequent analyses.

2.1 Extraction of Hom Pathum and Riceberry rice bran

Extraction of rice bran (Hom Pathum and Riceberry) was carried out with slight modifications based on the method of Sahoo et al. (2017). To remove interfering semi-polar compounds, rice bran was pre-extracted with ethyl acetate at a ratio of 1:3 (w/v). The mixture was shaken at 150 rpm for 60 min using a shaker (Wiggens, Germany) and subsequently filtered through filter paper No. 1 with a Buchner funnel system. The rice bran residue remaining after ethyl acetate extraction was dried at 50°C for 24 h using a hot air oven (Binder, Germany) and subsequently extraction with methanol. The extraction was carried out at a ratio of 1:3 (w/v), shaken at 150 rpm for 60 min, and filtered. The methanol extraction was repeated once under identical conditions. The combined methanol extracts were concentrated using a rotary evaporator (Buchi, Switzerland) under reduced pressure (150 mbar) at 70 rpm and 50°C, with the chiller maintained at 8°C. The obtained crude extracts were stored at -20°C.

2.2 Determination of residual solvent content from crude extracts

Quantification of residual solvents in crude extracts from Hom Pathum and Riceberry rice bran was performed with slight modifications to the method of Lim et al. (2023). Residual methanol in the crude extracts was determined using headspace gas chromatography–mass spectrometry (HS-GC-MS; Shimadzu TQ8050NX, Japan). Calibration curves were constructed using methanol standard solutions in the range of 1-250 ppm. Headspace–GC–MS analysis was performed using static mode with heat-ahead, injecting 15 µL of sample into a 20 mL vial. Samples were equilibrated at 80°C for 10 min before injection through a 0.2 mL loop pressurized at 100 kPa. The sample and transfer line temperatures were set at 90°C and 100°C, respectively. Split injection (50:1) was applied onto an Rxi-624 Sil MS capillary column (30 m × 0.25 mm, 1.40 µm film) with helium as the carrier gas (39.9 cm/s; 1.24 mL/min). The oven was programmed from 40°C (5 min hold), ramped at 10°C/min to 55°C (2 min hold), and then at 20°C/min to 200°C. The total GC runtime was 16.5 min (25 min cycle). Detection was performed in SIM mode (EI, 70 eV) with an ion source at 200°C and MS interface at 300°C; a 2 min solvent cut was applied to minimize interference.

2.3 Determination of lipid class from crude extracts by solid phase extraction

Lipids from crude extracts of Hom Pathum and Riceberry rice bran were fractionated into neutral lipids (NL), glycolipids (GL), and phospholipids (PL) using solid phase extraction (SPE) according to the method of Wadeesirisak et al. (2017). The SPE silica cartridge (900 mg silica 60, VertiPak, Thailand) was preconditioned by sequential rinsing with 2.7 mL of methanol followed by 2.7 mL of chloroform. Crude extract (30 mg in 0.5 mL of chloroform) was then loaded onto the activated SPE cartridge. Neutral lipids were eluted with 8.1 mL of chloroform in four successive fractions, followed by glycolipids and phospholipids, which were eluted with 8.1 mL of acetone:methanol (9:1, v/v) and 8.1 mL of methanol, respectively. The flow rate was controlled at approximately 3 mL/min using a vacuum pump. Each lipid fraction was evaporated to remove organic solvents, and the dry weight was recorded. The dried GL fraction was analyzed for GlcCer and SG by thin-layer chromatography (TLC), as described in Section 2.4.

2.4 Quantification of glucosylceramide and steryl glucoside from glycolipid fractions by thin-layer chromatography (TLC)

The glycolipid fractions were dissolved at a concentration of 10 mg/mL in chloroform:methanol (2:1, v/v). Aliquots of 10, 15, and 20 µL were applied onto TLC plates together with glucosylceramide and steryl glucoside standards (5 mg/mL; 1-5 µL). The plates were developed in a saturated chamber containing chloroform:methanol:water (95:20:2.5, v/v/v) until the solvent front reached the marked line, then air-dried under a gentle stream of warm air. Glycolipid bands were visualized by immersing the plates in an orcinol:ethanol:sulfuric acid solution (4:380:20, w/v/v) and heating at 100°C for 10 min. The developed plates were scanned using CLIQS software to determine band volume intensity. Quantification of glucosylceramide and steryl glucoside was performed using calibration curves generated by plotting band intensity against the amount of each standard applied per band (5-25 µg/band). The calibration equations were $y = 28025x + 108071$ ($R^2 = 0.993$) for glucosylceramide and $y = 30290x + 124698$ ($R^2 = 0.9663$) for steryl glucoside.

2.5 Evaluation of cytotoxicity in Riceberry rice bran extract

2.5.1 Sample preparation

The crude extract from Riceberry rice bran was dissolved in DMSO and subsequently diluted to different concentrations (200, 100, 50, 25, 12.5 and 6.25 µg/mL). The final concentration of DMSO in the treated cells was 0.2%.

2.5.2 Cell culture

HaCaT human epidermal keratinocytes (ATCC, USA) and BJ human dermal fibroblasts (ATCC, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA). The cells were incubated at 37ºC in 5% CO_2 with humidification and subcultured twice weekly using 0.25% trypsin-EDTA (Gibco, USA). Only cells with greater than 90% viability, confirmed by the 0.4% trypan blue (Gibco, USA) staining at density of 2 × 10^5 cells/mL, were selected for use in this experiment.

2.5.3 Resazurin reduction assay

The effects of the samples on cell viability were evaluated using a resazurin reduction assay, as described by Thungmungmee and Wisidsri (2025). Viable cells with active metabolism reduce resazurin (blue) to resorufin (pink), serving as an indicator of metabolic activity (Vieira-da-Silva & Castanho, 2023). HaCaT keratinocytes or BJ fibroblast cells were treated with various concentrations of the samples for 24 h. Following treatment, the cells were incubated with 50 µg/mL resazurin (Sigma-Aldrich, USA) at 37°C for 4 h. Absorbance was measured at 560 and 600 nm. The percentage of cell viability was calculated compared to the 0.2% DMSO treated cells (negative control) using the following equation: The results are presented as the mean±SEM of three independent experiments (N=3).

$$\%\ Cell\ viability = [(OD_{560} - OD_{600})_{sample} / (OD_{560} - OD_{600})_{control}] \times 100$$

Where *OD* control is the absorbance of 0.2%DMSO (Solvent control), and *OD* sample is the absorbance of sample.

2.6 Statistical analysis

The content in crude extracts was quantified and statistically evaluated using an independent t-test, whereas the cell viability of HaCaT keratinocytes and BJ fibroblasts was analyzed using one-way ANOVA. A p-value of < 0.05 was considered indicative of statistical significance.

3. Results and Discussion

3.1 Residual solvent content of crude extracts

The calibration curve of methanol standards in the range of 1-250 ppm exhibited a strong linear relationship (y = 36.404x + 6163.697) with a high coefficient of determination (R^2 = 0.997), demonstrating the excellent linearity of the method and supporting its reliability for

quantitative analysis. The mass spectrum of the methanol standard (Figure 1) showed characteristic fragment ions at *m/z* 31 ($[CH_2OH]^+$, base peak), *m/z* 29 ($[CHO]^+$), and *m/z* 32 ($[CH_3OH]^+$, molecular ion). These fragmentation patterns were consistent with the electron impact ionization of methanol and agreed with the National Institute of Standards and Technology reference library (NIST, 2025), thereby confirming the identification of methanol in the analysis.

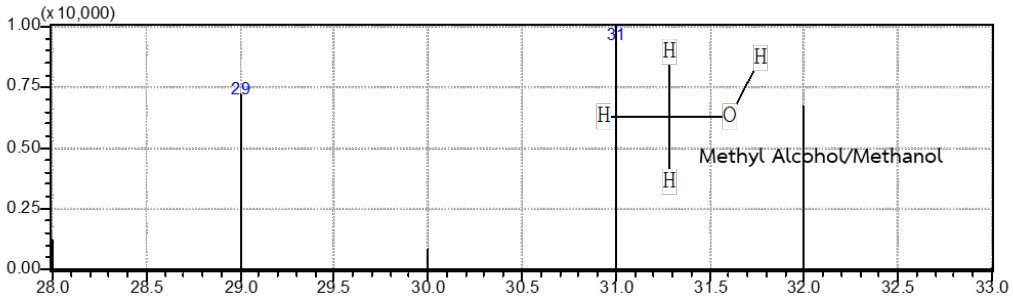

Figure 1. Mass spectrum of methanol standard using HS-GC-MS analysis

Methanolic crude extracts from Hom Pathum and Riceberry rice bran were analyzed for residual solvents. Residual methanol levels in extracts from Hom Pathum and Riceberry rice bran were found to be below the 10 ppm, as shown in Figure 2. This level is substantially lower than the concentration limit of 3,000 ppm established by the U.S. Food and Drug Administration (FDA) for drug products, as well as the permitted daily exposure (PDE) of 30 mg per day for methanol (U.S. Food and Drug Administration, 2018). The absence of detectable residual methanol above this threshold highlights the effectiveness of the extraction and solvent removal procedures. Furthermore, these findings indicate that crude extracts comply with stringent international safety standards for residual solvents and may thus be regarded as suitable candidates for further development in pharmaceutical, nutraceutical, and cosmetic applications. Although methanol was used in this study due to its high extraction efficiency for polar lipids such as glucosylceramides, ethanol may represent a safer alternative for food and cosmetic applications. Previous studies have demonstrated that ethanol can effectively extract glycolipids, albeit sometimes with lower efficiency compared to methanol. Therefore, future studies should explore ethanol-based extraction systems to improve the safety and scalability of the process while maintaining acceptable yields of target bioactive compounds.

3.2 Lipid classes from crude extracts

Fractionation of the crude extracts by solid-phase extraction yielded three lipid fractions: neutral lipids (NL), glycolipids (GL), and phospholipids (PL) (Figure 3). Analysis of lipid classes in the crude extracts from Hom Pathum and Riceberry rice bran revealed distinct compositional differences. Neutral lipids were more abundant in Hom Pathum extract (90.3% w/w) than in Riceberry extract (68.4% w/w). In contrast, Riceberry extract contained significantly higher levels of glycolipids (14.3% w/w) than Hom Pathum extract (5.8% w/w).

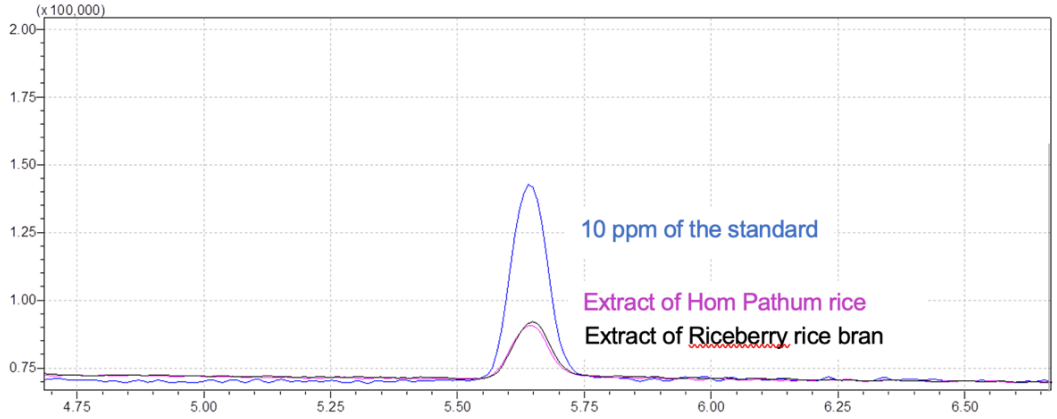

Figure 2. GC-MS Chromatogram of residual methanol in crude extracts from Hom Pathum rice bran and Riceberry rice bran compared with 10 ppm methanol standard

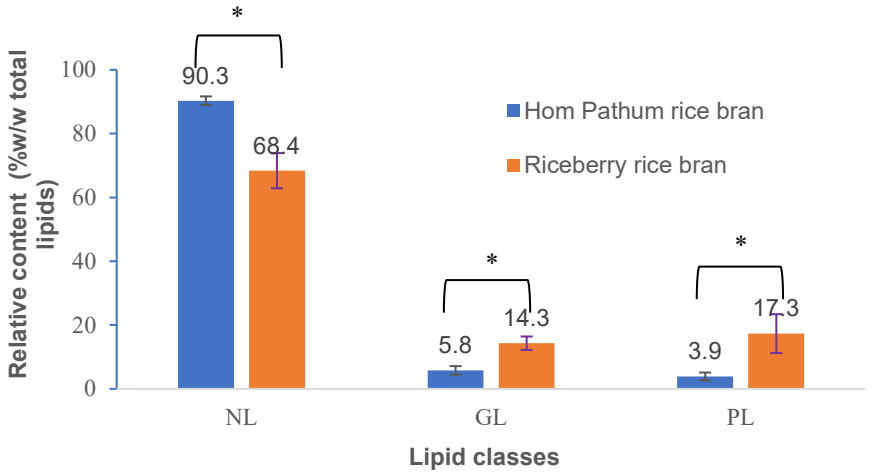

Figure 3. Relative content of lipid classes obtained from crude extracts of Hom Pathum and Riceberry rice bran using SPE. (*p-value < 0.05)

Phospholipid content was also greater in Riceberry (17.3% w/w) than in Hom Pathum (3.9% w/w). The substantially higher glycolipid content in Riceberry suggests it is a richer source of bioactive glucosylceramides. Glucosylceramides are sphingolipids essential for maintaining skin barrier integrity and water retention. Previous studies have shown that oral or topical administration of plant-derived GlcCer improves stratum corneum integrity, enhances ceramide synthesis, and reduces transepidermal water loss (TEWL) (Yong et al., 2025). Clinical trials using wheat-derived GlcCer have further demonstrated improved skin hydration and elasticity, along with reduced TEWL, underscoring its potential for functional food and nutraceutical applications (Bizot et al., 2017). Together, these findings indicate that Riceberry rice bran is a promising raw material for the development of value-added products targeting skin health.

3.3 Glucosylceramide and steryl glucoside contents from glycolipid fractions

The glycolipid fraction obtained from Hom Pathum and Riceberry rice bran extracts was analyzed for glucosylceramide and steryl glucoside contents using thin-layer chromatography (TLC). The samples were compared with glucosylceramide and steryl glucoside standards, which are glycolipids naturally occurring in rice bran. Quantitative analysis, based on spot intensities from TLC-image analysis (Figure 4), revealed that glucosylceramides were absent in the Hom Pathum extract, where only steryl glucosides were detected. In contrast, both glucosylceramides and steryl glucosides were present in the Riceberry extract. These results demonstrate a varietal difference: Riceberry rice bran contains detectable glucosylceramide, whereas Hom Pathum does not. This disparity likely reflects both genetic and structural differences between the rice types. The higher GlcCer level in Riceberry aligns with previous reports that pigmented rice varieties generally accumulate more glycosphingolipids than pale varieties (Paosila et al., 2020). Given that glucosylceramides are bioactive glycosphingolipids influencing skin hydration and related functions, these varietal differences may have practical implications for health and cosmetic applications.

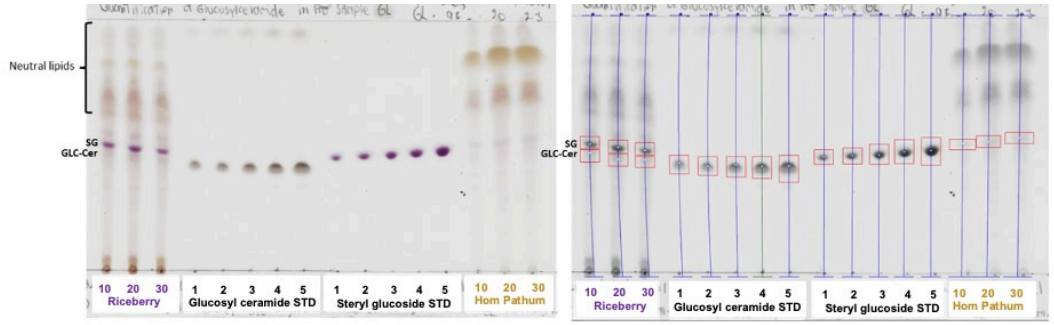

Figure 4. TLC profiles of glycolipid fractions (10 mg/mL; 10, 15, and 20 µL) compared with glucosylceramide and steryl glucoside standards (5 mg/mL; 1-5 µL). Images are shown before (1) and after (2) TLC-image analysis.

Based on TLC-image analysis, the contents of glucosylceramide (GlcCer) and steryl glucoside (SG) in rice bran glycolipid fractions were quantitatively determined (Table 1). In Hom Pathum rice bran, SG accounted for approximately 2% (w/w of glycolipids), corresponding to 0.004% (w/w of rice bran). In contrast, Riceberry bran contained significantly higher levels of both GlcCer and SG, measured at 0.027% and 0.055% (w/w of rice bran), respectively. These differences are attributable to the greater glycolipid fraction in Riceberry, consistent with previous findings that pigmented rice varieties generally accumulate higher levels of total lipids and bioactive compounds in the bran compared to non-pigmented rice (Zhao et al., 2025). The relatively high GlcCer content in Riceberry is notable due to its functional and nutritional significance. As a major plant sphingolipid, GlcCer contributes to skin barrier maintenance, anti-inflammatory activity, and intestinal homeostasis (Wang et al., 2022). Recent lipidomic studies have revealed that pigmented rice varieties possess diverse GlcCer species and related sphingolipids, further underscoring their biological relevance (Barros Santos et al., 2023; Guazzotti et al.,

2023). Although glucosylceramide spots in the TLC analysis were not visually prominent on the TLC plate, quantitative analysis based on image intensity suggested their presence in the Riceberry extract. This limited visibility may be attributed to the low analyte concentration or the limited detection sensitivity of glucosylceramides or the sensitivity of the detection method. To improve analytical confidence, future studies should consider using more sensitive techniques such as high-performance liquid chromatography (HPLC) or liquid chromatography–mass spectrometry (LC–MS) for confirmation and quantification. These findings corroborate our observation that Riceberry bran provides substantial GlcCer within its glycolipid fraction. Steryl glucosides, important phytosterol conjugates, also play roles in plant membrane stability and may exert health-promoting effects in humans, including cholesterol-lowering and modulation of intestinal barrier function (Guazzotti et al., 2023). The significantly higher SG content in Riceberry compared to Hom Pathum supports the notion that pigmented rice cultivars accumulate elevated levels of bioactive lipid species, including both sphingolipids and steryl derivatives (Barros Santos et al., 2023). Overall, these results indicate that pigmented rice varieties such as Riceberry are enriched in functional glycolipids, and especially when combined with other bioactive compounds such as phenolics, γ-oryzanol, and tocotrienols, Riceberry bran can be a promising source for nutraceutical applications. Investigation on optimized extraction methods, bioavailability, and *in vivo* efficacy should be investigated in the future to fully exploit the functional potential of GlcCer and SG from pigmented rice bran.

Table 1. Glycosyl ceramide and steryl glucoside content of glycolipid fractions

Sample	(%w/w of Glycolipid Fraction)		(% w/w of Extract)		(% w/w of Rice Bran)	
	Glycosyl Ceramide	Steryl Glucoside	Glycosyl Ceramide	Steryl Glucoside	Glycosyl Ceramide	Steryl Glucoside
Hom Pathum	nd*	2.11	nd*	0.12	nd*	0.004
Riceberry	3.08	6.34	0.44	0.91	0.027	0.055

*nd: not detected

3.4 Effects of Riceberry rice bran extracts on the viability of HaCaT keratinocytes and BJ fibroblast cells

The effects of Riceberry rice bran extract on the viability of HaCaT keratinocytes and BJ fibroblasts are summarized in Table 2. In HaCaT cells, all tested concentrations (6.25-200 µg/mL) maintained viability within a normal range, showing no statistically significant differences compared to the 0.2% DMSO control (97.37±1.44% to 102.98±3.03%; $p>0.05$). These findings indicate that the extract is non-cytotoxic to keratinocytes under the tested conditions, supporting its biocompatibility with epidermal cell models (Ronie et al., 2024). In contrast, BJ fibroblasts exhibited a concentration-dependent response, with significantly increased viability observed at 100 and 200 µg/mL (113.78±1.07% and 114.23±2.22%, respectively; $p\leq0.05$), while lower concentrations (6.25-50 µg/mL) showed no significant difference from the control. The elevated resazurin-reducing activity in fibroblasts suggests a potential stimulatory effect on cellular metabolic activity rather than cytotoxicity. Similar trends have been reported in studies of pigmented rice bran extracts, which contain phenolic compounds, γ-oryzanol, tocopherols, and ceramides, which are known to promote

dermal cell function (Linsaenkart et al., 2023; Hou et al., 2024). The modest increase in fibroblast viability may be attributed to antioxidant-mediated cytoprotection or hormetic activation of stress-response pathways, such as Nrf2/HO-1, which enhance cellular resilience and repair capacity (Ma et al., 2022). Previous reports have highlighted that rice-derived bioactives, particularly γ-oryzanol, possess the ability to modulate oxidative stress and support regenerative processes in skin and neural cells (Abate et al., 2024). Furthermore, the hormetic effect observed is consistent with plant-derived compounds that induce mild cellular stress, leading to adaptive enhancement of cellular function (Calabrese et al., 2024). These mechanisms collectively support the pro-regenerative potential of Riceberry rice bran extract (Linsaenkart et al., 2023). Therefore, the observed effects highlight not only the safety of Riceberry rice bran extract but also its potential utility in skin nourishment and regenerative applications, in agreement with emerging evidence demonstrating that rice bran phytochemicals can modulate extracellular matrix homeostasis and enhance dermal repair (Hou et al., 2024; Ronie et al., 2024).

Table 2. Effects of Riceberry rice bran extracts on the viability of HaCaT keratinocytes or BJ fibroblast cells

Sample Concentration (μg/mL)	Cell Viability (% of Untreated Control)	
	HaCaT Keratinocytes Cells*	BJ Fibroblast Cells**
0.2% DMSO	100.00±0.00	100.00±0.00[a]
200	102.20±2.92	114.23±2.22[b]
100	97.37±1.44	113.78±1.07[b]
50	101.56±3.23	105.69±1.05[a]
25	102.98±3.03	105.36±3.45[a]
12.5	100.96±2.22	105.43±2.48[a]
6.25	102.06±1.79	103.93±0.33[a]

Remark: *no significant difference. **different superscript letters in the same column indicate significant difference (p<0.05).

4. Conclusions

This study comparatively evaluated residual solvent levels, glucosylceramide and steryl glucoside contents, and cytotoxicity of rice bran extracts from Hom Pathum and Riceberry varieties. All crude extracts exhibited compliant safety profiles, with methanol residues well below international limits. Hom Pathum extract was dominated by neutral lipids (90.3% w/w) and lacked detectable glucosylceramide (GlcCer), whereas Riceberry contained higher glycolipid (14.3% w/w) and phospholipid (17.3% w/w) fractions and yielded measurable levels of GlcCer (3.08% of GL; 0.027% w/w of bran) and steryl glucosides (6.34% of GL; 0.055% w/w of bran). The GlcCer- and SG-enriched Riceberry extract was non-cytotoxic to HaCaT and BJ cells at 6.25-200 μg·mL^{-1} and increased resazurin-reducing activity in BJ fibroblasts (~113-114%) at 100-200 μg·mL^{-1}, indicating potential pro-regenerative activity. These findings highlight Riceberry rice bran as a superior source of bioactive glycolipids suitable for skin-targeted nutraceuticals, cosmeceuticals, and functional ingredients. Future studies should investigate mechanism-based effects, such as the regulation of skin barrier-related genes (e.g., filaggrin, involucrin) or antioxidant

pathways (e.g., Nrf2/HO-1 signaling) in keratinocytes and fibroblasts, to better understand the biological activities of glucosylceramides and steryl glucosides.

5. Acknowledgements

The authors gratefully acknowledge the Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani, Thailand, for providing the laboratory facilities essential to this study. This work was supported by the Fundamental Fund (FF) of the Science, Research and Innovation Promotion Fund (Fiscal Year 2025) [Grant Number. 209928].

6. Authors' Contributions

Duangduan Wattanuruk: Conceptualized the study, designed and managed the project, curated the data, and contributed to manuscript review and editing.
Phanwipa Pangsri: Contributed to study design, methodology development, conceptualization, resource provision, and manuscript revision and editing.
Pattamaporn Jaroennon: Contributed to study design, conducted the experiments, prepared visual data, and drafted the original manuscript as well as contributing to its revision and editing.

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7. Conflicts of Interest

The authors declare no conflict of interest.

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