Comparison of γ-Oryzanol Content Using HPLC Profiling and Bioactivity in Three Indonesian Brown Rice (*Oryza sativa* L.) Varieties

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Abstract: *Brown rice (BrR) is a nutrient-rich staple food and a potential source of γ-oryzanol, which has the potential to promote health effects in hypercholesterolemia. However, the availability and biological mechanism of γ-oryzanol in Indonesian BrR remain unexplored. This study aimed to characterize γ-oryzanol content in three Indonesian BrR varieties, Black Madras, Lawang, and UB BrR, and evaluate its biological functions for hypercholesterolemia. γ-Oryzanol was identified using HPLC, antioxidant activity by the ferric reducing antioxidant power (FRAP) method, and anticholesterol activity by the Lieberman-Burchard method. In silico analysis was performed to assess γ-oryzanol derivatives' interaction with microsomal triglyceride transfer protein (MTP). Black Madras BrR showed the highest γ-oryzanol content (0.3007 ± 0.0011 g). γ-Oryzanol was comprised of four main derivatives: cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate. Black Madras BrR exhibited strong antioxidant (IC_{50} 18.89 ± 0.37 μg/mL) and anticholesterol (IC_{50} 14.02 ± 0.23 μg/mL) activities. Docking simulations revealed that γ-oryzanol derivatives interact with MTP lipid-binding residues (Leu643, Ile666, Phe813, Val817) with the same binding energy as lomitapide. Molecular dynamics indicated γ-oryzanol stable interaction, closer to lomitapide. These findings proposed the potential bioactivity of γ-oryzanol from Black Madras BrR as antioxidant, anticholesterol, and a natural MTP inhibitor for hypercholesterolemia treatment.*

Keywords: *brown rice; γ-oryzanol; hypercholesterolemia;* in silico*; microsomal triglyceride transfer protein*

■ INTRODUCTION

Rice is the primary staple food commodity for people in most parts of Asia, including Indonesia. Consuming rice, especially pigmented rice, has good benefits in terms of biological function and control of metabolic mechanisms in the body [1]. Indonesia has a wide variety of pigmented rice that contains rich nutritional value for promoting health [2]. Pigmented rice, such as purple rice, contains ferulic acid, known for its anti-aging effects [3]. Brown rice (BrR, *Oryza sativa* L.) is rich in proanthocyanidins that offer antidiabetic benefits [4]. BrR also has strong anti-inflammatory properties [5]. BrR is a pigmented rice gaining popularity in Indonesia due to its minimal milling, which preserves the aleurone layer [6]. This offers BrR to contain higher vitamins,

fiber, protein, fat, minerals, phytochemicals, and insoluble fiber up to 16.51% than white rice [7]. BrR contains several bioactive compounds that have biological functions on health, including γ-oryzanol [8].

γ-Oryzanol is a typical phenolic compound widely distributed in the aleurone layer and rice bran [9]. γ-Oryzanol has various potential health functions, especially in treating hypercholesterolemia [10]. Hypercholesterolemia is a condition of elevated blood LDL cholesterol, increasing the risk of cardiovascular diseases like atherosclerosis and stroke [11]. National survey data recorded that hypercholesterolemia showed that more than 30% of adults in Southeast Asian countries have high total cholesterol levels. In Indonesia, in particular, the prevalence of high LDL cholesterol reached 41.9% of the adult population [12]. Several studies have shown that γ-oryzanol improves physiological health by stimulating liver antioxidant enzymes, reducing body weight, and decreasing triglyceride levels in hypercholesterolemic subjects [13-15]. HMG CoA Reductase has been widely targeted for treatment [14-15]. However, the molecular mechanism of γ-oryzanol in reducing LDL-cholesterol has not been fully reported [16]. Excessive microsomal triglyceride transfer protein (MTP) activity is one factor that increases LDL cholesterol by increasing cholesterol biosynthesis through HMG CoA Reductase [17].

MTP inhibition is a potential target for controlling hypercholesterolemia because it can reduce LDL cholesterol, increase high-density lipoprotein (HDL) cholesterol, and protect the liver from lipid overload [18]. Lomitapide is a commercial inhibitor that targets MTP, but it has some side effects, such as gastrointestinal disease and hepatic steatosis [19]. An alternative is needed, which is to use natural nutrients as natural MTP inhibitors. BrR is a food source and a potential candidate for antihypercholesterolemia due to its various compounds, especially γ-oryzanol, which has the potential to be a natural inhibitor of MTP. Yet, research on the γ-oryzanol profile of Indonesian brown rice and its bioactivity remains limited. Given its rising consumption and therapeutic potential, it is important to assess Indonesian BrR's γ-oryzanol content and bioactivity as a candidate for hypercholesterolemia therapy. This study aimed to identify and compare the γ-oryzanol content of Indonesian BrR using HPLC and evaluate its antioxidant and anticholesterol activities that hold the most potential for antihypercholesterolemia.

■ EXPERIMENTAL SECTION

Materials

The research materials used were purchased from Sigma-Aldrich: gold standard γ-oryzanol (cat. No. O0172), methanol, trichloroacetic acid (TCA), potassium ferricyanide ($C_6N_6FeK_3$), iron(III) chloride ($FeCl_3$), sulfuric acid, anhydrous acetate, and cholesterol (Sigma). This research used three varieties of BrR from three districts of East Java, Indonesia, i.e., Black Madras BrR from Pasuruan, Lawang BrR from Lawang, UB BrR from Kepanjen, and Mentik Wangi RR (*outlier*) from Ngawi.

Instrumentation

The instruments used in this study were a shaker water bath (Mammert), rotary evaporator (IKA HB 10), HPLC (Shimadzu® i-series LC-2030 LT), UV-vis spectrophotometer (SmartSpec Plus™, BioRad Laboratories Inc, Hercules, CA, USA), PyRx 0.8 Software, PyMol 3.0, BIOVIA Discovery Studio Visualizer 2024, and YASARA.

Procedure

Extraction of γ-oryzanol

As much as 10 g of each Indonesian BrR (Black Madras, Lawang, UB powder) was dissolved with 50 mL of distilled water (1:5 g/mL), then 2 g of ascorbic acid was added. Then, the sample was incubated at 40 °C for 40 min. The sample was mixed with 40 mL of *n*-hexane:propanol solvent (1:3 v/v) and stirred for 30 s. Then, the sample was precipitated with a centrifugation at 1,320 g for 15 min. The organic and aqueous phases of the sample were separated with a rotary evaporator. The crude γ-oryzanol BrR extract was weighed to determine the yield value by using Eq. (1). The crude γ-oryzanol BrR extract was stored at 4 °C [20].

$$\%\text{Yield value} = \left(\frac{\text{mass of extract}}{\text{mass of initial sample}}\right) \times 100\% \quad (1)$$

Characterization of γ-oryzanol with HPLC

The γ-oryzanol content of Indonesian BrR extracts was evaluated using a high-performance liquid chromatography (HPLC) test. A Shim-pack GIST C-18 column (150 mm × 4.6 mm × 5 μm), an inverted column with a UV detector was used for this analysis. A mobile phase consisting of acetonitrile, methanol, and isopropanol solvents in the ratio of 50:45:5 (v/v/v) was used. The mobile phase was slowed down at a 1 mL/min flow rate. The injection volume of the assay was 10 μL, and the detector wavelength was set at 330 nm. Retention times and peak areas were compared with standards for compound identification. Quantification was conducted based on the external standard method using a calibration curve prepared from a standard solution of γ-oryzanol objective [21].

Antioxidant activity assay with ferric reducing antioxidant power (FRAP) method

γ-Oryzanol extract from BrR Indonesia, gold standard γ-oryzanol, and ascorbic acid as a positive control were diluted to various concentrations (0, 1, 2, 4, 6, 8, and 10 μg/mL) using 0.1% methanol HCl. As much as 2.5 mL of 200 mM phosphate buffer (pH 6.6) was added for each diluted sample. Then, 1% $C_6N_6FeK_3$ was added. The reaction mixture was incubated at 50 °C for 30 min. After incubation, 2.5 mL of 10% TCA was added to quench the reaction and the mixture was homogenized. A solution of 5 mL was taken and transferred to a new test tube. Then, distilled water and 0.1% $FeCl_3$ solution 1 mL were added. The absorbance of the final solution was measured at 700 nm [22]. The antioxidant activity of the samples was calculated based on Eq. (2). IC_{50} values were obtained through linear regression analysis. Sample concentrations were plotted on the x and y axes, respectively. The straight line of the plotted data determined the IC_{50} value.

$$\%\text{Antioxidant} = \left(\frac{\text{Control}_{\text{abs}} - \text{Sample}_{\text{abs}}}{\text{Control}_{\text{abs}}} \right) \times 100\% \quad (2)$$

Anticholesterol determination with Lieberman-Burchard method

Simvastatin (positive control) was dissolved in distilled water at various concentrations (0, 1, 2, 4, 6, 8, and 10 μg/mL) and added up to 5 mL into a test tube containing blank water, which served as a positive control. A 5 mL of sample and gold standard γ-oryzanol were added to test tubes containing isopropanol blanks dissolved in isopropanol at concentrations of 0, 1, 2, 4, 6, 8, and 10 μg/mL. The samples were combined with 100 μg/mL standard cholesterol (2.5 mL) and shaken strongly for 2 min. Then, 0.1 mL of conc. H_2SO_4 and 1 M anhydrous acetate were added. Each sample was measured at 420 nm as the final value of absorbance [23]. Anticholesterol value was calculated with Eq. (3).

$$\%\text{Lowering cholesterol} = \left(\frac{\text{Control}_{\text{abs}} - \text{Sample}_{\text{abs}}}{\text{Control}_{\text{abs}}} \right) \times 100\% \quad (3)$$

In silico *analysis*

Molecular docking. Docking was performed between 4 γ-oryzanol derivatives of BrR based on the result of the HPLC test with MTP. A molecular dynamics simulation was performed to validate the stability of the inhibitory interaction on MTP compared to that of lomitapide as a control drug ligand. Four γ-oryzanol derivative ligands are cycloartenyl ferulate (CID 5282164), 24-methylenecycloartanyl ferulate (CID 9920169), campesteryl ferulate (CID 15056832), β-sitosteryl ferulate (CID 9938436), and lomitapide (CID 9853053) as the drug ligand control, were downloaded from the PubChem webserver. The canonical smile was duplicated and stored in SDF format. Ligand energies were minimized using Open Babel integrated with PyRx. MTP protein (PDB ID 6I7S) was downloaded from the RCSB Protein Data Bank web server, and then water molecules and ligand residues in the receptor structure were removed. Protein domains except lipid domain bindings were removed using BIOVIA Discovery Studio. Ligands and proteins were paired using AutoDock Vina software integrated with PyRx. The docked ligand configuration was coupled with the protein using PyMol. The ligand-protein complexes were analyzed using BIOVIA Discovery Studio software.

Molecular dynamics simulation. Molecular dynamics is examined with the YASARA software [24]. Environmental conditions were adjusted to match the physiological state of the virtual cell (water density 0.997 g/mL, 37 °C, 1 atm, pH 7.4, NaCl 0.9%). Molecular dynamics simulations were conducted for a duration of

40 ns. Snapshots of the simulation were captured at 25 ps intervals [25]. The simulation used the AMBER14 force field and the MD-run macro program. The RMSD values for all and ligand movements of value were analyzed to evaluate the stability of the interaction.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism 8 software. One-way analysis of variance (ANOVA) test followed by Tukey's HSD test was used to determine significant differences in yield values, γ-oryzanol content, and IC_{50} values for antioxidant and anticholesterol activities. Correlation between data was considered significant when $p < 0.05$ [26].

■ RESULTS AND DISCUSSION

Yield Value of γ-Oryzanol in Indonesian BrR

The yield value shows the ratio of the weight of the extract to the initial weight of the extracted powder [27]. The yield value of crude extract γ-oryzanol Indonesian BrR showed significant differences. The highest yield of crude γ-oryzanol extract was found in Mentik Wangi RR (11.2 ± 0.65%), and UB BrR (11.7 ± 0.24%) (Table 1). Lawang BrR has the lowest yield value (8.9 ± 0.12%). Different varieties of rice and plant locations affect the weight of the extract obtained [28]. A higher yield value of Mentik Wangi RR and UB BrR indicated that more γ-oryzanol compounds were successfully extracted from the sample.

HPLC Profile of γ-Oryzanol Indonesian BrR

HPLC aims to identify the γ-oryzanol profile and content of Indonesian BrR. HPLC analysis revealed 4 peaks corresponding to the dominant γ-oryzanol compound in the rice. In addition, the peak observed at a retention time of less than 10 min likely indicated the triterpene alcohol component of γ-oryzanol. γ-Oryzanol of Mentik Wangi RR showed 3 prominent peaks at retention times of 19.311, 21.354, and 22.984 min, with a smaller peak at 26.780 min (Fig. 1). The area under the highest peak at 21.354 min was measured. The compounds that appeared as the highest peak at each retention time of 19, 21, and 22 min were predicted as γ-oryzanol derivatives of cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesteryl ferulate (Fig. 1). The peak that appeared at the 26th minute was expected as a mixture of β-sitosteryl ferulate compounds [29]. Cycloartenyl ferulate is the main derivative of γ-oryzanol with the highest peak levels in Indonesian brown rice.

Table 1. Yield value of Indonesian brown rice extract

Sample	Yield value (%)
Mentik RR (*outlier*)	11.2 ± 0.65^{a}
Madras BrR	10.4 ± 0.52^{ab}
Lawang BrR	8.9 ± 0.12^{c}
UB BrR	11.7 ± 0.24^{a}

The different letters indicated significance differences (Tukey's HSD $p < 0.05$)

HPLC results showed that the γ-oryzanol content of Indonesian BrRs revealed significant differences. Mentik Wangi RR (*outlier*) has the highest average γ-oryzanol content (0.5324 ± 0.0006) per 1 g extract (Table 2). The highest average γ-oryzanol content of Mentik Wangi RR has followed the yield value obtained. Black Madras BrR has a higher average γ-oryzanol content, which was 30.07%, compared to Lawang and UB BrR. 1 g of Black Madras crude extract BrR contained 0.3007 ± 0.0011 g γ-oryzanol (Table 2). Black Madras BrR contains the highest concentration of γ-oryzanol among the other BrR samples. In Japanese rice, the average γ-oryzanol in black-purple rice, brown rice, green rice, and brown rice varieties was 54.2, 47.3, 44.3, and 43.3 mg per 100 g dry weight, respectively [30]. For another comparison, γ-oryzanol content in Korean rice varieties is 43.8 mg/100 g [21]. Rice γ-oryzanol levels are variable, influenced by factors such as extraction methods, the genetic makeup of rice, and environmental conditions [31].

In this study, the extraction method of γ-oryzanol followed by Heidtmann-Bemvenuti et al. [20], allows for a high yield of γ-oryzanol to be achieved by extracting at 40 °C for 40 min using 75 mL of a hexane:isopropanol mixture in a 1:3 ratio. Environmental conditions such as cooler temperatures, higher rainfall, and soil nutrients significantly influenced fitosterol levels in rice [32]. Suranto et al. [33] noted that altitude and related environmental factors in Ngawi District can influence

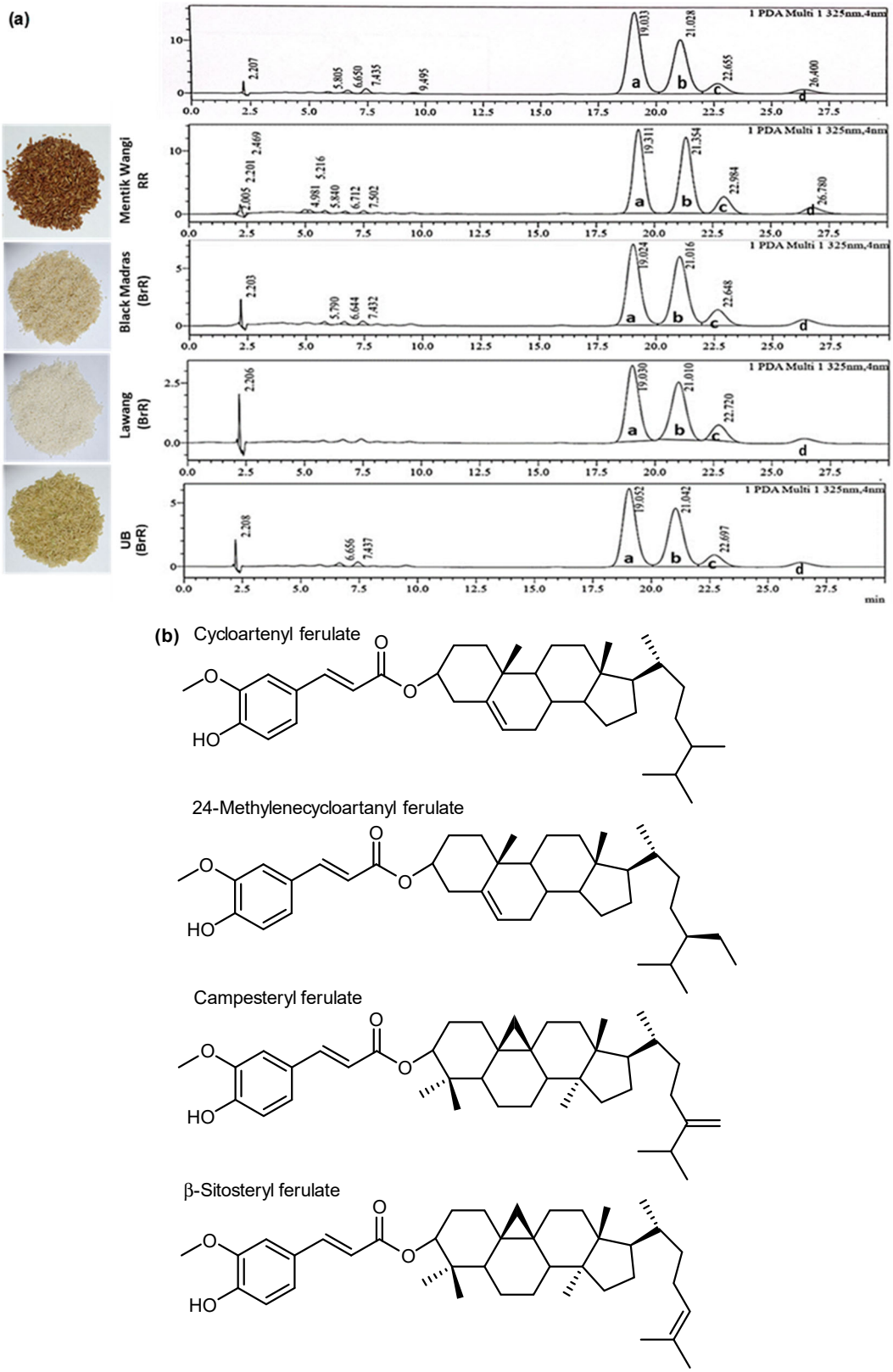


Fig 1. HPLC chromatographic profile of γ-oryzanol in Indonesian brown rice varieties. (a) Representative HPLC chromatogram; (b) γ-oryzanol derivatives identified from the chromatographic peaks

Table 2. γ-Oryzanol content of Indonesian brown rice from HPLC result

Sample	Retention time (min)	Average content γ-oryzanol (g/1 g)
Mentik wangi RR	21.354	0.5324 ± 0.0006[a]
Black Madras BrR	21.016	0.3007 ± 0.0011[b]
Lawang BrR	21.010	0.0402 ± 0.0001[d]
UB BrR	21.042	0.2370 ± 0.0004[c]

The different letters indicated significance differences (Tukey's HSD $p < 0.05$)

nutritional traits in rice, suggesting potential impact on bioactive compounds as well, even though γ-oryzanol was not directly measured. The γ-oryzanol content in rice is also influenced by particle size, rice variety, and pigmentation. Smaller rice bran particles (< 0.39 mm) yield more γ-oryzanol due to greater surface area for extraction [34]. Genotypic variation also plays a role, as glutinous rice varieties were reported to contain higher γ-oryzanol levels (213–686 mg/kg) than non-glutinous types (272–469 mg/kg) [35]. This aligns with our current results, where Mentik Wangi RR and Black Madras BrR exhibited significantly higher γ-oryzanol levels than other varieties. Additionally, compositional differences among rice types affect γ-oryzanol profiles; white rice showed absorbance at 325 nm, while pigmented rice peaked at 280 nm, indicating different compound compositions and potential antioxidant capacity [36].

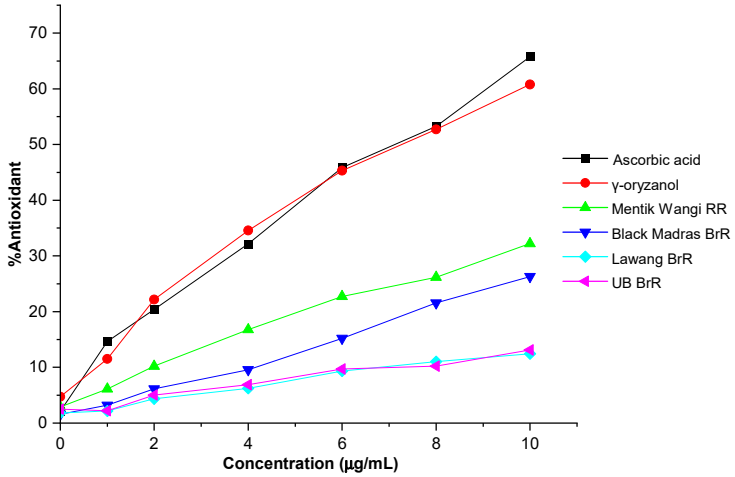
γ-Oryzanol Indonesian BrR comprises various compounds, primarily ferulic acid, and phytosterol (triterpenoid) components. Phytosterols are known for their unique structural characteristics, including methyl groups and double bonds, which are critical for their biological functions. The presence of certain functional groups is a key factor in identifying sterol class compounds. The O–H and C–H groups are important markers in sterol identification through techniques such as Fourier-transform infrared spectroscopy [37]. These groups play a crucial role in the biochemical activity of the compound, contributing to its potential health benefits, such as antioxidant and cholesterol-lowering effects [38].

The Antioxidant Activity of γ-Oryzanol in Indonesian Brown Rice

The antioxidant activity of γ-oryzanol Indonesian BrR was measured with the FRAP method. The ferric reducing antioxidant power (FRAP) assay is used to determine the antioxidant capacity of a compound by measuring its ability to reduce Fe^{3+} to Fe^{2+}, reflecting its reducing power. The OH group in γ-oryzanol facilitates the reduction reaction, highlighting its electron-donating ability, a crucial characteristic of antioxidants [39]. Black Madras BrR exhibited higher antioxidant activity (18.89 μg/mL) than Lawang (IC_{50} 56.08 μg/mL) and UB BrR (55.07 μg/mL), as measured by FRAP assay (Fig. 2).

The antioxidant assay showed that ascorbic acid had the highest antioxidant activity (7.78 μg/mL), followed by gold standard γ-oryzanol (8.83 μg/mL) and Mentik Wangi RR (*outlier*) (14.39 μg/mL). Black Madras has very strong antioxidants. IC_{50} value < 50 μg/mL is classified as very strong, 50–100 μg/mL is strong, 100–150 μg/mL is medium, and > 150 μg/mL is low [40]. According to prior research, Toorani et al. [41] showed that γ-oryzanol derived from rice bran oil exhibited the highest antioxidant efficiency compared to other vegetable oils, such as canola or olive. The molecular conformation of γ-oryzanol in rice bran enhances the interaction with unstable hydroperoxides, facilitates micelle formation, and inhibits lipid oxidation. In addition, Dewi et al. [36] reported differences in antioxidant profiles in white, red, and black rice varieties, indicating that pigmented rice contains higher concentrations, including γ-oryzanol derivatives. This is well correlated with the higher FRAP activity seen in Black Madras BrR in our study.

γ-Oryzanol of BrR worked as a chelating agent against Fe metal. Chelating agents prevent the formation of oxidative molecules by blocking catalytic activity on the metal and preventing the initiation of oxidant formation (Fig. 3) [29]. The derivatives of γ-oryzanol, such as cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesteryl ferulate, showed considerably



Sample	IC_{50} (µg/mL) ± STD	Antioxidant
Ascorbic acid	7.78 ± 0.42[a]	Very strong
Gold standard γ-Oryzanol	8.83 ± 0.46[a]	Very strong
Mentik Wangi RR (*Outlier*)	14.39 ± 1.02[b]	Very strong
Black Madras BrR	18.89 ± 0.37[b]	Very strong
Lawang BrR	56.08 ± 3.71[c]	Strong
UB BrR	55.07 ± 0.33[c]	Strong

Fig 2. Antioxidant activity by gold standard γ-oryzanol, Mentik Wangi RR, Black Madras BrR, Lawang BrR, UB BrR, at various concentrations

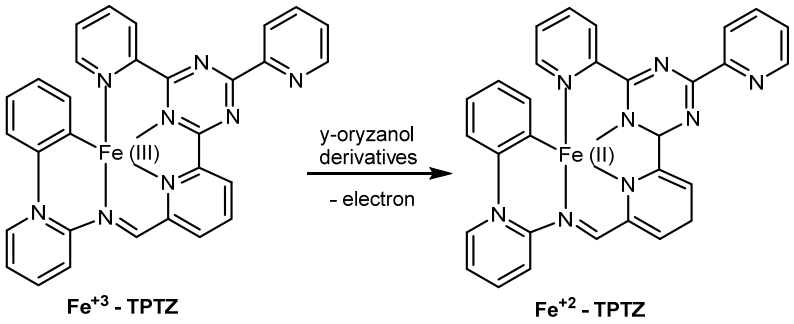
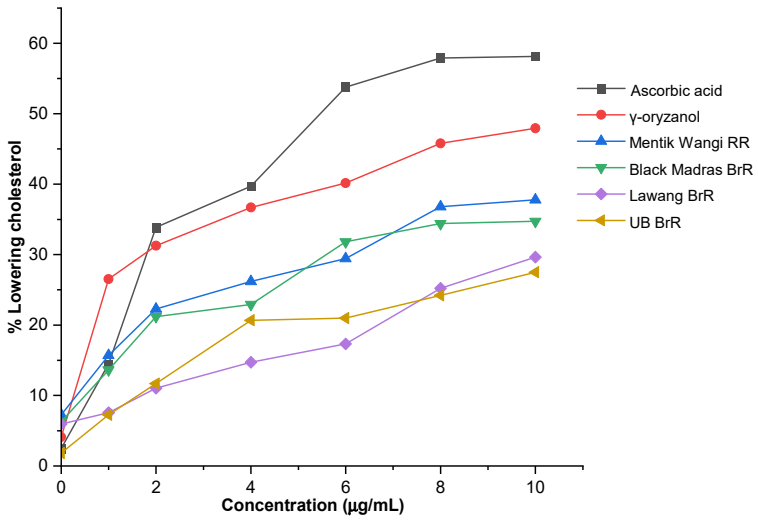


Fig 3. Antioxidant activity of γ-oryzanol derivatives in FRAP Assay. Illustration modified from [43]

higher antioxidant activity [42]. The higher antioxidant activity of γ-oryzanol suggested it may be more capable of neutralizing free radicals and potentially protecting cells from oxidative stress. These properties are invaluable in preventing chronic diseases and improving overall health [10].

The Anticholesterol Activity of γ-Oryzanol in Indonesian Brown Rice

In the anticholesterol test, γ-oryzanol of Black Madras BrR had higher anticholesterol bioactivity than Lawang (18.90 ± 0.42 µg/mL) and UB BrR (18.36 ± 0.12 µg/mL) with an IC_{50} value of 14.02 µg/mL (Fig. 4). Simvastatin, as a positive control, was found to have the highest anticholesterol ability (6.78 ± 1.34 µg/mL), followed by gold standard γ-oryzanol (9.23 ± 0.18 µg/mL). Compared to simvastatin and the gold standard γ-oryzanol, the γ-oryzanol of Black Madras BrR has a lower activity value. The –OH group on γ-oryzanol is thought to bind with cholesterol to form a γ-oryzanol-cholesterol complex. This complex inhibits the interaction of cholesterol with Lieberman-Burchard reagent, thus causing a decrease in the measured cholesterol rate. The anticholesterol activity of Black Madras BrR may be attributed to its higher γ-oryzanol content, particularly the dominant presence of cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesteryl ferulate, as confirmed by HPLC analysis.



Sample	IC_{50} (µg/mL) ± STD	Anticholesterol
Simvastatin	6.78 ± 1.34[a]	Very strong
Gold standard γ-Oryzanol	9.23 ± 0.18[a]	Very strong
Mentik Wangi RR (Out lier)	13.24 ± 0.1[ab]	Very strong
Black Madras BrR	14.02 ± 0.23[ab]	Very strong
Lawang BrR	18.90 ± 0.42[bc]	Very strong
UB BrR	18.36 ± 0.12[bc]	Very strong

Fig 4. Anticholesterol activity by gold standard γ-oryzanol, Mentik Wangi RR, Black Madras BrR, Lawang BrR, UB BrR at various concentrations

Various plant-derived phytosterols of γ-oryzanol compounds are known for their anticholesterol properties [44]. Stigmasterol from Sungkai leaves showed anticholesterol activity of 222.32 µg/mL with positive control simvastatin 24.68 µg/mL [23]. Moreover, supplementation of γ-oryzanol in hypercholesterolemic rats reduced oxidized LDL and improved HDL better than simvastatin [45]. These biological effects were associated with the upregulation of LDL receptors and downregulation of lipogenic enzymes, contributing to improved lipid metabolism. In addition, the phytosterol component of γ-oryzanol inhibits cholesterol absorption. Phytosterols compete with cholesterol in the intestinal lumen. This competitive inhibition reduces micellar solubilization and delays cholesterol uptake at the enterocyte level. γ-Oryzanol affects the expression of MTP, leading to cholesterol esterification, and the amount of cholesterol entering chylomicrons is reduced [46-47].

Inhibition γ-Oryzanol Derivatives Targeting Microsomal Triglyceride Transfer Protein in Hypercholesterolemia Mechanism

Docking methods were used to estimate ligand binding pose to the target protein, binding position, type of interaction, and binding affinity. It is important to predict the position of the native ligand within the binding site on the receptor to understand the molecular interactions based on the ligand's physicochemical properties. In the docking test, all γ-oryzanol derivatives were found to bind to the active residues of MTP protein, namely Leu643, Ile666, Phe813, and Val817 (Fig. 5). These residues are important triglyceride binding sites in VLDL formation by MTP [47]. Interestingly, the four residues also interact with lomitapide, a specific drug for controlling MTP (Table 3). This suggested that γ-oryzanol has a closed-inhibition mechanism of lomitapide. The γ-oryzanol derivative interacted with Val664, which was not observed in the lomitapide-MTP interaction. Lomitapide also interacted with Thr776, which was not found in the γ-oryzanol-lomitapide interaction. β-Sitosteryl ferulate has the most active site residue interactions compared to other γ-oryzanol derivatives, with ten interactions.

γ-Oryzanol-MTP interaction was dominated by a hydrophobic bond through the phytosterol group and one hydrogen bond by the ferulic group. The phytosterol group on γ-oryzanol has a nonpolar structure interacting

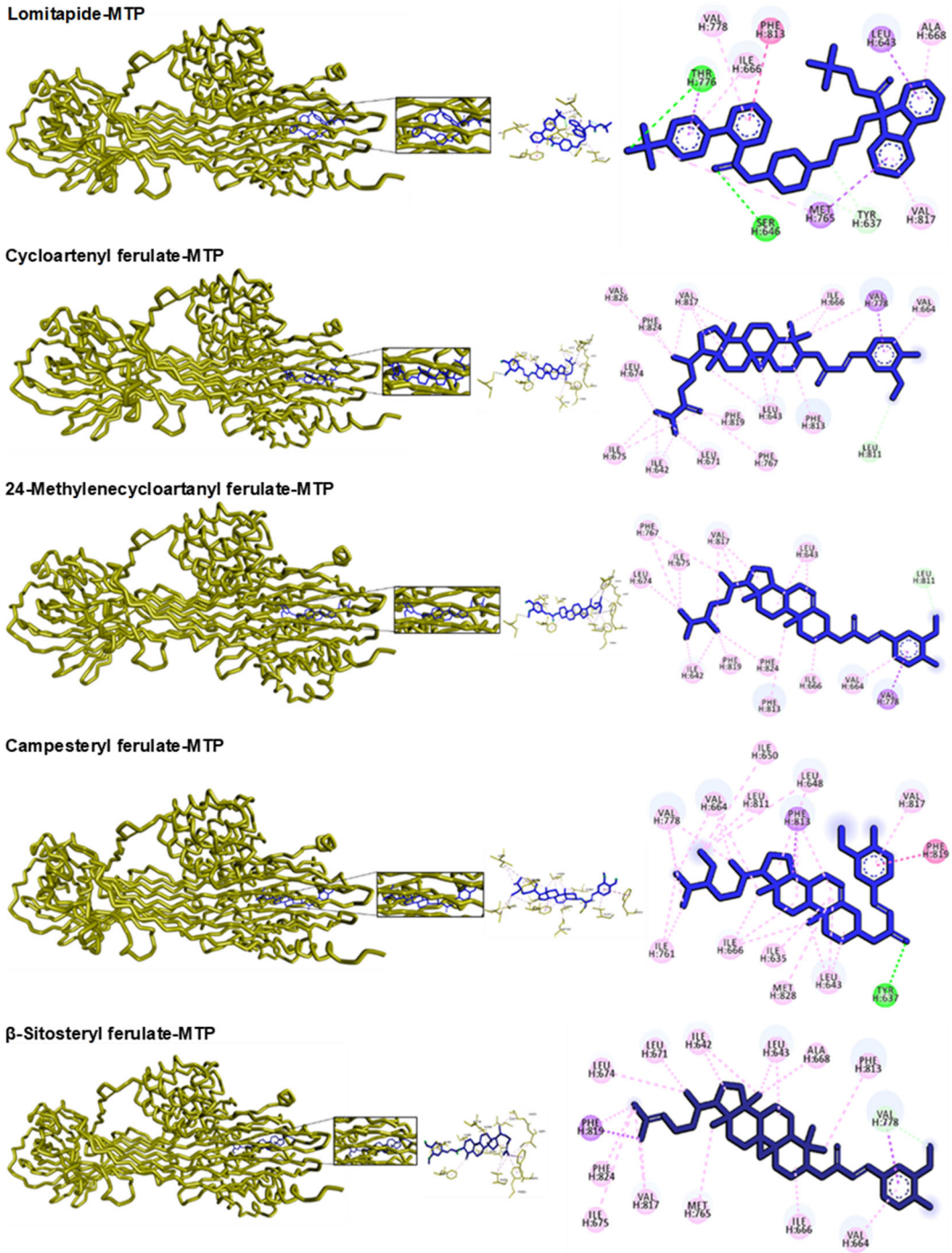


Fig 5. Docking inhibition derivatives of γ-oryzanol of brown rice on MTP. Ligand is shown in blue, MTP is shown in yellow

with nonpolar amino acid residues on MTP. This interaction was driven by the Van der Waals attraction, which occurred when the electron clouds of atoms in both molecules overlapped. Phytosterol groups interacted with Leu643, Ile666, Phe813, and Val817 residues on MTP. These residues have long hydrophobic side chains, allowing for strong Van der Waals interactions with the phytosterol groups. The ferulic group on γ-oryzanol has a O–H group that forms hydrogen bonds with amino acid residues that have C=O or N–H groups on MTP.

Table 3. Docking interaction derivatives of γ-oryzanol on MTP

Complex	Interaction		No. of residue interactions	No. of residue active site interactions	Binding affinity (kcal/mol)
	Hydrogen	Hydrophobic			
Lomitapide-MTP	Ser646, Tyr637, Thr776	Leu643, Ile666, Ala668, Met765, Val778, Phe813, Val817	10	7	−11.573
Cycloartenyl ferulate-MTP	Val778	Ile642, Leu643, Val664, Ile666, Ala668, Leu671, Leu674, Ile675, Met765, Phe813, Val817, Phe819, Phe824	14	6	−11.109
24-Methylenecycloartanyl ferulate-MTP	Leu811	Ile642, Leu643, Val664, Ile666, Leu671, Leu674, Ile675, Phe767, Val778, Phe813, Val817, Phe819, Phe824, Val826	15	8	−11.581
Campesteryl ferulate-MTP	Leu811	Ile642, Leu643, Val664, Ile666, Leu674, Ile675, Phe767, Val778, Phe813, Val817, Phe819, Phe824	13	8	−11.719
β-Sitosteryl ferulate-MTP	Tyr637	Ile635, Leu643, Leu648, Ile650, Val664, Ile666, Ile761, Val778, Leu811, Phe813, Val817, Phe819, Met828	14	10	−11.522

Note: underlined amino acids showed active site of MTP

This was found in γ-oryzanol, which interacted with hydrogen at residues Tyr637, Val778, and Leu811. The binding energy value of γ-oryzanol derivative on MTP was relatively the same, around −11 kcal/mol (Table 3). The binding energy was close to the binding energy of lomitapide. In molecular dynamics simulations, the low RMSD All value showed the stable interaction γ-oryzanol derivatives with MTP (below ≤ 3 Å) (Fig. 6(a)) [48]. RMSD is used to determine the stability of the protein-ligand complex [49]. RMSD of ligand movement showed that the movement of γ-oryzanol derivatives is better than lomitapide (Fig. 6(b)). At the initial simulation, γ-oryzanol derivatives of cycloartenyl ferulate interacted at relatively the same distance as lomitapide. However, at the end of the simulation, cycloartenyl ferulate. γ-Oryzanol derivatives interacted at a closer distance to the protein. A closer distance indicates stronger bonding of the ligand-protein complex [50]. γ-Oryzanol is a potential anticholesterol candidate that can inhibit MTP and is a safer alternative for controlling the mechanism of hypercholesterolemia.

MTP inhibition is a promising strategic pathway to control cholesterol elevation in hypercholesterolemic subjects [51]. This protein controls a significant metabolic regulation in the transport of triglycerides and cholesterol. Specifically, MTP forms very low-density lipoprotein (VLDL) in the liver. VLDL is converted into low-density lipoprotein (LDL) in the bloodstream, a major cholesterol carrier. Decreasing LDL cholesterol can increase the activity of the transcription factor SREBP-2, especially in LDL receptor gene expression [52]. MTP inhibition may affect SREBP-1c expression, potentially leading to increased triglyceride biosynthesis [53]. However, the potential increase in triglycerides due to SREBP-1c modulation must be addressed with appropriate dietary or pharmacological interventions. Increased LDL receptors can increase the uptake of LDL-cholesterol in plasma and increase HDL. HDL functions as a reverse cholesterol transporter, facilitating cholesterol efflux from peripheral tissues back to the liver for excretion. Consequently, elevated HDL levels contribute to a reduction in blood cholesterol [54]. MTP inhibition by γ-oryzanol of BrR offers a promising avenue for managing cholesterol levels and can be an attractive therapeutic strategy. This study only included three varieties of Indonesian BrR; thus, the findings have not been generalized to other BrR varieties that might have different γ-oryzanol content and biological activities. In addition, the biological activity of γ-oryzanol from three Indonesian BrR varieties was

assessed only through *in vitro* and *in silico* approaches. Therefore, *in vivo* validation is required to confirm its physiological relevance and therapeutic potential in hypercholesterolemia.

■ CONCLUSION

Indonesian BrR showed that the best rice is Black Madras BrR, which contained high γ-oryzanol, which has strong biological functions as an antioxidant and strong anticholesterol properties. γ-Oryzanol was indicated to have a stabilizing interaction and inhibited the lipid-binding domain of MTP protein. Thus, γ-oryzanol from Black Madras BrR has the potential as a nutraceutical and therapeutic agent for hypercholesterolemia. Further research is necessary to determine the health effects of γ-oryzanol of Black Madras BrR on the activity of cholesterol profile levels in animal models of hypercholesterolemia.

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■ CONFLICT OF INTEREST

The authors declare no conflicts of interest.

■ AUTHOR CONTRIBUTIONS

Ja'far Umar and Christine Natalia Palis were responsible for collecting primary data, analyzing the data, visualizing the data, and interpreting the findings. Ja'far Umar drafted the initial manuscript, reviewed, and edited. Eko Suyanto and Turhadi carried out formal analysis and supervision. Titin Andri Wihastuti conducted supervision. Fatchiyah was responsible for funding acquisition, methodology, supervision and approved the final version of the manuscript intended for publication.

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