Research Article

Phytochemical Screening and HMG-CoA Reductase Inhibitory Activity of Black Rice Extract as a Potential Treatment for Hypercholesterolemia

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ABSTRACT

Hypercholesterolemia is a significant risk factor for cardiovascular diseases, and the search for effective and natural alternatives to conventional treatments is crucial. Black rice, a pigmented rice variety, has gained attention for its potential health benefits, including its ability to modulate lipid metabolism. This study aimed to investigate the phytochemical profile and HMG-CoA reductase inhibitory activity of local black rice, called *Tadong* (TBR), evaluating its potential as a treatment for hypercholesterolemia. Methanol (80% v/v) and water were used to extract the phytochemicals from TBR. Qualitative phytochemical screening of TBR was conducted to screen the presence of saponins, tannins, alkaloids and steroids. Total phenolic content (TPC) was also measured using the Folin-Ciocalteu method. The HMG-CoA reductase inhibitory activity of the extract was assessed using an in-vitro enzymatic assay. Qualitative phytochemical screening revealed the presence of tannins, alkaloids, and steroids in the methanol extract. In contrast, these compounds were not detected in the water extract. The methanol extract (0.421 mg GAE/g extract) exhibited a significantly higher TPC when compared to the water extract (0.101 mg GAE/g) ($p<0.05$). The methanol extract (0.230±0.007 ng/ml) also demonstrated a potent inhibitory effect on HMG-CoA reductase activity, compared to the water extract (0.311±0.005 ng/ml) ($p<0.05$) indicating its potential as a natural cholesterol-lowering agent. The TBR's phytochemical profile and its ability to inhibit HMG-CoA reductase indicate its promising role as a natural and effective alternative for the management of hypercholesterolemia.

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1. Introduction

Cardiovascular disease (CVD) continues to be a major global health concern, with the Asia-Pacific region facing a rising prevalence of CVD risk factors, particularly plasma lipid disorders. The National Health and Morbidity Survey 2023 reveals that high cholesterol affects a substantial portion of the adult population in Malaysia. Specifically, 1 in 5 adults, approximately 20% of the population, have high cholesterol. Alarmingly, half of these individuals are unaware of their condition. The report further breaks down high cholesterol into its components: 27% of adults have low high-density lipoprotein cholesterol (HDL-C), 41% have high low-density lipoprotein cholesterol (LDL-C), and 23% have high triglycerides (TG). These findings highlight the significant prevalence of hypercholesterolemia in Malaysia and the considerable lack of awareness among those affected (Institute for Public Health Malaysia, 2024). Meanwhile, Lee *et al.* (2021) provide a crucial overview of the prevalence of these disorders, specifically high LDL-C, in several Asia-Pacific countries, utilising national survey data for a representative population overview. Their analysis reveals a wide range in the prevalence of high LDL-C, from approximately 17% in Indonesia to around 35% in Australia, with other countries like Thailand (around 20%), the Philippines (around 24%), and Vietnam (around 30%) falling in between. Similarly, high total cholesterol (TC) prevalence varies significantly, ranging from roughly 10% in Thailand to approximately 50% in Australia. Beyond prevalence, the study highlights a concerning gap in awareness, treatment, and control of dyslipidemia across the included countries, emphasising the need for improved public health strategies focused on early detection and optimised treatment. This highlights the urgency of addressing the growing burden of CVD risk factors in this diverse region through targeted interventions and increased awareness.

Black rice is a rich source of various phytochemicals, including flavonoids, anthocyanins, and phenolic compounds. These bioactive phytochemicals are abundant in this variety of *Oryza sativa*, the cultivated rice plant. The flavonoid content of black rice includes compounds such as proanthocyanidins, catechins, and quercetin derivatives, while the anthocyanin fraction is dominated by cyanidin and peonidin glycosides. Additionally, black rice is a particularly potent source of phenolic acids, including ferulic acid, gallic acid, and protocatechuic acid (Bhat *et al.*, 2020; Ciulu *et al.*, 2018; Limtrakul *et al.*, 2019; Xie *et al.*, 2023). This diverse phytochemical profile contributes to the many health-promoting properties associated with black rice consumption. These bioactive compounds in black rice have been shown to effectively lower TC, LDL-C and TG levels while increasing HDL-C levels in animal and human studies (Ito & Lacerda, 2019; Sangma & Parameshwari, 2021). The hypocholesterolemic activity of black rice is primarily attributed to its ability to inhibit the activity of HMG-CoA reductase, a key enzyme involved in the biosynthesis of cholesterol. Additionally, the antioxidant and anti-inflammatory properties of black rice phytochemicals may contribute to their cardioprotective effects by reducing oxidative stress and inflammation, which are known risk factors for the development of cardiovascular diseases (Ghasemzadeh *et al.*, 2018; Ito & Lacerda, 2019; Nurlaili *et al.*, 2020; Sangma & Parameshwari, 2021; Yoon *et al.*, 2014). Despite these promising findings, there is a lack of scientific research on the HMG-CoA reductase inhibitory activity of local black rice, specifically the *Tadong* variety originating from Sabah, Malaysia. The potential of this local black rice cultivar in treating hypercholesterolemia remains largely unexplored. Therefore, this study aims to conduct phytochemical screening and to investigate the HMG-CoA reductase inhibitory activity, to elucidate its potential as a natural and effective treatment for hypercholesterolemia.

2. Materials and Methods

2.1 Materials

Local black rice, called *Tadong* (TBR) was selected as the primary research material. The rice samples were obtained through a convenient sampling approach, with 3 kg of TBR acquired from a local market in Ranau, Sabah, Malaysia. To maintain the integrity and quality of the rice, the samples were securely packaged in a plastic sealed bag measuring 15.2 cm x 22.8 cm. Additionally, an accession number (Accession No: 2321) was provided by a research officer at the Agriculture Research Centre (ARC) in Tuaran, which served as a

key reference for tracking and verifying the authenticity of the TBR samples throughout the study. After the acquisition, the TBR samples were carefully stored at the Food Analysis Laboratory within the Faculty of Food Science and Nutrition at the Universiti Malaysia Sabah, where they were kept at ambient room temperature (23.5 ± 1.5°C). This storage protocol was essential to preserve the rice's natural characteristics and prevent any potential degradation of the phytochemicals before the extraction process.

2.2 Extraction Procedure

Methanol (80% v/v) and water were selected as extraction solvents due to their distinct polarity characteristics, allowing for a broader phytochemical extraction range. Methanol, particularly at 80% concentration, has been reported to be highly efficient in solubilising phenolic compounds and other moderately polar phytochemicals due to its intermediate polarity and protic nature (Rente *et al*., 2021; Khoo *et al*., 2012). Water, being highly polar, was included to assess the extraction of highly polar bioactive compounds. The combination of both solvents provides a comparative understanding of how solvent polarity influences the extraction efficiency of bioactive constituents from TBR.

2.2.1 Methanol Extraction

To perform the methanol extraction, the protocol outlined by Khoo *et al.* (2012) was followed. Initially, 1 gram of the TBR sample was finely ground using a Waring Commercial Blender (Model 7011HS, USA) equipped with a stainless-steel grinding chamber. The grinding process was performed at ambient room temperature (23.5 ± 1.5°C) at a speed setting of 18,000 rpm for 2 minutes. The grinding was performed in short bursts of 30 seconds to prevent overheating. The ground TBR powder was then passed through a 60-mesh sieve (250 µm) to standardise the particle size, ensuring uniformity and maximising the surface area available for extraction. The ground sample was placed in a 10 mL centrifuge tube and mixed with 10 mL of 80% methanol. This mixture was vortexed (INTLLAB, China) for 1 minute to ensure thorough mixing. Subsequently, the sample underwent sonication for 15 minutes using a SONIC 405 ultrasonic device (Hwashin, Korea) to enhance the extraction. The sonicated mixture was centrifuged (Centrifuge 5430 R Eppendorf, Germany) at 5000 rpm for 25 minutes to separate the extract from the solid residues. To eliminate residual methanol that could interfere with subsequent biochemical analyses, the methanol was removed by rotary evaporation under reduced pressure at 40°C using a Rotavapor R-300 (Büchi, Switzerland). The concentrated extract was then reconstituted with distilled water. Lastly, the extract was transferred to a sterile 5 mL tube and stored at 4°C in the Food Analysis Laboratory at Universiti Malaysia Sabah for further analysis.

2.2.2 Water Extraction

The water extraction method was adapted from the procedure described by Abdul Kadir *et al*. (2014). Briefly, 0.2 grams of ground TBR sample were combined with 10 mL of deionised water. The mixture was then subjected to sonication at room temperature for 15 minutes using a SONIC 405 ultrasonic device (Hwashin, Korea) to facilitate extraction. Following sonication, the mixture was filtered through a 0.45 µm nylon syringe filter with a 17 mm diameter, resulting in a clear extract. The filtered water extract was collected in a sterile 5 mL tube and stored at 4°C in the Food Analysis Laboratory at Universiti Malaysia Sabah, awaiting further analysis.

2.3 Phytochemical Screening

Qualitative phytochemical screening of the methanol and water extracts of TBR was performed using standard phytochemical tests to identify the presence of bioactive compounds, including saponins, tannins, alkaloids, and steroids.

2.3.1 Test for Saponins

To test for saponins, 1 mL of the TBR extract was mixed with 10 mL of distilled water in a test tube. The mixture was then vigorously agitated, and the formation of persistent, stable foam was observed. The presence of such foam lasting for 15 minutes indicated the presence of saponins in the extract (Salvamani *et al*., 2016).

2.3.2 Test for Tannins

To test for the presence of tannins, 1 mL of the TBR extract was combined with 3 mL of distilled water in a conical flask. Then, 3 drops of a 10% ferric chloride solution were added to the mixture. The development of a blue-green colour upon the addition of ferric chloride indicates the existence of tannins in the extract (Shaikh & Patil, 2020).

2.3.3 Test for Alkaloids

To test for alkaloids, 2 mL of the TBR extract was combined with 0.2 mL of diluted hydrochloric acid and 1 mL of Mayer's reagent in a conical flask. The formation of a yellowish colouration upon adding Mayer's reagent indicates the presence of alkaloids in the extract (Rao *et al*., 2016).

2.3.4 Test for Steroids

To test for the presence of steroids, 2 mL of the TBR extract was mixed with 2 drops of concentrated sulfuric acid and then observed. A red-rose colour in the lower layer indicates the presence of steroids, while a golden-yellow colour suggests triterpenes (Shaikh & Patil, 2020).

2.4 Determination of Total Phenolic Content

The total phenolic content (TPC) was determined using the well-established Folin-Ciocalteu method (Singleton & Rossi, 1965). The procedure involved the following steps: First, 200 µl of the TBR extract was transferred to a 25 ml volumetric flask. Then, 10 ml of distilled water and 1.5 ml of Folin-Ciocalteu reagent were added, and the mixture was incubated for 5 minutes. Next, 4 ml of a 20% w/v sodium carbonate solution was added, and the volume was brought up to 25 ml with distilled water. The mixture was left undisturbed for 30 minutes to allow the development of a blue colour, indicating the presence of phenolic compounds. The absorbance of the solution was then measured at 765 nm using a spectrophotometer (PerkinElmer, London, UK). A calibration curve was prepared using gallic acid solutions of known concentrations ranging from 0.05 mg to 1.5 mg, which served as a reference for quantifying the phenolic content in the TBR extract. Finally, the total phenolic content was calculated using the formula:

$$T = \frac{C \times V}{M} \quad (1)$$

where ***T*** is the total phenolic content (mg/g of extract as gallic acid equivalents, GAE), ***C*** is the concentration of gallic acid obtained from the calibration curve (mg/ml), ***V*** is the volume of the extract solution (ml), and ***M*** is the weight of the extract (g).

2.5 Determination of HMG-CoA Reductase Inhibitory Activity

The study evaluated the inhibitory potential of Tadong Black Rice (TBR) extracts on HMG-CoA reductase using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology Inc., United States). Both methanol and water extracts of TBR were prepared and tested. Simvastatin, a widely used cholesterol-lowering agent, was employed as the positive control due to its high inhibitory potency, lipophilic nature, and extensive clinical validation as an HMG-CoA reductase inhibitor (Ahmad, 2020; Sahebkar, 2014; Schointuch *et al*., 2014; Zhao *et al*., 2014). Its well-established role in suppressing cholesterol biosynthesis makes it a suitable benchmark for evaluating the inhibitory activity of natural extracts. Simvastatin was used at a concentration of 10 mg/mL, consistent with previous studies aiming to ensure measurable

inhibition in enzyme assays and to provide a robust comparative reference (Ahmad, 2020; Sahebkar, 2014). The standard curve for the HMG-CoA reductase activity assay was constructed using six concentrations of the provided standard, ranging from 0.63 ng/ml to 20 ng/ml. The standards ' optical density (OD) readings, TBR extracts, and simvastatin control were measured spectrophotometrically at 450 ± 2 nm (Meizheng Bio-Tech, China). A calibration curve was generated by plotting absorbance (Y-axis) against concentration (X-axis), yielding the regression equation $y = 0.0634x + 0.0288$ with a strong linear correlation ($R^2 = 0.99$), confirming the assay's reliability for quantitative evaluation. All assays followed the manufacturer's protocols to ensure precision and reproducibility. The results were expressed as ng/ml of HMG-Coa reductase activity.

2.6 Statistical Analysis

Data were expressed as mean ± standard deviation. One-way ANOVA using SPSS (IBM SPSS Inc., USA) for Windows 21 was utilised to analyse the data. The differences in TPC between the methanol and water extracts were analysed using an independent sample t-test. Additionally, Duncan's Multiple Range Test was used to compare the HMG-CoA reductase inhibitory activity of the methanol extract, water extract, and Simvastatin. The results were considered statistically significant when the $p<0.05$.

3. Results and Discussion

The phytochemical analysis of TBR demonstrated distinct variations in solvent efficacy, as evidenced by the diverse colourimetric responses from the methanol extract. The methanol extract confirmed the presence of tannins, indicated by a change from purple to blue-green, alkaloids, signified by a colour transformation from purple to yellowish, and steroids, denoted by the emergence of a red-rose colouration in the lower layer. In contrast, these compounds were not detected in the water extract. Meanwhile, the absence of colour change for saponins in both extracts suggests that these compounds are either inherently absent in TBR or present at levels not detectable by the colourimetric methods used.

Table 1. Phytochemical Screening of TBR Extract

Type	Methanol Extract	Water Extract
Saponins	-	-
Tannins	+	-
Alkaloids	+	-
Steroids	+	-

Note: (+) indicate the presence of phytochemical, (-) indicate the absence of phytochemical

Saponins are known for their pharmacological properties, including cholesterol-lowering effects (Juang & Liang, 2020). Their absence in TBR could suggest a variety-specific phytochemical profile or potentially low concentrations that fall below the detection threshold of standard colourimetric assays. Such findings highlight the need for more sensitive detection techniques, such as liquid chromatography coupled with mass spectrometry (LC-MS), which has been shown to offer greater specificity and sensitivity in the detection of saponins and other phytochemicals (Hassan *et al.*, 2022). Additionally, the variability in phytochemical composition across rice varieties is well-documented, which further indicates that the detection and quantification of these compounds may require tailored analytical approaches for each rice variety (Das *et al.*, 2023; Fatchiyah *et al.*, 2020; Ito & Lacerda, 2019; Sangma & Parameshwari, 2021).

Table 2. Total Phenolic Content in TBR Extract

Sample	Total Phenolic Content (mg GAE/g extract)
Methanol Extract	0.421 ± 0.040[a]
Water Extract	0.101 ± 0.025[b]

Different letters indicate a significant difference ($p < 0.05$) by using the Independent Samples T-test. Values are expressed as mean ± SD (n = 3). GAE: gallic acid equivalent.

The TPC of TBR was significantly higher in the methanol extract (0.421 mg GAE/g extract) than in the water extract (0.101 mg GAE/g) ($y = 0.0795x - 0.0429$; $R = 0.9975$) ($p < 0.05$). The difference in phenolic content between the methanol and water extracts is consistent with the trends observed in phytochemical screening, where methanol was more effective in extracting tannins, alkaloids, and steroids. This suggests that the higher efficacy of methanol in solubilising phenolic compounds contributes significantly to the overall phytochemical profile of the extract. Collectively, these comparative analyses affirm the role of methanol as a potent solvent for the extraction of phenolic compounds, likely attributable to its higher pKa value and protic nature, which enhance hydrogen bonding and acid-base interactions, thereby promoting the efficient extraction of mid-polar bioactive compounds (Jahromi, 2019; Rente *et al*., 2021). Methanol's intermediate polarity is particularly conducive to solvating low molecular weight organic molecules with functional groups such as –COOH and –OH, including phenolics, flavonoids, tannins, and alkaloids (Bowyer *et al*., 2015; Onyebuchi & Kavaz, 2020). Furthermore, its selective polarity facilitates the exclusion of highly non-polar impurities, resulting in a cleaner extract than that obtained with water. These physicochemical properties collectively contribute to the superior extraction of bioactive compounds and the enhanced HMG-CoA reductase inhibitory activity observed in the methanol extract. This discussion not only elucidates the complexities inherent in phytochemical extraction but also accentuates the significance of TBR as a functional food. The identified phytochemicals, tannins, alkaloids, and steroids, each hold promise for contributing to the rice's therapeutic potential, particularly in the context of hypercholesterolemia treatment.

Table 3. HMG-CoA Reductase Inhibition Activity in Experimental Samples

Sample	HMG-CoA reductase (ng/ml)
Methanol Extract	0.230±0.007[a]
Water Extract	0.311±0.005[b]
Simvastatin	0.357±0.011[c]

Different letters indicate significant differences ($p<0.05$) among samples by Duncan's multiple range test. Results are given as mean ± SD (n = 3).

Interestingly, the methanol extract showed the greatest inhibition of HMG-CoA reductase activity (0.230±0.007 ng/ml), when compared to water extract (0.311±0.005 ng/ml), and a standard, Simvastatin drug (0.357±0.0106 ng/ml) ($p < 0.05$). It is important to note that lower measured HMG-CoA reductase activity values indicate higher inhibitory capacity. Therefore, the methanol extract, which exhibited the lowest HMG-CoA reductase activity value, demonstrates the most potent inhibition compared to the water extract and the simvastatin control. This inverse relationship between enzyme activity and inhibitory effect confirms the potency of the phytochemicals extracted using methanol to suppress cholesterol biosynthesis. The phytochemicals in the methanol extract, such as tannins, alkaloids, and steroids, are believed to be crucial in inhibiting HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. Tannins can directly bind to and inhibit the HMG-CoA reductase enzyme, crucial for cholesterol synthesis. This inhibition

occurs through multiple mechanisms: 1) Tannins form stable complexes with the enzyme, physically blocking its active site and reducing access to the natural substrate, HMG-CoA, decreasing the enzyme's overall activity. 2) Tannins can also induce structural changes in HMG-CoA reductase, altering its shape and compromising its ability to effectively bind and convert HMG-CoA. 3) Additionally, tannins have been observed to interfere with the enzyme's cellular localisation and membrane association, sequestering it away from its site of action and diminishing its functionality within the cholesterol biosynthesis process (Chang *et al*., 2001; Radhia *et al*., 2018).

Alkaloids can also potently inhibit HMG-CoA reductase, with certain compounds directly competing with the enzyme's natural substrate, HMG-CoA, for binding to the active site, effectively blocking the conversion of HMG-CoA to mevalonate. Additionally, some alkaloids have been shown to induce structural changes in HMG-CoA reductase, impairing the enzyme's catalytic activity, and can also interfere with the enzyme's cellular translocation and membrane association (Hasanah *et al*., 2016; Ram *et al*., 2020). Steroids, with their structural similarities to cholesterol, can act as potent competitive inhibitors of HMG-CoA reductase. By closely mimicking the natural substrate, HMG-CoA, these phytochemicals can effectively compete for binding to the active site of the enzyme, disrupting the conversion of HMG-CoA into mevalonate, the essential precursor molecule for cholesterol synthesis (Ahmad, 2020; Jiang *et al.,* 2018). The synergistic effects of these diverse phytochemicals present in the methanol extract of TBR likely contribute to its potent HMG-CoA reductase inhibitory activity, making it a promising natural remedy for managing hypercholesterolemia.

However, it is important to note some limitations of this study. While providing valuable insights, the phytochemical screening method used in this study may be limited in detecting and quantifying the full spectrum of bioactive compounds present in TBR. The use of standard colourimetric assays, while commonly employed, can have limitations in sensitivity and specificity, potentially missing or underestimating the presence of certain phytochemicals, such as saponins, that may be present at low concentrations (Timilsena *et al*., 2023). Additionally, *in vitro* the HMG-CoA reductase inhibition assay, while demonstrating the potent inhibitory activity of the TBR methanol extract, may not fully capture the complex and synergistic mechanisms by which the individual phytochemicals contribute to the observed effects. Further in-depth analysis utilising more sensitive analytical techniques, such as LC-MS, would be beneficial to provide a more comprehensive understanding of the phytochemical composition and its relationship to the observed HMG-CoA reductase inhibitory activity. Further in vivo study would also be essential to validate the therapeutic potential of TBR in hypercholesterolemia. Such an investigation would allow for a more comprehensive assessment of the extract's efficacy, safety, and mechanisms of action in a whole-organism model, providing crucial data to support the development of TBR as a natural treatment option for hypercholesterolemia and associated cardiovascular conditions. Nonetheless, the findings reported in this study offer valuable insights into the phytochemical composition and HMG-CoA reductase inhibitory activity of TBR, positioning it as a promising functional food with potential therapeutic applications in managing hypercholesterolemia.

4. Conclusion

The phytochemical screening and HMG-CoA reductase inhibitory activity assessment of TBR extract provide valuable insights into its potential as a functional food and a natural therapeutic approach for managing hypercholesterolemia. The methanol extract of TBR exhibited a phytochemical profile, with the presence of tannins, alkaloids and steroids, which are known to possess cholesterol-lowering properties. The superior HMG-CoA reductase inhibitory activity of the methanol extract, even compared to a standard statin drug, highlights the therapeutic potential of TBR in treating hypercholesterolemia. These findings, combined with the ethnomedicinal history of black rice, suggest that TBR could be a valuable and accessible natural resource for developing novel, cost-effective, and potentially safer treatment options for individuals struggling with elevated cholesterol levels and associated cardiovascular risks.

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References

Abdul Kadir, N. A. A., Mutalib, M. A., & Rahmat, A. (2014). Comparison of antioxidant properties of tamarillo (*Cyphomandra betacea*), cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) and tomato (*Lycopersicon esculentum*). International Food Research Journal, 21(6).

Ahmad, V. (2020). Exploration of binding interaction of steroidal drugs with HMG-CoA reductase for the treatment of breast cancer patients who suffer with cardiovascular problems: in-silico studies. Asian Pacific Journal of Cancer Biology, 5(1), 27-33.

Bhat, F. M., Sommano, S. R., Riar, C. S., Seesuriyachan, P., Chaiyaso, T., & Prom-u-Thai, C. (2020). Status of bioactive compounds from bran of pigmented traditional rice varieties and their scope in production of medicinal food with nutraceutical importance. Agronomy, 10(11), 1817.

Bowyer, M. C., Van Vuong, Q., Van Altena, I. A., & Scarlett, C. J. (2015). Phytochemicals and antioxidant capacity of Xao tam phan (*Paramignya trimera*) root as affected by various solvents and extraction methods. Industrial Crops and Products, 67, 192-200.

Chang, J. J., Chen, T. H., Chan, P., Chen, Y. J., Hsu, F. L., Lo, M. Y., & Lin, J. Y. (2001). The in vitro inhibitory effect of tannin derivatives on 3-hydroxy-3-methylglutaryl-coenzyme A reductase on Vero cells. Pharmacology, 62(4), 224-228.

Ciulu, M., Cádiz-Gurrea, M. D. L. L., & Segura-Carretero, A. (2018). Extraction and analysis of phenolic compounds in rice: a review. Molecules, 23(11), 2890.

Das, M., Dash, U., Mahanand, S. S., Nayak, P. K., & Kesavan, R. K. (2023). Black rice: A comprehensive review on its bioactive compounds, potential health benefits and food applications. Food Chemistry Advances, 3, 100462.

Fatchiyah, F., Sari, D. R. T., Safitri, A., & Cairns, J. R. (2020). Phytochemical compound and nutritional value in black rice from Java Island, Indonesia. Systematic Reviews in Pharmacy, 11(7), 414-421. 1

Ghasemzadeh, A., Karbalaii, M. T., Jaafar, H. Z., & Rahmat, A. (2018). Phytochemical constituents, antioxidant activity, and antiproliferative properties of black, red, and brown rice bran. Chemistry Central Journal, 12, 1-13.

Hasanah, Q., Hasim, F. D., & Andrianto, D. (2016). Inhibition activity of HMG-CoA reductase by rice brain extract and its fraction as anticholesterole-mia In vitro study. Der Pharma Chemica, 8(23), 1-5.

Hassan, M., Bala, S. Z., Bashir, M., Waziri, P. M., Musa Adam, R., Umar, M. A., & Kini, P. (2022). LC-MS and GC-MS Profiling of Different Fractions of Ficus platyphylla Stem Bark Ethanolic Extract. Journal of Analytical Methods in Chemistry, 202*2*, 6349332.

Institute for Public Health Malaysia. (2024). National Health and Morbidity Survey (NHMS) 2023: Non-communicable Diseases and Healthcare Demand - Key Findings.

Ito, V. C., & Lacerda, L. G. (2019). Black rice (Oryza sativa L.): A review of its historical aspects, chemical composition, nutritional and functional properties, and applications and processing technologies. Food chemistry, 301, 125304.

Jahromi, S. G. (2019). Extraction techniques of phenolic compounds from plants. Plant physiological aspects of phenolic compounds, 1-18.

Jiang, S. Y., Li, H., Tang, J. J., Wang, J., Luo, J., Liu, B., ... & Song, B. L. (2018). Discovery of a potent HMG-CoA reductase degrader that eliminates statin-induced reductase accumulation and lowers cholesterol. Nature Communications, 9(1), 5138.

Juang, Y. P., & Liang, P. H. (2020). Biological and pharmacological effects of synthetic saponins. Molecules, 25(21), 4974.

Khoo, H. E., Azlan, A., Ismail, A., & Abas, F. (2012). Antioxidative properties of defatted dabai pulp and peel prepared by solid phase extraction. Molecules, 17(8), 9754-9773.

Lee, Z. V., Llanes, E. J., Sukmawan, R., Thongtang, N., Ho, H. Q. T., Barter, P., & Cardiovascular RISk Prevention (CRISP) in Asia Network. (2021). Prevalence of plasma lipid disorders with an emphasis on LDL cholesterol in selected countries in the Asia-Pacific region. Lipids in Health and Disease, 20, 1-12.

Limtrakul, P., Semmarath, W., & Mapoung, S. (2019). Anthocyanins and proanthocyanidins in natural pigmented rice and their bioactivities. In Phytochemicals in human health (pp. 1-24).

Nurlaili, E. P., Lestari, R. A. S., & Hartati, S. (2020, February). The influence of black rice bran aqoueus extract on blood and spleen profiles. In IOP Conference Series: Earth and Environmental Science (Vol. 443, No. 1, p. 012106). IOP Publishing.

Onyebuchi, C., & Kavaz, D. (2020). Effect of extraction temperature and solvent type on the bioactive potential of Ocimum gratissimum L. extracts. Scientific reports, 10(1), 21760. 5

Radhia, A., Hanen, N., Abdelkarim, B. A., & Mohamed, N. (2018). Phytochemical screening, antioxidant and antimicrobial activities of Erodium glaucophyllum (L.) L'Hérit. J Biomed Sci, 7(4), 13-9.

Ram, H., Jaipal, N., Charan, J., Kashyap, P., Kumar, S., Tripathi, R., ... & Abd Allah, E. F. (2020). Phytoconstituents of an ethanolic pod extract of Prosopis cineraria triggers the inhibition of HMG-CoA reductase and the regression of atherosclerotic plaque in hypercholesterolemic rabbits. Lipids in health and disease, 19, (1) 11.

Rao, U. S., Abdurrazak, M., & Mohd, K. S. (2016). Phytochemical screening, total flavonoid and phenolic content assays of various solvent extracts of tepal of Musa paradisiaca. Malaysian Journal of Analytical Sciences, 20(5), 1181-1190.

Rente, D., Paiva, A., & Duarte, A. R. (2021). The role of hydrogen bond donor on the extraction of phenolic compounds from natural matrices using deep eutectic systems. Molecules, 26(8), 2336.

Sahebkar, A., Simental-Mendía, L. E., Pedone, C., Ferretti, G., Nachtigal, P., Bo, S., ... & Watts, G. F. (2016). Statin therapy and plasma free fatty acids: a systematic review and meta-analysis of controlled clinical trials. British journal of clinical pharmacology, 81(5), 807-818.

Salvamani, S., Gunasekaran, B., Shukor, M. Y., Shaharuddin, N. A., Sabullah, M. K., & Ahmad, S. A. (2016). Anti-HMG-CoA Reductase, Antioxidant, and Anti-Inflammatory Activities of Amaranthus viridis Leaf Extract as a Potential Treatment for Hypercholesterolemia. Evidence-Based Complementary and Alternative Medicine, 2016(1), 8090841.

Sangma, H. C. R., & Parameshwari, S. (2023). Health benefits of black rice (Zizania aqatica)-a review. *Materials Today:* Proceedings, 80, 3380-3384.

Schointuch, M. N., Gilliam, T. P., Stine, J. E., Han, X., Zhou, C., Gehrig, P. A., ... & Bae-Jump, V. L. (2014). Simvastatin, an HMG-CoA reductase inhir, exhibits anti-metastatic and anti-tumorigenic effects in endometrial cancer. Gynecologic oncology, 134(2), 346-355

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies, 8(2), 603-608.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 16(3), 144-158.

Timilsena, Y. P., Phosanam, A., & Stockmann, R. (2023). Perspectives on saponins: food functionality and applications. International Journal of Molecular Sciences, 24(17), 13538.

Xie, F. Y., Bi, W. W., Wang, X. J., Zhang, X. L., Zhang, X. N., Zhao, G. X., & Liu, Q. Q. (2017). Extraction and Identification of Black Rice Polyphenolic Compounds by Reversed Phase High Performance Liquid Chromatography–Electrospray Ionization Mass Spectrometry. Journal of food processing and preservation, 41(5), e13119.

Yoon, J., Ham, H., Sung, J., Kim, Y., Choi, Y., Lee, J. S., ... & Kim, D. (2014). Black rice extract protected HepG2 cells from oxidative stress-induced cell death via ERK1/2 and Akt activation. Nutrition research and practice, 8(2), 125-131.