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Evaluation of Antioxidant Activity, α-Glucosidase Inhibition, and Malondialdehyde Reduction of Reformulated Galohgor Nutraceuticals from Sundanese-Indonesia

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ABSTRACT

Galohgor nutraceutical, a traditional herbal formulation from Sundanese-Indonesia, has long been used for its health benefits, including antidiabetic potential. However, studies on α-glucosidase inhibition in its new formulation remain limited. This study aimed to assess the antioxidant activity, α-glucosidase inhibitory potential of the reformulated (F2) of Galohgor Nutraceutical, and its effect on malondialdehyde (MDA) levels. The methods used a completely randomized design (CRD). Antioxidant activity and α-glucosidase inhibitory effect were evaluated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method and α-glucosidase inhibition assay. MDA levels were analyzed in vivo using serum samples from mice treated with the reformulated (F2) of Galohgor Nutraceutical. The statistical analysis used was an independent t-test. The results showed that the reformulated (F2) extract of Galohgor Nutraceutical exhibited antioxidant activity, with an IC_{50} value of 2904.06 ppm. In comparison, the positive control, ascorbic acid, showed a much lower IC_{50} value of 8.20 ppm, indicating stronger antioxidant capacity. The α-glucosidase inhibitory activity of the F2 extract was reflected by an IC_{50} value of 30.833,14 ppm, which was much higher than that of the positive control, acarbose (IC_{50} = 0.16 ppm), suggesting a relatively weak inhibitory effect. Administrating of the Galohgor extract at a dose of 5000 mg/kg body weight successfull reduced MDA levels. Although not statistically significant (p = 0.131), the observed reduction in MDA levels suggests a protective potential against oxidative stress. The reformulated (F2) of Galohgor Nutraceutical extract has potential as a natural antioxidant and antidiabetic agent.

Key Messages:

- The study investigates a simplified nutraceutical formulation —the reformulated (F2) Galohgor nutraceutical—composed of 10 selected Indonesian medicinal plants, offering a more practical and scalable approach for potential antidiabetic therapy.
- The findings of this study may support the development of Formula F2 as a scientifically validated, safe, and culturally relevant Galohgor Nutraceutical for reducing oxidative stress and managing diabetes.

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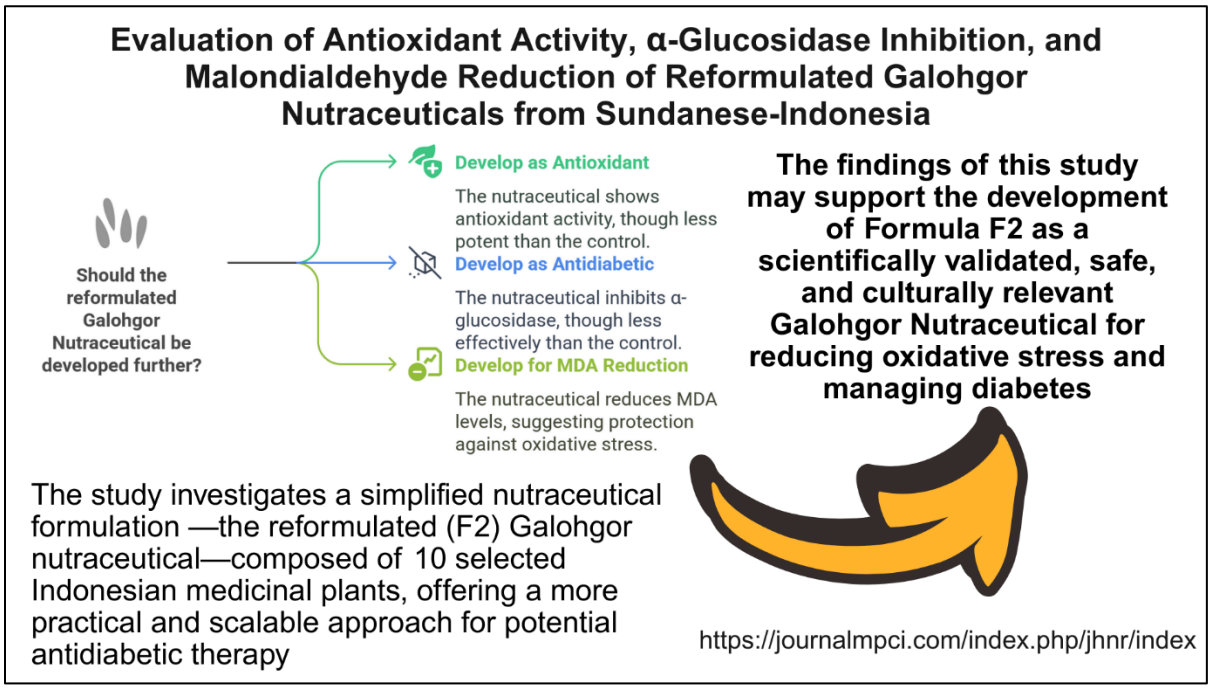
GRAPHICAL ABSTRACT



INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both (1). According to data from the International Diabetes Federation (IDF), the global prevalence of diabetes among individuals aged 20–79 years was estimated at 10.5% (536.6 million people) in 2021, and is projected to increase to 12.2% (783.2 million people) by 2045. In the Southeast Asia region, the prevalence in the same age group was estimated at 8.7% (90.2 million people) in 2021, with a projected increase to 11.3% (151.5 million people) by 2045. Meanwhile, in Indonesia, the prevalence among adults aged 20–79 years was estimated at 10.8% (19.5 million people) in 2021 and is projected to rise to 12.4% (28.6 million people) by 2045. These data indicate a continuous increase in the number of diabetes cases at global, regional, and national levels, highlighting the urgent need for enhanced prevention and management strategies.

Among such bioactive compounds, antioxidants play a significant role due to their ability to neutralize free radicals without becoming pro-oxidants themselves. These molecules protect cells from oxidative damage induced by reactive oxygen species (ROS), which are known contributors to the pathogenesis of diabetes (2). A diet rich in antioxidants is essential to prevent oxidative stress, thereby lowering the risk of oxidative stress-related diseases, including diabetes mellitus (3). Growing public awareness of the health benefits of antioxidants has spurred the development of antioxidant-rich nutraceutical products aimed at preventing or managing chronic metabolic disorders such as diabetes.

An effective strategy for diabetes treatment involves reducing postprandial blood glucose levels and preventing the development of advanced diabetic complications (4). However, the currently available anti-hyperglycemic drugs are often expensive and associated with serious side effects, which has led to growing interest in the search for safer, more affordable antidiabetic compounds derived from natural sources (5). One of the primary therapeutic approaches involves the inhibition of α-glucosidase, an enzyme responsible for the hydrolysis, digestion, and absorption of carbohydrates. In this context, there has been increasing attention toward nutraceuticals and bioactive compounds that can help regulate blood glucose levels and prevent diabetes-related complications (6).

In line with the growing interest in natural bioactive compounds, the use of nutraceuticals has emerged as a promising approach for the prevention and management of diabetes. Nutraceuticals are bioactive substances derived from plant or animal sources, formulated to promote health, prevent disease,

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or provide therapeutic benefits (7). They encompass a wide range of products, including dietary supplements, herbal preparations, probiotics, prebiotics, and medical foods designed to prevent and treat various diseases (8) One example of a promising nutraceutical is Galohgor, a traditional formulation composed of 56 plant species, including leafy greens, legumes, spices, and rhizomes (9). Consumption of Galohgor has been reported to improve oxidative stress conditions, reduce blood glucose levels, and decrease visceral fat in patients with type 2 diabetes mellitus (10, 11, 12).

As part of the continuous development of Galohgor-based nutraceuticals, a novel formulation has been introduced to enhance both practicality and efficacy. The latest innovation, known as Formula F2 Galohgor Nutraceutical, utilizes a simplified combination of 10 selected plant species, in contrast to the original formulation that comprised 56 different plants. This reduction aims not only to streamline the production process but also to improve product consistency, effectiveness, and safety. Moreover, the new formulation is expected to exhibit enhanced antioxidant activity and more potent α-glucosidase inhibitory effects, offering additional health benefits for diabetes management (13).

Although Formula F2 Galohgor Nutraceutical is expected to exhibit enhanced antioxidant activity and more effective α-glucosidase inhibition, scientific evaluation of these functional aspects remains limited. Therefore, further research is needed to complement and expand the evaluation of the safety and efficacy of Formula F2. This study aims to assess the antioxidant activity and α-glucosidase inhibitory potential of the Formula F2 Galohgor Nutraceutical, as well as its effect on MDA levels, a biomarker of oxidative stress. The findings are expected to provide scientific evidence supporting the development of Formula F2 as a safe and effective nutraceutical for diabetes management. This study is part of a BIMA grant-funded research (2024) entitled "*Pengembangan nutrasetikal antidiabetes galohgor berbasis pendekatan nutrigenomik dan metabolomik*".

METHODS

Materials and methods

An experimental study using a Completely Randomized Design (CRD) was conducted from January to February 2025. Sample preparation, powder and extract production were performed at the Food Experimentation Laboratory, IPB University. Antioxidant activity, Antioxidant activity, -glucosidase inhibition, and in vivo testing were analyzed at the Center for Tropical Biopharmaca Studies, IPB University."-glucosidase inhibition, and in vivo testing were analyzed at the Center for Tropical Biopharmaca Studies, IPB University.".

Product Preparation: the equipment used included a drying oven, simplicia grinder, 100-mesh sieve, cooking pot, stirrer, stove, water thermometer, cheese cloth, basin, blender, evaporator, digital scale, and plastic packaging. The materials used consisted of 10 plant ingredients: maize, Zingiber aromaticum (lempuyang), mung beans, guava (Psidium guajava), Kaempferia galanga (kencur), ginger (Zingiber officinale), nutmeg (Myristica fragrans), Pluchea indica (beluntas), Melastoma malabathricum (harendong), and cardamom (Amomum compactum). All plant materials were obtained from the Biopharmaca garden of IPB University. Distilled water (aqua destillata) was used as the extraction solvent.

Preparation of Galohgor Nutraceutical Extract

This study's first phase involved preparing Galohgor Nutrasetical powder and extract. The resulting extract was analyzed to evaluate antioxidant and α-glucosidase inhibitory activity. Powder preparation began with cleaning the ten selected plant materials, then drying at 70 °C for 24 hours. Once dried, the materials were ground into fine powder. The extraction process followed the decoction method (14), where the powder was mixed with distilled water at a ratio of 1:10, heated at 90 °C for 30 minutes, and then filtered using cheesecloth. The filtrate was evaporated at 90 °C, blended into a fine texture, and sieved using a 100-mesh filter, yielding the final Galohgor nutraceutical extract.

Two formulations, F2-1 and F2-2, were prepared with identical composition and extraction methods. Formulation F2-2 served as a replicate of F2-1, aimed at evaluating the reproducibility and consistency in the extract preparation process. The decoction method was employed for both formulations, as it not only reflects traditional preparation techniques but has also been demonstrated as the most optimal method for producing extracts with higher nutritional content such as carbohydrates, fats, and β-

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carotene compared to other extraction methods(9).

Antioxidant Activity Assay

Antioxidant activity was measured using the DPPH method (15), which was selected due to its simplicity, rapid execution, cost-effectiveness, and widespread application in assessing the free radical scavenging activity of plant-based antioxidants. Sample solutions were prepared in concentrations of 1000, 500, 250, 125, 62.5, and 31.25 ppm using methanol. The DPPH radical solution (0.2 mM) was prepared using DPPH from SRL (India) in ethanol. A total of 75 µL of the sample solution and 75 µL of the DPPH solution were pipetted into a microplate. The color change from purple to light purple indicated a reaction between DPPH radicals and antioxidants in the sample. The mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured using a microplate reader at 492 nm. Ascorbic acid (Merck KGaA, Germany) was used as the standard antioxidant. All measurements were performed in triplicate.

α-Glucosidase Inhibitory Assay

The α-glucosidase inhibitory activity was evaluated in vitro. This assay was chosen because α-glucosidase plays a key role in carbohydrate digestion and is highly relevant for postprandial (after-meal) blood glucose control. Extracts were dissolved in dimethyl sulfoxide (DMSO) at concentrations of 80.000, 40.000, 20.000, 10.000, 7.500, 5.000, and 0 ppm. The substrate, p-nitrophenyl-α-D-glucopyranoside (pNPG; Sigma-Aldrich, USA), was dissolved in 0.1 M phosphate buffer (pH 7). The α-glucosidase enzyme (Sigma-Aldrich, USA) was prepared by dissolving 1 mg in 100 mL of phosphate buffer containing 200 mg bovine serum albumin (BSA) and diluted 25-fold. The reaction mixture included 10 µL of extract, 50 µL phosphate buffer, 25 µL substrate, and 25 µL enzyme solution. After incubation at 37 °C for 30 minutes, the reaction was terminated by adding 100 µL of 0.2 M sodium carbonate. Absorbance was measured at 410 nm using a microplate reader. Acarbose (Glucobay) at concentrations of 0.1, 0.5, 1.5, and 10 µg/mL served as the positive control. All measurements were conducted in triplicate.

In Vivo Study

The study's second phase involved preclinical in vivo testing on experimental mice, following the Indonesian National Agency of Drug and Food Control (BPOM) Regulation No. 10 of 2022 concerning guidelines for preclinical toxicity testing. After in vivo testing, serum MDA levels were analyzed to evaluate the potential toxicity and physiological effects of the Galohgor nutraceutical extract.

The in vivo study was conducted using female ddy mice (8 weeks old, 27–31 grams), with five animals per treatment group. Mice were acclimatized for 7 days under laboratory conditions, fasted for 3–4 hours prior to treatment, and administered a single dose of the test formulation via oral gavage. Observations were made during the first 30 minutes post-administration, then every 4 hours for the first 24 hours, and once daily for 14 days. Body weights were recorded on days 7 and 14. At the end of the study, mice were euthanized, and blood samples were collected for malondialdehyde (MDA) analysis. The study protocol was approved by the Research Committee for Animal Care and Use of the National Research and Innovation Agency (BRIN).

MDA Analysis

MDA levels in serum samples were determined following (16). A total of 0.2 mL of serum was mixed with 1.2 mL of 0.082 N H_2SO_4 and allowed to stand for 10 minutes, followed by the addition of 0.15 mL of 10% phosphotungstic acid. After a 5-minute rest, the mixture was centrifuged at 3000 rpm for 20 minutes, and the resulting pellet was collected. The H_2SO_4 and phosphotungstic acid treatments were repeated. Then, 0.5 mL of 1% thiobarbituric acid (TBA) in 50% acetic acid was added. The mixture was incubated in a water bath at 95 °C for 60 minutes, cooled, and mixed with 2.5 mL of n-butanol: pyridine (15:1 v/v). After phase separation by centrifugation at 3000 rpm for 15 minutes, the upper pink phase was collected and measured at 532 nm using a UV-Vis spectrophotometer.

Data Analysis

Data were processed using Microsoft Excel 2019 and analyzed using IBM SPSS Statistics version 22.0 for Windows. The MDA level data were analyzed using an independent samples t-test. Results were considered statistically significant at $p < 0.05$ with a 95% confidence interval (CI).

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CODE OF HEALTH ETHICS

This study was approved by the Research Committee for Animal Care and Use of the National Research and Innovation Agency (BRIN), with the ethical approval number 186/KE.02/SK/07/2024.

RESULTS

Figure 1 shows that the Galohgor nutraceutical the IC_{50} value of the F2 extract of Galohgor nutraceutical was found to be 2904.06 ppm. In comparison, the positive control, ascorbic acid, exhibited a significantly lower IC_{50} value of 8.20 ppm.

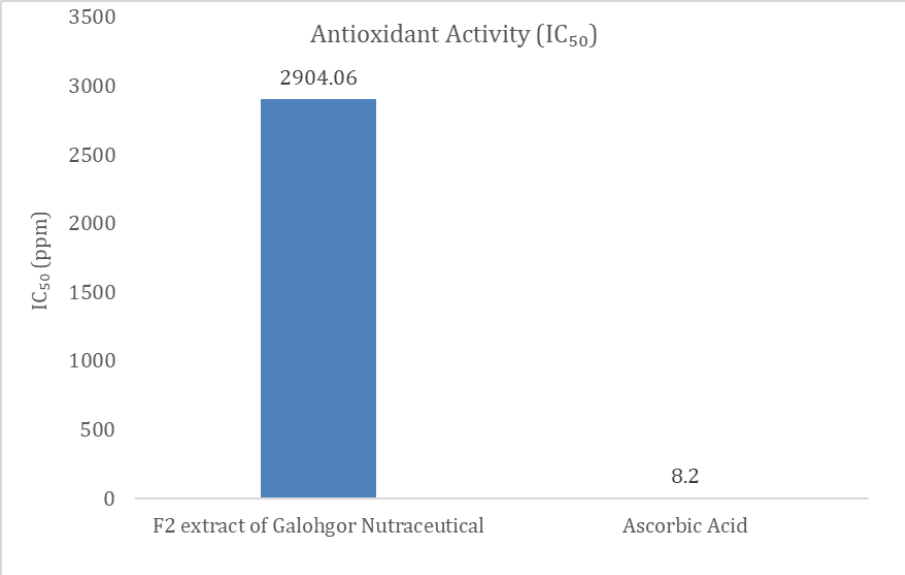

Figure 1. Antioxidant Activity (IC_{50}) of Galohgor Nutraceutical

Figure 2 shows that the α-glucosidase inhibitory activity of the F2 extract of Galohgor nutraceutical was found to be 30.833,14 ppm. In comparison, the positive control, acarbose, exhibited a significantly lower IC_{50} value of 0.16 ppm.

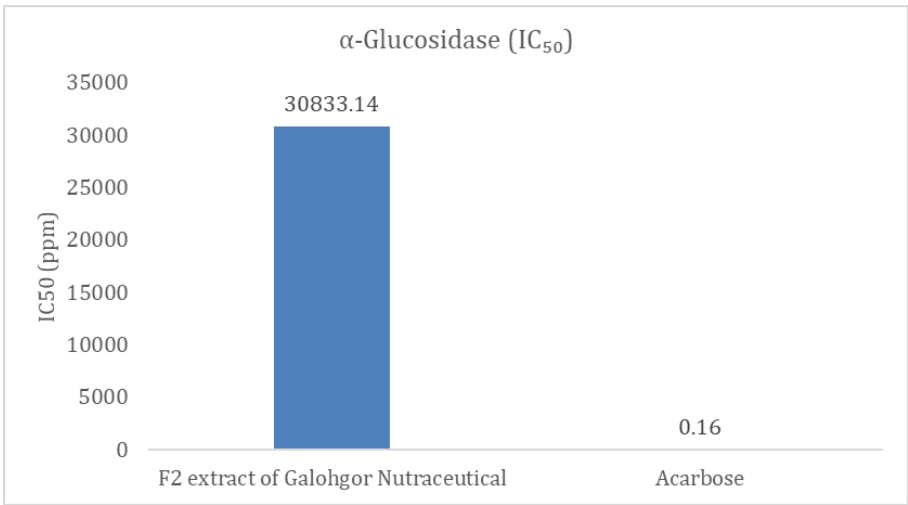

Figure 2. α-Glucosidase Inhibition (IC_{50}) of Galohgor Nutraceutical

Figure 3 show that the analysis of mean malondialdehyde (MDA) levels showed that the control group (without Galohgor nutraceutical treatment) had an MDA level of 2.49 µM, while the group treated with Galohgor nutraceutical at a dose of 5000 mg/kgBW showed a decreased MDA level of 1.88 µM.

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However, this difference was not statistically significant, as indicated by a p-value of 0.131 ($p > 0.05$), suggesting that the reduction in MDA levels in the treatment group was not strong enough to be considered significant. Nonetheless, the calculated effect size (Cohen's d = 1.55) indicates a large magnitude of difference between the groups.

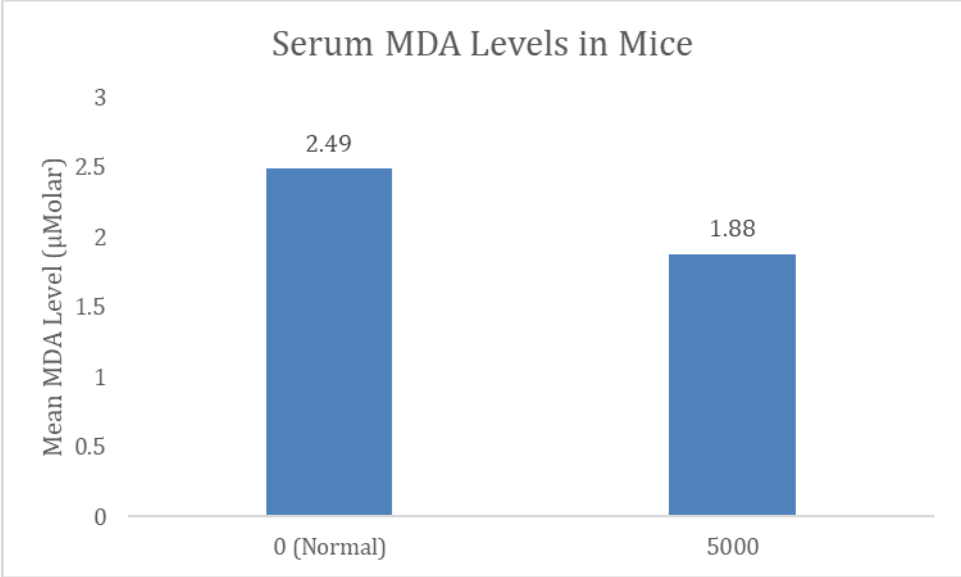

Figure 3. MDA serum levels in mice administered with Galohgor nutraceutical

DISCUSSUSSION

The antioxidant activity of a compound can be assessed using its IC_{50} value (17). This value represents the concentration required to inhibit 50% of DPPH radicals present. A lower IC_{50} indicates a stronger radical scavenging ability, thus reflecting higher antioxidant activity (18). Antioxidant activity testing revealed that the IC_{50} value of the F2 Galohgor nutraceutical extract was 2904.06 ppm. In comparison, the positive control, ascorbic acid, exhibited a significantly lower IC_{50} value of 8.20 ppm. According to general classification, IC_{50} values above 500 ppm are considered to indicate very weak antioxidant activity (19). These findings suggest that, in vitro, the antioxidant activity of the Galohgor nutraceutical is present but falls into the category of very weak when compared to ascorbic acid based on the DPPH assay.

However, the DPPH assay, which is a chemical and in vitro method, may not fully reflect the complex antioxidant mechanisms that occur in vivo. Therefore, malondialdehyde (MDA) analysis in animal models is required to provide a more biologically relevant assessment of oxidative stress. The MDA level in the treatment group (administered Galohgor Nutraceutical at a dose of 5000 mg/kgBW) was lower (1.88 µM) compared to the control group (2.49 µM). Although this difference was not statistically significant ($t = 1.89$; $p = 0.131$), the calculated Cohen's d effect size was 1.55, indicating a large difference between the control and treatment groups. This range of MDA values suggests that administration of Galohgor Nutraceutical at a dose of 5000 mg/kgBW may exert a biologically meaningful effect, despite the lack of statistical significance. The reduced MDA levels observed in mice treated with Galohgor Nutraceutical for 14 days indicate a potential antioxidant effect of the extract. The antioxidants contained in the extract may reduce oxidative stress, as evidenced by the decrease in MDA levels, a well-established biomarker of oxidative damage (20). This finding is consistent with previous studies in experimental rats administered Galohgor Nutraceutical for 14 days at various doses. At a dose of 0.375 g/kgBW (equivalent to the normal human dose), the MDA level was 0.57 µM. At 0.75 g/kgBW (2× the human dose), the MDA level was 0.60 µM, and at 1.5 g/kgBW (4×the human dose), the MDA level decreased to 0.52 µM. These results suggest that administration of Galohgor Nutraceutical may influence plasma MDA levels in rats, supporting its potential role in mitigating oxidative stress .

The presence of bioactive compounds such as alkaloids, glycosides, and triterpenoids in Galohgor

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is thought to contribute to its antioxidant activity (21) . Alkaloids are known to neutralize free radicals and enhance cellular defense mechanisms (22). Glycosides, such as aucubin and mangiferin, exhibit immunomodulatory and antioxidant properties (23). Triterpenoids are also recognized for their ability to scavenge reactive oxygen species such as superoxide and hydroxyl radicals, as well as to prevent lipid peroxidation (24) . In addition, Galohgor contains essential minerals including iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn), which serve as important cofactors for endogenous antioxidant enzymes such as superoxide dismutase (SOD) (21). These components may exert synergistic effects in strengthening the body's antioxidant defense system, which may not be fully captured by the DPPH assay.

The inhibition of the α-glucosidase enzyme reflects the in vitro antidiabetic potential of the extract, which was determined spectrophotometrically (25). The IC_{50} values (ppm) of the Nutrasetical Galohgor extract and the standard drug acarbose are presented in Figure 2. The study demonstrated that the α-glucosidase inhibitory activity of the F2 of the Nutrasetical Galohgor extract was 30,833.14 ppm. In comparison, the positive control, acarbose, exhibited a much lower IC_{50} value of 0.16 ppm. These findings are consistent with previous research indicating that Nutrasetical Galohgor has relatively weak α-glucosidase inhibitory potential (10). A higher IC_{50} value indicates that a greater concentration is required to inhibit 50% of the enzyme activity, thereby reflecting a lower inhibitory potency. Importantly, the potential synergistic effects with other antidiabetic mechanisms that may be exerted by compounds in Galohgor should not be overlooked. Compounds such as flavonoids and polyphenols present in Galohgor are known to influence glucose metabolism (10). This aspect warrants further investigation to fully understand the antidiabetic potential of Galohgor.

Statistical analysis of MDA levels indicated no significant difference between the control and treatment groups. This outcome may be attributed to inter-individual biological variability or the possibility that the administered dose was not sufficient to elicit a statistically significant effect. The 5000 mg/kgBW dose used in this study was based on the Indonesian Food and Drug Authority (BPOM) Regulation No. 10 of 2024 concerning acute toxicity testing standards in animals, which includes dosage levels of 0, 5, 50, 300, 2000, and 5000 mg/kgBW. The dose selection for the MDA analysis aimed to assess the effect of the highest recommended dose (5000 mg/kgBW) compared to the control (0 mg/kgBW).

CONCLUSION

Based on the findings of this study, it can be concluded that, in vitro, the F2 Galohgor Nutraceutical formula exhibits very weak antioxidant activity and α-glucosidase inhibitory capacity, as indicated by the high IC_{50} values observed in the DPPH and α-glucosidase assays. However, the in vivo test demonstrated a trend of decreased MDA levels, although not statistically significant, suggesting the potential biological effect of the F2 reformulation.

Therefore, further research is needed to explore the underlying mechanisms of action, expand the dose range, and integrate scientific approaches with traditional knowledge to comprehensively validate the therapeutic potential of the F2 Galohgor Nutraceutical formula as a health supplement and antidiabetic agent.

FUNDING

This study is part of a BIMA Grant-funded from the Ministry of Research, Technology, and Higher Education (Kemenristekdikti), Indonesia, in 2024, under the research project titled "Pengembangan nutrasetikal antidiabetes galohgor berbasis pendekatan nutrigenomik dan metabolomik".

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

There is no conflict of interest related to this research.

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