HYDROGEN
JURNAL KEPENDIDIKAN KIMIA
https://e-journal.undikma.ac.id/index.php/hydrogen/index

December 2025. 13(6)
e-ISSN: 2656-3061
p-ISSN: 2338-6487
pp. 1111-1116

Evaluating Alpha-Glucosidase Inhibitory Activity and Toxicity of Cacao Pod Husk (*Theobroma cacao* L.) Fractions

Iwan Sariyanto & Hartanti*
Department Medical Laboratory Technology, Poltekkes Kemenkes Tanjungkarang, Indonesia
* Corresponding Author e-mail: hartanti@poltekkes-tjk.ac.id

Article History
Received: 04-12-2025
Revised: 21-12-2025
Published: 31-12-2025

Keywords: CPH, cocoa, Alpha-Glucosidase, BSLT

Abstract
This study aimed to evaluate the anti-diabetic and cytotoxicity potential of ethyl acetate and acetone fractions from cocoa pod husk (*Theobroma cacao* L.). The cacao pod husks were extracted and fractionated using ethyl acetate and acetone solvent. The alpha-glucosidase inhibitory activity was determined using *Saccharomyces cerevisiae* alpha-glucosidase with p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate. Toxicity was evaluated against *Artemia salina* larvae to determine the Lethal Concentration (LC_{50}). The results showed that the ethyl acetate fraction and acetone fraction had LC_{50} values of 202.31 ppm and 360.07 ppm, respectively, indicating potential cytotoxic activity. Both fractions demonstrated weak alpha-glucosidase inhibitory activity, with inhibition percentages below 50% at the highest tested concentration (60 ppm), preventing the determination of IC_{50} values. These findings suggest that while the fractions possess bioactive potential indicated by toxicity, they may not be effective alpha-glucosidase inhibitors under the tested conditions.

How to Cite: Iwan Sariyanto, & Hartanti. (2025). Evaluating Alpha-Glucosidase Inhibitory Activity and Toxicity of Cacao Pod Husk (Theobroma cacao L.) Fractions. Hydrogen: Jurnal Kependidikan Kimia, 13(6), 1111–1116. https://doi.org/10.33394/hjkk.v13i6.18654

https://doi.org/10.33394/hjkk.v13i6.18654

INTRODUCTION

Diabetes Mellitus (DM) remains a significant global health challenge, with its prevalence increases rapidly worldwide. According to the International Diabetes Federation (IDF) reports, approximately 589 million adults were living with diabetes in 2024, and is projected to increase to 853 million by 2050 (Duncan et al., 2025). The burden of DM is higher in low and middle-income countries, including Indonesia, where it is often underrecognized, undiagnosed, and inadequately treated (Kengne & Ramachandran, 2024).

Diabetes is characterized by abnormally high plasma glucose levels resulting from insulin resistance and pancreatic beta cell dysfunction (Pham et al., 2014). Among the different types of DM, Type 2 diabetes mellitus (T2DM) represents the most common type in the adult population. One of the therapeutic strategies to control postprandial blood glucose levels in individuals with T2DM involves targeting carbohydrate-digesting enzymes, particularly alpha-glucosidase, in the small intestine. This key enzyme is responsible for the final step in the digestive process of carbohydrates, breaking down oligosaccharides into absorbable glucose (Lu et al., 2023). By inhibiting alpha-glucosidase, the rate of glucose absorption is retarded, blunting the post-meal spike in blood glucose levels and suppressing postprandial hyperglycemia. Synthetic inhibitors like voglitol, acarbose, and miglitol are commonly employed clinically for this purpose. However, the long-term use of these agents has some side effects, including diarrhea, abdominal pain, flatulence, and meteorism (Dirir et al., 2022). In recent decades, interest has increased in the use of natural products as therapeutic compounds, particularly for the prevention and treatment of T2DM. Historically, medicinal

plants and traditional treatments have been employed to address a wide range of medical conditions, including diabetes.

Cacao pod husk (CPH) is an agricultural waste product that can reach 70-75% of the whole cocoa fruit and cause an environmental problem (Indrianingsih et al., 2021). CPH is rich in bioactive compounds, like phenolic, flavonoids (especially catechins), alkaloids (theobromine), and dietary fibers. These secondary metabolites have the potential to be applied in the food industry and pharmaceuticals (as a candidate ingredient with therapeutic activity) (Vu et al., 2025). Water extract of CPH enriched with ethyl acetate fraction has antimicrobial properties, strong antioxidants, and low toxicity, so it has the potential to be used as a functional ingredient or natural additive in food products (Yahya et al., 2021). Although crude extracts of CPH have demonstrated potential in various bioassays, the antidiabetic properties of its semi-polar (ethyl acetate) and polar (acetone) fractions remain insufficiently investigated, especially in terms of their comparative inhibitory effects on alpha-glucosidase. The evaluation of pharmacological safety is a prerequisite for the development of any herbal medicinal product. The Brine Shrimp Lethality Test (BSLT) utilizing *Artemia salina* larvae serves as a rapid, reliable, and cost-effective preliminary screening method for toxicity. Significantly, research has established a positive correlation between toxicity in the BSLT assay and cytotoxicity against human solid tumor cell lines (e.g., lung, colon, and breast carcinomas). Thus, BSLT results not only provide an index of acute toxicity but can also indicate potential antitumor bioactivity (Hamidi et al., 2014).

This study was designed to fill the gap in knowledge by testing the alpha-glucosidase inhibitory activity of ethyl acetate and acetone fractions of cocoa pod husks, while assessing their toxicity profiles through the BSLT test. Through a systematic comparison between these fractions, this study sought to determine whether polarity-based fractionation could enrich bioactive compounds that play a role in antidiabetic and toxic effects, thereby confirming cocoa pod husk waste as a potential raw material for pharmaceutical product development.

METHOD

Materials

Fresh cacao pod husk was obtained from Lampung Province. Chemical reagents used methanol, acetone, ethyl acetate, aquades, dimethyl sulfoxide, acarbose, *Saccharomyces cerevisiae* alpha-glucosidase, p-nitrophenyl-α-D-glucopyranoside (pNPG), sodium carbonate (Na_2CO_3), phosphate buffer (pH 7), and brine shrimp eggs for BSLT.

Extraction and Fractination

Fresh cacao pod husk were washed, sliced, and dried for 3 days. The dried sample was ground and sieved to form a 100-mesh simplicia powder. The simplicia was extracted using methanol by maceration for 3 days. Methanol exctract was partioned by liquid-liquid extraction with acetone and ethyl acetate solvent. Its fractions were concentrated using roraty evaporator to yieldid acetone and ethyl acetate fractions for BSLT and alpha-glucosidase inhibitory activity test.

Alpha-Glucosidase Inhibitory Activity Assay

The alpha-glucosidase inhibitory activity was measured according to (Indrianingsih *et al,* 2021) with slight modifications. A sample fractions was dissolved using 10% DMSO at various concentrations (0-60 ppm). The samples were treated with *Saccharomyces cerevisiae* alpha-glucosidase and incubated at 37^{o}C for 5 min. To each reactions add p-nitrophenyl-α-D-glucopyranoside (pNPG) and the mixture ware incubated for 20 min. The reaction was stopped

by adding Na_2CO_3. The absorbance of solutions test was measured at 405 nm using microplate reader. The assay was performed in triplicate.

Brine Shrimp Lethality Assay

The assay was performed using brine shrimp (Artemia salina Leach) as previously described by (Mayer et al,1982). The eggs were hatched for 48 hours under constant aeration and illumination. Ten nauplii were transferred into a tube test containing the fractions at concentrations 0, 10, 100, 500, and 1000 ppm, with three replicates for each concentration. After 24 hours, the number of survivors at each concentration was counted and the LC_{50} was calculated using a computer programme.

RESULT AND DISCUSSION

Alpha-Glucosidase Inhibitory Activity

The alpha-glucosidase inhibitory activity of the fractions is presented in Table 1. The result showed that ethyl acetate and acetone fractions did not reach 50% inhibition at the highest tested concentration of 60 ppm. The maximum inhibition was approximately 27% at 60 ppm for the fractions. Consequently, the IC_{50} values for these fractions could not be determined within the tested range.

Table 1. Alpha-Glucosidase Inhibition of cacao pod husk fractions

Sample	Concentration Range (ppm)	IC_{50}	Note
Ethyl Acetate fraction	10 - 60	undetermined	Inhibition < 50%
Acetone fraction	10-60	undetermined	Inhibition < 50%

The present study found that both fractions of cacao pod husk demonstrated weak alpha-glucosidase inhibitory activity, with maximum inhibition not exceeding 27% at the highest tested concentration of 60 ppm. This finding is noteworthy because previous phytochemical screening of cacao pod husk has confirmed the presence of polyphenolic compounds, flavonoids, and tannins, which are typically known as potent alpha-glucosidase inhibitors in other plant species (Indrianingsih et al., 2021). Several factors may account for this result.

Polyphenolic composition and concentration in these specific fractions may differ substantially from those in the total extract. Although cacao pod husk is known to contain high levels of bioactive compounds, the ethyl acetate and acetone fractionation processes may have selectively isolated compounds with structural characteristics that result in weak alpha-glucosidase binding affinity. The three-dimensional structure and hydroxyl group positioning of phenolic compounds are critical determinants of enzyme inhibition efficacy (Montenegro et al., 2024). Xanthones and certain flavonoid subclasses have been identified as potent alpha-glucosidase inhibitors, whereas benzophenones and other polyphenolic variants exhibit weak or negligible effects. The obtained fractions may be enriched in the latter group of compound.

The concentration of the test fractions may have been insufficient to achieve clinically relevant enzyme inhibition. This pronounced difference in potency suggests that the active compounds in the cacao pod husk fractions are either present at very low concentrations or that the fractionation process may have excluded the most bioactive constituents. Additional concentration and purification steps, such as chromatographic separation, may be required to isolate individual bioactive compounds and achieve inhibition thresholds comparable to those of synthetic drugs.tic drugs. Extraction and fractionation method are another factor may affect the bioavailability and enzymatic interactions of the compounds. Ethyl acetate is semi-polar

solvents and polar solvents such as acetone may extract compounds that form complexes or exist in forms that diminish their capacity to interact with the alpha-glucosidase active site. Optimizing extraction conditions, including solvent composition, extraction temperature, and duration, has been shown to significantly enhance the alpha-glucosidase inhibitory activity of plant extracts.

These findings indicate that although cacao pod husk fractions exhibit notable bioactive properties, substantial refinement is necessary before they can be considered as antidiabetic agents based solely on alpha-glucosidase inhibition. The tested concentration range of 10-60 ppm for alpha-glucosidase inhibition may have been suboptimal. Although this range was selected based on standard protocols, testing at higher concentrations or with more finely spaced intervals could reveal IC_{50} values beyond the initially tested range. Additionally, incubation time and temperature parameters should be optimized to maximize enzyme-substrate interaction. The relatively weak alpha-glucosidase inhibition observed in the present study may be attributable to the specific solvent fractionation approach used. Standardizing extraction conditions across research institutions remains a major challenge in natural products research. The development of standardized quality control parameters for cacao pod husk fractions, including quantification of key polyphenolic markers and standardized bioassay protocols, would enable more meaningful comparisons between studies and help establish benchmarks for pharmaceutical development.

Toxicity Brine Shrimp Lethality Test

The ethyl acetate and acetone fractions of the cocoa pod husk were studied for their toxicity activity by means brine shrimp lethality assay. Both fractions exhibited notable brine shrimp toxicity, with lethality increasing proportionally to the administered concentration. The LC_{50} of these fractions are shown in Table 2. Based on these results, both fractions have LC_{50} values <500 ppm. According to (Mackeen *et al*, 2000), extracts with LC_{50} values <500 ppm are classified as toxic, indicating strong potential for bioactivity. This significant lethality to brine shrimp is indicative of the presence in this plant of a potent cytotoxic component which warrants further investigation.The Brine Shrimp Lethality Assay is a highly practical method for assessing the biological activity of various plant species. This technique is often used as a first step in evaluating the potential toxicity of a plant extract. Its speed, simple procedure, and minimal equipment requirements are some of the main advantages of this method (Hamidi et al., 2014).

The ethyl acetate fraction has greater toxicity (202,31 ppm) than the acetone fraction (360.07 ppm), suggesting differential bioactive sencondary metabolite content. Based on the results of GCMS analysis, 22 metabolites were identified from the ethyl acetate fraction and 12 compounds in the acetone fraction (Hartanti & Sariyanto, 2023). The difference in toxicity levels between the ethyl acetate and acetone fractions is likely related to variations in the types of compounds successfully extracted by each solvent. The ethyl acetate fraction, which has medium polarity, tends to concentrate flavonoids and a number of alkaloids, a group of compounds that often exhibit cytotoxic activity. Conversely, the more polar acetone fraction can attract compounds with high polarity that generally have lower toxicity. This pattern is in line with the phytochemical profile of cocoa pod husk fractions reported in previous studies.

Table 2. LC_{50} values of cocoa pod husk fractions

Sample	LC_{50} (ppm)
Ethyl Acetate fraction	202.31
Acetone fraction	360.07

Based on these findings, the ethyl acetate and acetone fractions of CPH have strong toxicity that can be further investigated for their pharmacological effects, such as anticancer. In the context of anticancer drug development, initial toxicity tests such as the Brine Shrimp Lethality Test (BSLT) are widely used as preliminary screening methods to identify compounds or fractions that have potential cytotoxic activity against cancer cells. Meyer et al. (1982) reported a good correlation between toxicity against Artemia salina and cytotoxic activity in various cancer cell lines, so that fractions with moderate LC_{50} values can be considered as initial candidates for anticancer agents. This cytotoxic activity is potentially related to the mechanisms of apoptosis induction, inhibition of cell proliferation, and cell cycle disruption, which are the main targets of modern cancer therapy (Fulda & Debatin, 2006).

CONCLUSION

The ethyl acetate and acetone fractions of *Theobroma cacao* L. pod husk demonstrated moderate toxicity in the Brine Shrimp Lethality Test, with LC_{50} values of 202.31 ppm and 360.07 ppm, respectively. These findings suggest potential pharmacological bioactivity, possibly of a cytotoxic nature. Both fractions exhibited weak alpha-glucosidase inhibitory activity compared to Acarbose, with IC_{50} values that could not be determined (greater than 60 ppm). While the toxicity profile confirms the presence of bioactive compounds, these fractions do not appear suitable as direct alpha-glucosidase inhibitors under the tested conditions

RECOMMENDATIONS

Further research should investigate alternative mechanisms of antidiabetic action, optimize extraction procedures, and assess the cytotoxic potential of these fractions for broader pharmaceutical applications beyond glycemic control.

BIBLIOGRAPHY

Dirir, A. M., Daou, M., Yousef, A. F., & Yousef, L. F. (2022). A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. In *Phytochemistry Reviews* (Vol. 21, Issue 4, pp. 1049–1079). Springer Science and Business Media B.V. https://doi.org/10.1007/s11101-021-09773-1

Duncan, B. B., Magliano, D. J., & Boyko, E. J. (2025). IDF Diabetes Atlas 11th edition 2025: global prevalence and projections for 2050. *Nephrology Dialysis Transplantation*. https://doi.org/10.1093/ndt/gfaf177

Fulda, S., & Debatin, K. M. (2006). Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene*, 25(34), 47984811. https://doi.org/10.1038/sj.onc.120960.

Hamidi, M. R., Jovanova, B., & Panovska, K. (2014). Toxicological evaluation of the plant products using Brine Shrimp (Artemia salina L.) model. In *Macedonian pharmaceutical bulletin* (Vol. 60, Issue 1).

Hartanti, & Iwan Sariyanto. (2023). Comparative analysis of total flavonoid, total phenolic content, and antioxidant activity of Theobroma cacao Pod Husk extract and its derived fractions. *GSC Biological and Pharmaceutical Sciences*, *25*(3), 159–166. https://doi.org/10.30574/gscbps.2023.25.3.0525

Indrianingsih, A. W., Wulanjati, M. P., Windarsih, A., Bhattacharjya, D. K., Suzuki, T., & Katayama, T. (2021). In vitro studies of antioxidant, antidiabetic, and antibacterial

activities of Theobroma cacao, Anonna muricata and Clitoria ternatea. *Biocatalysis and Agricultural Biotechnology*, *33*. https://doi.org/10.1016/j.bcab.2021.101995

Kengne, A. P., & Ramachandran, A. (2024). Feasibility of prevention of type 2 diabetes in low- and middle-income countries. In *Diabetologia* (Vol. 67, Issue 5, pp. 763–772). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s00125-023-06085-1

Lu, H., Xie, T., Wu, Q., Hu, Z., Luo, Y., & Luo, F. (2023). Alpha-Glucosidase Inhibitory Peptides: Sources, Preparations, Identifications, and Action Mechanisms. In *Nutrients* (Vol. 15, Issue 19). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/nu15194267

Mackeen, M. M., Khan, M. N., Samadi, Z., & Lajis, N. H. (2000). Brine shrimp toxicity of fractionated extracts of Malaysian medicinal plants. Natural Product Sciences, 6(3), 131–134

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*, 45(05), 31-34.

Mihardja, L., Siswoyo, H., Ghani, L., & Soegondo, S. (2009). *Prevalence and Determinants of Diabetes Mellitus and Impaired Glucose Tolerance in Indonesia (A Part of Basic Health Research/Riskesdas)*.

Montenegro, M. F., Teruel, J. A., García-Molina, P., Tudela, J., Rodríguez-López, J. N., García-Cánovas, F., & García-Molina, F. (2024). *Molecular Docking Studies of Ortho-Substituted Phenols to Tyrosinase Helps Discern If a Molecule Can Be an Enzyme Substrate***.** International Journal of Molecular Sciences, 25, 6891. https://doi.org/10.3390/ijms25136891

Pham, A. T., Malterud, K. E., Paulsen, B. S., Diallo, D., & Wangensteen, H. (2014). α-Glucosidase inhibition, 15-lipoxygenase inhibition, and brine shrimp toxicity of extracts and isolated compounds from Terminalia macroptera leaves. *Pharmaceutical Biology*, *52*(9), 1166–1169. https://doi.org/10.3109/13880209.2014.880486

Vu, V. N. H., Cao, T. Q., Nguyen, T. T. H., Nguyen, L. T. N., Le, P. H., & Nguyen, V. (2025). Extraction of Bioactive Compounds from Cocoa Pod Husk (Theobroma cacao L.) Using Deep Eutectic Solvent Assisted with Ultrasound. *Natural Product Communications*, *20*(4). https://doi.org/10.1177/1934578X251333026

Yahya, M., Ginting, B., & Saidi, N. (2021). In-vitro screenings for biological and antioxidant activities of water extract from theobroma cacao l. Pod husk: Potential utilization in foods. *Molecules*, *26*(22). https://doi.org/10.3390/molecules26226915