INHIBITION OF A-AMYLASE ENZYME WITH EXTRACT AND FRACTION OF *ZINGIBER ZERUMBET* RHIZOME

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Abstract

Diabetes mellitus is a non-communicable disease that attacks many people today. This disease is characterized by blood glucose levels that are higher than normal. α-amylase inhibitors slow down the final stages of carbohydrate digestion and prevent glucose from entering the bloodstream. Natural herbal α-amylase inhibitors are becoming increasingly important in the treatment of diabetes due to their efficacy with fewer side effects. *Zingiber zerumbet* is one type of zingiber that is often used. The ethanol extract of *Zingiber zerumbet* rhizome was fractionated through liquid-liquid extraction using n-hexane, ethyl acetat, butanol, and water. Preliminary phytochemical screening of the extract and fractions identified the presence of alkaloids, flavonoids, saponins, tannins, phenolics and steroid/triterpenoid. Inhibition of α-amylase was carried out to determine the inhibitory activity of lempuyang rhizome extracts and fractions on the enzyme α-amylase. This test is carried out using the DNS method. Lempuyang rhizome extract contains alkaloids, flavonoids, phenolics, tannins and terpenoids. The hexane fraction only contains terpenoids. The ethyl acetate fraction contains flavonoid and triterpenoid compounds. The butanol fraction contains alkaloids, phenolics, tannins and flavonoids while the water fraction contains phenolics and alkaloids. The research results showed that the % inhibition in the extract, hexane fraction, ethyl acetate fraction, butanol fraction and water fraction was 72.15% each; 81.96%; 86.47%; 77.81%, and 80.52%.

Keywords: diabetes mellitus, α-amylase enzyme, *Zingiber zerumbet*, DNS method

1. INTRODUCTION

Diabetes mellitus (DM) is a non-communicable disease and is a common non-communicable disease that attacks society today. This disease is characterized by higher than normal blood glucose levels. Typical symptoms of diabetes sufferers include polyphagia, polyuria, and polydipsia. Treatment for diabetes includes lifestyle changes and medication prescribed by a doctor. However, traditional medicine is also believed to be able to lower blood sugar levels in diabetes patients (Sagita et al., 2020). The prevalence of diabetes in Indonesia ranks fifth in the world. According to the IDF study, the number of people with diabetes is estimated to reach 19.5 million in 2021 (Alberti, 1990). Diabetes is usually characterized by postprandial hyperglycemia. Alpha amylase inhibitors slow down the final stage of carbohydrate digestion, preventing glucose from entering the bloodstream, and are considered an effective method of preventing hyperglycemia. Natural herbal alpha amylase inhibitors are

becoming increasingly important in the treatment of diabetes due to their efficacy with fewer side effects (Gong et al., 2020). *Z. zerumbet* is one of the zingiber species that is often used for medicine. *Z. zerumbet* has three varieties. The first variety is americans (blume) which is often called bitter ginger or 'lempunyang emprit'. The second variety is the aromaticum variety or fragrant ginger or 'lempuyang wangi' and the third is the zerumbet variety known as 'lempuyang gajah'. Of the three varieties, the one that is often used for medicine is 'lempuyang gajah'. *Z. zerumbet* is often used as an anti-inflammatory, antipyretic, analgesic, antimicrobial (Silalahi, 2018) while one of the essential oils contained has activities such as anti-inflammatory, antioxidant, anti-diabetic, anti-cancer, analgesic and antiviral (Maddeppungeng et al., 2023). Based on research (Suhendi et al., 2023) extract *Zingiber* leaf has α-amylase enzyme activity. Ethanol extract of *Zingiber* rhizome has antidiabetic activity in alloxan-induced white mice (Sakika et al., 2014). The main components of *Z. zerumbet* are essential oils or volatile oils, namely cyclic sesquiterpene *zerumbone* or 2,6,9-humulatrien-8-one and humulene camphene. The rhizome contains α-pinene, camphene, 3-carene, β-cymene, limonene, eucalyptol, linalool, camphor, borneol, 4-cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propan-1-1-yl)-1-vinyl, 1-6-10-dodecatrien-3-ol, 3-7-11-trimethyl, caryophyllene oxide, 1-2-dihydropyridine, anisole, α-caryophyllene, etc (Silalahi, 2018). This study analyzed the active fraction for inhibition of α-amylase enzymes in vitro. The method used is the DNS (3,5-Dinitrosalicylic acid) method as the basis is the reduction of the amount of reducing sugar resulting from the hydrolysis reaction of dissolved starch by the α-amylase enzyme in the presence of inhibitor compounds

2. METHOD

Materials used in this study include: 96% ethanol, n-Hexane (Brataco), Ethyl acetate (Brataco), n-butanol (Merck), α-amylase enzyme (Sigma), corn starch, 3,5-dinitrosalicylic acid (Aldrich), Acarbose tablets 100mg, Buffer pH 7.4, NaOH (Supelco), KNa Tartrate (Supelco), amyl alcohol (Merck), HCl (MKR), $FeCl_3$ (Supelco), Glacial acetic acid (Smart Lab), Anhydrous acetic acid (Smart Lab), Mg powder, TLC plates, *Zingiber zerumbet*. The tools used in this study include: Multimode Reader (BioTek).

2.1 Remaceration Process

Z. zerumbet was weighed as much as 250 g then added 96% ethanol with a ratio of 1:10, then left for 3 x 24 hours. Next, the dregs were filtered, 96% ethanol was added again with the same ratio, left for 2 x 24 hours after which filtering was carried out. The filtrate from filtering

1 and 2 were combined then concentrated by evaporation followed by a water bath until a thick extract was obtained (Alqahtani dkk., 2020)

2.2 Fractination

The thick extract from the maceration was weighed as much as 20 g then added with distilled water until completely dissolved. Put it in a separating funnel, add n-hexane until the solution is colorless in the n-Hexane layer, so that the n-hexane fraction and water fraction are obtained. The water fraction is put back in the separating funnel, adding ethyl acetate until the ethyl acetate layer is colorless, so that the ethyl acetate fraction and water fraction are obtained. The water fraction is put back in the separating funnel, add n-Butanol until the n-butanol layer is colorless, so that the butanol fraction and water fraction are obtained. Each fraction is concentrated on a water bath until a thick fraction is formed (Sogandi et al., 2019).

2.3 Phytochemical Screening Test

Alkaloid Test

Extract and fraction samples were added with 1 mL of 2N HCl and distilled water, then heated and filtered. The filtrate formed was used for the experiment.

- The filtrate is added with Mayer's reagent to form a white or yellowish white precipitate.
- Dragendorff's reagent is added to the filtrate to form an orange-red precipitate.
- The filtrate was added with bouchardat reagent to form a blackish-brown precipitate (Ar. et al., 2019).

Flavonoid Test

The dissolved extract and fraction samples were added with Mg powder, then concentrated HCl and amyl alcohol were added. The sample was declared positive for flavonoids if the amyl alcohol layer was red or orange (Karlina and Nasution, 2022).

Saponin Test

Extract and fraction samples were added with 10 mL of hot distilled water and then shaken vertically for 15-20 min. Observe the foam formed. The sample was declared positive for saponin if stable foam was formed (Mulyani et al., 2024).

Steroid/terpenoid test

Extract and fraction samples were added with n-hexane and then filtered. The filtrate was evaporated and then the remaining evaporation was added with anhydrous acetic acid and concentrated $H^2SO^{4\cdot}$ The sample was declared to contain steroids if a blue or blue-green color was formed, while if the sample contained triterpenoids if a red, pink or purple color was formed (Karlina and Nasution, 2022).

Tannin Test

The dissolved extract and fraction samples were added with $FeCl^3$ and the color formed was observed. The sample was declared positive for tannin if blue-black, blue-green, and green colors were formed (Mulyani et al., 2024).

2.3.6 In vitro α-amylase enzyme inhibition test

96-well UV Transparent microplate was prepared. The amount of substrate solution is pipetted 20μL and 50 μL of extract/fraction/acarbose sample then incubated at 37^0C for 20 min. α-Amylase enzyme is pipetted 25 μL then incubated at 37^0C for 30 min then add 100 μL of DNS then incubated at 100^0C for 10 min. Absorbance was measured at a wavelength of 540 nm using a Multimode reader (Alqahtani et al., 2020).

3. RESULTS AND DISCUSSION

Extraction of *Zingiber zerumbet* used remaceration where repeated addition of solvents was carried out. The first soaking was carried out for 3 x 24 hours with stirring then filtered after that the dregs were added with the same solvent, soaked for 2 x 24 hours. The remaceration method was used because compounds that are not heat resistant will all be extracted. The solvent used was 96% ethanol. Because ethanol solvents are universal, they are able to attract all active substances contained in *Zingiber zerumbet* and are easy to obtain. The yield results obtained by remaceration were greater than maceration. After obtaining a thick extract, liquid-liquid fractionation was carried out using solvents that have different polarity values, namely water, butanol, ethyl acetate and n-hexane. The principle of fractionation is Like dissolve like where the solvent will dissolve compounds that have the same polarity as the solvent (Ramdhini, 2023). Based on the phytochemical results shown in **Table 1**, the extract of rhizome *Z. zerumbet* contains alkaloids, flavonoids and steroids/triterpenoids. The hexane fraction only contains steroids/triterpenoids. The ethyl acetate fraction contains flavonoids and steroids/ triterpenoids. The butanol fraction contains alkaloids and flavonoids. The water fraction contains alkaloids. In the preliminary flavonoid test, Mg powder, HCl and amyl alcohol were used. Magnesium and hydrochloric acid are used to reduce the benzopyrone core in flavonoids and produce salts to form a red or orange color (Karlina and Nasution, 2022).

Table 1. Phytochemical test results

No	Types of Phytochemical Tests	Test Result				
		Extract	**n-Hexane fraction**	**Ethyl acetate fraction**	**Butanol fraction**	**Water fraction**
1	Alkaloids	+	-	+	+	+
	a. Phosphomolybdic acid	+	-	-	-	-
	b. Bouchardat	+	-	-	+	+
	c. Mayer	+	-	+	+	+
	d. Hager	+	-	+	-	+
2	Flavonoids	+	–	+	+	-
3	Saponin	-	-	-	-	-
4	Steroid / terpenoid	+	+	+	+	-
5	Tannin	+	+	+	+	+
6	Polyphenols	+	+	+	+	+
7	Phenolic	+	+	+	+	+

In the steroid and triterpenoid test, the extract, n-hexane fraction, ethyl acetate fraction and butanol fraction were positive for containing steroids /terpenoids using the *Liebermann-burchard* method. Triterpenoids will produce a red-purple color while steroids are green-blue. The addition of a little acetic anhydride in the test will absorb water and help oxidize the acid by sulfuric acid, because the reaction will not take place if it still contains water in it. The heating process to accelerate the process of water absorption by acetic anhydride. Then the hydrogen group and its electrons are released, as a result the compound undergoes conjugation extension which shows the appearance of a red-purple color (Mulyani et al., 2024).

The α-amylase enzyme inhibition test was conducted to determine the inhibitory activity of extracts and fractions of rhizome *Z. zerumbet* against the α-amylase enzyme. This test was conducted using the DNS method. The DNS method was chosen because this method is most commonly used to measure the activity of the α-amylase enzyme by measuring the amount of reducing sugar formed. The principle of the DNS method is that 3,5 dinitrosalicylic acid is reduced to 3-amino-5-nitrosalicylic acid. The aldehyde group in the polysaccharide chain is oxidized to a carboxyl group, at the same time the aldehyde group of the sugar will reduce

dinitrosalicylic acid. The reaction will continue as long as there is reducing sugar in the test solution. The color change that occurs in the DNS reagent is from yellow to reddish orange. The α-amylase enzyme used comes from the porcine pancreas, sigma Aldrich. The α-amylase enzyme used comes from the porcine pancreas because it is almost similar to the human pancreas. The resulting absorbance is read using a Multiplate Reader. The results of the enzyme inhibition test are expressed in percent inhibition, which means that the greater the percent inhibition result, the greater the inhibitory effect of the α-amylase enzyme (High et al., 2022).

The α-amylase enzyme inhibition test was performed on extracts and fractions. The positive control used was acarbose 100 mg because acarbose is one of the drugs that functions as an α-amylase enzyme inhibitor. In addition, acarbose is also easier to obtain and is widely used as a comparison. The negative control used was the solvent used to dissolve the sample and the positive control was DMSO 1% (Gaspersz et al., 2022). From the results of the study in **Figure 1**, it was obtained that the % inhibition in the extract, n-hexane fraction, ethyl acetate fraction, butanol fraction and water fraction were sequentially 72.15 ± 1.23 %; 81.96 ± 1.34%; 86.47 ± 0,51%; 77.81 ± 0.68% and 80.52 ± %.

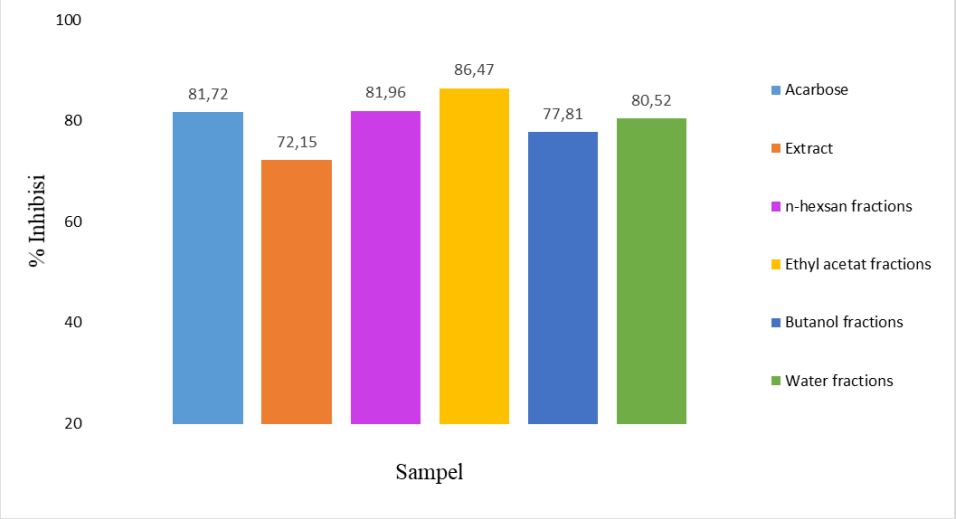

Figure 1. % Inhibition of α-amylase enzyme acarbose, extracts and fractions of *Zingiber zerumbet* rhizomes.

The greatest inhibition was the ethyl acetate fraction. where the fraction contains terpenoids and flavonoids. Terpenoids are known to have antidiabetic activity with a mechanism that stimulates and stabilizes the release of insulin from β cells of the islets of Langerhans of the pancreas. Terpenoid compounds are also known to have inhibitory activity against the enzyme α-glucosidase (Kusumawati et al., 2021).

4. CONCLUSION

Based on the research that has been done, it can be concluded that the greatest inhibition activity of the α-amylase enzyme from the ethyl acetate fraction is 86.47% which has terpenoid and flavonoid content. For further research, it is expected to be able to determine the content of compounds in the ethyl acetate fraction.

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6. REFERENCES

Alberti, KGMM 1990. Diabetes around the world. Current status of prevention and treatment of diabetic complications: proceedings of the Third International Symposium on treatment of Diabetes Mellitus. ICS821, 116–122.

Alqahtani, AS, Hidayathulla, S., Rehman, MT, Elgamal, AA, Al-Massarani, S., Razmovski-Naumovski, V., et al. 2020. Alpha-amylase and alpha-glucosidase enzyme inhibition and antioxidant potential of 3-oxolupenal and katononic acid isolated from Nuxia oppositiveolia. Biomolecules, 10: .

Ar., NI, Kadang, Y., and Permatasari, A. 2019. Identification Test of Alkaloid Compounds of Methanol Extract of Moringa Leaves (Moringa oleifera Lamk) from Ende Regency, East Nusa Tenggara by Thin Layer Chromatography. Sandi Karsa Pharmaceutical Journal, 5: 52–56.

Gaspersz, N., Fransina, EG, and Ngarbingan, AR 2022. Inhibitory Activity Test of α-Amylase and Glucoamylase Enzymes from Ethanol Extract of Kirinyuh Leaves (Chromolaena odorata L.). Mulawarman Chemical Journal, 19: 51.

Gong, L., Feng, D., Wang, T., Ren, Y., Liu, Y., and Wang, J. 2020. Inhibitors of α-amylase and α-glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia. Food Science and Nutrition, 8: 6320–6337.

Karlina, VR and Nasution, HM 2022. Phytochemical Screening and Antibacterial Activity Test of Ethanol Extract of Kaffir Lime Leaves (Citrus hystrix DC) Against Staphylococcus Aureus and Escherichia Coli Bacteria. Journal of Health and Medical Science, 1: 132–139.

Kusumawati, N., Haryoto, H., and Indrayudha, P. 2021. Inhibition of Alpha-Glucosidase /

Maddeppungeng, NM, Tahir, KA, Nurdin, NC, and Wahyuni, S. 2023. Formulation and Evaluation of Dermal Patch of Methanol Extract of Galangal Rhizome (Zingibe zerumbet L.) as Antibacterial Against Staphylococcus aureus Bacteria In Vitro and In Vivo. Jurnal Mandala Pharmacon Indonesia, 9: 621–631.

Mulyani, E., Noviyanti, Y., Fauziah, DW, and Haque, AF 2024. Identification of secondary metabolites compounds of ceremai landa (Eugenia uniflora L) leaves growing in bengkulu city. JFM (Jurnal Farmasi Malahayati), 7: 258–265.

Ramdhini, Rizki Nisfi. 2023. Standardization of the quality of simplisia and ethanol extract oF Clitoria ternatea L. FLOWERS. Health Journal: Multi Sciences Scientific Journal, 13: 32–38.

Sagita, P., Apriliana, E., Mussabiq, S., and Soleha, T. 2020. The Effect of Giving Soursop Leaves on Diabetes. Jurnal Medika Hutama, 3: 1266–1272.

Sakika, KA, Hanwar, D., Suhendi, A., Trisharyanti, I., and Santoso, B. 2014. antidiabetes activity of ethanol extract of lempuyang emprit rhizome (Zingiber amaricans BL) IN white rats induced by alloxan. Journal of Pharmaceutical Sciences and Clinical Pharmacy, 0: 10–16.

Silalahi, M. 2018. Botany and Bioactivity of Galangal (*Zingiber zerumbet* (L.) Smith.). EduMatSains Journal, 2: 147–160.

Sogandi, G., Darma, WST, and Jannah, R. 2019. Potential of Antibacterial Compounds from Sweet Root Extract (*Glycyrrhiza glabra* L) on Bacillus cereus. Journal of Chemical Science and Applications, 22: 105–111.

Suhendi, A., Hanwar, D., Santoso, B., and Abidi1, SR 2023. Inhibition Activity of Indonesian *Zingiber zerumbet* Leaves Extract on α-Glucosidase Enzyme. Proceeding Univerity Research Collaquim, 59–65.