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Alpha-Glucosidase Inhibitory Activity of Tiger Milk Mushroom (*Lignosus rhinocerus*) Ethanolic Extract

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Abstract

Tiger's milk mushroom (TMM) is a plant that can only grow in certain regions in Asia. Indonesia is the country with the most TMM plants in the world. This plant grows wild in the forests of Kalimantan and Papua. This plant is widely used by people to treat fever, breast cancer, and asthma. TMM is also known to have various pharmacological activities such as anticancer. The research about TMM originating from Indonesia is yet still rarely carried out. Hence, this research aims to carry out an alpha-glucosidase inhibition activity test of TMM ethanolic extract. The method employed for the extraction of the TMM was the maceration method using ethanol solvent. To identify the secondary metabolite compounds from TMM, phytochemical tests and thin layer chromatography (TLC) tests were carried out. An inhibition test of the alpha-glucosidase enzyme activity of TMM ethanolic extract was conducted using a p-nitrophenyl-alpha-D-glucopyranoside reagent. The results of this phytochemical test revealed that the ethanol extract of TMM contains flavonoid, alkaloid, terpenoid, and phenolic compounds. This is confirmed by the TLC test, indicating the presence of flavonoid and alkaloid compounds. The results of the alpha-glucosidase enzyme inhibition activity test also demonstrated that the ethanol extract of tiger milk mushrooms had an IC 50 value of 39.96 ppm, which is categorized as strong antidiabetic activity. From this research, it can be concluded that TMM ethanolic extract has very potential as an antidiabetic drug.

Keywords: Antidiabetic; Tiger milk mushroom; Alpha-glucosidase enzyme

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INTRODUCTION

Diabetes is one of the biggest causes of death in the world. In 2021, diabetes triggered the deaths of 6.7 million people worldwide.[1] Based on data from the International Diabetes Federation (IDF),[2] in 2030, diabetes sufferers will increase to 643 million people. Diabetes is a metabolic disease where there is an increase in glucose in the blood. Type II diabetes mellitus is a global crisis that threatens the world's health and economy.[3] As many as 90% of patients with diabetes are patients with type II diabetes.[3] Patients with type II DM must maintain postprandial glucose levels because this is the main factor in complications in patients with DM. Controlling these levels is done by delaying the body's glucose absorption by inhibiting the enzyme that hydrolyzes carbohydrates, namely the alpha-glucosidase enzyme in the digestive organs. This inhibition causes the enzyme to be unable to break down carbohydrates

to produce sugar for absorption in the body. This can reduce the increase in postprandial glucose levels in patients with DM.[4]

The drugs used to inhibit the activity of this enzyme are acarbose, mannitol, and voglibose.[5] Nevertheless, oral use of this drug can cause undesirable effects, which is why it is important to develop safer and easier treatment for the community with smaller effects, namely the use of traditional medicine.[7] Some of the side effects of this oral medication are impaired liver function, diarrhea, nausea, and vomiting.[6] An Indonesian plant that can be used for type II DM therapy is TMM (*Lignosus rhinocerus*). This plant has pharmacological effects, including anti-asthma,[8] antibacterial S, anti-breast cancer,[9] anticoagulant,[10] antioxidant,[8] anti-viral,[11] and immunomodulator.[12]

The compounds contained in tiger milk mushrooms (*L. rhinocerus*) are alkanes, acids, fats, carbohydrates, phenolic compounds and flavonoids[8]. Flavonoid compounds can inhibit the activity of the alpha-glucosidase enzyme so that glucose levels in type II diabetes sufferers can decrease.[13] Research[14] shows that tiger milk mushrooms (*L. rhinocerus*) contain total flavonoid levels of 33.041 mgEQ/g. This value is twice as large as the total flavonoid value from apple juice, which is 15.55 mg EQ/g,[15] where it is widely known that apples have high antioxidant activity due to the presence of flavonoid compounds. This indicates that TMM has a very big opportunity to be used as an antidiabetic drug.

Specifically, Indonesia is one of the largest producers of TMM (*L. rhinocerus*) in the world.[16] This plant grows abundantly in Indonesia's tropical forests, namely in Kalimantan and Papua.[14] Ethnobotanically, this plant is used by people to treat diarrhea, asthma, and cancer.[17] The many benefits of TMM (*L. rhinocerus*) make people cultivate mushrooms outside their habitat.[18]

There have not been many studies examining the pharmacological activity of tiger milk mushrooms originating from Indonesia, especially research examining the antidiabetic activity of TMM *(L. rhinocerus*). Therefore, this research was conducted to determine the activity of TMM ethanolic extract (*L. rhinocerus*) on the inhibition of alpha-glucosidase enzyme, which is an enzyme associated with diabetes. This research is based on research where it was found that the total flavonoid content of TMM (*L. rhinocerus*) is twice as high as apple juice, which is known to have high levels of flavonoids. Flavonoids play a role in inhibiting the glucosidase enzyme so that it can reduce blood sugar levels in patients with DM.

The sample of TMM in this study was a tiger's milk mushroom (*L. rhinocerus*) from the forests of Kalimantan. This is intended to examine the natural resources of Indonesia's tropical forests which have great potential for society, particularly in the health sector. As far as the authors' literature review, this research is the first to focus on the antidiabetic activity of TMM originating from Indonesia, especially the activity of this plant in inhibiting the alpha-glucosidase enzyme. Consequently, this research can be used as the basis for developing natural antidiabetic drugs that have fewer side effects than synthetic antidiabetic drugs that are already on the market. Transformations also later become the basis for isolating pure compounds that have antidiabetic activity in tiger TMM, and functional group transformations of the isolated compounds can also be carried out to increase their antidiabetic activity.

METHOD

Tools and Materials

Tools

The tools used in this research were Glassware, a Rotary Evaporator, a microplate, an incubator, a microplate reader, and an oven.

Ingredients

Ethanol, water, chloroform, ammonia, sulfuric acid, Mayer's reagent, TLC plate, acetic acid, butanol, methanol, hexane, ethyl acetate, alpha-glucosidase enzyme, phosphate buffer, p-nitrophenyl-alpha-D-glucopyranoside, sodium carbonate, and acarbose were used.

Research Procedure

Plant samples were obtained from Kalimantan. The fungal sclerotium was washed with water and dried. Then, the sample was cut thinly, dried in the sun, covered with a black cloth, and grounded with a grinder to produce a fine powder (dry simplicia). Dried simplicia was macerated to produce the extract. The solvent used was 70% ethanol. The maceration process was carried out within 3 x 24 hours, where every 1 x 24 hours, the maceration results were filtered. The solvent was evaporated with the macerate using a rotary evaporator at a temperature of 40°C to obtain a concentrated extract of TMM.

Phytochemical Test

Phytochemical tests carried out included alkaloid, flavonoid, and terpenoid tests.

a. Alkaloid Test
   A total of 4 mL of ethanol extract was added to each of Mayer's and Wagner's reagents. The presence of alkaloids is indicated by the production of a white precipitate for the Mayer test and a brown precipitate for the Wagner test.[19]

b. Flavonoid Test
   1 mL of ethanol extract was taken, and 66% sulfuric acid was added. As many as 5 drops. The presence of flavonoids is indicated by the formation of a yellow, reddish black, or orange.[19]

c. Terpenoid Test
   A total of 5 mL of ethanol extract was put into a test tube, and a mixture of concentrated sulfuric acid and acetic acid anhydride was added. The formation of a purplish-red solution indicates a positive result for this test.[20]

Thin Layer Chromatography Test

The TLC tests carried out are as follows:

a. Identification of flavonoid compounds
   The stationary phase in this TLC was silica gel GF254 with a length of 8 cm and a width of 2 cm. The mobile phase was glacial acetic acid: butanol: water (1: 4:5) with ammonia vapor as a staining agent. Positive results are indicated by the formation of a yellow-brown stain after the TLC plate is passed through ammonia vapor.[21]

b. Identification of alkaloid compounds
   The stationary phase in this TLC was silica gel GF254 with a length of 8 cm and a width of 2 cm. The mobile phase was chloroform: methanol (9.5: 0.5). Positive results are indicated by the formation of a yellow stain under 254 nm UV light.[22]

c. Identification of terpenoid compounds
   The stationary phase in this TLC was silica gel GF254 with a length of 8 cm

and a width of 2 cm. The mobile phase was n-hexane: ethyl acetate with a ratio of (6: 4). Color detection was carried out by spraying with Lieberman Burchard reagent, heated within 5 minutes at a temperature of 105°C. The presence of terpenoids is indicated by the formation of a red-violet color in visible light.[23]

Alpha Glucosidase Enzyme Inhibition Test

Enzyme solution containing 20 µL of alpha-glucosidase enzyme and 120 µL of 0.1 M phosphate buffer solution pH 6.8. p-nitrophenyl-alpha-D-glucopyranoside 10 mM in the same buffer was used as the substrate solution. A total of 10 µL of TMM ethanol extract with enzyme solution in a microplate was incubated for 15 minutes at a temperature of 37°C. 20 µL of the substrate was added, and incubation continued for 30 minutes. The reaction was ended by adding 80 µL of 0.1 M sodium carbonate. The absorbance was then detected using a *microplate reader* at a wavelength of 415 nm, where a solution without extract was used as a control, a solution without glucoside was used as a blank, and acarbose was used as a positive control. The experiment was carried out three times. Inhibitory activity against glycoside enzymes is expressed in % inhibition using the formula 1:[24]

$$\%inhibisi = \frac{c-s}{c}\ 100\% \qquad (1)$$

Where c = control absorbance
S = sample absorbance

The $IC_{50\ value}$ was determined using a plot of percent inhibition (y) versus inhibition concentration (x) and calculated using linear regression analysis. The equation (2) y = bx + c was used to determine IC_{50}:[24]

$$\%inhibition = \frac{50-a}{b}\ 100\% \qquad (2)$$

Data Analysis

Data analysis was used with One-way ANOVA. Significant differences were considered at a p-value <0.05.

RESULTS AND DISCUSSION

TMM extraction process

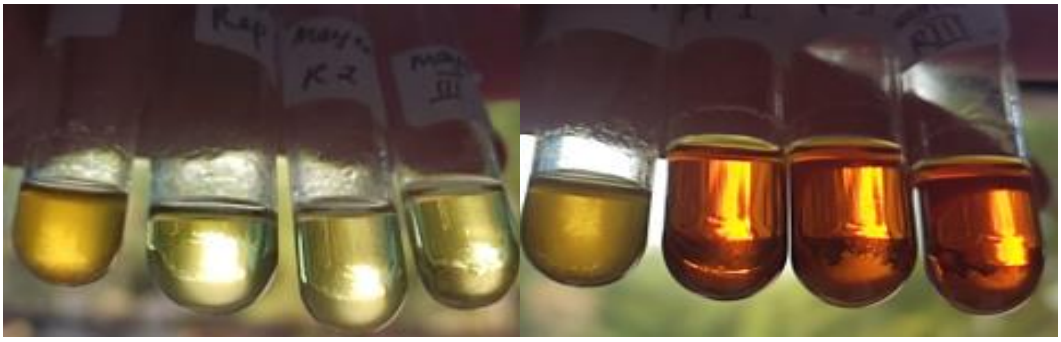
The stages of this research were making TMM ethanolic extract, phytochemical and TLC testing of TMM ethanolic extract and testing the inhibitory activity of tiger milk mushroom ethanol extract on the alpha-glucosidase enzyme. TMM sample were from Kalimantan (2.1 Kg). The sample was sorted to remove impurities. After that, it was washed to remove stuck dirt by water. Next, the TMM was chopped to make the sample size smaller. The smaller the sample, the larger the area of contact between the extracting solvent and the sample so that the compounds contained in the sample can come out maximally. Following that, the sample was dried in the sun and covered with a black cloth so that the sun did not damage the compounds in the sample. The sample was then powdered using a grinder, and 800 grams of powder was obtained. The TMM powder was then macerated using ethanol. Ethanol is a polar solvent that can extract compounds in plants optimally.

In this research, maceration was used for the extraction technique. The advantage of maceration is that it uses simple equipment, is easy to do, and is cheap.[25] A total of 500 grams of TMM powder was macerated with ethanol for 3 x 24 hours. This repeated maceration aimed to

maximize the compounds obtained because every 1 x 24 hours, the extracting solvent was replaced. The macerate was then filtered using a Buchner with the help of a vacuum. This vacuum would speed up the filtering process. The filtrate was then put in a rotary evaporator to evaporate the ethanol solvent. From this process, a concentrated extract of TMM weighing 3.1 grams was obtained. The yield of the ethanol extract obtained was 0.7%. This result was much smaller than the yield of tiger milk mushroom extract extracted with ether, chloroform, methanol, and water, namely 1.16%, 2.84%, 9.4% and 15.4%, respectively.[26] This small yield is possible because TMM contains many compounds with large molecular weights and are very polar, such as carbohydrates (8.84 grams/Kg), protein (0.38 grams/Kg), and others.[16]

Phytochemical Test

Phytochemical testing is a qualitative test to determine the profile of secondary metabolite compounds in samples.[27] Phytochemical tests in this research included tests for alkaloids, flavonoids, terpenoids, steroids, phenolics, and tannins. For the alkaloid test with Wagner and Mayer's reagent, positive results were obtained, namely the formation of this precipitate, indicating that tiger milk mushrooms contain alkaloids. The results of the alkaloid test can be seen in Figure 1.



(a) (b)

Figure 1. Alkaloid test results (a) alkaloid test results of ethanol extract of TMM with Mayer's reagent (b) alkaloid test results of ethanol extract of TMM with Wagner reagent

Mayer's reagent was formed from the reaction between mercury (II) chloride and potassium iodide to produce mercury (II) iodide, where this compound reacted further with excess potassium iodide to produce potassium tetraiodomercurate (II). Alkaloids have a nitrogen atom with a lone pair of electrons (PEB). This PEB binds with metal ions to produce complex compounds. In alkaloid identification, the nitrogen atom of the alkaloid compound is bound to potassium metal to produce a potassium tetraiodomercurate (II) complex, which is characterized by the formation of a precipitate.[28] A positive result for Wagner's reagent is the presence of a brown precipitate. This precipitate is thought to originate from an alkaloid-potassium complex.

In the Wagner reaction, iodine binds to I - ions to produce I 3- ions (brown solution). In this test, the K + ion will covalently bond with the nitrogen atom of the alkaloid to produce an alkaloid-potassium complex precipitate.[29] The flavonoid test in this study was carried out by adding 3 drops of

66% sulfuric acid to the extract solution. A positive result was obtained when a green solution was formed (Figure 2). The formation of this solution is due to the formation of chalcone compounds. Sulfuric acid protonates the oxygen atom in flavonoids so that the C ring in the flavonoid opens and chalcone is produced.[30]

Figure 2. Flavonoid test results of ethanol extract of TMM

Terpenoid and steroid tests were carried out on the ethanol extract solution of TMM. In this study, a red-brown solution was obtained (Figure 3). This indicates that the sample contains terpenoids. Based on research,[31] the formation of a blue to green solution indicates positive for the steroid test, and a brownish red to purple solution indicates positive for the terpenoid test.

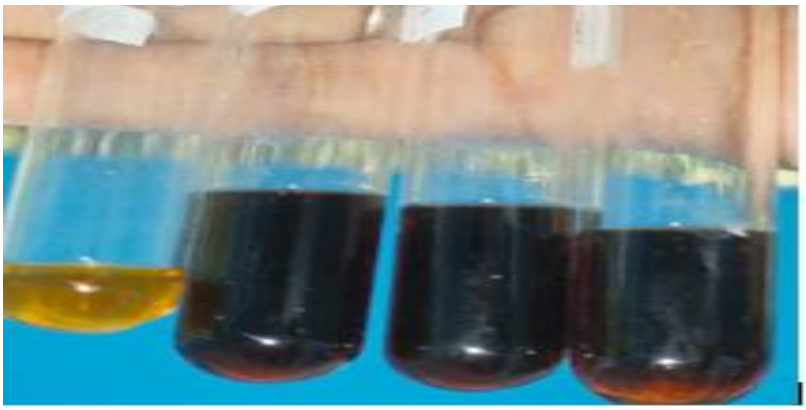


Figure 3. Terpenoid and steroid test results of TMM ethanol extract

The phenolic test was conducted by adding FeCl 3 solution to the extract solution. The result of this test is that a dark green solution was obtained (Figure 4). Phenolic compounds are aromatic compounds with hydroxy group (-OH). The delocalization of benzene electrons causes all electrons to be attracted to the O atom so that the compound will produce phenolic anions, which is followed by the release of hydrogen atoms from the hydroxy group in the form of protons (H +). The phenolate formed binds to the Fe 3+ cation, and then a coordinating covalent bond is formed to produce a Phenol-Fe complex. This compound causes the solution to change to dark green.[32]

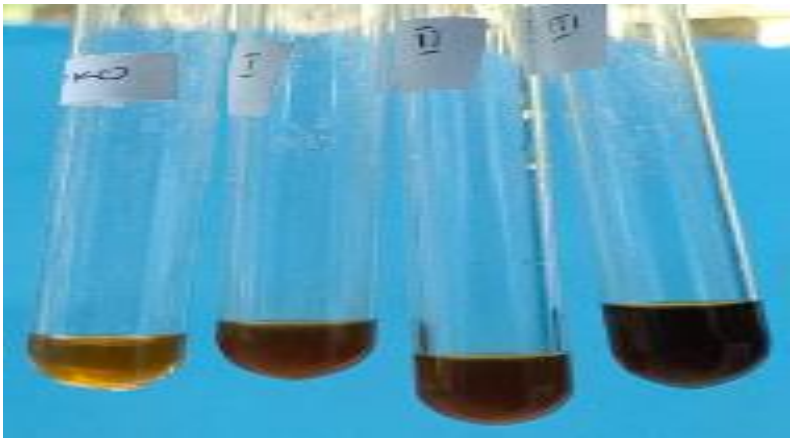


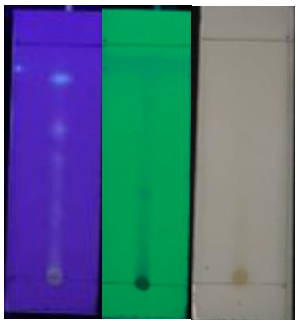
Figure 4. Phenolic test results of TMM ethanol extract

From the phytochemical tests carried out, the ethanol extract of TMM contains phenolic compounds, terpenoids, flavonoids, and alkaloids. The results of this research align with research[14] with the results that TMM ethanolic extract contains flavonoid compounds. A summary of the phytochemical tests in this research can be seen in Table 1.

Table 1. Phytochemical Test Results of Tiger Milk Mushroom Ethanolic Extract

Secondary metabolite compounds	Positive results	Conclusion
Alkaloids	Mayer: white precipitate	+
	Wagner: red precipitate	+
Flavonoids	Blue or green color solution	+
Phenolic	color change from bluish-black to dark black	+
Terpenoids/steroids	A blue to green solution indicates a positive steroid test result, and a brownish-red to purple color indicates a positive terpenoid test result.	A red solution is formed. (+ terpenoids)

The results of this phytochemical test were then confirmed by the TLC test. The extract was spotted onto a TLC plate and then eluted with the mobile phase ethyl acetate n-hexane (4: 6). The TLC plate was then sprayed with Lieberman Burchard for terpenoid testing. The results are seen in Figure 5. The results of this TLC test uncovered that the ethanol extract of TMM contains terpenoids, alkaloids, and flavonoids.



(a) (b) (c)

Figure 5. TLC terpenoid test results of TMM ethanol extract. Viewed in a 366 nm UV lamp (a) 254 nm UV lamp (b) after spraying with Lieberman Burchard reagent (c)

The ethanol extract of TMM was then tested for its inhibitory activity against the alpha-glucosidase enzyme. This enzyme has a role in breaking down carbohydrates, where the monosaccharide glucose is formed as a result of this breakdown in the digestive tract.[33] Thus, this enzyme helps absorb glucose in the body. In other words, this enzyme helps increase blood glucose levels. Inhibitors of this enzyme can inhibit the work of the enzyme where the inhibitor works competitively to compete for the active site of the enzyme and inhibits glucose hydrolysis. The mechanism of action of the alpha-glucosidase enzyme and its inhibitors is illustrated in Figure 6.[34]

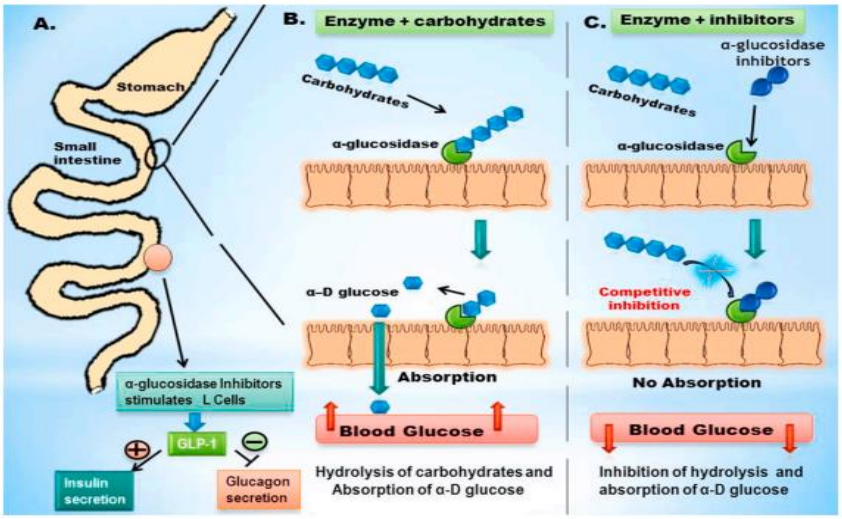


Figure 6. The role of alpha-glucosidase enzyme inhibitors in controlling hyperglycemia. (A) Schematic diagram of the small intestine: alpha-glucosidase enzyme inhibitor stimulates L cells and glucagon-like peptide (GLP-1) secretion from the lower small intestine. (B) Hydrolysis of carbohydrates by the alpha-glucosidase enzyme causes blood glucose levels to rise. (C) Competitive inhibition of the alpha-glucosidase enzyme by its inhibitor causes the enzyme's work to be disrupted, thereby limiting carbohydrate hydrolysis and blood glucose absorption.[34]

The method was used to test the activity of ethanol extract of TMM on glucopyranosides. The working principle of this test is that the alpha-glucosidase enzyme hydrolyzes p-nitrophenyl-α-D-glucopyranoside so that p-nitrophenol

(yellow solution) and glucose are produced (Figure 7). Enzyme activity was obtained based on the absorption of the yellow p-nitrophenol produced. The fainter the yellow color produced, the better the inhibitory activity against the alpha glucoside enzyme.[35]

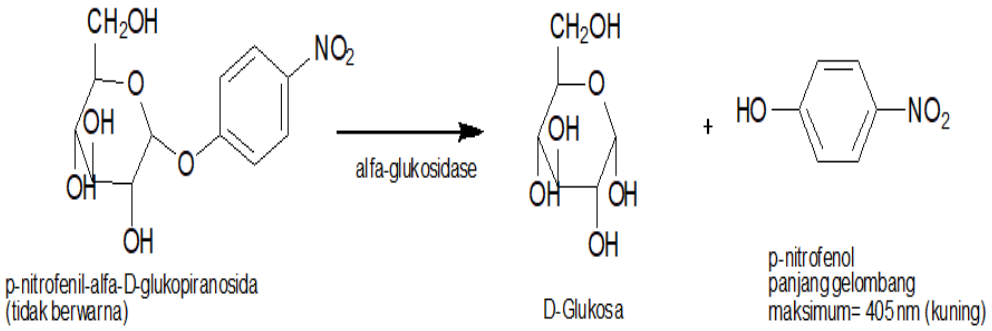


Figure 7. The principle of testing the inhibitory activity of p-nitrophenyl-α- D-glucopyranoside[35]

The results of the inhibition test can be seen in Table 2, Table 3, and Figure 8. The ethanol extract of TMM has an inhibitory activity against the alpha-glucosidase enzyme. The statistical test indicated a significant increase of inhibition value from lower to higher concentration of the sample. Analysis of IC50 value of sample and acarbose each measured in 39.94 ppm and 0.19 ppm. While the sample has lower inhibitory activity than acarbose, the IC50 of the sample is still in the range of strong inhibition groups.

Table 2. Inhibition value of TMM ethanolic extract against the alpha-glucosidase enzyme. The IC50 value of TMM was measured at 39.94 ppm.

Extract Concentration (ppm)	Inhibition (%)			Mean (%)	S.E
	R1	R2	R3		
10	17.040	16.704	17.152	16.96	**0.23**
5	8.857	8.520	8.296	8.558	**0.28**
2	7.960	7.063	7.511	7.511	**0.45**
1	6.614	6.839	6.614	6.689	**0.13**

Table 3. Inhibition value of acarbose (positive control) against the alpha-glucosidase enzyme. The IC50 value of acarbose was measured at 0.19 ppm.

Extract Concentration (ppm)	Inhibition (%)			Mean (%)	S.E
	R1	R2	R3		
10	98.676	98.529	99.265	98.824	**0.27**
5	93.235	94.853	93.529	93.873	**0.61**
1	72.353	72.206	72.059	72.206	**0.10**
0.5	59.559	60.882	61.029	61.209	**0.65**

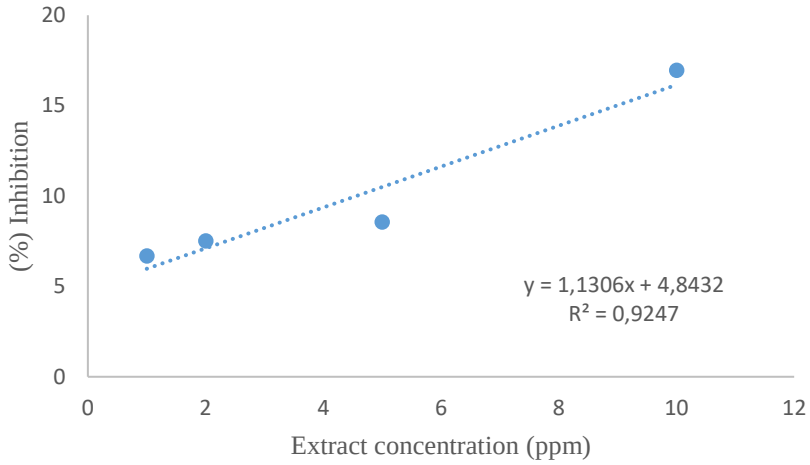


Figure 8. Inhibition of TMM ethanolic extract against the alpha-glucosidase enzyme

The IC 50 value of the ethanol extract of TMM was 39.94 ppm in the strong category. This strong inhibitory activity of the alpha-glucosidase enzyme is thought to be due to the content of flavonoid compounds and phenolic compounds in the ethanol extract of TMM, whose existence has been proven through phytochemical tests in this study and based on research from Sari et al. that the content of compounds quercetin from the ethanol extract of tiger milk mushrooms is twice as large (33.041 mgEQ/g) as from apples (15.55 mg EQ/g).[14] It uses quercetin as a standard, and the results of flavonoid levels are expressed in quercetin equivalents (mg EQ/g). This denotes that the quercetin content is high in the ethanol extract of TMM.[14]

The mechanism of action of the α-glucosidase enzyme inhibitor in the ethanol extract of TMM is thought to be the same as the mechanism of acarbose inhibition, namely competitive inhibition of the α-glucosidase enzyme in the lumen of the small intestine. This inhibition occurs because the inhibitor can bind to the.[36] Flavonoid compounds can inhibit the activity of the alpha-glucosidase enzyme by competing for the active site of the enzyme. Molecular docking studies have been carried out, and it was found that the amount and position of -OH contained in flavonoids greatly determines this inhibitory activity. The flavonoid compound with the most potential for this inhibitory activity is quercetin. -OH group at positions C-5, C-7 and/or C-8 on ring A and hydroxyl at positions 3' and 4' on ring B and hydroxyl on ring C at position 3 and alkene at positions C-2 and C- 3 in ring C is the most important functional group for flavonoid activity in inhibiting the alpha-glucosidase enzyme.[36] The results of this research suggest that TMM has a great opportunity to be developed as an antidiabetic drug.

CONCLUSION

Based on the phytochemical tests carried out, the ethanol extract of TMM contains alkaloids, terpenoids, phenolics, and flavonoids, and the ethanol extract of TMM has an IC 50 value of 39.94 ppm in the strong category. The results of this research imply that TMM has a great

opportunity to be developed as an antidiabetic.

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CONFLICT OF INTEREST

Authors declare that there is no potential conflict of interest with the research, authorship, and/or article publication.

REFERENCES

1. World Health Organization. Global Report on Diabetes. Isbn. 2016;978:88.
2. Webber S. International Diabetes Federation. Vol. 102, Diabetes Research and Clinical Practice. 2013. 147–148 p.
3. Widiasari KR, Wijaya IMK, Suputra PA. Diabetes Melitus Tipe 2: Faktor Risiko, Diagnosis, Dan Tatalaksana. Ganesha Medicine. 2021;1(2):114.
4. Arsiningtyas IS, Gunawan-Puteri MDPT, Kato E, Kawabata J. Identification of α-glucosidase inhibitors from the leaves of Pluchea indica (L.) Less., a traditional Indonesian herb: Promotion of natural product use. Vol. 28, Natural Product Research. Taylor & Francis; 2014. p. 1350–3. 10.1080/14786419.2014.904306
5. Dinicolantonio JJ, Bhutani J, O'Keefe JH. Acarbose: safe and effective for lowering postprandial hyperglycaemia and improving cardiovascular outcomes. Open Heart. 2015;2(1):e000327. 10.1136/openhrt-2015-000327
6. Isfa M, Walid M. Uji Kombinasi Antidiabetik antara Ekstrak Kulit Durian dan Acarbose dengan Perhitungan Combination Index dalam Penghambatan Kerja Enzim α-Amilase. PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia). 2019;16(1):85. 10.30595/pharmacy.v16i1.4482
7. Hati K, Setiawan M, Yuliarta D. The Effect of Red Betel Leaf (Piper Crocatum) Decoction On Reducing Blood Sugar Level in White Rats (Alloxan-induced Rattus norvegicus). Saintika Medika. 2017;9(1):59.
8. Johnathan M, Gan SH, Ezumi MFW, Faezahtul AH, Nurul AA. Phytochemical profiles and inhibitory effects of Tiger Milk mushroom (Lignosus rhinocerus) extract on ovalbumin-induced airway inflammation in a rodent model of asthma. BMC Complementary and Alternative Medicine. 2016;16(1):1–13. 10.1186/s12906-016-1141-x
9. Nesi N, Delourme R, Brégeon M, Falentin C, Renard M. Genetic and molecular approaches to improve nutritional value of Brassica napus L. seed. Comptes Rendus Biologies. 2008 Oct;331(10):763–71. 10.1016/j.crvi.2008.07.018
10. Nallathamby N, Serm LG, Raman J, Abd Malek SN, Vidyadaran S, Naidu M, et al. Identification and in vitro evaluation of lipids from sclerotia of lignosus rhinocerotis for antioxidant

and anti-neuroinflammatory activities. Natural Product Communications. 2016;11(10):1485–90. https://pubmed.ncbi.nlm.nih.gov/30549604/

11. Ellan K, Thayan R, Raman J, Hidari KIPJ, Ismail N, Sabaratnam V. Anti-viral activity of culinary and medicinal mushroom extracts against dengue virus serotype 2: An in-vitro study. BMC Complementary and Alternative Medicine. 2019;19(1):1–12. https://bmccomplementmedtherapies.biomedcentral.com/articles/10.1186/s12906-019-2629-y
12. Liu C, Chen J, Chen L, Huang X, Cheung PCK. Immunomodulatory Activity of Polysaccharide-Protein Complex from the Mushroom Sclerotia of Polyporus rhinocerus in Murine Macrophages. Journal of Agricultural and Food Chemistry. 2016;64(16):3206–14. 10.1021/acs.jafc.6b00932
13. Santa-Cecília F V, Vilela FC, da Rocha CQ, Dias DF, Cavalcante GP, Freitas LAS, et al. Anti-inflammatory and antinociceptive effects of Garcinia brasiliensis. Journal of ethnopharmacology. 2011 Jan 27;133(2):467–73. https://doi.org/10.1016/j.jep.2010.09.036
14. Sari DY, R W, AN T. Penentuan Kadar Flavonoid Total Ekstrak Etanol Jamur Susu Harimau (Lignosus rhinocerus). Jurnal Farmasi Udayana. 2021;10(1):23. 10.24843/JFU.2021.v10.i01.p03
15. Pavun L, Uskoković-Marković S, Jelikić-Stankov M, Dikanović D, Durdević P. Determination of flavonoids and total polyphenol contents in commercial apple juices. Czech Journal of Food Sciences. 2018;36(3):233–8. 10.17221/211/2017-CJFS
16. Nallathamby N, Phan CW, Seow SLS, Baskaran A, Lakshmanan H, Abd Malek SN, et al. A status review of the bioactive activities of tiger milk mushroom Lignosus rhinocerotis (Cooke) Ryvarden. Frontiers in Pharmacology. 2018;8(JAN). 10.3389/fphar.2017.00998
17. Lau BF, Abdullah N, Aminudin N, Lee HB, Yap KC, Sabaratnam V. The potential of mycelium and culture broth of lignosus rhinocerotis as substitutes for the naturally occurring sclerotium with regard to antioxidant capacity, cytotoxic effect, and low-molecular-weight chemical constituents. PLoS ONE. 2014;9(7). 10.1371/journal.pone.0102509
18. Mahyar UW, Burley JS, Gyllenhaal C, Soejarto DD. Medicinal plants of Seberida (Riau Province, Sumatra, Indonesia). Journal of Ethnopharmacology. 1991 Feb;31(2):217–37. 10.1016/0378-8741(91)90007-z
19. Rumagit HM, Runtuwene MR, Sudewi S. UJI FITOKIMIA DAN UJI AKTIVITAS ANTIOKSIDAN DARI EKSTRAK ETANOL SPONS Lamellodysidea herbacea. PHARMACONJurnal Ilmiah Farmasi-UNSRAT. 2015;4(3):2302–493.

https://doi.org/10.35799/pha.4.2015.8858

20. Dwisari F, Harlia, Andi Hairil Alimuddin. Isolasi dan Karakterisasi Senyawa Terpenoid Eekstrak Metanol Akar Pohon Kayu Buta-Buta (Excoecaria agallocha L.). Jurnal Kajian Komunikasi (JKK). 2016;5(3):25–30. https://jurnal.untan.ac.id/index.php/jkkmipa/article/view/14909
21. Yuda PESK, Cahyaningsih E, Winariyanthi NPY. SKRINING FITOKIMIA DAN ANALISIS KROMATOGRAFI LAPIS TIPIS EKSTRAK TANAMAN PATIKAN KEBO (Euphorbia hirta L.). Jurnal Ilmiah Medicamento. 2017;3(2):61–70.
22. Putu N, Ayuni S, Sukarta N. Isolasi dan Identifikasi Senyawa Alkaloid pada Biji Mahoni (Swietenia mahagoni Jacq). Seminar Nasional FMIPA UNDIKSHA III Tahun. 2013;1(1):387–95. https://ejournal.undiksha.ac.id/index.php/semnasmipa/article/view/2734
23. Fajriaty I, Ih H, Setyaningrum R. Skrining fitokimia dan analisis kromatografi lapis tipis dari ekstrak etanol daun bintangur (Calophyllum soulattri Burm. F.). Jurnal Pendidikan Informatika dan Sains. 2018;7(1):54–67. 10.31571/SAINTEK.V7I1.768
24. Christianty FM. In vitro α-glucosidase inhibitory activity of various tea (Camellia sinensis L.) extracts. Proceeding ICMHS. 2016;104–7. https://jurnal.unej.ac.id/index.php/prosiding/article/view/3905
25. Badaring DR, Sari SPM, Nurhabiba S, Wulan W, Lembang SAR. Uji Ekstrak Daun Maja (Aegle marmelos L.) terhadap Pertumbuhan Bakteri Escherichia coli dan Staphylococcus aureus. Indonesian Journal of Fundamental Sciences. 2020;6(1):16.
26. Mohanarji S, Dharmalingam S, Kalusalingam A, Science A. Screening of Lignosus rhinocerus Extracts as Antimicrobial Agents against Selected Human Pathogens. Journal of Pharmaceutical And Biomedical Sciences Screening. 2012;18(18):1–4.
27. Jafar W, Masriany, Sukmawaty E. Uji Fitokimia Ekstrak etanol Bunga Pohon Hujan (Spathodea campanulata) secara In Vitro. Prosiding Seminar Nasional Biotik. 2020;(2019):328–34. https://jurnal.ar-raniry.ac.id/index.php/PBiotik/article/view/9658
28. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Vol. 6, Plants. MDPI AG; 2017. 10.3390/plants6040042
29. Parbuntari H, Prestica Y, Gunawan R, Nurman MN, Adella F, Padang N. Preliminary Phytochemical Screening (Qualitative Analysis) of Cacao Leaves (Theobroma Cacao L.). 10.24036/eksakta/vol19-iss02/142
30. Fransina EG, Tanasale MFJDP, Latupeirissa J, Malle D, Tahapary R. Phytochemical screening of water extract of gayam (Inocarpus edulis) Bark and its amylase inhibitor activity assay. IOP Conference Series: Materials Science and Engineering.

2019;509(1):0–7. 10.1088/1757-899X/509/1/012074

31. Septia Ningsih D, Henri H, Roanisca O, Gus Mahardika R. Skrining Fitokimia dan Penetapan Kandungan Total Fenolik Ekstrak Daun Tumbuhan Sapu-Sapu (Baeckea frutescens L.). Biotropika: Journal of Tropical Biology. 2020 Dec 3;8(3):178–85. https://doi.org/10.21776/ub.biotropika.2020.008.03.06
32. Tjitda PJP, Nitbani FO. Skrining Fitokimia Ekstrak Metanol, Kloroform Dan N-Heksan Daun Flamboyan. Jurnal Sains dan Terapan Kimia. 2019;13(2):70. 10.20527/jstk.v13i2.5949
33. Farmasi M, Farmakologi D, Ayu I, Kusuma P, Stikes S, Waluya P, et al. PENGHAMBATAN AKTIVITAS α-GLUKOSIDASE OLEH EKSTRAK ETANOL, AIR, DAN SERBUK PARE. 2020; https://doi.org/10.20956/mff.v24i3.11547
34. Hossain U, Das AK, Ghosh S, Sil PC. An overview on the role of bioactive α-glucosidase inhibitors in ameliorating diabetic complications. Food and Chemical Toxicology. 2020;145(August):111738. 10.1016/j.fct.2020.111738
35. Lankatillake C, Luo S, Flavel M, Lenon GB, Gill H, Huynh T, et al. Screening natural product extracts for potential enzyme inhibitors: protocols, and the standardisation of the usage of blanks in α-amylase, α-glucosidase and lipase assays. Plant Methods. 2021;17(1):1–20. 10.1186/s13007-020-00702-5
36. Proença C, Freitas M, Ribeiro D, Oliveira EFT, Sousa JLC, Tomé SM, et al. α-Glucosidase inhibition by flavonoids: an in vitro and in silico structure–activity relationship study. Journal of Enzyme Inhibition and Medicinal Chemistry. 2017;32(1):1216–28. 10.1080/14756366.2017.1368503