ANTIHIPERGLICEMIC ACTIVITY TEST OF NANOCARRIER LIPID COMBINATION OF SWEET WOOD (Cinnamomum burmanii) AND PEGAGAN (Centella asiatica) ETHYL ASETATE FRACTION AGAINST ALFA GLUCOSIDASE ENZYMES

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ABSTRACT

Hyperglycemia is a major sign of a complex metabolic disease known as diabetes mellitus (DM). Cinnamon (Cinnamomum burmanii) and Centella asiatica (Centella asiatica) have antidiabetic activity, which means that they are able to lower blood sugar levels, and inhibit enzymes α-glucosidase. The purpose of this study was to obtain lipid nanocarrier combination of ethyl acetate fraction of cinnamon (Cinnamomum burmanii) and centella asiatica (Centella asiatica) which is expected to increase enzyme inhibition α-glucosidase. This antihyperglycemic research uses the mechanism of inhibition of the enzyme α-glucosidase, to determine the amount of inhibition that can be given by Lipid Nanocarrier combination of ethyl acetate fractions of cinnamon (Cinnamomum burmanii) and centella asiatica (Centella asiatica) against the enzyme α-glucosidase. Data analysis was performed by measuring the absorbance of the solution using a UV-Vis spectrophotometer at a wavelength of 405 nm. Then, the percentage of sample inhibition is calculated using the absorbance value from the analysis data. The results IC50 LNC cinnamon, gotu kola LNC and combination LNC were 4.54 ± 0.05 mg/mL, respectively; 3.13 ± 0.14 mg/mL; and 1.93 ± 0.02 mg/mL. While the value of IC50 acarbose as a standard is 1.80 ± 0.05 mg / mL. Based on statistical tests using one-way ANOVA and tukey test showed that between LNC combination with standard acarbose showed no significant difference (p = 0.663). So, it can be concluded that LNC combination between cinnamon and gotu kola has the inhibitory ability of the enzyme α-glucosidase which is equivalent to acarbose.

Keywords: *Diabetics, antidiabetics, cinnamon, gotu kola, nanocarrier*

INTRODUCTION

One of the characteristics of diabetes mellitus (DM) is hyperglycemia, a condition in which blood glucose levels increase continuously, leading to abnormal conditions (Banday, 2020). One of the most dangerous health problems in the world is diabetes, and its population continues to increase (Banday, 2020). Diabetics in Indonesia in 2021 amounted to 19.47 million people. This number has increased rapidly by 167% over the past 10 years. According to the International Diabetes Federation (IDF), by 2045, the number of people with diabetes in Indonesia will increase by 47% from 2021 (Anonymous, 2021). The Special Region of Yogyakarta will have 747,712 diabetic patients in 2020 (Yogyakarta Special Region Health Office, 2020).

Seeing the increasing prevalence of diabetes and WHO recommendations on the use of traditional medicine for the treatment of a disease and in several other studies it was also revealed that patients did not comply with taking antidiabetic drugs because they were afraid of the side effects of the chemical drugs given, the most side effects caused by diabetic drugs are gastrointestinal disorders and hypoglycemia (Hasfika et al, 2020), therefore there is a need for an intervention step to reduce hyperglycemia without causing side effects for long-term use.

One of the techniques used in diabetes intervention is the manufacture of antihyperglycemic lipid nanocarrier combinations. Lipid nanocarrier is one of the colloidal dispersion systems of nanoparticles measuring 40 to 1000 nm (Pamudji et al, 2016). Some plants that have the potential to reduce blood glucose levels are Cinnamon and Gotu Kola.

Research by Novendy et al (2020), Roswiem et al (2015), and Bernardo et al (2015) showed that ethanol extract of Cinnamon (Cinnamomum burmanii) has activity as an antidiabetic that can reduce blood glucose levels, inhibiting the αglucosidase enzyme, Arifin et al (2019) and Apriani (2012) also said that the ethyl acetate fraction of Cinnamon (Cinnamomum burmanii) has inhibitory activity against the α-glucosidase enzyme and Tulini et al (2016) also showed that the single LNC of Cinnamon (Cinnamomum burmanii) has good enough activity as an antidiabetic, which is able to reduce blood glucose levels, inhibit the αglucosidase enzyme, and control glucose metabolism. Sinamaldehyde, methyl hydroxy ketone polymers (MHCP), and type-A procyanidin or proanthocyanidin polymers are the main compounds considered as antidiabetic agents. Research by Tulung (2021), Khairurrizki et al (2022), Muhlishoh et al (2019) showed that ethanol extract of Gotu Kola (Centella asiatica) has antidiabetic properties, which can help reduce blood sugar and inhibit α-glucosidase enzyme, Widodo (2022) and Udhiyati (2017) also said that the ethyl acetate fraction of Pegagan (Centella asiatica) has inhibitory activity against the α-glucosidase enzyme and Minarno et al (2021) also showed that the single LNC of Pegagan (Centella asiatica) has activity as an antidiabetic that can reduce blood glucose levels, and inhibit the α-glucosidase enzyme quite well.

The step taken in the antihyperglycemic testing of lipid nanocarrier combination of ethyl acetate fraction of cinnamon and gotu kola is by inhibiting the enzyme α-glucosidase. This herbal medicine innovation is expected to increase the antihyperglycemic potential so that it can reduce the prevalence of diabetes in Indonesia.

MATERIALS AND METHODS

Lipid Nanocarrier

In this study Lipid Nanocarrier ethyl acetate fraction of cinnamon, Lipid Nanocarrier ethyl acetate fraction of gotu kola and Lipid Nanocarrier combination of ethyl acetate fraction of cinnamon and ethyl acetate fraction of gotu kola is a new preparation made at Ahmad Dahlan University which is expected to increase the inhibition of α-glucosidase enzyme. The concentration of Lipid Nanocarrier was made with a concentration of 1 mg/mL; 1.5 mg/mL; 2 mg/mL; 2.5 mg/mL; 3 mg/mL, 3.5 mg/mL.

Enzim α-glucosidase

In this study, the α-glucosidase enzyme was used as an inhibitor of Lipid Nanocarrier, before using the αglucosidase enzyme, it was first prepared in 50% DMSO as much as 1 U/mL (Koh, 2020).

PBS 0,1 M pH 6,8

A total of 1.4196 grams of Na2HPO4; 1.5601 grams of NaH2PO4 and 1.1688 grams of NaCl into 200 mL of CO2-free water. Then set the pH of the solution to 6.8.

50% DMSO

Made 50% DMSO stock by adding 20 mL of 100% DMSO to 20 mL of 0.1 M sodium phosphate buffer pH 6.8.

p-nitrophenyl α-glucopyranoside (pNPG) 5 mM

A total of 6.075 mg of p-nitrophenyl α-glucopyranoside (pNPG) was put into 6 mL of 0.1 M PBS pH 6.8 (Koh, 2020).

Na_2CO_3 10%

A total of 10 grams of Na2CO3 was added to 100 mL of 0.1 M PBS pH 6.8.

Positive Control Test Solution (Sample)

A total of 250 µL of each sample solution level series was put into a test tube, then 250 µL of α-glucosidase enzyme solution was added. After that, the solution was incubated for 15 minutes. Then 250 mL of 5 mM p-nitrophenyl αglucopyranoside (pNPG) solution was added, after which the solution was incubated for 20 minutes. Finally, to stop the reaction, 3 mL of 10% Na2CO3 solution was added (Ahda, 2023).

Blank/Correction Test Solution

A total of 250 µL of each sample solution level series was put into a test tube, then 250 µL of 50% DMSO solution was added. After that, the solution was incubated for 15 minutes. Then 250 mL of 5 mM p-nitrophenyl αglucopyranoside (pNPG) solution was added, after which the solution was incubated for 20 minutes. Finally, to stop the reaction, 3 mL of 10% Na2CO3 solution was added (Ahda, 2023).

Negative Control Test Solution (Without Sample)

A total of 250 mL of 50% DMSO solution was put into a test tube, then 250 mL of αglucosidase enzyme solution was added. After that, the solution was incubated for 15 minutes. Then 250 mL of 5 mM p-nitrophenyl αglucopyranoside (pNPG) solution was added, after which the solution was incubated for 20 minutes. Finally, to stop the reaction, 3 mL of 10 Na2CO3 solution was added (Ahda, 2023).

Each positive control and blank/correction test solution was made as much as 3 replicates. After that, all solutions were analyzed with a UV-Vis spectrophotometer at a wavelength of 405 nm. The absorbance value obtained from the analysis data was used to calculate the percent inhibition of the sample (Ahda, 2023).

Data Analysis

The absorbance data of the sample solution, positive control and blank obtained were calculated by calculating the percent inhibition with the formula:

$$\%\ Aktivitas\ Inhibisi = 100\ x\left(1 - \left(\frac{As - Ab}{Ac}\right)\right)$$

Description:
As = Absorbance of the sample
Ab = Absorbance of the blank
Ac = Control absorbance

IC50 was calculated using linear regression of sample concentration (x-axis) and % inhibition (y-axis). The regression equation y = bx + a obtained was then used to determine IC50 using the formula:

$$IC50 = \frac{50 - a}{b}$$

The data obtained was tested for normality. If the data is normally distributed, the analysis is continued with the one-way ANOVA test with a confidence level of up to 95%. Data results are mean ± SD (standard deviation) (Ahda, 2023).

RESULTS AND DISCUSSION

Antidiabetic activity test was conducted by αglucosidase enzyme inhibition method. The α-glucosidase enzyme inhibition activity test of LNC ethyl acetate fraction of cinnamon, gotu kola and the combination aims to determine the potential of the fraction used as antidiabates with the IC50 value parameter. α-Glucosidase inhibitors such as acarbose work by delaying glucose absorption in the intestine so as to prevent the increase in post-prandial blood sugar levels. Therefore, α-glucosidase enzyme is one of the target enzymes for the treatment of type II diabetes mellitus (Yuniarto and Selifiana, 2018).

Testing of cinnamon LNC, gotu kola LNC and combined LNC (cinnamon + gotu kola) was analyzed by inhibition of α-glucosidase enzyme using concentration variations of 1; 1.5; 2; 2.5; 3; 3.5 mg/mL which aims to determine the effect of concentration variations on α-glucosidase enzyme inhibition. The ability to inhibit the α-glucosidase enzyme can be seen by looking at the IC50 value obtained in each LNC sample, this value is the concentration value needed in the 50% inhibition process. The α-glucosidase inhibitory activity in vitro was determined from the calculation of the absorbance value of the sample with the absorbance of the control and blank obtained from the analysis using a UV-Vis spectrophotometric instrument at a wavelength of 405 nm. The IC50 value of the sample was then compared with acarbose standard solution. The lower the IC50 value, the higher the inhibitory activity of the sample against α-glucosidase enzyme.

Acarbose is used as a comparison or standard solution in this test, because according to McGown (2006) acarbose is one of the αglucosidase enzyme inhibitors produced by Actinoplanes sp. which is a microbe isolated from Kenya.

Absorbance testing of LNC sample solution (As) was carried out to determine the ability of LNC samples to inhibit the α-glucosidase enzyme. Testing the absorbance of the LNC (As) sample was carried out by adding the αglucosidase enzyme solution, while testing the absorbance of the blank/correction (Ab) was carried out as a correction of the LNC (As) sample solution without the addition of the α-glucosidase enzyme to the solution. Then each solution was added p-nitrophenyl α-glucopyranoside (pNPG).

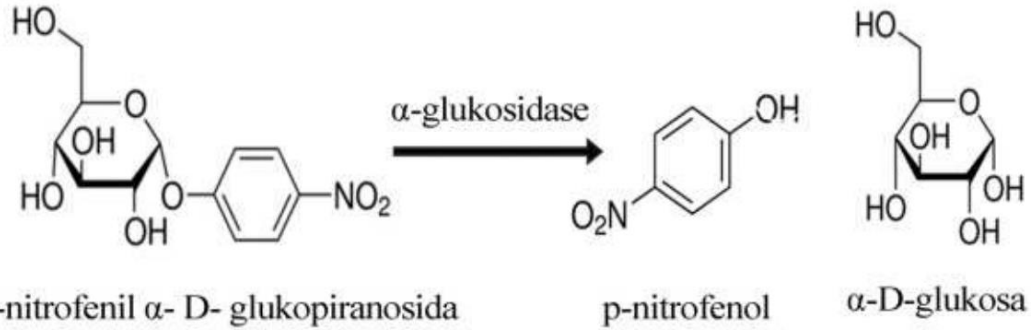

Figure 1. Reaction equation of α-glucosidase and p-nitrophenyl-α-D glucopyranoside (Pratama et al, 2015)

Figure 3 shows that p-nitrophenyl α-glucopyranoside (pNPG) will react with the α-glucosidase enzyme, then produce p-nitrophenol with a yellow color. The higher the ability of LNC to inhibit the α-glucosidase enzyme, the less p-nitrophenol compound produced (Puspitasari, 2024). The control solution (Ac) is an inhibitory test solution without using LNC samples, but has the same treatment as the sample test solution (As).

The results of testing the antidiabetic activity of LNC cinnamon, gotu kola and the combination obtained can be seen in Table 2.

Table 2. Results of LNC In Vitro Antidiabetic Activity Testing

Sampel	IC_{50} (mg/mL)	Aktivitas Penghambatan α-glukosidase
LNC Kayu Manis	$4{,}54 \pm 0{,}05^{b}$	Sangat kuat
LNC Pegagan	$3{,}13 \pm 0{,}14^{c}$	Sangat kuat
LNC Kombinasi	$1{,}93 \pm 0{,}02^{a}$	Sangat kuat
Acarbose	$1{,}80 \pm 0{,}05^{a}$	Sangat kuat

Notes: Small letters on different IC50 values indicate statistically significant differences ($p < 0.05$), while the same letters indicate no statistically significant differences ($p > 0.05$).

Table 2 shows that all LNC samples are categorized as LNCs that have very strong activity in inhibiting α-glucosidase enzyme activity. Samples that have antidiabetic activity are classified as very strong if the IC50 value is < 10 mg/mL, classified as strong if the IC50 value is 10 - 30 mg/mL and classified as not strong if the IC50 value is > 30 mg/mL (Qusti, 2010). According to Tulini et al (2016) and Minarno (2023), LNC derived from cinnamon and gotu kola extracts has the ability as an antidiabetic with inhibition of the α-glucosidase enzyme. This is in accordance with the test results obtained, namely the combined LNC sample (cinnamon + gotu kola) has the strongest antidiabetic activity value with an IC50 value of 1.93 ± 0.02 mg/mL compared to cinnamon LNC and gotu kola LNC. However, when compared to the standard acarbose which has an IC50 of 1.80 ± 0.05 mg/mL, the activity of acarbose is almost comparable to the antidiabetic activity of the combined LNC but, still better than the antidiabetic activity of acarbose.

The IC50 results obtained were also tested statistically using one-way ANOVA analysis and Tukey test. Data from statistical tests can be seen in appendix 3, LNC

cinnamon and gotu kola have a higher IC50 than LNC combination between cinnamon and gotu kola, this is stated to be significantly different with a value of $p < 0.05$. While the IC50 value of LNC combination is not much different from acarbose, it is stated that LNC combination with acarbose has inhibitory activity on α-glucosidase enzyme is not significantly different with p value > 0.05.

The IC50 value of antidiabetic activity of LNC combination of cinnamon and gotu kola is thought to be due to the content of active compounds such as flavonoids, tannins and quercetin contained in cinnamon and gotu kola. The high value is also caused by the use of ethanol solvent when making LNC, so that compounds that act as antidiabetics are more soluble than other solvents (Wulandari et al, 2020).

CONCLUSION

Based on the research that has been done, it can be concluded that the LNC sample of cinnamon and gotu kola combination has the best antidiabetic activity value with an IC50 value of 1.93 ± 0.02 mg/mL and is equivalent (not significantly different) to the positive control acarbose which has an IC50 value of 1.80 ± 0.05 mg/mL ($p > 0.05$) from in vitro testing.

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