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EVALUATION OF THE ANTIDIABETIC MECHANISM OF BLACK TURMERIC ETHANOL EXTRACT (CURCUMA CAESIA) THROUGH INHIBITION OF α-AMYLASE ENZYME AND α-GLUCOSIDASE ENZYME

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Abstract

Introduction: Indonesia is a tropical country that has biodiversity of natural materials. *Curcuma caesia* or black turmeric is a plant that has many properties and has many chemical compounds that are good for the body. The objective of this research is to investigate the inhibitory potential of the extract from black turmeric (Curcuma caesa) on α-amylase and α-glucosidase enzymes. **Method:** Preparation of black turmeric samples was carried out by maceration extraction technique with 96% ethanol solvent. Inhibition analysis on α-amylase enzyme was determined by UV-VIS spectrophotometer and inhibition on α-glucosidase enzyme was determined by *ELISA reader* using acarbose as comparison. **Results:** inhibition activity of black turmeric extract close to IC_{50} value at a concentration of 800 mg / L can inhibit α-amylase activity by 45.68% with IC_{50} value of 918.0108 mg / L; inhibitory activity of black turmeric extract close to IC_{50} value at a concentration of 250 mg / L can inhibit α-glucosidase activity by 51.967% with IC_{50} value of 224.292 mg / L. **Conclusion:** Black turmeric extract showed the ability to inhibit α-amylase (45.68% at 800 mg/L, IC_{50}: 918.0108 mg/L) and α-glucosidase (51.967% at 250 mg/L, IC_{50}: 224.292 mg/L). Its potential as an inhibitor of this enzyme signifies potential benefits in the control of blood sugar levels.

Keywords: Curcuma Caesia, α-amylase, α-glucosidase, Acarbose.

1. INTRODUCTION

Diabetes mellitus (DM) is a persistent metabolic condition characterized by elevated levels of blood glucose, leading to potential long-term complications affecting the heart, blood vessels, nerves, eyes, and kidneys [1]

Basic Health Research Data (Riskesda) states that the prevalence of DM in Indonesia is 80.5% or as many as 20.4 million Indonesians affected by DM. People with DM often also experience serious acute and chronic complications that can cause death. Other problems related to handling diabetes mellitus are geographical, cultural, and socially diverse [2]

One approach to diabetes therapy is to lower glucose levels in the blood, this can be achieved by inhibiting enzymes that hydrolyse carbohydrates in the digestive tract so that glucose absorption becomes slow. These enzymes include alpha glucosidase and alpha amylase which are enzymes that can break down polysaccharides, oligosaccharides, and disaccharides into monosaccharides. Therefore, the absorption

and digestion time of glucose becomes slower and lowers glucose levels in the blood [3]

Plants have been widely used as the main source of medicines, currently many drugs are available derived from plants both directly and indirectly. The black turmeric plant (*Curcuma caesia*) is a species of turmeric, family *Zingberaceae.* The bioactive components found in the Curcuma caesia plant exhibit various pharmacological effects, including anti-inflammatory, antioxidant, antimicrobial, anticoagulant, hypoglycemic, and smooth muscle relaxant properties[4], anti-diabetic activity [5], free radical clearance action against reactive oxygen, antitumor activity [6],[7], anti-mutagenic activity [8], antimicrobial activity [9] and antitoxicity against cyclophosphamide [10].

This study was conducted to see the antidiabetic mechanism of black turmeric ethanol extract *(Curcuma caesia*) in the inhibition of α-amylase and α-glucosidase enzymes.

2. MATERIALS AND METHODS

2.1 Location and design of the study

This research was conducted at the Biochemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, to be tested for inhibition of α-amylase enzyme using a Spectrophotometer, Research and Testing Laboratory of Pharmacy, Universitas Muslim Indonesia, to be tested for inhibition of α-glucosidase enzyme using *ELISA reader* (Biotech®), and for the collection and processing of black turmeric samples (*Curcuma caesia*) carried out in Tanarigella Village, Bua District, Luwu Regency, South Sulawesi. The design of this study is an experimental study by determining the IC_{50} value, the smaller, the stronger the inhibitory activity of the enzyme activity of α-amylase and α-glucosidase enzymes [11]

2.2 Tools and materials

This study were maceration vessels, porcelain cups, separator funnels, stirring rods, beaker glass, measuring cups, arlojim glasses, drip pipettes, measuring flasks, volume pipettes, knives, horn spoons, vial tubes, test tubes, tube racks, micropipettes, rotary evoporators, waterbaths, analytical balances, blenders, hotplates, UV-VIS spectrophotometers, ELISA readers, refrigerators, while the ingredients used in this study were samples of black turmeric extract (*Curcuma caesia),* ethanol 96%, N-Hexan fraction, water, dragendorf solution 0.1 mL, Pb acetate 0.1 mL, $FeCl_3$ 0.1 mL, NaOH 4 grams, amylum 0.5 grams, aquadest, K_2HPO_4 0.2 M 100 mL, α-amylase insert kit (Hunan Inen Biotech Co. Ltd), α-glucosidase from recombinant Saccharomyces cerevisiae (Sigma Aldrich, USA), dapar phosphate solution pH 7, Na_2CO_3 solution (Sigma Aldrich, USA), substrates p-nitrophenyl-α-D-glucopyranoside (PNPG) (Wako Pure Chemical Industries, Ltd, Japan), tip micropipettes and aluminum foil.

2.3 Work procedure

2.3.1 Black turmeric (Curcuma caesia) extraction procedure

Samples of black turmeric (Curcuma caesia) are cleaned from the ground and then washed under running water, then made small cuts after which they are dried. After drying, the sample was mashed using a blender, then sifted using a mesh 60 sieve,

after that the sample of black turmeric (Curcuma caesia) weighed 277 grams, then put into a maceration container. Subsequently, 3000 mL of 96% ethanol solvent was introduced to immerse the plant material, and the mixture was allowed to stand for three days in a sealed container shielded from direct sunlight. Periodic stirring occurred during this period. Following three cycles of 24 hours each, a screening process was implemented to obtain the liquid ethanol extract with a concentration of 96%.. After that, the filtering results obtained were then evaporated using a rotavapor with a temperature of 70 C, until a thick extract of °black turmeric *(Curcuma caesia)* was obtained, followed by the fractionation process using the liquid-liquid extract method.

2.3.2 Black turmeric (Curcuma caesia) partition procedure

Black turmeric thick extract *(Curcuma caesia)* weighed as much as 10 grams then prepared with aquadest as much as 50 mL and then put into the separator funnel and added N-Hexan as much as 50 mL and shaken periodically, then the separator funnel was allowed to stand until 2 layers were formed. The N-Hexan layer is accommodated into the container and the aquadest layer is put back into the separator funnel and the process is repeated, until the layer on the separator funnel is colorless. After that, it is evaporated again using a water bath to obtain a thick extract of N-Hexan fractionation of black turmeric *(Curcuma caesia),* then weighed and put into a container.

2.3.3 Phytochemical tests

Alkaloid

One milliliter of black turmeric (Curcuma caesia) sample was transferred into two separate tubes. In Tube 1, a few drops of Dragendorff reagent were added, with a positive test indicated by the initiation of an orange/red/yellow precipitate. In Tube 2, a few drops of Mayer reagent were added, and a positive test was indicated by the development of a yellowish cream precipitate.

For flavonoid analysis, a 1 mL sample of black turmeric was pipetted into two tubes. In Tube 1, some Pb reagent (CH3COO)2 was added, with a positive test identified by the emergence of a yellow precipitate. In Tube 2, a small amount of Mg powder was added, followed by shaking and the addition of a few drops of concentrated HCl. A positive test result was characterized by the formation of a red cream-colored precipitate.

Phenolic

A sample of black turmeric *(Curcuma caesia)* as much as 1 mL added 1 mL aquadest then added a few drops of $FeCl_3$ (positive test results indicated by the emergence of green/black deposits).

2.3.4 The procedure of action of inhibition of the enzyme α-amylase

Making a 1000 ppm black turmeric sample parent solution and a concentration series of black turmeric samples (Curcuma caesia)

Samples of black turmeric *(Curcuma caesia)* weighed 0.01 grams, then dissolved in 10 mL DMSO so that a sample of 1000 ppm was obtained. The parent solution of black turmeric *samples (Curcuma caesia)* 1000 ppm was pipette as much as 0.05; 0.1; 0.2; 0.4; and 0.8 mL into different test tubes, then sufficient volume with DMSO

up to 1 mL, so that a series of test sample concentrations of 50; 100; 200; 400; and 800 ppm.

Antidiabetic testing of black turmeric (Curcuma caesia) samples (IC_{50})

The concentration series of black turmeric samples (*Curcuma caesia*) 50; 100; 200; 400; and 800 ppm as much as 0.2 mL, reacted with the enzyme α-amylase 0.4 mL, then added 0.2 mL phosphate buffer pH 6.9 and incubated for 20 minutes then added 0.2 mL starch 1% and incubated for 5 minutes, then added 1 mL DNS, then heated for 10 minutes, then cooled. After that, the absorbance is measured at the maximum wavelength (λmax = 526 nm) λmax.

Antidiabetic activity formula:

$$\text{Percent Inhibition } (\%) = \frac{\text{A control - A sample}}{\text{A control}} \times 100$$

The IC_{50} value is calculated based on the linear equation obtained from the graph between concentration and percent inhibition, so the equation for calculating IC_{50} is as follows:

$$y = ax + b$$

Information:

y : percent inhibition (to calculate IC_{50}, y = 50)

x : Concentration (IC_{50})

then to calculate IC_{50}:

x = (y-b)/a

x = (50-b)/a.

Preparation of 1000 ppm acarbose master solution and acarbose concentration series

Acarbose was weighed as much as 100 mg, then dissolved in 100 mL DMSO so that a sample of 1000 ppm was obtained. Pipetted 0.5 mL from 1000 ppm acarbose solution and then added 9.5 ml DMSO to make 50 ppm Acarbose solution.

Acarbose solution 50 ppm pipette as much as 0.01; 0,02; 0,04; 0.08; and 0.16 mL into different test tubes, then sufficient volume with DMSO up to 1 mL, so that a test sample concentration series of 0.5 is obtained; 1; 2; 4; and 8 ppm.

Acarbos antidiabetic testinge (IC_{50})

Acarbose concentration series 0.5; 1; 2; 4; and 8 ppm as much as 0.2 mL, reacted with 0.4 mL α-amylase enzyme, then added 0.2 mL phosphate buffer pH 6.9 and incubated for 20 minutes then added 0.2 mL starch 1% and incubated for 5 minutes, then added 1 mL DNS, then heated for 10 minutes, then cooled. After that, the absorbance is measured at the maximum wavelength (λmax = 526 nm) λ_{max}.

Antidiabetic activity formula:

$$\text{Percent Inhibition } (\%) = \frac{\text{A control - A sample}}{\text{A control}} \times 100$$

The IC_{50} value is calculated based on the linear equation obtained from the graph between concentration and percent inhibition, so the equation for calculating IC_{50} is as follows:

$$y=ax + b$$

Information:

y : percent inhibition (to calculate IC_{50}, y = 50)

x : Concentration (IC_{50})

then to calculate IC_{50}:

x = (y-b)/a

x = (50-b)/a.

2.3.5 The procedure of action of inhibition of the enzyme α-glucosidase

Blank testing

Put into the well can phosphate pH 7 as much as 36 µl and substrate p-nitrogenyl-α-D-glucopyranoside (PNPG) 5 mM 17 µl, then incubated in a water bath for 5 minutes at a temperature of 37 C. After the incubation period is complete, then added to the °well enzyme α-glucosidase as much as 17 µl, then incubated again in a water bath for 15 minutes at a temperature of 37°C. After incubation is complete, 100 µl of 200 mM Na_2CO^3 solution is added to stop the reaction.

Subsequently, the measurement of absorbance was conducted using an ELISA reader at a 405 nm wavelength..

Blank control testing

Add 36 µl of phosphate buffer at pH 7 and 17 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) substrate into a well, followed by a 20-minute incubation in a water bath at 37°C. Subsequently, 100 µl of 200 mM Na_2CO_3 is introduced to halt the reaction. The absorbance is then assessed using an ELISA reader at a 405 nm wavelength.

Comparator testing (acarbose)

Place 36 µL of phosphate buffer at pH 7 into a well, followed by the addition of 30 µL of a 0.2 ppm concentration, as well as varying concentrations of 0.4 ppm, 0.6 ppm, 0.8 ppm, and 1 ppm for comparison. Add 5 mM of PNPG substrate, and incubate the mixture in a water bath for 5 minutes at 37°C. After the initial incubation, introduce 17 µL of α-glucosidase enzyme to each well and incubate again for 15 minutes at 37°C. Following this, add 100 µL of 200 mM Na2CO3 to terminate the reaction. Measure the absorbance of the samples using an ELISA reader at a wavelength of 405 nm.

Comparator control testing

Place 36 µL of phosphate buffer with a pH of 7 into a well, followed by the addition of 30 µL for a concentration of 0.2 ppm and varying concentrations of 0.4 ppm, 0.6 ppm, 0.8 ppm, and 1 ppm for comparison. Add 17 µL of 6 mM PNPG substrate, and incubate the mixture in a water bath for 20 minutes at a temperature of 37°C. After the incubation period, introduce 100 µL of 200 mM Na2CO3 to halt the reaction. Measure the sample using an ELISA reader at a wavelength of 405 nm.

Sample testing of black turmeric extract (Curcuma caesia)

Place 36 µL of phosphate buffer with a pH of 7 into a well, followed by the addition of 30 µL of black turmeric ethanol extract with a concentration of 100 ppm and varying concentrations of 125 ppm, 150 ppm, 175 ppm, and 200 ppm for comparison. Add 17 µL of 5 mM PNPG substrate, and incubate the mixture in a water bath for 5 minutes at a temperature of 37°C. After the initial incubation, introduce 17 µL of α-glucosidase enzyme into each well and incubate again for 15 minutes at 37°C. Following this, add 100 µL of Na2CO3 to terminate the reaction. Measure the sample's absorbance using an ELISA reader at a wavelength of 405 nm

Sample control testing of black turmeric extract (Curcuma caesia)

Place 36 µL of phosphate buffer with a pH of 7 into a well, followed by adding 30 µL of black turmeric ethanol extract with a concentration of 100 ppm, and varying concentrations of 125 ppm, 150 ppm, 175 ppm, and 200 ppm. Then, introduce 17 µL of 5 mM PNPG substrate and incubate the mixture in a water bath for 20 minutes at a temperature of 37°C. After the incubation period, add 100 µL of 200 mM Na2CO3 to halt the reaction. Measure the sample's absorbance using an ELISA reader at a wavelength of 405 nm.

3. RESULTS

Table 1: Percent Yield of Black Turmeric Extract Bath (*Curcuma caesia*)

Sample	Weights before extraction (g)	Amount of solvent (L)	Extract weights (g)	% rendamen
Extract black turmeric (*Curcuma caesia*)	277	3	8,9	8,55

Table 2: Phytochemical Test Results of Black Turmeric Extract (*Curcuma caesia*)

No	Senyawa	Result
1	Alkaloid	Positive
2	Flavonoid	Negative
3	Flavonoid	Negative

Table 3: The outcomes of inhibiting the activity of the α-amylase enzyme were compared between acarbose and black turmeric extract *(Curcuma caesia)*

Name	Concentration (mg/L)	Inhibitory Activity (%)	Value IC_{50} (mg/L)
Black turmeric extract (*Curcuma caesia*)	50	14,76	918,0108
	100	20,74	
	200	26,82	
	400	28,96	
	800	45,68	
Checklists (acarbose)	0,5	8,23	6,6306
	1,0	12,68	
	2,0	27,88	
	4,0	36,51	
	8,0	56,07	

Table 4: The findings regarding the inhibition of α-glucosidase enzyme activity were compared between acarbose and black turmeric extract *(Curcuma caesia)*

Name	Concentration (mg/L)	Inhibitory Activity (%)	Value IC_{50} (ppm)
Black turmeric extract (*Curcuma caesia*)	150	38,156	224.292
	200	45,892	
	250	51,967	
	300	58,479	
	350	71,722	
	400	83,741	
Checklists (acarbose)	0,2	31,144	0,745
	0,4	37,830	
	0,6	44,340	
	0,8	51,906	
	1,0	59,179	

4. DISCUSSION

The research aimed to assess the inhibitory effects of black turmeric extract (Curcuma caesia) on α-amylase and α-glucosidase enzymes, with a focus on determining its potential as an antidiabetic agent through IC_{50} values, using acarbose as a reference. Diabetes mellitus (DM), a prevalent chronic disease affecting approximately 8.3% of the global population, has prompted the exploration of active compounds like curcumin for potential DM treatment [12].

Curcuma caesia plant or known as black turmeric in Indonesia is a plant that has many benefits. Perennial plants from the Curcuma genus within the Zingiberaceae family are widely distributed across tropical and subtropical regions worldwide. [13]

Based on Table 2. Shows that phytochemical compounds contained in the black turmeric plant (*Curcuma caesia*) are alkaloids with positive results, while flavonoid and phenolic compounds show negative results. Research by Vasavda, et al (2013) suggests that turmeric hita methanol extract is known to contain chemical compounds such as alkaloids, flavonoids, penols, curcuminoids, terpenoids, tannins and proteins [14]. Based on the results of research by Wahyu Udayani, N, N., et al (2022) that the quantitative determination of levels for the total content of flavonoids, tannins, and alkaloids in black turmeric ethanol is 2775.65 mg / 100 grams; 2714.75 mg/100grams; and 1470.58 mg/100 grams [15].

Based on Table 3. The results of research on the inhibitory activity of black turmeric *extract (Curcuma caesia) and comparison (acarbose) in α-Amylase, showed that concentrations close to the* IC_{50} value of black turmeric extract (Curcuma caesia) namely 800 mg / L concentration can inhibit α-Amylase by 45.68% with an IC_{50}

Value of 918.0108 mg / L. While the comparison (acarbose) showed results at a concentration of 8.0 mg / L can inhibit α-Amylase by 56.07% with an IC_{50} value i.e. 6.6306 mg/L. Based on Table 4. The results of research on the inhibitory activity of black turmeric extract (Curcuma caesia) *in α-glucosidase, showed that concentrations close to* the IC_{50} value of black turmeric extract (Curcuma caesia) which is a concentration of 250 mg / L can inhibit α-glucosidase by 51.967% with an IC_{50} value of 224,292 mg / L. While the comparison (acarbose) shows results at a concentration of 1.0 ppm can inhibit α-glucosidase by 59.179% with an IC_{50} value of 0.745 mg/L.

Jain and Parihar (2018) explored the potential antidiabetic properties of black turmeric extract (Curcuma caesia). The ethyl acetate extract exhibited notably higher α-amylase inhibitory activity (97.72% ± 0.28, IC_{50} value: 63.96 µg/mL) examine the standards of curcumin and camphor.

In a study by Majumder et al. (2017), C. caesia rhizomes were identified as promising antidiabetic agents in STZ-induced diabetic rats. The rhizomes significantly reduced fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), and oral glucose tolerance tests (OGTT), while also promoting weight gain compared to the diabetes control group. Methanol from C. caesia demonstrated effective inhibition of α-amylase and α-glucosidase.

The methanol extract from C. caesia rhizomes exhibited antidiabetic effects by reducing blood glucose levels in in vivo studies and regulating intestinal monosaccharide absorption through the inhibition of alpha-amylase and alpha-glucosidase. Additionally, it enhanced glucose uptake in yeast cells, indicating improved glucose utilization. The extract displayed effective antioxidant activity, successfully scavenging free radicals like superoxide and hydroxyl ions. Pancreatic histopathological studies revealed gradual healing post-treatment. In conclusion, the methanol extract of Curcuma caesia rhizomes demonstrates antidiabetic activity and effective reduction of oxidative stress due to its antioxidant properties [16]

5. CONCLUSION

The inhibitory activity of black turmeric extract close to the IC_{50} value at a concentration of 800 mg / L can inhibit α-amylase activity by 45.68% with an IC_{50} value of 918.0108 mg / L. While the inhibitory activity of black turmeric extract which is close to the IC_{50} value at a concentration of 250 mg / L can inhibit α-glucosidase activity by 51.967% with an IC_{50} value of 224.292 mg / L.

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Conflict of Interest

There is no conflict interest

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