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Phytochemical Properties, Antioxidant Activity and α- Amilase Inhibitory of Curcuma Caesia

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia, which produces free radicals, particularly reactive oxygen species (ROS), through glucose auto-oxidation and glycosylation. Administration of antioxidants and polyphenolic compound components can capture free radicals and reduce oxidative stress. Polyphenols are also able to reduce blood glucose levels by inhibiting the action of amylase enzymes as carbohydrate catabolism so that glucose absorption does not occur. One of the plants that contain polyphenols is Black Turmeric (Curcuma caesia). Some studies on the rhizomes of this plant include methanol extracts containing phenolics, Flavonoids, alkaloids, triterpenoids, saponins, and tannins. Dry powders and wet rhizome powders were studied to have antioxidant activity. Research on the inhibition of the enzyme alfa amylase is still lacking. This study aims to determine the content of chemical compounds using 70% ethanol solvent. The total phenolic and flavonoid assay with spectrophotometry, determine antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) at 490 nm and inhibition of α-amylase enzyme using 3,5- dinitro salicylic acid (DNS) at 529 nm. The results showed that phenolic levels were 235 mgGAE/100gr, flavonoids were 61 mg QE/100gr, Alkaloids 30 mg Quinine /100gr and saponin 158 mg Quillaja bark/100gr, dan tannin 535mg tannic acid/100gr. DPPH antioxidant activity obtained IC50 value of 299,18 µg/mLµg/mL and α- amylase inhibitory 50,26 µg/mLµg/mL. The research showed that 70% ethanol extract Curcuma caesia roxb contains phenolic, flavonoids, alkaloids, quinine, and saponins constituents and have been antioxidant activity and high α-amylase inhibitory

1. Introduction

Spices are a natural source of antioxidants. Black turmeric (Curcuma caesia Roxb.) is a new variant that appears as a newcomer in herbal medicine, especially in India, Pakistan, and Turkey. In Indonesia, black turmeric (Curcuma caesia Roxb.) comes from a less well-known curcuma species [1].

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Black turmeric (Curcuma caesia Roxb.) has potential as a medicinal plant because it contains polyphenolic bioactive compounds such as flavonoids, phenols, and alkaloids [2]. The active compounds of the polyphenol group in plants have antioxidant activity [3-6]. Giving antioxidants and components of polyphenolic compounds can capture free radicals, reduce oxidative stress, reduce TNF-α expression [7] and lower blood glucose [8]. By inhibiting the action of the amylase enzyme as a carbohydrate catabolist so that glucose absorption does not occur [9], with this condition it is expected that hyperglycemia which produces free radicals, especially reactive oxygen species (ROS) which results in oxidative damage can be prevented.

Research on black turmeric that has been carried out includes antifungal [10], antioxidant and antimutagenic [11], anxiolytic, locomotor depressant, anti-convulsant, and muscle relaxant [12]. Black turmeric (Curcuma caesia) besides having the same phytochemical content as another turmeric, namely curcumin, it turns out that black turmeric (Curcuma caesia) contains flavonoids, alkaloids and tannins which are not owned by Curcuma longa or Curcuma amada which are suspected of being able to lower blood glucose levels [13]. Rhizomes from various regions in India have very high antioxidant activity [14] equivalent to ascorbic acid (86.91% [15]. It has an IC50 value of 94.03 g/ml [16], and antioxidant activity in dry rhizomes is higher than in fresh rhizomes [17] Ethyl acetate extract has alpha-amylase inhibitory activity [18] To the best of the author's knowledge, no research has been conducted on the chemical content and antioxidant activity of DPPH and the inhibition of alpha-amylase enzymes on the rhizome of Curcuma caesia roxb in Pekanbaru using 70% ethanol. the safest compared to a series of organic solvents and the addition of 30% water is expected to increase the polarity of the solvent so that more polar compounds can be extracted.

Based on Molyneux's research [19], one of the antioxidant tests is the DPPH method using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a free radical using a microplate reader. The DPPH method is an easy, simple, and accurate method and uses a small number of samples in a short time. Inhibition test of the alpha-amylase enzyme by determining the level of starch hydrolysis using the 3,5-dinitro salicylic acid (DNS) spectrophotometer method. Antioxidant and alpha-amylase activities are expressed in IC50.

2. Materials and Methods

The materials used are Spectrophotometer T60 UV-Vis, Microplate Reader Rayto RT-2100C, Top Centrifuge PLC-03, Evaporator EYELAN-1300, Hot Plat with Magnetic stirrer VELP SCIENTIFICA F20520162, Analytical balance, glassware, aluminum foil, Whatman no 1 filter paper, hot plate DAHAN Scientific Co., Ltd HP-20D, Thermometer, water Bath and Glassware.

The apparatus used are 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Merck), Ethanol p.a (Sigma), methanol p.a (Sigma), Phosphate buffer (pH 6,8), Amylum (Merck), Natrium Hidroksida (Merck), Dinitrosalicilic Acid (DNS)(Sigma), alfa Amylase enzyme, Acarbose, Vitamine C (Merck), Sodium hydroxide, Gallic acid, Quercetin, Follin Ciocalteus, reagent phenol, Na 2CO3 (Merck), Sodium Nitrite (Merck) and Aluminum Nitrate (Merck) and Rhizome Curcuma caesia Roxb from Pekabaru city Riau Province Indonesia.

2.1 Preparation of Extract Curcuma caesia Roxb

Simplisia was extracted as described in [20] (Alam *et al.*, 2014) 4 g of simplicia powder were mixed with 40 ml of 70% ethanol in a closed flask. Chemicals extracted from natural sources with ethanol or propanol are preferable for culinary applications [21]. The mixture will be allowed to stand for 24 hours at room temperature, stirring occasionally. After that, the mixture is allowed to

settle for 10-20 minutes. Then the supernatant was filtered through Whatmann filter paper. The extraction was repeated twice using the combined fresh solvent and extracted solvent. The extracted samples were concentrated using a rotary evaporator (Buchi Rotavapor) at 37 °C until 70% of the total volume was reduced. Finally, the extracted samples were stored in a refrigerator.

2.2 Determination of Total Phenolics

The total phenolic content of the extracts was tested according to the method of [22] with slight modifications. 0.2 mL of the sample was mixed with 0.5 mL of Folin-Ciocalteu phenol reagent and 7.5 mL of aquous solution and shaken for 10 minutes before adding 1.5 mL of 20% sodium carbonate solution and shaking for 10 minutes before adding 10 mL of distilled water and diluting 10 times, and the absorbance was measured at 760 nm. The Folin Ciocalteu Phenol Reagent was used to create a standard curve of total phenol equivalent gallic acid.

2.3 Determination of Total Flavonoids

The flavonoid content of the sample was determined according to [22], which was slightly modified, and quercetin was used as the standard. A total of 0.5 mL of the sample was added along with 0.3 mL of 5% sodium nitrite. After being left for 5 minutes, 0.6 mL of 10% aluminum nitrate was added and left for 5 minutes. Then, 2 mL of 1 M sodium hydroxide was added and diluted 10 times. The absorption was read at a wavelength of 510 nm. Quercetin standard curves were made with a concentration range of 0.5–100 ppm.

2.4 Determination of Alkaloid

Sebanyak ± 100 mg sampel digojog dg 5 mL HCl 2N dilakukan ekstraksi menggunakan 10 ml Chloroform dan fase Chloroform dibuang, ektrak dinetralkan dengan NaOH selanjutnya ditambahkan 5 mL Larutan BCG dan 5 mL Buffer Phosphat. Dilakukan ekstraksi kembali dengan 5 mL Chloroform dan aduk dengan magnetic stirrer kecepatan 500 rpm selama 15 menit. Fase Chloroform evaporasikan dengan Gas Nitrogen dan addkan dengan Chloroform hingga volume 5 mL serapan dibaca pada panjang gelombang 470 nm, kadar Alkaloid dinyatakan dalam total Equivalent dengan Quinine.

2.5 Determination of Total Saponin

The ether dry filtrate of Curcuma caesia roxb extract was added to 1 ml of water and vortexed for 5 minutes. Added 50 µl anisaldehyde, shaken, and let stand for 10 minutes. Then 2 ml of 50% sulfuric acid was added and heated in a water bath at 60oC for 10 minutes, diluted with water up to 10 ml, and diluted five times. Uptake was measured at λ 435 nm, and saponin levels were expressed in equivalent Quillaja bark.

2.6 Determination of Total Tanin

The tannin content of the sample was determined according to the [23] used Folin Ciocalteu. Add 1 mL of sample solution with 0.1 mL of Folin Ciocalteu reagent and vortex, wait 5 minutes. Add 2 mL of 20% sodium carbonate and vortex for 5 minutes, then dilute with distilled water. The absorbance

was read at λ 760 nm after incubation for 30 minutes at room temperature, and the tannin content was expressed in total equivalent to tannic acid.

2.7 Determination of Total Curcuminoid

A number of samples were extracted with 2 ml of ethanol, then vortexed and sonicated for 60 minutes, macerated for 24 hours, and centrifuged. The filtrate was transferred to a 5 ml measuring flask and ethanol was added to the mark, the sample absorption was read at 425 nm.

2.8 Antioxidant Activity Assay with 1,1-Diphenyl-2-picrylhydrazyl (DPPH) with 96 Well

DPPH as a free radical model in the test was carried out using the method shown by [24] (Zhang et al., 2006) and (Endrini, 2011) [25] 1,1 -Diphenyl-2-picrylhydrazyl free radical scavenging assay (DPPH) was carried out according to the following procedure. Each 70% ethanol at various concentrations (31.25, 62.5, 125, 250, and 500 ppm) has been added to a 1.5 x 10−4 M solution of DPPH in methanol and the reaction mixture will be shaken vigorously. The amount of DPPH remaining will be determined at 490 nm, and the radical scavenging activity obtained from the following equation:

Radical scavenging activity (%) = {(OD control –OD sample) / OD control} x 100

The antioxidant activity of plants extracts was partially expressed as IC50, which was defined as the concentration (ppm) of extract required to inhibit the formation of DPPH radicals by 50%.

2.9 α -Amylase Inhibitory Assay

Test with Amylase enzyme following the method [26] (Nair, Kavrekar, & Mishra, 2013). The test mixture containing 200 L of sodium phosphate buffer pH 5.9 200 L of enzyme and 70% ethanol extract in the concentration range of 20-100 g/mL was incubated for 10 minutes at room temperature, followed by the addition of 200 g/ml. 200µL starch in all test tubes. The reaction was stopped by adding 400 L of DNS reagent and placed in a boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water, and the absorbance was measured at 540 nm using a UV-Vis spectrophotometer. Control samples were prepared without plant extracts. The formula was used to calculate the percentage of inhibition.

Inhibition (%) = Abs 540 (Control) – Abs 540 (Extract) * 100 Abs 540 (Control)

The IC 50 value was determined from the plot of percent inhibition versus log inhibitor concentration and calculated by non-linear regression analysis of the mean value average inhibition. Acarbose was used as a reference for alpha amylase inhibitors.

2.10 Data Analysis

The absorption value of the DPPH solution and the inhibition of the amylase enzyme before and after the addition of the extract was calculated as the percent inhibition (% inhibition). Then it was entered into the regression equation and the IC50 value was calculated when the % inhibition was 50%.

3. Result and Discussion

The Phiyochemical Contens 70% Ethanol *Curcuma caesia* roxb extract, can be seen Figure 1 below.

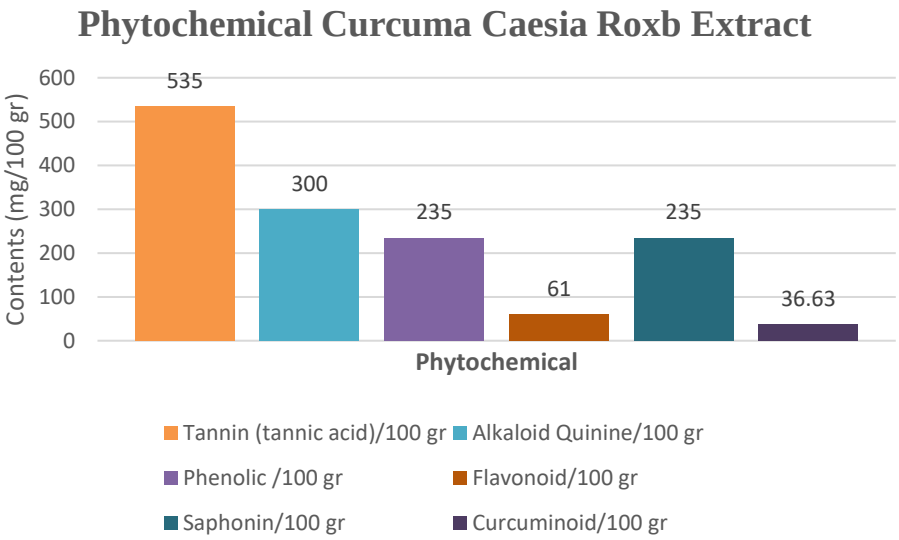

Fig. 1. Grafic of Phiyochemical Contens 70% Ethanol Curcuma caesia roxb extract

From Figure 1 it can be seen that the main content of the 70% ethanol extract of Curcuma caesia roxb is tannin compound 535 mg/100gr, alkaloids 300 mg/100gr, phenolics 235 mg/100gr, Flavonoids 61 mg/100gr, saphonins 235 mg/10gr and curcumimoid 36.63 mg/100gr.

Phenolic levels in this study were 235 mgGAE/100gr. Not the highest level compared to alkaloids and tannins and the lowest level of cucuminoid, namely 36.63 mg/100gr The compound content in plants greatly influences the antioxidant activity of these plants. Phenolic compounds play a major role in antioxidant activity, the more phenolic compounds the higher the antioxidant activity [27]. The higher the phenolic content obtained, the higher the curcuminoids, so that the antioxidant activity is also stronger.

In the healing mechanism of diabetes mellitus, flavonoids are assumed to play a significant role in increasing the activity of antioxidant enzymes and being able to regenerate damaged pancreatic beta cells so that insulin deficiency can be overcome [28]. Flavonoids, in particular quercetin, have been reported to have antidiabetic activity [29]. Quercetin works through inhibition of α-glucosidase by inhibiting the transport of glucose and fructose in the GLUT 2 transporter. Inhibition of GLUT 2 causes a reduction in glucose absorption in the lumen of the small intestine so that it will lower blood glucose levels [30].

From the results of testing the levels of secondary metabolites obtained alkaloids of 300 mg and quinine alkaloids of 300 mgQuinine/100gr. Alkaloids work by stimulating the hypothalamus to increase the secretion of Growth Hormone Releasing Hormone (GHRH), so that the secretion of Growth Hormone (GH) can be increased properly. High Growth Hormone (GH) levels will stimulate the results to secrete Insulin-like Growth Factor-1 (IGF-1). IGF-1 has the effect of inducing hypoglycemia and reducing gluconeogenesis so that glucose levels in the body and insulin requirements decrease [31]. In the world of medical and organic chemistry, the term alkaloid has long been an important and inseparable part of research that has been carried out so far, either to search for new alkaloid compounds or to trace bioactivity.

Saponins work by inhibiting the increase in vascular permeability thereby preventing inflammation in kidney cells and saponins inhibit superperoxides through the formation of hydroperoxide intermediates, thus preventing biomolecules from damage by free radicals. Saponins can improve kidney function by reducing urea and creatinine levels by increasing urea and creatinine excretion in the urine. The mechanism of action of saponins in reducing blood glucose levels is by inhibiting the transport of glucose in the digestive tract and stimulating insulin secretion in pancreatic beta cells [32]. Saponins have pharmacological activities, which include lowering cholesterol, having antioxidant, antiviral and anti-carcinogenic properties [33]. Saponin content of 70% ethanol extract Curcuma caesia 158 from quillaja bark/100gr.

3.1 Antioxidant Activity and Amylase Inhibitory 70% Ethanol Extract of Curcuma Caesia

The antioxidant activity test was carried out using the DPPH method to evaluate antioxidants using a spectrophotometer [34] with the working mechanism of the purple DPPH color reducing to light yellow due to antioxidants donating their hydrogen atoms to form stable DPPH radicals [35]. The α-amylase inhibitory test uses the DNS method, where alpha amylase hydrolyzes amylylum to maltose as a reducing sugar which reduces 3,5-dinitrosalicylic acid (brownish yellow) to 3-amino-5-nitrosalicylic acid (reddish orange) and reducing sugar. to form D-glucoseic acid. The intensity of the reddish orange color was measured by its absorption. The mechanism of inhibition of the alpha amylase enzyme belongs to compounds with hydroxyl (OH) functional groups in flavonoids and phenolics [36]. The activity of the alpha amylase enzyme is expressed in IC50 values. The smaller the value, the stronger it is in inhibiting the work of the enzyme. The value of % inhibition and IC50 can be seen in Table 1 and Figure 2 below.

Table 1
Antioxidant activity and α- amilase inhibitory of *Curcuma caesia* Roxb

Antioxidant activity			α-amilase Inhibitory		
Conct	%inhibition		Conct	%inhibition	
(µg/mL)	70% ethanol	Ascorbic acide	(µg/mL)	70% ethanol	Acarbose
500	70.152 ± 0.36	91,883 ± 1.80	100	73.196 ± 4.87	91.455 ± 1.80
250	41.354 ± 0.50	89,923 ± 0.20	80	53.150 ± 2.76	88.845 ± 0.20
125	27.477 ± 0.54	84,370 ± 1.96	60	37.514 ± 0.36	86.693 ± 1.96
62.5	18.087 ± 0.60	83,608 ± 0.20	40	25.086 ± 1.72	83.975 ± 0.96
31.5	12.635 ± 0.43	83,390 ± 0.19	20	15.693 ± 1.10	74.929 ± 0.19
15.625	8.436 ± 0.49	49,257 ± 0.20	10	2.567 ± 1.16	64.275 ± 0.10

The consentration versus % Inhibition grafics, regression to antioxidant activity and amiles inhibitory can be seen in the Figure 2 below.

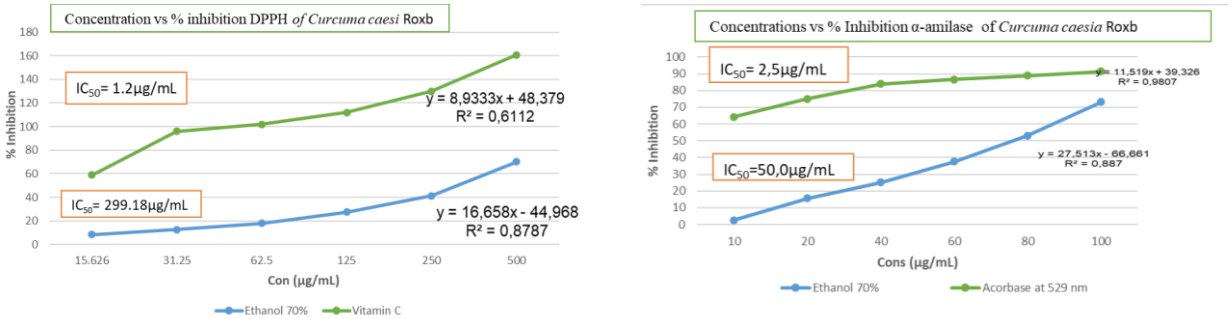

Fig. 2. Garfic Antioxidant activity and α- amilase inhibitory of *Curcuma caesia* Roxb

From the table and graph it can be seen that the IC50 value for the antioxidant black turmeric extract obtained a value of 299.18µg/mL with R^2 = 0.8787 classified as very weak and the IC50 for vitamin C was 1.2 µg/mL (very strong) with R^2 = 0.6142. The antioxidant activity of black turmeric is influenced by the presence of secondary metabolites it contains. The low value of antioxidant activity in this study is thought to be due to the low levels of phenolics (235 mgGAE/100gr), flavonoids (61 mgQE/100gr), and curcuminoids (36.63mg/100gr), namely 36.63mg/100gr. According to [26] the higher the phenolic content obtained, the higher the curcuminoid level so the antioxidant activity is also stronger. This study is similar to Mukunthan's research (2017) Curcuma caesia samples from India which were extracted using polar solvents resulted in lower antioxidant activity in polar solvents, and yields in polar solvents were smaller than non-polar solvents [14]. Research [41] in hexane solvent Calmination yielded a yield that is higher than petroleum ether solvent and produces a quite strong antioxidant activity. in this study the yield was 1.6% and relatively small. In addition to the solvent, the extraction temperature will affect the antioxidant activity.

The inhibitory activity of the α-amylase enzyme was obtained by IC50 of 50.27 µg/mL with R^2 = 0.8656 and IC50 of acarbose 2.535 µg/mL with R^2 = 0.9807. The inhibitory activity of the amylase enzyme is thought to be due to the highest alkaloid tannin content. Tannins are macromolecular compounds produced by plants and act as antinutrients and enzyme inhibitors, resulting in low starch hydrolysis and reduced response to blood sugar in animals [37]. Alkaloids can lower blood glucose by inhibiting glucose absorption. Competitive inhibition of the α-amylase enzyme by resembling the substrate and binding to the active site of the enzyme so that the enzyme decreases activity or does not carry out activity [38].

Phenol compounds that have an inhibitory effect on the α-amylase enzyme through hydroxylation bonds and substitution on the β ring. The principle of this inhibition is similar to that of acarbose, namely by delaying the hydrolysis of carbohydrates and disaccharides and the absorption of glucose and inhibiting the metabolism of sucrose into glucose and fructose [39]. Although the low content of flavonoids in the 70% ethanol extract of Curcuma caesaia may also affect the strength of action in inhibiting enzymes. Flavonoids act as non-competitive inhibitors on enzymes by way of inhibitors, and substrates can bind enzymes together on different bonding sites so that enzymes cannot move. Competitive inhibition of the α-amylase enzyme by resembling the substrate and binding to the active site of the enzyme so that the enzyme decreases activity or does not carry out activity [40]

4. Conclusion

The results showed that the 70% ethanol extract of Curcuma caesia Roxb contained 535 mg/100gr tannins, 300mg/100gr alkaloids, 235 mg/100gr phenolics, 61mg/100gr flavonoids, 235mg/10gr safonins and 36.63 mg/100gr curcumimoid constituents and have been low antioxidant activity and high α-amylase inhibitory.

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