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ANTIDIABETIC ACTIVITY TEST OF BLACK TURMERIC EXTRACT (CURCUMA CAESIA) ON ALOKAN-INDUCED WHITE RATS (RATTUS NORVEGICUS) USING GOD-PAP METHOD AND STRIP METHOD

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Abstract

Curcuma caesia or black turmeric is a plant that has many properties and has many compounds that can be used as medicinal ingredients. The purpose of this study was to determine the antidiabetic activity of giving black turmeric extract (*Curcuma caesia*) to reduce blood glucose levels in alloxan-induced white rats (*Rattus norvegicus*), the methods used were *the GOD-PAP* method and *the Strip* method. This study used 5 groups of white rats. Group 1 negative control NaCMC 1%, group II black turmeric extract concentration 100mg/kgBB, group III black turmeric extract concentration 200mg/kbBB, group IV black turmeric extract concentration 400mg/kbBB and group V positive control drug Metformin 200mg/kgBB, in white rats that had previously been induced with Aloxane 150mg/kgBB. The results showed a decrease in blood glucose in group (I) strip method there was a decrease in blood glucose 0.436%±2.294 and GOD-PAP method 0.3216%±1.348, After administration of black turmeric extract group (II) concentration of strip method there was a decrease in blood glucose 25.847%±1.595 and GOD-PAP method 0.3216%±1.348, group (III) decreased blood glucose by strip method 35.402%±4.826 and GOD-PAP method 39.500%±0.889 and for Group (IV) decreased blood glucose by method strip 53.727%±1.882 and for GOD-PAP method 67.827%±2.152. And for group (V) as a positive control strip method there was a decrease in blood glucose 81.462%±2.191 and the GOD-PAP method 87.146%±0.598. So it can be concluded that black turmeric extract can reduce blood glucose levels in white rat test animals by using 2 paremeters, GOD-PAP parameters and Strip parameters.

Keywords: Rhizome of Black Turmeric *(Curcuma caesia*), Diabetes mellitus, GOD-PAP Reagent, Aloxane, Metformin.

INTRODUCTION

High levels of blood glucose, exceeding normal ranges, are a hallmark of various illnesses, notably diabetes mellitus and several related conditions collectively termed hyperglycemia. Presently, diabetes mellitus stands as a significant global health threat. Depending on the underlying factors, diabetes mellitus can be categorized into four main groups: type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes mellitus, and other forms of diabetes mellitus [1]. Based on projections from the World Health Organization (WHO), the prevalence of type 2 diabetes mellitus (DM) in Indonesia is expected to surge from 8.4 million individuals in 2000 to approximately 21.3 million by 2030. Similarly, estimates provided by the International Diabetes Federation (IDF) indicate a rise in the number of diabetes mellitus cases from 10.7 million in 2019 to 13.7 million by 2030 [1].

Based on a report by the International Diabetes Federation, the prevalence of diabetes mellitus worldwide is 1.9% and has made it the seventh leading cause of death in the world. In 2013, there were 382 million cases of diabetes worldwide, with the prevalence of type 2 diabetes accounting for 95% of the global population, and the prevalence of type 2 diabetes cases at 85–90% [2].

Relative or absolute lack of insulin is the cause of diabetes mellitus. There are three different ways to experience insulin deficiency, pancreatic cell damage due to external factors such as viruses, chemicals, desensitation or decrease in glucose receptors in the pancreas gland and desensitation or damage to insulin receptors in peripheral tissues [3]. Complications of DM can include problems with blood vessels, both macrovascular and microvascular, as well as problems with the nervous system or neuropathy. Macrovascular complications usually include blood vessels such as the heart, brain, and kidneys. DM patients also often experience neuropathy, including motor, sensory, or autonomic neuropathy [4].

Aloxane is an unstable, highly hydrophilic compound with weakly acidic properties. Aloxane has a half-life of 1.5 minutes at pH 7.4 with a temperature of 37^{o}C. This compound is stable at acidic pH [5]. Aloxane as one of the induction compounds of diabetes mellitus rats can be administered intravenously, intraperitoneally and subcutaneously. Different places of administration of alloxane also have an effect on the dose given. Intravenous administration is usually 65 to 75 mg/kg/kg body weight, while intraperitoneal and subcutaneous administration is 2 to 3 times. In rats made diabetes mellitus can be induced intraperitoneally at a dose of 150 mg / kg body weight. The majority of herbal plants contain flavonoid compounds that are known to function as antioxidants, able to fight free radicals. This plant also has antidiabetic properties that can lower blood glucose levels and is used as a preventive measure and therapy for diabetes[6]. States that certain classes of compounds such as flavonoids, phenolics, alkaloids, and terpenes, are involved in the production of drugs.

Curcuma caesia Roxb. or known as black turmeric is a plant that has many benefits. Plants belonging to the Zingiberaceae family are perennial plants belonging to the Curcuma Genus distributed throughout tropical and subtropical regions of the world [7], bronchodilating activity [8], antioxidant activity [9], anxiolytic and CNS depressant activity, locomotor depressants, anti-convulsants [10], antielmintic activity [11] ,anti-bacterial activity [12]. Various types of turmeric play a role and have benefits in the health sector play a role and have benefits in the health sector. Throughout history, turmeric is believed to have the ability to cure various types of diseases, the ability to cure several types of diseases. It has been reported that black turmeric contains various phytochemical compounds, including carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids, and tannins. Higher concentration of phytochemicals compared to other types of turmeric [13]

Curcuma caesia, an herbal plant, is increasingly drawing attention from researchers due to its potential health benefits. The interest in Curcuma caesia stems from its observed antioxidant, antibacterial, antifungal, antiproliferative, anticancer, anti-inflammatory, analgesic, anticonvulsant properties, along with its muscle relaxant, locomotor depressant, antidepressant, and thrombolytic activity. Several scientific studies have highlighted these diverse medicinal properties, contributing to the growing popularity of Curcuma caesia as a subject of research in the scientific community [14].

Checking glucose levels has now been signaled by enzymatic means, no longer with the principle of reduction to avoid participating in other substances that would give false high yields. Enzymatic ways can be done in automatic ways such as with GOD-PAP and strip ways [15] So this study was conducted as one of the parameters or data regarding the benefits of black turmeric extract against antidiabetic diseases where the author conducted a study with the thesis title of antidiabetic activity test of black turmeric extract (*Curcuma caesia*) in alloxan-induced white rats (*Rattus norvegicu* s) using the GOD-PAP method and the Strip method.

MATERIALS AND METHODS

Location and design of the study

Research location

1. Sampling location

Black Turmeric Rhizome sampling was obtained from Baleendah District, Bandung Regency, West Java Province

2. Working location for dry extract of black kunyi rhizomes

The research was conducted at the chemical engineering laboratory of Ujung Pandang State Polytechnic University to carry out a process to obtain dry extracts from maceration of black turmeric rhizome samples.

3. Testing of dry extract samples of black turmeric rhizomes

Location Test of antidiabetic activity test of black turmeric extract (*Curcuma caesia*) on white rats (rattus norvegicus) induced by alokan using the GOD-PAP method and the Strip method was carried out by the Biopharmaceutical Laboratory, Faculty of Pharmacy, Hasanuddin University Makassar

4. Location of GOD-PAP method measurement

Conducted in the laboratory of the Makassar Health Office Center

5. Test location for the compound content of black turmeric extract

Conducted in the laboratory of mathematics and natural sciences, faculty of Mathematics and Natural Sciences, Hasanuddin University Makassar

Assurance Design

This research is an experimental study using Posttest Only Control Group Design [16].

TOOLS AND MATERIALS

Tool

Maceration tools Analytical scales, measuring cups, glass crackers, stirring rods, funnels, flasks, filter paper, electric stoves, measuring flasks, *erlenmeyer*, rat cages, drinking water bottles, 1.0 ml injection syringes, 5 ml oral syringes, spoits to take 5 ml blood, Blood sugar activity measurement Blood test (Nesco), rotary evaporator, freeze drying and GOD-PAP (UV-Vis Spectrophotometry) measurements.

Material

Black Turmeric rhizome, Ethanol 70% , white rat wistar, Feed, NaCl 0.9%, aquadest, Alcohol 96%, Aloxane, Na-CMC, GOD-PAP Reagent, metformin.

WORKING METHODS

Procedure for extracting black turmeric (Curcuma caesia)

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)* was weighed as much as 500 grams, then put into a maceration container, then added 70% ethanol solvent as much as 3000 mL until the simplisia was submerged, left for 3 days in a closed vessel container and protected from direct sunlight while stirring periodically. After 3 x 24 hours, screening was carried out to obtain 70% liquid ethanol extract. After that, the filtering results obtained were then evaporated using a rotavapor with a temperature of 60°C, until a thick extract of black turmeric *(Curcuma caesia)* was obtained, followed by the fresdraying process to obtain dried black turmeric extract.

Phytochemical screening of black turmeric extract

1. Flavonoid Test

Black turmeric rhizome extract is pipetted as much as 1 mL, added 6 drops of concentrated HCl and 0.1 grams of magnesium powder then shaken slowly, if red, orange and green colors indicate the presence of flavanoid compounds (Endarini, 2016)

2. Alkaloid test

Black turmeric rhizome extract was pipetted as much as 1 ml, put into a test tube, then added 0.1 ml hydrochloric acid 2 N then tested with alkaloid reagent with mayer reagent and dranendrof reagent. Positive results when a yellow reagent precipitate is formed with mayer reagent and red precipitate with dragendrof reagent (Ministry of Health RI, 1995, endarini, 2016)

3. Saponin test

Black turmeric rhizome extract is pipetted 1 ml, add with 0.1 ml hot water, then shaken for 1 minute and add 0.1ml HCL 2 N. if foam is formed shows positive containing saponin compounds.

4. Tannin Test

Black turmeric rhizome extract is pipetted as 1ml, added 0.5 mL FeCL3 1% . If it is formed, blackish-green and dark blue colors indicate positive for tannins.

5. Terpenoid test and Steroid test

Black turmeric rhizome extract is pipetted as much as 3ml, add 0.5 ML of Buchard liquemen, positive reaction when formed brown ring indicates the presence of terpenoids and positive reaction when formed blue or green ring indicating the presence of steroids (Ministry of Health, 1979)

Determination of total flavonoid and polyphenol compounds Levels of black turmeric extract

Analysis of total flavonoid levels using UV-Vis spectrophotometry method. The standard used is quercetin. Maximum wavelength measurements are carried out in the wavelength range of 400-800 nm. The maximum wavelength of quercetin measured is at a wavelength of 441 nm.

The maximum wavelength was used to determine the curve of the quercetin series and the total flavonoid levels in Black Turmeric Extract. In determining the quercetin standard curve, quercetin is made with concentration series of 2, 4, 8, 16 and 32 ppm. And for polyphenolic compounds The maximum wavelength of tannic acid measurement results are at a wavelength of 760 nm.

The maximum wavelength is used to determine the curve of the tannic acid series and the total polyphenol content in Black Turmeric Extract. In determining the quercetin standard curve, quercetin is made with concentration series of 1, 2, 4, 8 and 16 ppm

Testing of black turmeric extract on research test animals

This research received approval from the Research Ethics Commission obtained from the faculty of public health sciences, Hasanuddin University, Makassar with Number: 328/UN4.6.4.5.31/PP36/2023. A total of 40 male wistar rats aged 4.5 – 6 months, weighing 200 – 300 grams.

All groups measured normal glucose levels by the Strip method and GOD-PAP (enzymatic photometric test) and after that induced alloxane 150 mg / kg body weight intra peritoneal and blood glucose measurement with > levels of 135 mg / dl 3 - 4 days, remeasured blood glucose levels with the Strip method and GOD-PAP for hyperglycemic mice. Rats were grouped into five groups and each group of 5 groups each, with treatment in accordance with the treatment of each group.

The control group (-) was given NaCMC treatment as a negative control, the treatment for group 2, group 3 and group 4 respectively received doses of 100 mg / KgBB, 200 mg / KgBB and 400 mg / KgBB dry black turmeric extract, while group 5 was given treatment as a positive control (metformin) with a dose concentration of 200mg / kg BB rats.

The administration of black turmeric extract was carried out orally for 14 days. At the end of the study, blood was taken through the orbital vein using microhematocrit and inserted into the ependorf containing EDTA to prevent clotting and lysis of blood, then the blood sample that had been taken was centrifuge for separation of blood serum and analyzed using spectrophotometry.

Data testing analysis

Analysis using the method using Graphad Prism vol 10 from the *two-way anova* analysis was then continued with *multiple comparisons test* where comparisons between treatments and groups obtained significant or significantly different results between the Control group and the extract group.

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RESULTS AND DISCUSSION

1. Research Results

Table 1: Percentage Yield of Drying Shrinkage of Black Turmeric Extract *(Curcuma caesia)*

Plant Name	Wet Weight (grams)	Dry Weight (grams)	Shrinkage Drying (%)
Black Turmeric (Curcuma caesia)	4700 gram	462 gram	9,83%

Table 2: The yield of Black Turmeric Extract *(Curcuma caesia)*

Plant Samples	Weight of Viscous Extract (grams)	Simplicia Weight (grams)	Renderment (%)
Kunyit Hitam	35 gram	462 gram	7,57%

Table 3: Qualitative Phytochemical Screening Test of Black Turmeric Extract (*Curcuma caesia)*

No	*Compound*	*Result*
1	Alkaloids	Positive
2	Flavonoids	Positive
3	Polyphenols	Positive
4	Tannin	Positive
5	Saponin	Negative
6	Triterpenoids/ Steroids	Negative

Table 4: Total Flavanoid Levels of Black Turmeric Extract (*Curcuma caesia)*

Sample Code	A (=441	Measurable Flavonoids (ppm)	Sample Mass (g)	Volume of Sample Solution (L)	Mg Quercetin equivalent/gram Sample	Flavonoid Levels (%)	Average Flavonoid Levels (%)
Black Turmeric Simplo	0,062	6,0202	0,0510	0,01	1,18043	0,11804	
Black Turmeric Duplo	0,066	6,4242	0,0516	0,01	1,24501	0,12450	0,1213

Table 5: Quercetin Standard Series Measurement

Quercetin (ppm)	Abs (441 nm)
2	0,022
4	0,04
8	0,085
16	0,161
32	0,32

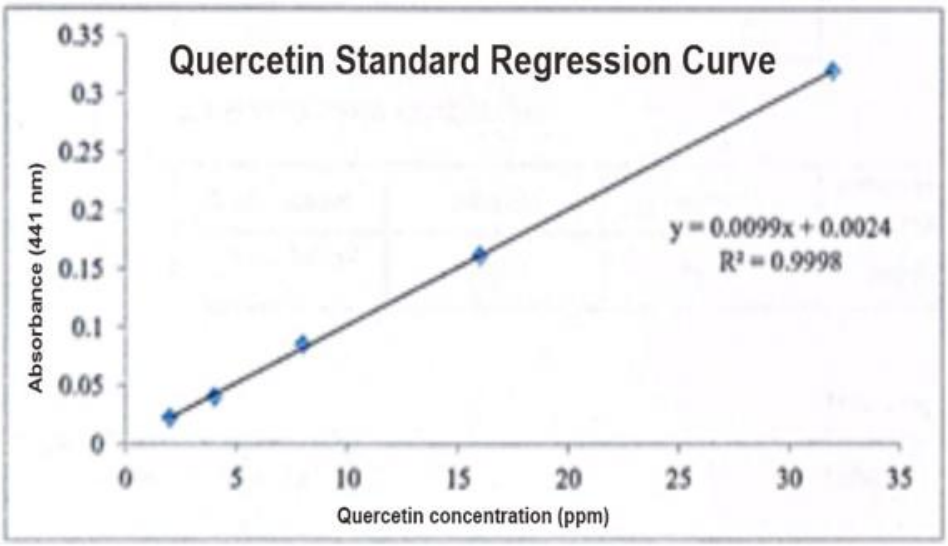

Figure 1: Standard Regression Curve of Quercetin

Table 6: Standard Series Measurement of Tannic Acid

Tannic Acid (ppm)	Abs (760nm)
1	0,05
2	0,1
4	0,21
8	0,387
16	0,739

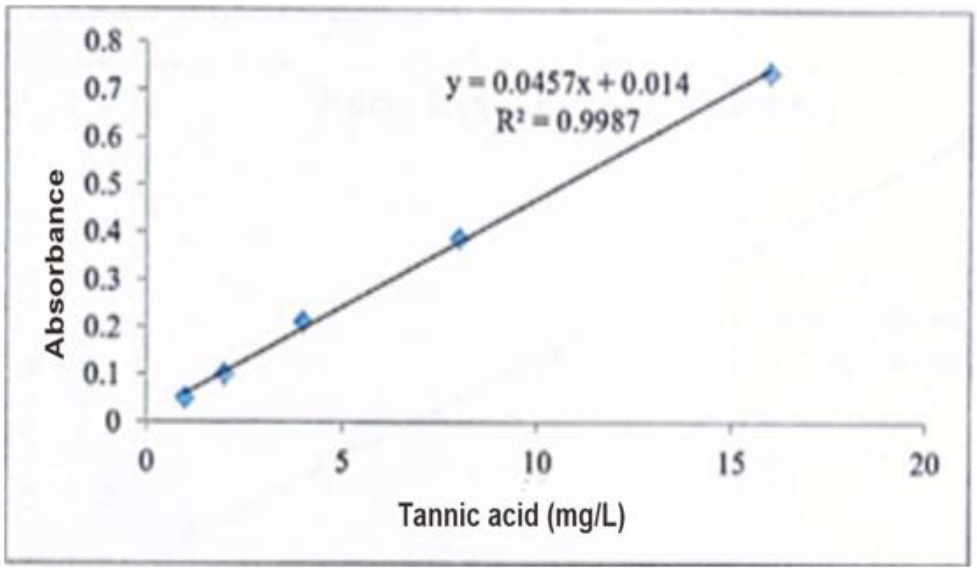

Figure 2: Standard Regression Curve of Tannic Acid

Table 7: Polyphenol LevelsTotal Black Turmeric Extract (*Curcuma caesia)*

Sample Code	A (=760 mm	Dilution Factor	Measurable Polyphenols (mg/L)	Sample Mass (mg)	Volume of Sample Solution (L)	Mg Tannic Acid Equivalent/mg Sample	Polyphenol Levels (%)	Average Polyphenol Content (%)
Black Turmeric Simplo	0,287	50	298,687	52,5	0,01	0,05689	5,69	5,2760
Black Turmeric Duplo	0,242	650	249,453	51,3	0,01	0,04863	4,86	

Table 8: Blood Sugar Drop Black Turmeric Extract *(Curcuma caesia)*

NO	STRIP		GOD-PAP	
	% Average Decline	Standard Deviation	% average decrease	Standard Deviation
Control Negative	0,436 %	2,294	0,3216 %	1,348
Concentration 100mg/kg/bb	25,847 %	1,595	17,724 %	1,515
Concentration 200mg/kg/bb	35,402 %	4,826	39,500 %	0,889
Concentration 400mg/kg/bb	53,171 %	1,882	67,827 %	2,152
Control Positive (metformin)	81,462 %	2,191	87,146 %	0,598

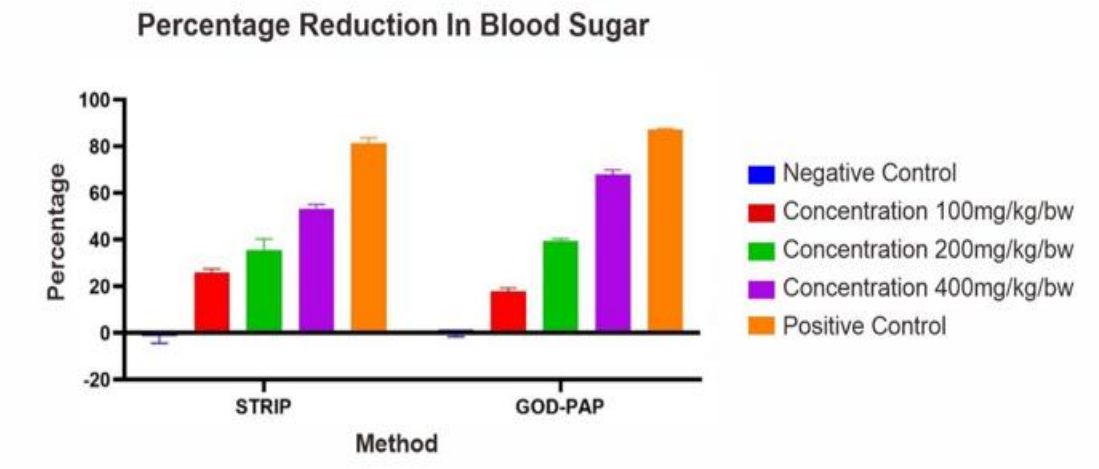

Figure 3: Blood Sugar Reduction Percentage Diagram

DISCUSSION

Diabetes mellitus (DM) refers to a cluster of metabolic disorders marked by elevated levels of blood sugar resulting from issues with insulin secretion, insulin responsiveness, or both. Typically, there are two primary types: type I and type II. Type I, known as insulin-dependent DM, is characterized by a complete absence of insulin production. Type II, termed insulin-independent DM, is characterized by reduced insulin secretion and compromised insulin function [17]

As many as 80.5%, or 20.4 million people in Indonesia, are affected by DM, according to Basic Health Research (Riskesdas) data. The disease often leads to serious acute and chronic complications, it can even lead to death. Social, cultural, and geographical variables are additional challenges in the treatment of diabetes mellitus [1]

Despite the introduction of antidiabetic medications, sourced from both natural and synthetic origins, diabetes and its associated micro and macro complications remain a significant global health concern. Modern pharmaceuticals available for diabetes treatment often come with limitations such as insufficient effectiveness, high expenses, and a range of side effects. Given these drawbacks linked with conventional drugs, medicinal plants purported to possess antidiabetic properties can serve as an alternative approach for managing diabetes, particularly in developing nations. This alternative presents advantages including cost-effectiveness, accessibility, widespread cultural acceptance, and reduced risk of adverse effects. One such plant with potential medicinal use is Black Turmeric (Curcuma caesia).[18].

Black turmeric (*Curcuma caesia*) is a family of *Zingiberaceae* is a perennial herb with a variety of medicinal properties. In Chinese medicine, oil derived from black turmeric containing metabolites such as curcumol, germacrone, beta-elemene and curdione is used to cure various diseases such as malignant tumors Used to treat hemorrhoids, leprosy, asthma, menstrual disorders, fever, cancer, inflammation, epileptic wounds, vomiting, anthelmintic disorders, aphrodisiacs, gonorrhea, etc. [19]This black turmeric plant contains steroids, phenols, alkaloids, flavonoids and tannins as well as essential oils [14]

The purpose of this study was to determine the effectiveness of black turmeric rhizome extract in preventing increased blood glucose levels in alloxan-induced white rats using the GOD-PAP and strip methods. Aloxane damages the pancreas by forming reactive oxygen which becomes superoxide radicals through the redox cycle which will produce hydroxyl compounds that are very reactive, these hydroxyl compounds that will cause damage to pancrea β cells [20] Aloxane can also interfere with the process of cell oxidation due to the presence of calcium ions released by mitochondria which causes homeostatic disorders resulting in the death of pancreatic β cells [21]

In this study, Black Turmeric Rhizome was carried out the process of making simplisia by wet sorting, dry sorting and drying so that the water content in the rhizome was reduced and met the requirements of the drying loss standard, which is 10%. from the results of research conducted the wet weight of black turmeric rhizomes amounted to 4700 grams, while the weight after drying was 462 grams so that the drying shrinkage obtained was 9.83%. The extraction method used in this study is the maceration method. Simplisia black turmeric as much as 462 grams using 70% ethanol solvent.the extraction results are then consumed with an evavorator and freeze drying to obtain dry extract as much as 35 grams. The yield calculation result of the extract is 7.57%.

To ascertain the chemical compounds of black turmeric rhizomes, phytochemical screening tests are carried out using appropriate reagents. From the results of research conducted for alkaloid tests using Meyer reagents with the results obtained there is a yellowish-white precipitate. The positive reaction of alkaloids with Mayer's reagent is the occurrence of bonds between N atoms that have free electron pairs in alkaloids with Hg atoms in Mayer's reagents so that yellowish-white nonpolar complex deposits are formed (Sandy, Susilawati and Ramadhania, 2020). flavonoid test using concentrated Magnesium and Hydrochloric acid reagents with red results. Flavonoid

compounds will be oxidized with magnesium ions to form complexes. The compound that gives it such color is flavonon. Polyhydroxy from flavonons will be reduced by magnesium and hydrochloric acid to form red benzopyrylium salts or flavonoid flavilium salts (Khairunnisa, Hakim and Audina, 2022). polyphenol test using 5% FeCl3 reagent with the formation of black-bluish color. due to the formation of complexes between phenol and Fe groups found in FeCl3 reagents. This reaction is analogous to the reaction between phenol groups and AlCl3 compounds because Fe is also a metal [24]. Tannin test using 1% FeCl3 reagent with blue-black results obtained due to the formation of complex compounds between tannins and Fe3+ ions (Datu, Hasri and Pratiwi, 2021).

In this study, the total flavonoid content was determined based on the UV-Vis colorimetry/spectrophotometry method in the treatment of the test material, AlCl3 added to form a stable acid complex with C-4 ketone groups, then with C-3 or C-5 hydroxyl groups from flavones and flavonols. In addition, AlCl3 also forms a labile acid complex with orthodihydroxyl groups on the A or B ring of flavonoids so that it will have maximum absorption at a wavelength of 430 nm and the addition of potassium acetate which aims to maintain wavelengths in visible areas In determining total flavonoid levels of black turmeric test material (*Curcuma caesia* Using quercetin as a standard solution that will be used as a comparison because it is one type of flavonoid flavanol group found in many types of plants. quercetin is also among the compounds that most effectively capture free radicals and inhibit various oxidation reactions because it can produce phenolic radicals that are stabilized by the resonance effect of the aromatic ring (Bachtiar, Handayani and Ahmad, 2023)

Measurement of quercetin standard solution absorbance for calibration curves using concentrations of 2 ppm, 4 ppm, 8 ppm, 16 ppm and 32 ppm allowed to stand for operating time. The results of measuring the absorbance of quercetin standard solutions at various concentrations of the calibration curve at a wavelength of 441 nm obtained a linear regression equation, namely y = 0.0099x + 0.0024 with a value of correlation coefficient (r) = 0.9998. An r value close to one indicates that the calibration curve is linear and there is a relationship between quercetin solution concentration and absorption value (Khairunnisa, Hakim and Audina, 2022)

Furthermore, sample measurements were made, made in two replications where the sample solution was added AlCl3 which serves for complex formation In addition, CH3COOK reagents are also used which aim to maintain the wavelength in the visible area. Then incubation is carried out for 30 minutes so that the resulting color index is maximized. From the results of measurements made, the total flavonoid content results were obtained as much as 0.1213%.

Determination of total phenolic levels quantitatively in black turmeric test material was carried out using the UV-Vis spectrophotometer method. Standard tannic acid curve data. Tolal polyphenol levels in black Turmeric samples can be calculated using the linear regression equation y = 0.0457x + 0.014. The linearity value of the determination of total polyphenols is 0.9987. This linearity value shows the relationship between sample concentration and sample absorbance. If the value of linearity or r is close to one or equal to one, it shows that the equation is getting better and is positively correlated or linear. The higher the concentration used, the higher the absorbance obtained. The maximum wavelength for measuring absbyteration of standard tannic

acid solutions and multiple sample concentrations is 700 nm (Manongko, Sangi and Momuat, 2020)

The reduction of total phenol levels in black turmeric samples was carried out twice in the hope of obtaining better data. Determination of total polyphenol content using spectrophotometric methods. Proving the polyphenol content uses the Follin-ciocalteu reagent Ciocalteau reagent because this reagent can react with groups of polyphenolic compounds to form a concentrated solution whose absorbance can be measured. The higher or more concentrated the sample concentration, the higher the absorbance value Folin reagent will oxidize the polyphenol functional group The phenol compound group will react with the Folin-Ciocalteau reagent if the conditions formed are in an alkaline atmosphere. Base conditions allow the dissociation of protons in phenol compounds into phenolic ions. Alkaline conditions can be formed using Na2CO3. As a result of this reaction, a blue color is formed Based on the results of quantitative tests using a spectrophometer, data showed that betung bamboo shoots or shoots contained a total of 8,065 mg / L polyphenol compounds [28].

In antidibestes testing using the GOD-PAP method and strips on pituh rats induced with alloxane.in this study rats were divided into 5 groups consisting of group 1 negative control group 2 black turmeric extract 100 mg / kgBB, group 3 black turmeric extract 200 mg, group 4 black turmeric extract 400 mg / KgBB. Group 5 positive controls using metformin. White rats measured baseline blood sugar levels then induced with alloxane for 14 days and measured blood glucose, after which given extract administration and positive control for 14 days after which it was measured

From the results of research carried out the percentage of lowering blood glucose by the strip method, for group 1 negative control was obtained by 0.436% ± 2.294. While group 2 amounted to 25.847%± 1.595, group 3 amounted to 35.402%±4.826, group 4 obtained results of 53.272%±1.882. While group 5 positive controls obtained results of 81.462% ±2.191.

For measurements using the GOD-PAP method, the average percentage of blood glucose reduction for group 1 control group 1 negative control was obtained at 0.3216%±1.348. For group 2 description of black turmeric extract 100 mg / KgBB of 17.724% ± 1.515, group 3 description of black turmeric extract 200 mg / KgBB of 39.500% ±0.889, group 4 description of extract 400 mg / KgBB of 67.827% ± 2.152, while group 5 description of metformin decreased blood glucose by 87.146% ± 0.598. From these data, then analyzed by the method using Graphad Prism vol 10 from the *two-way anova analysis*, a $p<0.05$ value of 0.0023 was obtained, which means that each treatment, both extract administration and control, was significantly different. In *the multiple comparisons test* where comparisons between treatments and groups were obtained significant or significantly different results between the control group and the extract group. So it can be concluded to show that black turmeric extract has blood glucose lowering activity in white rat test animals.

According to research [29] black turmeric extract Black turmeric extract showed the ability to inhibit α-amylase (45.68% at 800 mg / L, IC50: 918.0108 mg / L) and α-glucosidase (51.967% at 250 mg / L, IC50: 224.292 mg / L). Its potential as an inhibitor of this enzyme signifies potential benefits in controlling blood sugar levels. On research [30] Studies exploring the activity of C. caesia have concluded that the plant has the ability to reverse all sorts of pathological changes that occur in diabetic neuropathy. Thus, it can be proven as an advantage of natural therapies for treating

diabetes-related complications. Research (Aini, Herdwiani and Wijayanti, 2023) entitled "The effectiveness of black turmeric rhizomes (*curcuma caesia* roxb.) Against the decrease in blood glucose and kidney improvement of diabetic nephropathy rats" where white rats were induced with STZ-Na it was found that the percentage of reduction in blood glucose levels was 16.75% and renal histopathology improvement was 6.67% with mild damage category.

Polyphenols found in black turmeric exhibit potential as antidiabetic agents. Particularly, polyphenolic flavonoids play a crucial role in carbohydrate metabolism by impeding key enzymes responsible for breaking down carbohydrates into glucose, such as ÿ-glucosidase and ÿ-amylase. They also disrupt the process by inhibiting sodium-dependent glucose transporters, namely Sodium/glucose cotransporter (SGLT)1. Research has indicated that polyphenols can regulate postprandial glycemia and hinder the onset of glucose intolerance by facilitating insulin response and suppressing the release of glucose-dependent insulinotropic polypeptides (GIPs) and glucagon-like polypeptide-1 (GLP-1). Various polyphenols can regulate fundamental pathways of glucose homeostasis and carbohydrate metabolism, including glycolysis, glycogenesis, and gluconeogenesis, processes commonly impaired in diabetes. [32]. While flavonoid compounds are able to suppress the decrease in blood glucose by restoring insulin sensitivity secreted by pancreatic β cells, flavonoids can reduce blood glucose levels with some mecanime. First, flavonoids inhibit the production of Reactive Oxygen Species (ROS). Second, flavonoids inhibit GLUT 2 intestinal mucosa so that it can reduce glucose absorption. This leads to a reduction in glucose and fructose absorption from the intestine which can lower blood glucose levels [15]

CONCLUSION

Based on the results of research that has been conducted and it can be concluded that dry extract of black turmeric can reduce blood glucose levels in male white rat test animals with the parameters used GOD-PAP and Strip methods

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