

Activity of Antioxidant and Inhibitor α -Glucosidase Instant Granul Ethanol Tea Leaf Extract (*Camellia sinensis*) and Tea Benalu (*Scurulla atropurpurea* BL Dans)

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Abstract. Tea leaves are plants that are widely spread especially in the area of West Java, while parasites are parasitic plants in tea trees that empirically have various properties such as antioxidants and anti diabetic. The α -glucosidase inhibitor is one of the therapeutic drugs for diabetic that works by inhibiting carbohydrate metabolism. Instant granule preparation is one form of dosage used by dissolving in water. This study aims to determine the antioxidant activity and inhibitors of instant granular α -glucosidase ethanolic extract of Tea and Tea Tea leaves. The extract was obtained from extraction with 60% ethanol solvent-assisted extraction (MAE) method. Research was done by making 3 formula (Formula 1, 2 and 3) with comparison of tea leaf extract, parasite, Polivinil pyrrolidone (PVP) and cyclodextrin ie 26,88%, 1,8%, 2%, 0% (Formula 1) 26.88%, 0%, 3%, 12% and 0% (Formula 2), 1.8%, 4%, 15% (Formula 3). Formula 2 is active as an antioxidant and α -glucosidase enzyme inhibitor with IC50 values of 68.21 and 56.76 ppm.

Keywords: Tea Leaves, Benalu Tea, Antioxidants, α -glucosidase Inhibitors, Instant Granules

I. INTRODUCTION

Diabetes mellitus (DM) or known as sugar disease is one of the degenerative diseases caused by chronic metabolic disorders (especially carbohydrates), characterized by chronic hyperglycemia (hyperglycemia) ie high blood sugar levels exceeded normal, due to absolute and relative insulin hormone disorders (Sratton, 2000). According to International Diabetes Federation (IDF) data in 2013, the number of diabetics in the world reaches 382 million people and is expected to increase to 592 million by 2035. Indonesia is ranked 7th with the number of people with diabetes ranging from 8.5 million people.

Free radical imbalance causes oxidative stress on β -pancreatic cells that can cause hyperglycemia. Antioxidants are compounds that can inhibit oxidation reactions by binding to free radicals and highly reactive molecules (Winarsih, 2007). The resulting antioxidant activity plays an important role in the prevention of degenerative diseases by maintaining immune system function and potentially maintaining cell membrane against oxidant attacks so as to counteract free radicals (Widowati, 2008).

α -glucosidase inhibitors are used to treat type II DM. The antihyperglycemic action of α -glucosidase inhibitors stems from competitive reversible inhibition of pancreatic α -amylase hydrolase enzymes and digestive enzymes in the small intestine such as isomaltase, sucrase and maltase. Inhibition of this

enzyme can lead to inhibition of glucose absorption in DM patients so as to decrease the state of hyperglycemia after meals (Sugiwati, 2005).

West Java has a very large biological wealth, the utilization of tea leaves and parasites has been widely developed, but the activity as antioxidants and type 2 antidiabetes has not been much research. One of the food diversification efforts in an effort to improve public health is to develop a mineral in the form of instant granule. Instant granule advantages compared more practical, both in terms of packaging and presentation, easy to eat and taste better (Ansel, 2005).

Tea leaves containing polyphenols such as catechins and derivatives have been reported to have a variety of beneficial physiological effects on health, such as antioxidants, anticancer, and prevent degenerative diseases such as cardiovascular, arthritis, and diabetes (Naghma, 2008). Some tea comes from the species of *Scurulla atropurpurea* BL. Dans is a parasitic plant in tea trees. In the case of dried tea boiled water can be drunk to cure uterine cancer and other cancers. Cancer patients given a parasitic extract from the *Viscum album* showed improvements in DNA in lymphocytes and immune cells. Therefore, it is necessary to do more intensive research so potential of parasite tea as raw material of medicine can be further developed.

Microwave-assisted extraction (MAE) is a method of extraction using radiation. The content of catechins extracted by MAE method for 6 minutes with

ethanol solvent was higher (82.46%) than the maceration method for 24 hours at room temperature (49.39%) (Quan et al., 2006).

From the above exposure it will be irradiated on tea leaf simplicia and parasite tea, extracting the two simplicia with 60% ethanol solvent MAE, making instant granule formula, testing the antioxidant activity and inhibitor of α -glucosidase enzyme in vitro.

II. RESEARCH METHODS

Materials

The ingredients used are Tea leaves (*Camellia sinensis* L.), tea seed (*Scurulla atropurpurea* BL Dans), free aqua demineralisata CO₂, FeCl₃, ethanol 60%, dilute ammonia, chloroform, Mayer reagent, Wagner reagent, Dragendorf reagent, methanol, Mg powder, concentrated HCl, 1% gelatin in 10% NaCl, KH₂PO₄, NaOH, p-nitrophenol- α -D-glucopyranoside (PNPG) powder, α -glucosidase enzyme, 1,1-diphenyl-2-picrylhydrazyl (DPPH), vitamin C, bovine albumin serum, DMSO, Na₂CO₃, acarbose tablets, sucralosa, PVP, citrus essence, citric acid, and lactose.

Methods

Simplisia Tea Leaves and Benalu Tea is extracted using MAE method with 60% ethanol solvent for 6 minutes. Then the instant granules are formulated by wet granulation method and then sieved using a mesh sieve 12 and after dried diayak using mesh screen 16. Instant granule quality test extract includes granule flow test, angle break test, moisture content, solubility test and hedonic test including taste, smell and color. Further testing of antioxidant activity and α -glucosidase enzyme inhibitors in vitro.

Table 1. Formulation of Instant Granules Tea Leaf Extract and Tea Benalu

Ingredients	Formula 1	Formula 2	Formula 3
White Tea Leaf Extract	26,88 %	26,88%	-
Benalu Tea Extract	1,8 %	-	1,8 %
PVP	1 %	1 %	1 %
Cyclodextrin	15%	15%	15%
Maltodextrin to	100%	100%	100%

Antioxidant Activity Test

Antioxidant Testing was conducted on extract and instant granules of Tea Leaves and Tea Benalu by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The percentage value of resistance to DPPH is calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorban blanko} - \text{Absorban sample}}{\text{Absorban blanko}} \times 100\%$$

The value of IC₅₀ (Inhibition Concentration 50) is obtained from the intersection of the line between 50% inhibitory power with the concentration axis using

the linear equation ($y = bx + a$), where $y = 50$ and x denote IC₅₀.

Test of α -Glucosidase Inhibition

Solution Making

The acarbose solution was prepared by weighing 10.0 mg, dissolved in 10.0 mL phosphate buffer pH 6.8 to obtain a solution concentration of 1000 ppm (standard parent solution), diluted to obtain a concentration of 0.1; 0.5; 1; 5; and 10 ppm.

Sample solution \pm 10.0 mg dissolved in 2 mL DMSO then sufficient volume with phosphate buffer pH 6.8 at 10.0 mL measuring flask to obtain a solution of sample with a concentration of 1000 ppm. Then diluted with concentrations of 25, 50, 100, and 200 ppm. • The enzyme solution was prepared by dissolving 5.056 mg of α -glucosidase enzyme dissolved in 50 mL buffer solution containing Bovine Serum Albumin (BSA) in cold conditions to obtain a parent solution of 2.5 U / mL enzyme. Then plucked 1 mL of mother liquor and diluted with 17 mL phosphate buffer pH 6.8 containing BSA until 0.15 U / mL enzyme solution was obtained.

Optimization of Substrate Concentration The reaction mixture consisted of 100 μ L phosphate buffer pH 6.8 and 50 μ L PNPG with concentrations of 1, 2, 3, 4, and 5 mM, incubated for 5 min at 37 ° C and 50 μ L α -glucosidase solution for test solution and Preparation of Reagent Solution for Inhibition Test 200 μ L 200-ml sodium carbonate enzyme for a blank solution, then incubated for 15 min at 37 ° C. 200 μ L sodium carbonate 200 mM was then added for the test solution and 50 μ L α -glucosidase solution for the blank solution. The result of the reaction of p-nitrophenol produced is read by absorbance at λ 405 nm with microplate reader.

Table 2. Procedure Test Inhibition Activity α -Glucosidase Instant Granul Tea Leaves and Tea Benalu

Reagen	Volume (μ L)			
	B ₁	B ₀	S ₁	S ₀
Sampel	-	-	2	2
DMSO	2	2	-	-
Dapur	98	98	98	98
Substrat	50	50	50	50
Inkubasi 37°C selama 5 menit				
Enzim	50	-	50	-
Na ₂ CO ₃	-	200	-	200
Inkubasi 37°C selama 15 menit				
Enzim	-	50	-	50
Na ₂ CO ₃	200	-	200	-
Absorbansi diukur pada λ = 405 nm				

B₁ = blanko

B₀ = kontrol blanko

S₁ = sampel dan standar (acarbose)

S₀ = kontrol sampel dan kontrol standar (acarbose)

Data analysis was done by calculating percentage of α -glucosidase inhibition. The data obtained from the absorbance measurement results can be calculated using the equation:

$$\%Reduction = \frac{Absorbance\ control - Absorbance\ test\ sample}{Absorbance\ control} \times 100\%$$

Information : Absorbance control (DMSO) = no sample (control-blank) Absorbance test sample = S1-S0
 S1 = Absorbance of sample with addition of enzyme S0 = Absorbance of sample without addition of enzyme
 Calculation of IC₅₀ uses linear regression equation, sample concentration as x axis and % inhibition as y axis. The value of IC₅₀ can be calculated from the equation $y = bx + a$ by using the formula:

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

(Sugiwati *et al.*, 2009)

III. RESULTS AND DISCUSSION

Extraction

Advantages of the MAE method, among others, can attract compounds better than conventional maceration methods. As long as the temperature extraction is maintained not exceeding 80°C to avoid damaging the active compound on the simplicia. Preparation of dry extract of white tea and parasite tea is done by vacuum dry method. This method is a method of drying using heating and vacuum. Dry extract produced 102.35 g and tea parasite 20.28 g. The yield of white tea extract yielded 30,012% tea parasite equal to 19,99%. 60% ethanol extracted the best catechin compounds on tea leaves at ethanol concentrations up to 99.5% (Quan *et al.*, 2006). The use of 60% less ethanol can extract the compound well in tea parasites, this is thought to be because the compound content of tea parasites dissolved in ethanol is less resulting in a smaller yield than in white tea.

Granuloid Antioxidant Activity

Examination of instant granule antioxidant activity of tea leaf extract and tea parasite was done on all granule formula with positive control of vitamin C and got IC₅₀ value in picture.

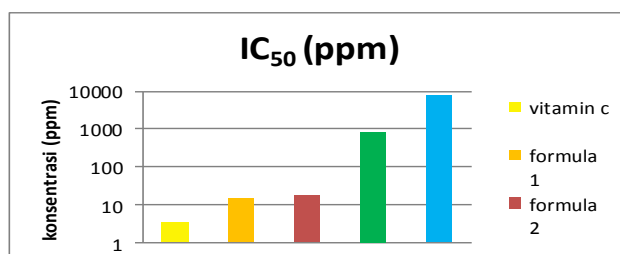


Figure 1 . Comparison of Antioxidant Activity Formula

Formula 1 antioxidant activity is stronger than formula 2 and 3. The synergistic effect on formula 1 of the active compound in white tea and parasite tea is

quercetin and epigallocatechin error, both of which can improve peripheral blood function of mononuclear membrane cells, not only by providing antioxidant activity, but also membrane fluidity modulation and trans membrane potential (Margina *et al.*, 2012).

Formula 3 has low antioxidant activity with IC₅₀ 863,75 ppm, because it contains one kind of extract of parasitic tea with content ± 15 times less than tea leaves. Flavonoid content in tea parasite based on UV spectrum and IR isolation on parasite is a compound a class of flavonoids substituted by aliphatic groups and carbonyl groups namely quercetin flavonoids (Fitrya, 2011). Quercetin has more activity as an anticancer because quercetin is an aglycone which, when bound to its glycos, becomes a glycoside. This compound can act as an anticancer to cell cycle regulation, interacting with estrogen receptor (ER) type II and inhibiting the enzyme tyrosine kinase (Lamson and Brignall., 2000).

The antioxidant activity of white tea granules and tea parasites is largely contributed by flavonoid content in both extracts. This compound has activity as an antioxidant because it can transfer an electron to free radical compounds (Kandaswami and Middleton, 1997). These compounds that have antioxidant activity can have an equivalence effect on the treatment of degenerative diseases. In tea leaf and parasite tea there is compound Epigallocatechin Error which has inhibition activity against cancer cell equal to 72,8% at concentration 10 ppm (Ohashi, K *et al.*, 2003). Epigallocatechin and quercetin have antioxidant activity that provides the effect of stabilizing cell membranes that contribute to the protection of cells from various pathologies (Margina *et al.*, 2012).

α -Glucosidase Enzyme Activation Test

The concentration of extract formulas used in this test is 0.1; 0.5; 1; 5; and 10 ppm. The increasing concentration of the inhibition power is also increasing. The linear regression equation between the concentration of the exhaust and the inhibitory power will be used to determine the value of IC₅₀. The enzyme activity of alpha-glucosidase which can be inhibited by 50% by the concentration of inhibitor is called IC₅₀. Inhibition of alpha-glucosidase enzyme is better if IC₅₀ value is smaller (Mohan *et al.*, 2013). The average value of IC₅₀ of the assay of this test was 0.044 ppm, whereas the mean values of IC₅₀ formula 1,2 and 3 were 68,212; 56.758 ppm and no activity. If seen the amount of IC₅₀ on tea leaf extract formulas and tea parasite has the potential to inhibit alpha-glucosidase enzyme. This is in line with the results of studies performed by Christianty *et al.*, where the IC₅₀ value in white tea leaves has the highest value compared with other tea plant extracts of 43.42 ppm (Christianty *et al.*, 2016).

Table 3. IC₅₀ granule data of tea leaf extract, parasite and acarbose

	AverageIC ₅₀
Acarbose (ppm)	0,044
Formula 1	68,212
Formula 2	56,758
Formula 3	> 1000

The test results show that acarbose has an inhibitory effect of alpha-glucosidase activity with IC₅₀ value = 0.044 ppm. Acarbose a pseudotetrasakarida (BM = 645) that acts inhibiting pancreatic alpha-amylase enzyme and alpha-glucosidase. Working as a competitive inhibitor of alpha-glucosidase enzymes in the gut are sucrase, dextrinase, maltase and glucoamylase (Lee, S.M., 1982).

At 370C and pH = 6.8 the alpha-glucosidase enzyme will catalyze the conversion of 4-nitrophenyl- α -D-glucopyranoside (PNPG) substrate into α -D-glucopyranoside and 4-nitrophenol (PNP), the yellow color formed by spetrophotometry at 405 nm. The enzyme activity is expressed in IU / L or mIU / mL. One unit is capable of releasing 1 μ molar PNP from PNPG per minute under pH = 6.8, 370C.

The results of Formula I have lower IC₅₀ values than formula II, indicating the ability to inhibit the weaker α -glucosidase enzyme than formula II. Formula II with white tea leaves extract content of 26,88% showed that alpha-glucosidase inhibition activity was stronger than combination with parasite tea extract. Benalu tea is a parasitic plant whose utilization in traditional herbs inhibits cancer cell invasion in vitro with 99% inhibition value at 10 μ g / mL concentration (Ohashi, et al. 2003) and as antihypertensive with dose of 200 mg / kg BW in rats (Nour and Erna, 2013). Flavonoids are known to inhibit alpha-glucosidase enzyme activity (Gu et al., 2015). Isolation of tea parasite shows the flavonoid content of quercetin (Fitriya, 2011). Flavanoid tea is a polyphenol compound with catechol as its main constituent and commonly called catechins (Martono and Rudi, 2014). In this study, catechins in white tea had an inhibitory effect on alpha-glucosidase enzyme by hydroxylation bond and substitution on β ring. So the formula II produces a stronger IC₅₀ value than if in combination with the parasite of tea.

IV. CONCLUSION

Formula II instant granules of white tea leaf extract and tea parasite are active as antioxidants and alpha glucosidase inhibitors, with a resistance value (IC₅₀) of 17.99 \pm 0.01 ppm and 56.758 ppm respectively.

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