IN VITRO α-GLUCOSIDASE INHIBITORY ACTIVITY OF VARIOUS TEA (*Camellia sinensis* L.) EXTRACTS

Fransiska Maria Christianty, Faculty of Pharmacy, University of Jember, Jl. Kalimantan I/2 Jember, East Java, Indonesia, fransiska.farmasi@unej.ac.id**; Diana Holidah,** Faculty of Pharmacy, University of Jember, Jl. Kalimantan I/2 Jember, East Java, Indonesia**; Yasmin,** Graduated student, Faculty of Pharmacy, University of Jember, Jl. Kalimantan I/2 Jember, East Java, Indonesia

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia [1]. DM is classified based on the pathogenic process that leads to hyperglycemia as opposed to earlier criteria such as age of onset or type of therapy. There are two broad categories of DM, designated type 1 and type 2 [2]. There were 10 million cases of diabetes in Indonesia in 2015. The number of these cases is predicted to continue rising [3].

Therapeutic approaches for the treatment of type 2 diabetes, such as diet, sulphonylurea, metformin and insulin therapy, are all successful in decreasing fasting glucose levels. The importance of postprandial hyperglycaemia has led to the development of new agents to control this important aspect of diabetes.Thus, α-glucosidase enzyme has become one of important target in the treatment of type-2 diabetes [4,5].

α-glucosidase inhibition will slow the digestion of carbohydrate and absorbtion of monosaccahrides in the proximal jejenum [6]. Acarbose is an oligosaccharide which reversibly inhibits intestinal α-glucosidase enzymes responsible for digestion of complex carbohydrates and disaccharides to absorbable monosaccharides [7]. The most common adverse effect of acarbose is abdominal discomfort associated with flatulence and diarrhoea [8]. The undesirable side effects that exist underlie the emergence of research for alternative therapy of type 2 diabetes, particularly through the mechanism of inhibition enzyme α-glucosidase.

Tea (*Camellia sinensis* L.) is annual plant that widely spread in Southest Asia, India, South China, Northwest Laos, Thailand and Burma. Tea is one of the most popular beverages consumed worldwide [9]. Various studies have reported beneficial effect of tea extract, including for diabetes mellitus therapy. Previous studies showed that tea extract have antidiabetic activity in mice induced by streptozotocin [10]. Antidiabetic activity of tea extract have been reported by Yang and Koh. The results showed that black tea extract had higher activity to inhibit α-glucosidase than green tea extract. The present study was designed to compare in vitro α-glucosidase inhibitory activity of various tea extracts, such as black tea, green tea, oolong tea, and white tea.

MATERIALS AND METHODS

Materials

Black tea, green tea, oolong tea and white tea were obtained from PT Perkebunan Nusantara XII Jember, Indonesia, α-glucosidase enzyme (from *Saccharomyces cerevisiae*, Sigma-Aldrich, Singapore), acarbose (Sigma-Aldrich, Singapore), *p*-nitrophenyl-α-d-glucopyranoside (PNPG) (Sigma-Aldrich, Singapore), sodium carbonate (Na_2CO_3) sodium hydrogen phosphate (Na_2HPO_4) (Merck-USA), aquadest.

Methods

Preparation of tea extract

100 g of each various tea was soaked separately in 1.000 mL boiled water (90 ^{0}C) for 15 min. Filtrate was filtered using a Buchner funnel. Then, dry extracts were made using freeze dryer [12]. The tea dry extracts were further diluted in phosphate buffer (pH 6.8) to obtain serial dilution of tea extracts (16, 32, 48, 64, dan 72 µg/mL).

Preliminary test of enzymatic reactions

Optimization was done to determine enzyme concentration that has an optimum activity. The maximum wavelength, concentration of substrate, and incubation tme were refers to the previous research that has been conducted by Zuhro *et al.* [13]. The maximum wavelength, concentration of substrate, and incubation time were chosen as 415 nm, 10 mM, and 60 min, respectively.

α-glucosidase inhibition assay

α-Glucosidase inhibitory activities were evaluated according to the method described by Moradi-Afrapoli *et al* [14], with some modifications. The enzyme solution contained 20 µL α-glucosidase and 120 µL 0.1 M phosphate buffer (pH 6.8). p-Nitrophenyl-α-D-glucopyranoside (10 mM) in the same buffer (pH 6.8) was used as substrate solution. Ten microliters of test samples were mixed with enzyme solution in microplate wells and incubated for 15 min at 37^{0}C. Twenty microliters of substrate solution were added and incubated for an additional 30 min. The reaction was terminated by adding 80

µL of 0.2 M sodium carbonate solution. Absorbance was measured with a microplate reader at 415 nm, while reaction solution without tea extracts was used as control. The solution without glucosidase was used as blank, and acarbose was used as positive control. Each experiment was conducted in triplicate. The glucosidase inhibitory activity was expressed as % inhibition and was calculated as follows:

$$\alpha\text{-glukosidase inhibition (\%)} = \frac{C-S}{C} \times 100$$

(C= absorbance of control, S= absorbance of sample).

The IC_{50} values were determined from plots of percent inhibition (y) versus inhibitor concentration (x) and were calculated by linear regression analysis. The linear equation y= bx + a was used to determine IC_{50} as follows:

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

Statistical analysis

All data were expressed as the mean ± SD. Statistical analysis was performed by one way Anova. Significant difference was considered at p value < 0.05 [15].

RESULTS

Rendemen of Tea Extract

Infusion method was used for preparing tea extract. Rendemen of black tea extract was the highest, as seen in **Table 1**.

Table 1. Rendemen of tea extract

No	Varian	Rendemen (% w/w)
1	Black tea	22,1
2	Green tea	18,25
3	Oolong tea	17,09
4	White tea	14,9

Optimization of Enzyme Concentration

Selected enzyme concentration was determined by its maximum absorbance as shown in **Table 2**. Concentration of enzyme 0,4 U/mL was chosen in order to get optimum activities.

Table 2. Absorbance in different enzyme concentrations

Concentration of Enzyme (U/mL)	Absorbance
0,1	0,0063
0,2	0,0163
0,3	0,0240
0,4	0,4347
0,5	0,6470

α- glucosidase inhibition assay

Selected The inhibitory potency of black tea, green tea, oolong tea, and white tea extracts on α-glucosidase activity were summarized in **Table 3**. Results showed that white tea extract has the highest α-glucosidase inhibitory activity, followed by green tea, black tea, and oolong tea. There was a significant difference observed at p=0.004. LSD's post-hoc tests were then performed, showing that IC_{50} of white tea extract was statistically significant compared to black tea (p=0,03) and oolong tea (p=0,02) extracts, but it was not significant compared to green tea extract (p=0.635). IC_{50} of black tea extract was also not significant (p=0,833) compared to oolong tea extract. Unexpectedly, acarbose as positive control showed the highest IC50.

Table 3. IC_{50} of various tea extract

Groups	Sample	$IC_{50} \pm$ SD (µg/mL)*
1	Black tea	$54,86 \pm 1,19^a$
2	Green tea	$44,79 \pm 1,64^b$
3	Oolong tea	$55,46 \pm 6,21^a$
4	White tea	$43,42 \pm 1,88^b$
5	Acarbose	$7.111,11 \pm 82,28$

*** The same superscript indicated no significant difference (n=3)**

DISSCUSSION

α-Glucosidase activity was measured by the release of p-nitrophenol from α-PNPG (**Figure 1**). The yellow p-nitrophenol released was read at 400 nm in a spectrophotometer [16].

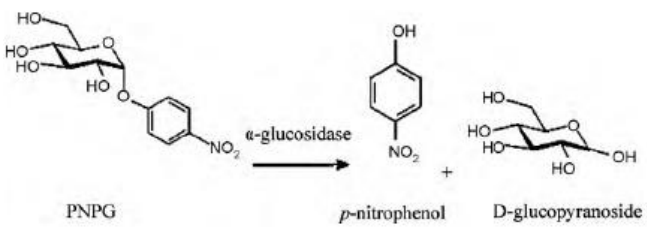

Figure 1. The α-glucosidase-catalyzed reaction

Results showed that white tea extract has higher α-glucosidase inhibitory activity with an IC_{50} of (43,42 ± 1,88) µg/mL, followed by green tea (IC_{50} = 44.79 ± 1,64 µg/mL), black tea (IC_{50} = 54,86 ± 1,19 µg/mL), and oolong tea (IC_{50} = 55,46 ± 6,2 µg/mL). In line with the results, Bait compared the activity of two tea extract in alloxan induced diabetic rat and it was shown that green tea is more effective than black tea in decreasing blood glucose level [17]. In different study, oolong tea had lowest hypoglycemic activity, compared with black tea and green tea extract [12]. So far, green tea was the highest activity in reducing blood glucose level. It may be correlated with total polifenol composition of each tea extract. Compared with

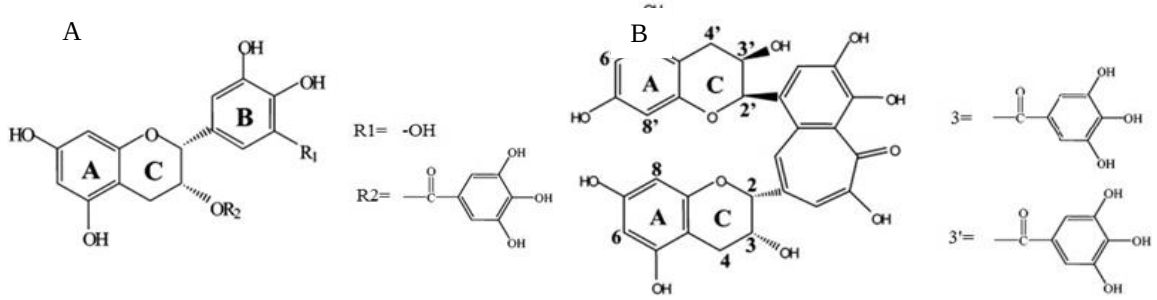

Figure 2: The structure of (A) epigallocatechin gallate dan (B) theaflavin digallate

black tea and oolong tea, green tea extract had the highest total polifenol. [12].

Different degree of enzymatic oxidation or fermentation in tea manufacturing process will affect the phytological components. Concentration level of polyphenols in unfermented tea leaf, such as in white tea and green tea is higher than oolong tea (partially fermented)
and black tea (fully fermented). The simply manufacturing process of green tea and white tea leads to the high concentration of catechins. Fermentation process in black tea and oolong tea will oxidase catechins into theaflavin [18]. In contrast, other previous reports showed that water extract of black tea has the highest α-glucosidase inhibitory activity, followed by white tea and oolong tea [19]. Environment factors, such as climate and soil can contribute to the different quality of plant, thus will affect the activity of phytochemical component [20,21,22].

The major polyphenol components of tea, in particularly flavonoids, that has an important role in α-glucosidase inhibitory activity is catechins. Gallated catechins (catechin 3-gallate (CG), gallocatechin-3-gallate (GCG), epicatechin 3-gallate (ECG), epigallocatechin 3-gallate (EGCG)) and ungallated catechins (catechin, gallocatechin (GC), epicatechin (EC), and epigallocatechin (EGC)) showed good rat intestinal α-glucosidase inhibition with IC_{50} value ranging from 40 to 53 μM. Moreover, theaflavins, the oxidative coupling product of catechins, found only in oolong and black tea also showed maltase inhibitory effect. Both, catechin and theaflavin were have α-glucosidase inhibitory effect [22,23,24].

Although the exact inhibition mechanisms are still unclear, binding mode analyses using molecular docking simulations, have been performed to predict the binding interactions between the polyphenols and the enzymes [11,24]. The interaction between flavonoids and yeast α-glucosidase (YAHG) using a flexible docking method showed that 3', 4' dihydroxyl groups of B ring and 3-OH of C ring played a more important role in the inhibition activity than other hydroxyl groups. Both hydroxyl groups of B ring directly interacted with the active-site residues of YAGH to inhibit enzyme activity and 3-OH of C ring seemed to be necessary to maintain the proper binding orientation of flavonoid molecules, thereby making the hydroxyl groups of B ring interact with active-site residues tightly in the hydrophobic pocket of YAGH [25,26].

In different studies, plant extracts containing catechin 3-gallates, in particular epigallocatechin gallate, are potent inhibitors of α-glucosidase activity. The presence of a gallate group esterified to the 3-position of the C-ring has been suggested to be critical for the interaction of flavan-3-ols with the enzyme [27]. The following structure enhanced the inhibitory activity: the unsaturated C ring, 3-OH, 4-CO, the linkage of the B ring at the 3 position and the hydroxyl substitution on the B ring [28].

Acarbose, a potent α-glucosidase inhibitors gave the highest IC_{50} (7.111,11 $\pm$ 82,28 μg/mL). That was mean that acarbose can not be compared as a positive control because of low potency of inhibition. Previous researches explained that acarbose strongly inhibited mammalian α-glucosidase, whereas no or less inhibition was observed in yeast α-glucosidase [29,30,31].

CONCLUSION

The result showed that various tea extracts (black tea, green tea, oolong tea, and white tea) could inhibit the activity of α-glucosidase with different IC_{50}. This findings suggested that tea extracts, especially white tea and green tea extracts may have a potential effect in treatment of type-2 diabetes.

REFERENCES

1. American Diabetes Association [Internet]. America: American diabetes association (2015) [cited 2015 December 28]. Available from: http://www.diabetes.org/
2. Kasper DL, Hauser SL, Jameson JL, Fauci AS, Longo DL, Loscalzo J. *Harrison's: principles of internal medicine*. New York: McGraw-Hill (2015).
3. International Diabetes Federation [Internet]. Belgia: International diabetes federation (2015) [cited 2015 December 28]. Available from: http://www.idf.org/

4. Badan Pengawas Obat dan Makanan Republik Indonesia. Diabetes mellitus. *Informasi Produk Terapetik*. **19,** 1-12 (2009).
5. Baron AD. Postprandial hyperglycaemia and α-glucosidase inhibitors. *Diabetes Res Clin Pr*. **40,** 51-55 (1998).
6. Lebovitz HE. Alpha-glucosidase inhibitors. *Endocrin Metab Clin*. **26,** 539-551 (1997).
7. Balfour JA, McTavish D. Acarbose. *Drugs*. **46,** 1025-1054 (1993).
8. Walker R, Whittlesea C. *Clinical pharmacy and therapeutics*. Edinburgh: Elsevier (2012).
9. Effendi IDS, Syakir M, Yusron M, Wiratno. *Budidaya dan pasca panen teh*. Bogor: Pusat Penelitian dan Pengembangan Perkebunan (2010).
10. Tang W, Li S, Liu Y, Huang MT, Ho CT. Anti-diabetic activity of chemically profiled green tea and black tea extracts in a type 2 diabetes mice model via different mechanisms. *J Funct Foods*. **5,** 1784-1793 (2008).
11. Yang X, Kong F. Evaluation of the in vitro a-glucosidase inhibitory activity of green tea polyphenols and different tea types. *J Sci Food Agr*. **96,** 777-782 (2016).
12. Holidah D, Christianty FM. Uji aktivitas antidiabetes ekstrak teh hitam, teh olong dan teh hijau secara in vivo. Prosiding Seminar Nasional Current Challenges in Drug Use and Development: Tantangan Terkini Perkembangan Obat dan Aplikasi Klinis (2015).
13. Zuhro F, Puspitasari E, Muslichah S, Hidayat MA. Aktivitas inhibitor α-glukosidase ekstrak etanol 70% daun kenitu (*Chrysophyllum cainito L*.). *Pustaka Kesehatan*. **4,** 1-7 (2015).
14. Moradi-Afrapoli F, Asghari B, Seidnia S, Ajani Y, Mirjani M, Malmir M, et al. In vitro α-glucosidase inhibitory activity of phenolic constituent from aerial parts of *Polygonum hyrcanicum*. *DARU J Pharm Sci*. **20,** 1-6 (2012).
15. Dahlan MS. *Statistika untuk kedokteran dan kesehatan: uji hipotesis dengan menggunakan SPSS Seri 1: evidence based medicine*. Jakarta: Arkans Entertainment and Education in Harmony (2004).
16. Li KB, Chan KY. Production and properties of alpha-glucosidase from *Lactobacillus acidophilus. Appl Environ Microb*. **46,** 1380-1387 (1983).
17. Bait Y. Efektivitas pemberian seduhan teh hitam, teh hijau (*Camelia sinensis* var. assamica), teh daun murbei (*Morus kanva*) dan campurannya dalam aktivitas hiploglikemik pada tikus (*Rattus norvegicus*) diabetes. Institut Pertanian Bogor (2010).
18. Harbowy, Balentine, Davies & Cai. Tea Chemistry. *Critical Reviews in Plant Sciences*. **16,** 415-480 (1997).
19. Kwon YI, Apostolidis E, Shetty K. Inhibitory potential of wine and tea against α amylase and α-glucosidase for management of hyperglycemia linked to type 2 diabetes. *J Food Biochem*. **32,** 15-31 (2008).
20. Setyamidjaja D. *Teh: Budidaya dan pengolahan pascapanen*. Yogyakarta: Kanisius (2000).
21. Woodward, FI. *Climate and plant distribution*. Melbourne: Cambridge University Press (1987).
22. Haslam E. Thoughts on thearubigins. Phytochemistry. **64,** 61-73 (2003).
23. Matsui, Tanaka, Tamura, Toshima, Tamaya, Miyata et al. α-Glucosidase inhibitory profile of catechins and theaflavins. *J Agr Food Chem*. **55,** 99-105 (2007).
24. Kamiyama, Sanae, Ikeda, Higashi, Minami, Asano et al. In vitro inhibition of α-glucosidases and glycogen phosphorylase by catechin gallates in green tea. *Food Chem*. **122,** 1061-1066 (2010).
25. de Melo EB, da Silveira Gomes A, Carvalho I. α- and β-glucosidase inhibitors: chemical structure and biological activity. *Tetrahedron*. **62,** 10277-10302 (2006).
26. Xu H. Inhibition kinetics of flavonoids on yeast α-glucosidase merged with docking simulations. *Protein Peptide Lett*. **17,** 1270-1279 (2010).
27. Yilmazer-Musa, Griffith, Michels, Schneider, Frei. Grape seed and tea extracts and catechin 3-gallates are potent inhibitors of α-amylase and α-glucosidase activity. *J Agr Food Chem*. **60,** 8924-8929 (2012).
28. Tadera, Minami, Takamatsu, Matsuoka. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. *J Nutr Sci Vitaminol*. **52,** 149-153 (2006).
29. Shinde J, Taldone T, Barletta M, Kunaparaju N, Hu B, et al. α-Glucosidase inhibitory activity of *Syzygium cumini* (Linn.) skeels seed kernel in vitro and in goto–kakizaki (GK) rats. *Carbohyd Res*. **343,** 1278-1281 (2008).
30. Kim YM, Wang MH, Rhee HI. A novel a-glucosidase inhibitor from pine bark. *Carbohyd Res*. **339,** 715-717 (2009).
31. Oki T, Matsui T, Osajima Y. Inhibitory effect of a-glucosidase inhibitors varies according to its origin. *J Agr Food Chem*. **47,** 550-553 (1999).