The effect of combination of active fraction *Andrographis paniculata* (Burm.f) Ness and *Centella asiatica* (I) Urban on the alpha glucosidase inhibitor and antioxidant activities

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**ABSTRACT**

The *Andrographis paniculata* and *Centella aistica* extract have been reported that had an anti-diabetic effect. However, the specific mechanism and the effect combination of both were not yet reported. This study was purposed to determine the potency of extract, fractions and the combination of *Andrographis paniculata* (AP) and *Centella asiatica* (CA) active fraction to inhibit alpha glucosidase enzyme and its ability to reduce DPPH radical. AP and CA were extracted using 50% ethanol then fractionated with solvents under different polarity levels. The inhibiting activity to alpha glucosidase enzyme and antioxidant activity of each fractions was tested. The most active fractions from AP and CA were then combined and re-tested for activity. The results report that both of AP and CA had inhibition of alpha glucosidase activity and antioxidant activity. Based on calculation combination index (CI) of of active fraction of AP and CA showing in alpha glucosidase activity had a antagonist action and antioxidant had a sinercic action. Therefore, combination of AP and CA not has not recommended for alpha glucosidase inhibitor but the combination has ability to reduce DPPH radical.

**Keywords:** Diabetes mellitus, *Andrographis paniculata* (Burm. F.) Nees, *Centella asiatica* (I) Urban, alpha glucosidase, antioxidant

**INTRODUCTION**

Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson’s and Alzheimer’s diseases, cancers. Antioxidants can reduce free radicals so that can reduce the incidence of several degenerative diseases. Secondary metabolite from plant such usphenolic acids, polyphenols and flavonoids can act as antioxidant (Mahdi-Pour et al., 2012). The antioxidants from plants is highly recommended because it has very minimal effects compared to synthetic antioxidants.

Hyperglycemia is a medical condition in the form of an increase in the level of glucose in the blood that exceeds the normal limitcaused by abnormalities in insulin secretion, insulin action and other things (Shaw et al., 2010). This condition is one of the characteristics of diabetes mellitus (DM). Some synthetic drugs have been developed to reduce glucose levels but still cause side effects that are not desirable if used continuously. Alpha glucosidase enzyme is an enzyme that plays a role in the digestive system. This enzyme plays a role in turning polysaccharides into glucose. Inhibition of this enzyme, is a treatment to reduce blood glucose levels for diabetics (Alam et al., 2017). *Andrographis paniculata* (Burm. F.) Nees (AP) is one of some plant who developed for diabetes drugs. AP is reported to be able to reduce blood glucose levels in diabetic rats (Nugroho et al., 2012; X-F. Zhang and Tan, 2000). In addition, other herbs that have hypoglycemic activity and the potential as antidiabetic are *Centella asiatica* (I) Urban (CA). Ethanol extract of CA was able to reduce blood glucose levels and inhibit significant pancreatic β cell damage in rats that had been induced by alloxan (Chauhan et al., 2010; Sasikala, et al., 2015).
The inhibition of alpha glucosidase enzymes by AP and CA extracts has been carried out by (Subramanian et al., 2008) and (Supkamonseni et al., 2014) both extracts of this plant have alpha glucosidase inhibitory ability. Based on the results of the literature search, no research has been found on the inhibition of alpha glucosidase enzymes by AP or CA fractions, so that which fraction plays a role as an alpha glucosidase enzyme inhibitor. This research was conducted to explore which fraction of the two plants that acted as alpha glucosidase enzyme inhibitors and see its ability to reduce free radicals and then see the effect of the combination of the active fractions of the two plants on the inhibition of alpha glucosidase enzyme

MATERIAL AND METHODS

Plant Material
AP and CA were harvested from Kulon Progo (Yogyakarta, Indonesia). Andrographolide 98% was purchased from Sigma Aldrich (St. Louis, Missouri), asiaticoside 98.5% was purchased from Fluka (Switzerland), TLC Silica gel 60G F254 was obtained from merck (Darmstadt, Germany), p-nitrofenil-alpha-D-glukopiranoside, alpha glucosidace from VSaccharomyces cerevisiae, 2,2-Diphenyl-1-picrylhydrazyl were purchased from Sigma Aldrich (St. Louis, Missouri).

Extraction and fractionation
AP and CA were extracted by a maceration method using 50% (v/v) ethanol for 5 days then filtered and the residue was eliminated. The filtrate was collected and concentrated using a rotary evaporator. The extracts were then fractionated using hexane-chloroform (1:1) v/v and chloroform solvents for AP extract and chloroform and ethyl acetate to CA extract.

Qualitative analysis extract and fraction of AP and CA
To know the major component secondary metabolite of AP and CA analyzed using TLC. TLC extract and fractions AP were carried out using silica gel 60 F254 as stationary phase and mobile phase of chloroform-methanol (9:1) while CA extract and fraction using silica gel 60 F254 as stationary phase and mobile phase of chloroform:acetone:formic acid (10:2:1).

Alpha glucosidase Inhibitory assay
Alpha glucosidase enzyme inhibition test using the method performed by Xu et al (2018) with a minor modification. Each fraction test solution was taken 60μL and then added 60μL of a solution containing alpha glucosidase with a concentration of 0.2U/mL and in 0.1M phosphate buffer pH 7. The solution was then added 30μL of a solution containing 0.5mM p-nitrofenyl-alpha-D-glucopiranoside as a substrate and incubated for 30min at 37°C. The reaction was stopped by adding 40μL of a solution containing 0.2M sodium carbonate solution. The absorbance solution is measured at wavelength (399 nm). The absorbance value obtained is then used to measure the presentation value of enzyme activity

Inhibitory activity = \( \frac{s_1 - s_0}{s_1} \) \times 100%

S1 = absorbance without the addition of extract; S0 = absorbance with the addition of extract. The combination is based on IC50 calculations from AP and CA active fractions. Dose combination was used, 0.5; 1; and 2 times IC50 Value. Calculation of combination index (CI) followed Ichite et al. (2009)

DPPH Radical Scavenging Assay
Antioxidant activity extract, fraction of AP and CA fraction was measured using DPPH radical capture test (1,1-diphenyl-2-picryl-hydrazyl). A total of 50μL of sample was added with 1mL of 0.4mM DPPH and 3.95mL of methanol. The mixture was incubated at room temperature for 30min and then the absorbance was measured at a wavelength of 517nm. Control or blank methanol (without extract) and ascorbic acid as standard were analyzed in the same way. All analyzes were replicated three times. Percent (%) The fishing activity is calculated as follows:

% Antiradical activity = \( \frac{A_0 - A_1}{A_0} \times 100\% \)

Where A0 is the absorbance of the control and A1 is the absorbance of the test sample(Sa'dar et al., 2017). Calculation of combination index (CI) followed Ichite et al.(2009)

RESULT AND DISCUSSION
Alfa glucosidase is an enzyme in the digestive system which functions to convert disaccharide molecules into glucose molecules through the breaking of 1.6 glycoside bonds. Inhibition this enzyme can suppress the release and absorption of glucose in the blood so as to reduce the incidence of hyperglycemia which can indirectly be used as a therapy to reduce blood glucose levels in diabetics.

Inhibition of alpha glucosidase enzyme by extracts and fraction of AP (Figure 1a) indicate that the extract and fraction of AP have the ability to inhibit the action of the alpha glucosidase enzyme.
The highest potential alpha glucosidase inhibitory activities were found in the HKAP (Table 1). Andrographolide, which is a marker of bitter active compounds and found in the chloroform fraction (Figure 2) does not show an inhibitory effect, this can be seen from the IC\textsubscript{50} value in the chloroform fraction. Andrographolide is a compound which has a ring of γ lactone subsidized hydroxy group. The hydroxy group substitution in the lactone ring can reduce the inhibitory effect of alpha glucosidase enzyme (Prabhakar Reddy \textit{et al.}, 2009). The presence of lactone groups in these compounds is thought to be the cause of this compound having an inhibitory effect on alpha glucosidase enzymes (Yin, \textit{et al.}, 2014). Lactone compounds have been widely reported to have pharmacological effects, one of which is the ability to inhibit alpha glucosidase enzymes (Oki \textit{et al.}, 1999; Yang \textit{et al.}, 2015; Y. Zhang \textit{et al.}, 2013) (Oki \textit{et al.}, 1999; Zhang \textit{et al.}, 2013; Yang \textit{et al.}, 2015).

Table 1. The IC\textsubscript{50} value of alpha glucosidase inhibitory extract and fraction of AP and fraction of CA

| No. | Extract and fraction of AP | IC\textsubscript{50} (µg/mL) | Extract and fraction of CA | Group | IC\textsubscript{50} (µg/mL) |
|-----|---------------------------|-----------------|---------------------------|-------|-----------------|
| 1   | AP extract                | 1870±264        | CA extract                | 1345±57|
| 2   | HKAP                      | 310,25 ± 18,36  | KCA                       | 924,27 ± 47,53 |
| 3   | KP                        | >2000           | EACA                      | 346 ± 8,62 |
| 4   | WAP                       | >2000           | WCA                       | >2000  |
| 5   | Acarbose                  | 78,99 ± 9,84    | Acarbose                  | 78,99 ± 9,84 |

Figure 1. Inhibitory effect extract and fraction of AP (a) and CA (b) on alpha glucosidase enzyme

Figure 2. TLC Profile AP extract (1), HKAP (2), KAP (3), WAP (4), and andrographolide (5) using the stationary phase of silica gel 60 F\textsubscript{254} mobile phase chloroform: methanol (9: 1)

Figure 3. Inhibitory effect alpha glucosidase enzyme by combination ½ IC\textsubscript{50}HKAP + ½ IC\textsubscript{50} EACA (1), ½ IC\textsubscript{50} HKAP + IC\textsubscript{50} EACA (2), ½ IC\textsubscript{50} HKAP + 2X IC\textsubscript{50} EACA (3), IC\textsubscript{50} HKAP + ½ IC\textsubscript{50} EACA (4), IC\textsubscript{50} HKAP + IC\textsubscript{50} EACA (5), IC\textsubscript{50} HKAP + 2X IC\textsubscript{50} EACA (6), 2X IC\textsubscript{50}HKAP + ½ IC\textsubscript{50} EA (7), 2X IC\textsubscript{50} HKAP + IC\textsubscript{50} EACA (8), 2X IC\textsubscript{50} HKAP + 2X IC\textsubscript{50} EACA (9)

The highest potential alpha glucosidase inhibitory activities were found in the HKAP (Table 1). Andrographolide, which is a marker of bitter active compounds and found in the chloroform fraction (Figure 2) does not show an inhibitory effect, this can be seen from the IC\textsubscript{50} value in the chloroform fraction. Andrographolide is a compound which has a ring of γ lactone subsidized hydroxy group. The hydroxy group substitution in the lactone ring can reduce the inhibitory effect of alpha glucosidase enzyme (Prabhakar Reddy \textit{et al.}, 2009). The presence of lactone groups in these compounds is thought to be the cause of this compound having an inhibitory effect on alpha glucosidase enzymes (Yin, \textit{et al.}, 2014). Lactone compounds have been widely reported to have pharmacological effects, one of which is the ability to inhibit alpha glucosidase enzymes (Oki \textit{et al.}, 1999; Yang \textit{et al.}, 2015; Y. Zhang \textit{et al.}, 2013) (Oki \textit{et al.}, 1999; Zhang \textit{et al.}, 2013; Yang \textit{et al.}, 2015).
Inhibition of alpha glucosidase enzymes by extracts and fractions CA is shown in Figure 1. The highest potential alpha glucosidase inhibitory activities were found in the EACA (Table I). The flavonoid content found in this fraction plays a role in the inhibition process of alpha glucosidase enzyme. Flavonoid compounds have been reported well in vitro (Hong et al., 2013) or in silico about its ability to inhibit alpha glucosidase enzymes (Proença et al., 2017). The combination of HKAP and EACA paw fraction has an antagonistic effect (CI = 1.45-1.33) (Figure 2) The same action target by these two fractions is thought to be the cause of the antagonistic effect.

DPPH radical scavenging by extracts and fractions of AP and CA shows that as concentration increases, DPPH radical scavenging are greater. The lowest IC50 value on AP is indicated by the water fraction and CA in the ethyl acetate fraction (Table II). Flavonoids are a group of secondary metabolites that play a role in reducing free radicals. Flavonoid compounds can provide proton donors to reduce radical DPPH (Brunetti, Di Ferdinando, Fini, Pollastri, and Tattini, 2013). AP and CA are reported to have flavonoids. Based on the quantitative analysis, it was found that the EA fraction of CA had contained flavonoids which were indicated the EACA fraction contained a spot that glowed on UV 366 light.

CONCLUSION

The combination of active fraction of AP and CA has not recommended for alpha glucosidase inhibitor but the combination has ability to reduce DPPH radical.
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