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

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ABSTRACT

The *Andrographis panicullata* and *Centella asistica* extract have been reported that had a 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 panicullata* (AP) and *Centella asistica* (CA) active fraction to inhibit alpha glucosidase enzymeand 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 result reported 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 glucocidase activity had a antagonist action and antioxidant had a sinergic action. Therefore, combination of AP and CA not has not recomended for alpha glucocidase 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 thet can reduce the incidence of several degenerative diseases. Secondary metabolite from plant such usphenolic acids, polyphenols and flavonoidscan 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).

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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, O, asiaticoside 98,5% was purchased from Fluka (Switzerland),TLC Silica gel 60G F254 was obtained from merck (Darmstadt,m Germ any) , p-nitrofenil-alfa-D-glukopiranoside, alpha glucosidace from vSaccharomyces cerevisiae. 2,2-Diphenyl-1-picrylhydrazyl were purchased from Sigma Aldrich (St. Louis, MO).

Extraction and fractination

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 filtrat 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 40fL 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

$$\text{Inhibitory activity} = \frac{S_1 - S_0}{S_1}\ x\ 100\ \%$$

S1 = absorbance without the addition of extract, S0 = absorbance with the addition of extract. The combination is based on IC_{50} calculations from AP and CA active fractions. Dose combination use 0,5; 1; and 2 times IC_{50} Value. Calculation of combination index (CI) followed Ichite *et al.* (2009)

DPPH Radical Scavenging Assay

Antioxidant activity extract, fraction ofAP 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 asorbic acid as standard were analyzed in the same way. All analyzes were replicated three times. Percent (%) The fishing activity is calculated as follows:

$$\%\ \text{Antiradical activity} = \frac{A^\circ - A1}{A^\circ} X 100\%$$

Where A_0 is the absorbance of the control and A1 is the absorbance of the test sample(Safdar *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.

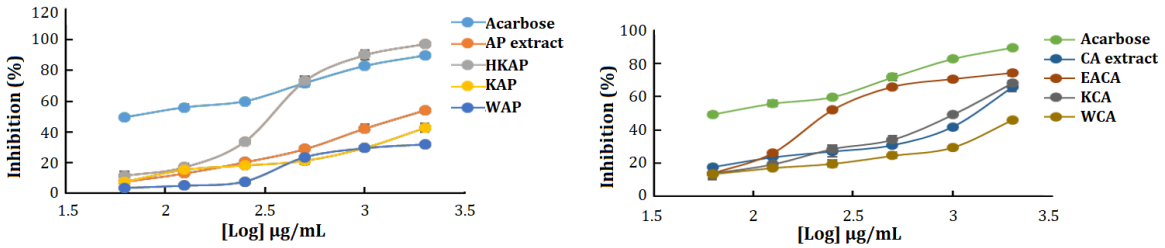

Figure 1. Inhibitory effect extract and fraction of AP (a) and CA (b) on alpha glucocidase enzyme

Table I. The IC_{50} value of alpha glucosidase inhibitory extract and fraction of AP and fraction of CA

No.	Extract and fraction of AP		Extract and fraction of CA	
	Group	IC_{50} (µg/mL)	Group	IC_{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

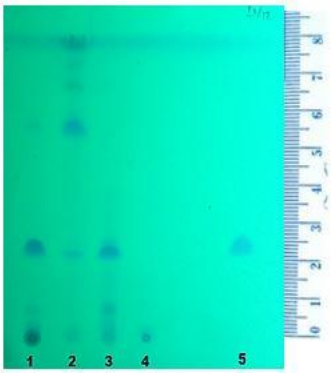

Figure 2. TLC Profile AP extract (1), HKAP (2), KAP (3), WAP (4), and andrographolide (5) using the stationary phase of silica gel 60 F_{254} mobile phase chloroform: methanol (9: 1)

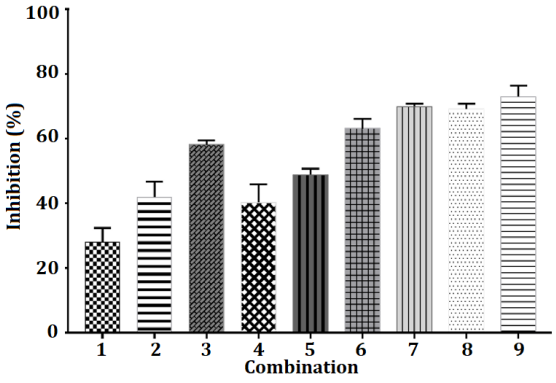

Figure 3. Inhibitory effect alpha glucosidase enzyme by combination ½ IC_{50}HKAP + ½ IC_{50} EACA (1), ½ IC_{50} HKAP + IC_{50} EACA(2), ½ IC_{50} HKAP + 2X IC_{50} EACA (3), IC_{50} HKAP + ½ IC_{50} EACA (4), IC_{50} HKAP + IC_{50} EACA (5), IC_{50} HKAP + 2X IC_{50} EACA (6), 2X IC_{50} HKAP + ½ IC_{50}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

The highest potential alpha glucosidase inhibitory activities were found in the HKAP (Table I). 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_{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 *et al.*, 2009). The presence of lactone groups in these compounds is thought to..p be the cause of this compound having an inhibitory effect on alpha glucosidase enzymes (Yin, *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 *et al.,* 1999; Yang *et al.*, 2015; Y. Zhang *et al*., 2013) (Oki *et al*., 1999; Zhang *et al*., 2013; Yang *et al*., 2015)

Table II. IC_{50} value DPPH radical scavenging of extract and fraction AP and fraction CA

No	Extract and fraction AP		Extract and fraction CA	
	Group	**IC_{50} ± SD (µg/mL)**	**Group**	**IC_{50} ± SD (µg/mL)**
1.	AP Extract	215.07±17.44	CA Extract	31.16±5.01
2.	HKAP	313.65±34.27	KCA	155.30±9.57
3.	KAP	472.73±13.99	EACA	17.86±6.49
4.	WAP	117.06±21.09	WCA	100.81±17.93
5.	Ascrorbic acid	4.18±0.1	Ascorbic acid	4.18±0.1

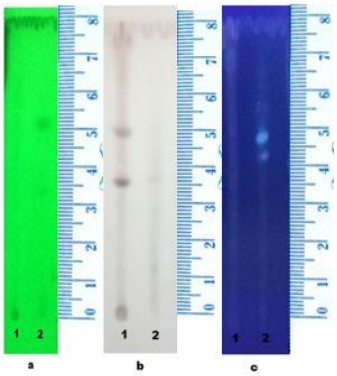

Figure 4. TLC profile of KCA (1) and EACA (2) CA fraction on UV_{254} nm (a), visible light after Liebermann-Burchard reagent spray (b), and at UV_{366} nm after sitroborate reagent spray (c) using silica gel 60 F254 and mobile phase chloroform:acetone:formic acid (10:2:1)

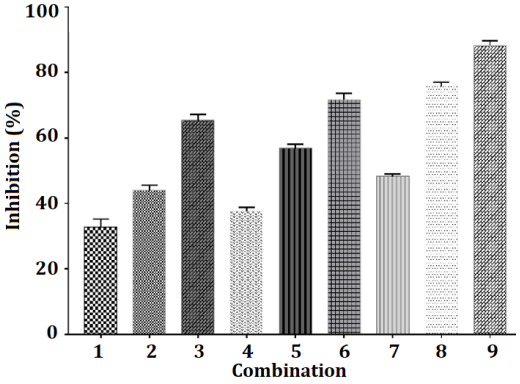

Figure 5. DPPH radical scavenging bycombination ½ IC_{50}WAP + ½ IC_{50} EACA (1), ½ IC_{50}WAP + IC_{50} EACA (2), ½ IC_{50}WAP + 2X IC_{50} EACA (3), IC_{50}WAP + ½ IC_{50} EACA (4), IC_{50}WAP + IC_{50} EACA (5), IC_{50}WAP + 2X IC_{50} EACA (6), 2X IC_{50} WAP + ½ IC_{50} EA (7), 2X IC_{50}WAP + IC_{50} EACA (8), 2X IC_{50}WAP + 2X IC_{50} EACA (9)

Inhibition of alpha glucosidase enzymes by extracts and fractions CA is shown in Figure 1.b. 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 and shows that as concentration increases, DPPH radical scavenging are greater. The lowest IC_{50} 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} after spraying with sitroborate (Figure 4). Flavonoid compounds will glow when observed under UV_{366} light. The antioxidant activity of the combination of WAP and EACA fractions (Figure 3). The scavenging effect of DPPH radical by the combination of these two active fractions showed that these two extracts showed a synergistic effect when combined (CI = 0.27-0.43).

CONCLUSION

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

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