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Inhibition Activity of Alpha Amylase and Antioxidant Activity of Breadnut (*Artocarpus camansi*) Leaf Extract from Different Leaf Position

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Diabetes mellitus is a disorder which is linked to alpha amylase function in hydrolyzing complex carbohydrates into monosaccharides. Plants bioactive component that act as an alpha amylase inhibitor has a potency to answer the problem. This research is a descriptive research to observe alpha amylase inhibition activity and antioxidant activity of Breadnut (*Artocarpus camansi*) leaf extract (BLE) from different leaf position. Breadnut leaf from different position were extracted with water, concentrated by rotary vacuum evaporator and freeze dried to obtain dried extract. A potential to inhibit carbohydrates hydrolysis was shown by Breadnut leaf dried extract with alpha amylase activity of inhibition (IC_{50}) of young leaf, mature leaf and old leaf, respectively, 1888.83 µg/ml, 2353.84 µg/ml, and 3199.35 µg/ml; antioxidant activity (IC_{50}) of 748.02 µg/ml, 1266.17 µg/ml, 1377.00 µg/ml and moisture content of Breadnut leaf dried extract of 10.17%, 10.13% and 10.15% Breadnut leaf dried extract has potential to inhibit activity of alpha amylase and antioxidant activity.

Key words: Amylase, antioxidant, breadnut, enzyme, inhibition

Diabetes mellitus is a disease which indicated by above normal of blood glucose level (fasting >126 mg/dl) (1), which is also familiar by the name of hyperglycemia. Chronical hyperglycemia in Diabetes will result in free radicals production as the effect of gluco autooxidation may cause body cell and tissue damage, including beta cells of pancreas (2). Diabetes is treated mostly by taking antidiabetical drugs and medicinal herbs. Furthermore, preventing the disease is also possible by consuming functional food such as crackers with mulberry fresh leaf and fresh leaf extract (3), dragon scale (*P. piloselloides*), leaf tea (4) and instant powder drink of *Phyllanthus niruri* (5). One of the potential antihyperglycemic herbs but less noticeable is Breadnut (*Artocarpus camansi*). Dried extract of breadnut leaf contains alkaloid, flavonoid, tannin, phenolics, triterpenoid and saponin (6) as it is ethanolic extract of Breadnut that is capable of decreasing blood glucose level (7). Thus far, some bioactive components that reported to act as antihyperglycemic agents are alkaloid (8), flavonoid (9, 10) and tannin (9). According to Harborne and Williams (11), flavonoid also acts as antioxidant.

A common practice in using medicinal herbs is boiling the herbs with water. Water was also used for extraction in this experiment. The leaf from various position were chosen as the object for extraction. Permata and Asben (6) found that total polyphenol of BLE of young leaf, mature leaf and very old leaf, were 11175 mgGAE/g, 10238.89 mgGAE/g and 7858.33 mgGAE/g, respectively. Several researches also demonstrated the influence of leaf position on total polyphenols and antioxidant activity of Kawa coffee (12), Cacao leaf tea (13) and Indian Camphorweed *(P. indica)* extract (14).

Materials and Methods

This research was a descriptive experiment to observe inhibition capacity of alpha amylase and antioxidant activity of Breadnut Leaf Dried Extract (BLDE) of different leaf position (young, mature and old). Each observation was done in 3 repetitions.

Materials and equipment: Materials used were Breadnut leaf (young, mature and old), alpha amylase, Na- phosphate buffer, HCl, methanol, DPPH, acarbose, starch, distilled water, and DNS. Equipments for the

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research were hot air oven (YCO-N01), pH meter, freeze dryer (Edwards), oven, desiccator, spectrofotometer (UV-1800 Shimadzu), analytical balance (ABJ-NM/ABS-N), water bath, ultrasonic bath (Elma Ultrasonic), rotary vacuum evaporator (Buchi) and micro pipette.

BLDE production: 200 g of Breadnut leaves were added to 1 L of water and boiled until the volume reach 100mL. The solution was filtered and the dreg were washed with 400 mL of water. Both were collected and concentrated by rotary vacuum evaporator until the volume of 100 mL was reached. The concentrates were dried in freeze dryer.

Biochemical parameters: Parameters such as moisture content, alpha amylase inhibition activity and actioxidant activity of BLDE were determined.

Moisture content: Moisture was determined as per method given by Yenrina (15). Empty dish and lid was dried in the oven for 20 minutes and placed in desiccator for 10 minutes for cooling. Empty dish and lid was weighted (Wo). 5g of samples that spread equally on the closed dish was weighted (W1). It was then placed in the oven for 6 hours. The dried sample weight was determined after constant mass was reached (W2).

$$\text{Moisture content} = \frac{W_1 - (W_2 - W_0)}{W_1} \times 100\%$$

Alpha amylase enzyme inhibition: It was determined by the method given by Thalapeneni *et al.* (16). The reaction mixture was prepared from the dilution of 125 µL BLDE solution and 125 µL of enzyme solution. 125 µL of soluble starch was added after 10 minutes of incubation at 37°C. The mixture was incubated on 37°C for 10 minutes. 500 µL of DNS was added and incubated on boiled water for 5 minutes. The addition of 5 ml was done prior to absorbancy measurement on wavelength of 540 nm (Table 1).

Table 1: Mixture composition on the analysis of alpha amylase inhibition activity

Solution	Blank (µl)	A Control (µl)	B Control (µl)	Sample (µl)
Sample Solution	-	-	125	125
Buffer	250	125	125	-
Enzyme	-	125	-	125
Starch	125	125	125	125
DNS	500	500	500	500
Distilled water	5000	5000	5000	5000

Activity of inhibition of sample was calculated by the formula given below.

$$\%\ \text{of inhibition} = \frac{A1 - A2}{A1} \times 100\%$$

where:

A1 = Absorbancy of A Control – Blank Absorbancy

A2 = Sample absorbancy – Absorbancy of B Control

IC_{50} value was determined by finding regression equation based on a chart of inhibition percentage versus concentration. Based on the equation, extract concentration with 50% of α-amylase, inhibition was identified.

Antioxidant Activity: 2 mL of BLDE was mixed with 2mL of methanol containing 80 µg/ml of DPPH. The mixture was shaken and allowed to stand for 30 minutes in the dark room. The measurement was done with spectrophotometer at wavelength of 517 nm. The blank used was methanol. The formula was as follows.

$$\text{DPPH scavenging activity} = 1 - \frac{\text{sample absorbancy}}{\text{blank absorbancy}} \times 100\%$$

IC50 value is calculated by plotting a curve of inhibition percentage and concentration percentage. By the graph, regression equation was obtained and BLDE concentration which was able to inhibit oxidation at the level of 50%.

Results and Discussion

Moisture content of BLDE from different position: Moisture content found in Breadnut fresh leaf were 39.45% in young leaf, 25.72% in mature leaf and 14.76% in old leaf. Fresh leaf of breadnut was extracted with distilled water and concentrated with rotary vacuum evaporator prior dried with freeze dryer. BLDE had moisture content of ± 10% (Fig. 1). Low moisture content will prolong the shelf life of product.

Inhibition activity of alpha amylase of BLDE: In this experiment, the result expected was the decrease in starch digestibility as the effect of alpha amylase activity reduced in starch hydrolysis. Alpha amylase will hydrolyze starch into simple sugars. The less monosaccharide such as glucose and maltose produced

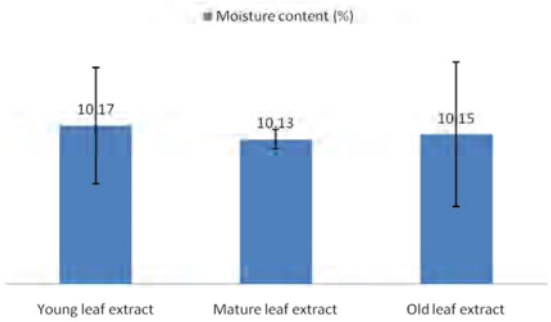

Fig. 1: Moisture content of Breadnut Leaf Dried Extract in different Position

by starch hydrolysis is equal to the decrease of starch digestibility. Glucose and maltose reacted to DNS (Dinitrosalicylic acid) so the amount of both monosaccharides was measured by spectrophotometer at wavelength of 540 nm. Based on the result, BLDE had a potency to inhibit carbohydrate hydrolysis with its alpha amylase inhibition activity. Alpha amylase inhibition activity (IC_{50}) of young leaf, old leaf and very old leaf were 1888.83 µg/ml, 2353.84 µg/ml and 3199.35 µg/ml (Fig. 2), respectively. The highest alpha amylase inhibition activity was obtained from BLDE of young leaf (Fig. 2). This result was lower than several medicinal herbs such as methanolic extract of *C. auriculata* (flower), *T. arjuna* (tree bark) and *P. zeylanicum* (rhizome) with IC_{50} of 37.28 µg/ml, 48.75 µg/ml, and 68.66 µg/ml; methanolic extract of *C. rotundis*, *A. calanus*, *M. ferra*, *B. Aristata*,and *A. heterophyllum*had IC_{50} of 89.54 µg/ml, 133.6 µg/ml, 146.8 µg/ml, 177.9 µg/ml, and 323.1 µg/ml, in sequential order (17). The methanolic extract of *A. alitis*, *A. heterophyllus*, *C. zeylanicum*, *Piper betle* had inhibition activity IC_{50} of 118.88 µg/ml, 70.58 µg/ml, 130.55 µg/ml, and 84.63 µg/ml, respectively (18). *Artocarpus itegrifolia*, a closely related plant within the same genus with *Artocarpus camansi*,was also reported to demonstrate capability in inhibiting alpha amylase (19).

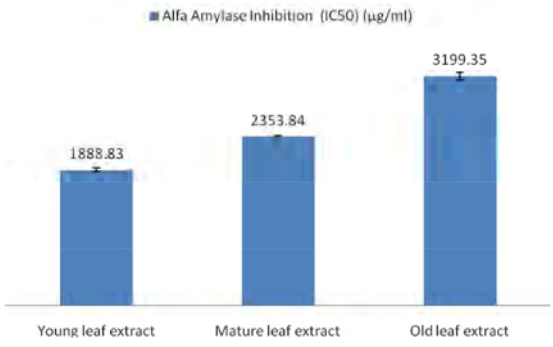

Fig. 2: Alpha amylase inhibition activity of BLDE of different position

Antioxidant activity of BLDE of different leaf position: DPPH was chosen as a method to measure radicals scavenging activity of various compounds on BLDE. Antioxidant activity (IC_{50}) of BDLE of young leaf, mature leaf and very old leaf were found to be 748.02 µg/ml, 1266.17 µg/ml and 1377.00 µg/ml, respectively (Fig. 3). Consumption of BLE will prevent damaging activity of free radicals from inside and outside of the body. Thus, lowering the chance to develop diabetes. During diabetic condition, *radical oxygen species* production is elevating, resulted in oxidative stress of the body. Oxidative stress is a condition of imbalance, where oxidant and free radicals is more numerous than antioxidant (20). Non-enzymatic protection system against oxidative stress is possible in the form of vitamin C, vitamin E, carotenoid and polyphenols (21). Polyphenols have antioxidant characters for their ability to act as hydrogen donors, free radicals scavengers and metal chelating agents. Thus, polyphenol has a role on antioxidant properties in BLDE.

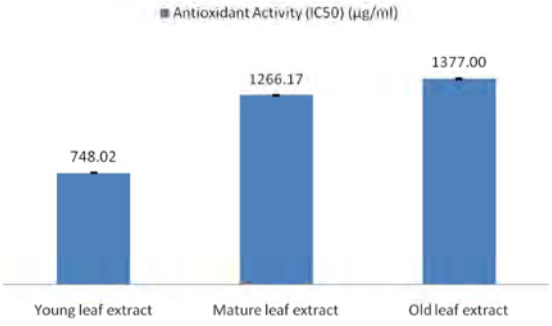

Fig. 3: Antioxidant activity of BLDE based on leaf position

Hari *et al.* (19) reported that *Artocarpus itegrifolia*, a species within the same genus with *Artocarpus camansi*, also posessed antioxidant activity. Antioxidant activity of *Artocarpus itegrifolia* was 38.81 µg/ml and it was lower than methanolic extract of *Artocarpus heterophylulus* (16). Leaf position had an influence on antioxidant properties of the Indian camphor weed (*P. indica*) leaf

with IC_{50} value of 1st-3rd segment, 4th – 6th segment and >6th segment, respectively, 3.71 mg/L, 6.85 mg/L and 49.62 mg/L (14).

Breadnut leaf dried extract (BLDE) has a potency in inhibiting carbohydrates hydrolysis with alpha amylase inhibition activity (IC_{50}) of young, mature and old leaf the values being 1888.83 µg/ml, 2353.84 µg/ml, and 3199.35 µg/ml, respectively. Antioxidant activity (IC_{50}) of 748.02 µg/ml in young leaf, 1266.17 µg/ml in mature leaf and 1377.00 µg/ml in old leaf was observed with moisture content of 10.17%, 10.13% and 10.15% in young, mature and old leaf, respectively. BLDE has potential of inhibition activity of alpha amylase and antioxidant activity.

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