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Some Physiological Characteristics and Bioactive Compounds of Sweet Potato Leaves

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Abstract: ***Sweet potato leaves have been reported to content an appreciable amount of bioactive compounds. Over the years, phenolic including flavonoids are intensely studied for their physiological health benefits as functional foods. This study was aimed to reveal some physiological properties of sweet potato leaves. The leaves of locally grown three sweet potato genotypes used in this study were harvested six weeks after planting. The results showed methanol extract had 63-79% DPPH radical scavenging activity 55-59 % inhibitory effect on α amylase , 0.81-1.75g GAE/100 g (dw) of total phenolic content, and 7.57-10.51mg QE/100 g (dw) of flavonoids.***

Keywords: flavonoids, Ipomoea batatas, phenolic, radical scavenging activity, sweet potato leaves

1. Introduction

Sweet potatoes are cultivated widely around the world primarily for their underground tubers utilized as a raw material for starch production, staple and snack foods . They can be harvested up to three times a year. In addition to be regarded as biomass waste after harvesting the tubers, sweet potato leaves are regularly harvested and consumed in some Asian countries mainly because it has a high content of protein, mineral substances, vitamins and bioactive compounds as well as soluble and non-soluble dietary fiber. Sweet potato leaves contain up to 15-27% protein on dry matter basis depending on the varieties [1] , and are a valuable source of iron, vitamins E, beta carotene lutein and polyphenol [2]. These compounds have been reported to have antioxidant properties, inhibit α glucosidase and α amylase activities [3] which is very potential to manage blood glucose level in diabetic patients.

Bioactive components, namely polyphenol, of sweet potato leaves have been intensively studied because of their ability to protect against free radicals. The over production of free radicals and nitrogen species could result in oxidative stress that increases the onset of chronic non-communicated diseases (NCDs) such as some types of cancers, neurogical problem, and cardiovascular[4,5] The main polyphenol contents of sweet potato leaves are caffeoylquinic acid (CQA) derivatives[6]. The amount of polyphenol in leaves is higher than those of in the tuber flesh and the peel[7]. Eugenio et al.[8]reported sweet potato leaves contain high amount of phenolic compounds mainly quercetin, chlorogrenic acid, and rosmarinic.

In Lampung areas, although has not been reported quantitatively, sweet potatoes are grown widely among small growers. However, despite of their valuable content of protein and bioactive compounds , sweet potato leaves have no economic value, they are either fed to cattle or utilized as compost. Therefore it is important to provide information on various health-promoting biological activities of sweet potato leaves to encourage people to utilize sweet potato leaves as part of their healthy diet.This study revealed the antioxidant property, α amylase inhibitor, flavonoid and total phenolic contents of three sweet potato genotypes namely LPG-01, LPG-06, and LPG-07 grown at PoliteknikNegeri Lampung Experimental Field. These sweet potatogenotypes are stillon-going experiment and have not been grown commercially.

2. Material and Methods

2.1 Materials

Three sweet potato genotypes namely LPG-01, LPG-06, and LPG-07 grown at PoliteknikNegeri Lampung Experimental Field were used in this study. Sweet potato leaf tips used in this study had the desirable attribute for human consumption. They had small stems and petioles they were glabrous, tender and showed some vein purple color. The 15 cm long tips were harvested 6 weeks after transplanting. For each genotype, harvested fifteen sweet potato tip stems wererinsed with tap water, then steam blanched for 3 min, and cooled at room temperature. They were then cut into small pieces, put into plastic bags, and frozen at -15°C until analysis was performed. The samples were evaluated for their DPPH radical scavenging activity ,αamylase inhibition, total phenolic, and total flavonoid contents. The chemicals such as Folin-Ciocalteau reagent, DPPH, methanol were purchased form Sigma-Aldrich via local supplier.

2.1 Sweet Potato Leaf Extraction

Samples of frozen blanched sweet potato leaves (50g) were macerated in 100 ml of 60% methanol in a closed container for 24 h at 4°C in the dark room, then filtered through Whatman No. 42 filter paper. The remaining residues were washed with 100 ml of methanol, both filtrates were collected as the crude extract filtrate. The methanol in the crude extract was evaporated by the rotary evaporator (0.1 MPa, 40°C) until the volume was 50 mL, kept in a dark brown bottle and stored at -20°C until further analysis.

2.2 Scavenging Radical Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity of leaf extract was determined using method described by Chung et al. [9] with slight modification. Two µL of the methanol extracted leaves (as described in above procedure) were mixed with 2.0 mL of 2×10-4 M DPPH in methanol. The mixture was shaken vigorously and left in the dark at 25°C for 30 min. The absorbance of mixture was read immediately at 517 nm using a GENESYS 10S S UV-Visible spectrophotometer (A_{sample}). The mixture of 95% methanol (2 mL) and sample (2 mL) serve as blank (A_{blank}). The control solution was prepared by mixing methanol (2 mL) and DPPH radical solution (2 mL) ($A_{control}$). The ability of sweet potato leaf extract to scavenge DPPH radical or antioxidant activity was calculated by the following equation:

$$\text{Scavenging Radical Activity} = [100 - \frac{A_{sample} - A_{blank}}{A_{control}}] \times 100\ \%$$

2.3 Invitro α-amylase Inhibition Study

The α-amylase inhibitory activity was determine using method described by Zengin[10] with modifications. Briefly, 250 µl of leaf extracts with varying concentrations (125–500 µg/ml) and 250 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase (from Aspergillusoryzae , Merck) solution (0.5 mg/ml) were incubated for 10 min at 25°C. After preincubation, 250 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at 5 s intervals. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 500 µldinitrosalicylic acid (Sigma, St. Louis, USA) color reagent. The tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 5 ml of distilled water, and absorbance was read at 540 nm using a GENESYS 10S S UV-Visible spectrophotometer. the α-amylase inhibitory activity was calculated as percentage inhibition, using the formula.

% Inhibition =([$Abs_{control}$ - $Abs_{samples}$]/ $Abs_{control}$)×100

2.4Total Phenolic

Total phenolic content in sweet potato leaf extract was determined by colorimetry method using Folin-Ciocalteu reagent assay as modified from Singleton and Rossi [11]. SP leaf extract (1ml) was mixed with 1ml of Folin-ciocalteu's phenol reagent and allowed to react for 3 minutes. Then, 0.8ml of 7.5% (w/v) sodium carbonate was added. The mixture was agitated and allowed to stand for further 30 minutes in the dark. The absorbance of leaf extracts and a prepared blank were read at 765 nm using GENESYS UV-Visible spectrophotometer. Gallic acid with the concentration range from 10 µg/mL to 100 µg/mL, was used for the standard curve and the total phenolic content was expressed as mg gallic acid equivalents per gram of dried sample (g GAEs/100g dry weight).

2.5 Total Flavonoids

Total flavonoid content was determined using the method described by Vuong et al.[12]with slight modification. Extract (0.5 mL) was added 2 mL of deionized (DI) water and 0.15 mL of 5% (w/v) $NaNO_2$, kept at 25°C for 6 min. Then, 0.15 mL of 10% (w/v) of $AlCl_3$ was added and allowed to stand for 6 min, followed by the addition of 2 mL of 4% (w/v) NaOH and 0.7 mL of DI water. The mixture was mixed thoroughly and left at 25°C for 15 min. The absorbance was read at 380 nm using GENESYS UV-Visible spectrophotometer. Quercetin with the concentration range from 10 µg/mL to 100 µg/mL was used as a standard and the total flavonoid content was expressed as mg quercetin equivalents per gram of dried sample (mg QEs/100g dry weight).

2.6 Statistical Analysis

All the analysis were conducted in triplicates for each genotype, and the data were reported as means ± standard deviation

3. Result and Discussion

The results of scavenging activity, enzyme inhibition , total phenolic and total flavonoid contents are summarised in Table 1.

3.1 Scavenging Radical Activity

The DPPH radical scavenging activity of the three sweet potato genotypes can be ranked as LPG-7 > LPG-6 > LPG-2. The difference in total antioxidant activity among sweet potato varieties grown under similar condition might be influenced by the plants tolerance against viruses and fungi[13]. DPPH is a free radical generating compound used to measure the scavenging ability of sweet potato leaf extract. The radical scavenger of the leaf extract will decolorize the color of DPPH in methanol solution (purple) to yellow color because of reduction of the stable DPPH radicals to diphenyl-pricrylhydrazine in the presence of H-donating antioxidant. In this study, the antioxidant property of the sweet potato leaf extract was determined using only single method (DPPH assay). The decision was based on its availability and simplicity. Furthermore Steed and Truong [14] reported that the antioxidant activities assayed by DPPH and ORAC (oxygen radical absorbance capacity) showed a significant correlation.

3.2 α-AmylaseInhibition

The inhibitory effect on α-amylase of three genotypes sweet potato leaf extracts were between 55 to 60%. LPG-7 leaf extract showed the strongest inhibitory effect , followed by LPG-2 and LPG-6. The variation of inhibitory effect on α-amylase could be due to variation in the phenolic content. It was claimed that the higher levels of chlorogenic acid (one type of phenolic) may contribute to α-amylase and α-glucosidase inhibitory effect[15].

3.3 Total Phenolic

The total phenolic contents among leaf extract from three genotypes sweet potato were between 0.82 and 1.75g GAEs/100 g extract. Phenolic compounds contribute to the ability to scavenge the reactive oxygen species (ROS)[12], and therefore are important to keep healthy body. Phenolics also has important role in modulating the carbohydrate absorption[16], which may be used to manage blood glucose in diabetic patients.

3.4 Total Flavonoids

The flavonoids contents were between 7.57 – 10.51 mgQEs/100g dry weight. The highest was found in LPG-6 leaf extract, followed by those of LPG-2 and LPG-7. Flavonoids have been intensely studied mainly because of their function as antioxidant . The mechanism of flavonoids as antioxidant or in removing free radicals can be divided in to two, which is scavenging and chelating [17].

Table 1: Antioxidant ability, enzyme inhibition, total phenolic and total flavonoid of sweet potato leaf extracts

Sample	DPPH Scavenging radical activity (%)	α-amylase inhibition (%)	Total Phenolic (g GAEs/100g dry weight)	Total Flavonoid (mg QEs/100g dry weight)
LPG-2	79.68±0.13	57.95±005	1.75±0.27	8.23±0.51
LPG-6	75.72±0.24	54.92±0.03	0.81±0.09	10.51±0.72
LPG-7	63.39±0.36	59.84±0.15	1.49±0.05	7.57±0.18

4. Conclusion

The methanol extract of sweet potato leaves from 3 genotypes have shown to contain considerable level of antioxidant properties, enzyme inhibition, total phenolic and total flavonoid. Based on these findings, sweet potato leaves are very potential to be promoted as promising natural source of antioxidant, and α-amylase inhibition that could be used to overcome hyperglycemia problem.

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