POTENTIAL ANTIDIABETIC AND ANTICANCER ACTIVITIES IN THE LEAVES OF IPOMOEA BATATAS (L.) LAM. GENOTYPES

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Abstract- This study investigated the antidiabetic, antimutagenicity and antioxidant activity from sweetpotato (Ipomoea batatas L.) leaves in vitro. The sweetpotato leaves extract showed stronger inhibitory activities for all the physiological functions studied. The α-glucosidase inhibition assay evaluated the antidiabetic activity, and the extract showed a considerable α-glucosidase inhibitory activity. Among the genotypes, UAPB-2 exhibited the highest a-glucoside inhibitory activity, followed by UAPB 1, and UAPB-3. The leaf extract showed the inhibitory activity ranging from 28.76% to 70.60% on a-glucosidase from 10 to 1000 mg/ml, which was increased steadily with increasing sample concentrations. Antimutagenicity in the leaves was explored using the Salmonella typhimurium TA 98. The Ipomoea batatas genotypes effectively decreased the reverse mutation induced by Trp-P-1, and the mutagenic activities were dose dependent. Furthermore, the extract also capable of reduced the reverse mutation persuaded by Trp-P-2, IQ, and DEGB extract of grilled beef. The fallouts demonstrate that antioxidant capacity (4.01 to 6.89 µmole Trolox g^{-1} DW) and total phenolic contents (7.89 to 17.63 µmole TA g^{-1} DW) broadly fluctuate among the genotypes. Our data demonstrate that all the genotypes have the physiological functions studied, and UAPB-2 exhibited the highest activities. Therefore, Sweetpotato leaves had a high concentration of naturally active biological compounds that show significant physiological functions, which might have values in the anticipation of certain human health conditions.

Index Terms- antidiabetic activity, antimutagenicity, antioxidant, sweetpotato leaves

I. INTRODUCTION

The eating of Ipomoea batatas L. leaves as a vegetable in many parts of the world[1–3] indicates that they are acceptable as edible like other traditional leafy vegetables. They are rich in phytonutrients, and are further tolerant of diseases and pests than numerous other green vegetables [1] - [4]. Phytonutrients are acted as bioactive composites and a diverse group of secondary metabolites commonly present in higher plants [5]-[12]. They play important roles and contribute to the structure of the plants and complicated by a significant number of metabolic pathways [13] - [14]. Thus, the phenolic plant complexes, because of their diversity and widespread distribution, are the greatest talented group of natural antioxidants, and add to the organoleptic and nutritional qualities of fruit and vegetables.

Diabetes mellitus is a common disease with many complications such as atherosclerosis, cardiac dysfunction, retinopathy, neuropathy, and nephropathy [15]. α-glucosidase (EC 3.2.1.20) catalyzes the final step in the digestive process of carbohydrates. Its inhibitors can delay the uptake of dietary carbohydrates and suppress postprandial hyperglycemia and could be useful for treating diabetic and/or obese patients [16]. α-Glucosidase inhibitors such as acarbose, miglitol, and voglibose are recognized to decrease postprandial hyperglycemia mainly by interfering with the carbohydrate digestive enzymes and by delaying glucose absorption. Free radicals can lead to a variety of physiological and biochemical lesions [17] and induce degenerative diseases such as coronary disease, diabetes, stroke and cancer [18]. The new expansion of screening approaches for environmental carcinogens by determining their mutagenicity has allowed detecting numerous types of mutagens and carcinogens in foods [18] - [22]. On the other hand, it is now recognized that several types of inhibitors act against mutagens and carcinogens in food. They show a substantial part in plummeting the dangers of mutagenesis and carcinogenesis [23]. Several authors described that the nutritive constituents of sweetpotato tops are comparable to those of commercial leafy vegetables [6] - [10], 12, [20] - [21], [24] - [25]. However, the physiological function of sweetpotato leaves has not yet been deliberate synthetically. In the current article, the effects of the extracts of the sweetpotato genotypes with the diverse polyphenolic levels on the antidiabetic activity, mutagenicity, and antioxidant capacity are explored.

II. MATERIALS AND METHODS

A. Materials

The leaves from three Ipomoea batatas L. three Ipomoea batatas L. (sweetpotato) genotypes were used for this study. After harvest, the leaves were washed softly, moved into pre-labeled separate vinyl bags and directly frozen at –85^{O}C. The next day all the frozen samples were freeze-dried for 48 h in a freeze dryer. The freeze-dried samples were ground in a blender and used for laboratory analysis. The extract was prepared from the lyophilized flour (1g) using 20 mL of ice-cold water for 1h. The suspension was centrifuged at 18000 x g for 20 min and the resultant precipitate was re-extracted under the same

conditions. The collected supernatant was lyophilized and used for analysis.

B. α-Glucosidase inhibitory assay

One hundred microliters of 3 mM pNPG in 0.2 M sodium phosphate buffer (pH 6.8) was added as a substrate to the mixture of 50 µl of α-glucosidase (0.15 unit/ml) and 50 µl of sample to start the reaction. The reaction was conducted at 37°C for 15 min and clogged by the addition of 750 µl of 0.1 M Na_2CO3. α-Glucosidase activity was assessed by measuring the release of p-nitrophenol from pNPG at 405 nm. Acarbose was used as a positive control. All tests were performed in independent triplicate (n=3) and data were expressed as mean ± SD.

C. Assay of antimutagenicity

The antimutagenic activity was assessed for Salmonella typhimurium TA 98 using a mutagen, Trp-P-1. These mutagens need metabolic activation to induce mutation in TA 98. The s-9 mix containing 50 µmol of sodium phosphate buffer (pH 7.4), 4 µmol of MgCl2, 16.5 µmol of KCl, 2.5 µmol of glucose-6-phosphate, 2 µmole of NADH, 2 µmol of NADPH, and 50 µL of the S-9 fraction in a total volume of 0.5 mL. For the inhibition test, 0.1 mL of mutagen, 0.1 mL DMSO-dissolved polyphenolics solution, and 0.5 mL of S-9 mix or phosphate buffer were concurrently incubated with 0.1 mL of bacterial suspension at $37^{O}C$ for 20 min and then dispensed into minimal-glucose-agar plates with 2 mL of soft agar. The colony number of each plate was accounted after 48 h cultivation at $37^{O}C$. The antimutagenicity assay was performed as described in earlier papers [9], [22].

D. Extraction and Measurement of Total Phenolic

The lyophilized sweetpotato leaf extract was forcefully mixed with ten times its equivalent volume of 80% ethanol. The mixture was boiled for 5 min and centrifuged at 5000g for 10 min, and the supernatant was composed. The residue was mixed with additional 80% ethanol and boiled for 10 min to re-extract the phenolics, and centrifuged under the similar conditions. The extracts were pooled and made up to 10 mL and used for to quantity of total phenolics. The alcohol extract was diluted to achieve an absorbance reading at the range of the standard tannic acid (TE). The results were stated as µmol TE g^{-1} DW (dry weight).

E. Antioxidant capacity in the DPPH assay

The radical-scavenging activity of the extracts was measured according to a slightly modified method described by Islam et al. 926]. A stock solution of DPPH (6 mM) was prepared by dissolving 0.0263 g in 10 ml of ethanol (or methanol). The stock solution is diluted to make a 60 µM working solution. Again, a 10 mM stock solution of Trolox was prepared for every sample tested. Dilutions were made for each sample tested. Dilution strength was dependent upon each extract's relative antioxidant capacity. For each dilution, 20 µL were added to 2.5 ml of DPPH· Solution and incubated in a dry bath at 37°C for 30 min. Absorbances were measured at 520 nm on an ASYS UVM 340 plate reader. TEAC values were measured by comparing the slope of sample plots to the slope of Trolox. Antioxidant activity was reported as µmoles Trolox equivalent per gram dry weight sample (µmol TE/g DW).

F. Statistical analysis

A randomized complete block design with three replications was adopted. Data for the different parameters were analyzed by analysis of variance (ANOVA) procedure, and the level of significance was calculated from the F value of ANOVA.

III. RESULTS AND DISCUSSION

A. α-Glucosidase inhibitory effect

The antidiabetic activity was evaluated by the α-glucosidase inhibition assay, and the extract showed a considerable α-glucosidase inhibitory activity (Table 1). The leaf extract demonstrated a moderate to high inhibitory activity on α-glucosidase, Among the genotypes, UAPB-2 exhibited the highest a-glucoside inhibitory activity, followed by UAPB 1, and UAPB-3. The leaf extract showed the inhibitory activity ranging from 28.76% to 70.60% on a-glucosidase from 10 to 1000 mg/ml, which was increased steadily with increasing sample concentrations.

Table 1. The ∞-glucosidase inhibitory activity of Ipomoea batatas L. leaves extract.

Genotypes	Concentration (µg/ml)	Inhibition rate (%)
UAPB – 1	10	31.37± 2.51
	50	36.57± 5.50
	100	39.66± 2.75
	500	61.96± 2.62
	1000	63.10± 4.25
UAPB – 2	10	49.33± 2.52
	50	52.57± 4.04
	100	58.01± 4.58
	500	70.60± 3.93
	1000	69.03± 1.95
UAPB – 3	10	28.76± 2.93
	50	44.63± 3.84
	100	48.07± 3.52
	500	59.36± 2.52
	1000	57.33± 4.08
Acarbose (control)		98.01± 4.81

Data are the mean of three replications ± SD.

On the other hand, the control treatment (Acarbose) showed 98.01% inhibitory activity at the concentration of 0.1 µg/ml. One therapeutic approach to treating diabetes is to increase the postprandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzyme α-glucosidase in the in the digestive tract. Inhibitors of these enzyme delay carbohydrate digestion, causing a reduction in

the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise [27]. The results suggest that the Ipomoea batatas leaf extracts have the potentiality for treating diabetes by inhibiting α-glucosidase activity.

B. Effects of water extract of leaves on the mutagenicity

The antimutagenic impact of the water extracts from sweetpotato leaves of three genotypes was determined by antimutagenicity assays using Trp-P-1 at a dose of 0.075 µg/plate, and using three different doses of the sweetpotato leaves extracts such as 100, 50 and 10 µg/plate (Table 2).

Table 2. Effect of Ipomoea batatas leaf extract on the mutagenicity of Trp-P-1 against Salmonella typhimurium TA 98[a]

Genotypes	Added volume (µL)	His^+ revertants (per plate[b])	Inhibition (%)
UAPB-1	100	23 ± 3	77
	50	51 ± 5	69
	10	159 ± 8	63
UAPB-2	100	27± 4	91
	50	55 ± 5	88
	10	162 ± 7	71
UAPB-3	100	31 ± 3	80
	50	57 ± 2	71
	10	176 ± 8	68

[a]Trp-P-1 was added at a dose of 0.075 µg/plate. The mutagenicity was tested with S-9 mix.[b]Each value represents the mean ± SD of triplicate plates. The values shown have had the spontaneous mutation frequency subtracted. The His^+ revertant values of the controls were 689 ± 17 per plate.

The results found that inhibitory activity was higher at higher doses in all genotypes studies. The inhibitory activity (%) ranged from 77 to 91 at 100µg/plate, 69 to 88 at 50µg/plate and 63 to 71 at 10 µg/plate doses. The highest activity found in the genotypes UAPB-2 followed by UAPB-1 while UAPB 3 had the lowest.

Table 3. Effcets of caffeoylquinic acid derivatives on the mutagenicity of Trp-P-2, IQ, and DEGB against Salmonella typhimurium TA 98.

Mutagen[a] (µg or µl/plate)	Inhibition (%)[b]		
	UAPB-1	UAPB-2	UAPB-3
Trp-P-2 (0.20 µg)	47± 5.03	69± 4.50	57± 4.55
IQ (0.20 µg)	29± 3.66	58± 5.35	34± 3.10
DEGB (0.20 µl)	49± 3.51	67± 4.30	56± 4.61

[a]Mutagenicity was tested with S-9 mix. [b]The leaf extract was used at a concentration of 0.5 mg/plate. Each value represents the mean of triplicate ± SD. The value shown have had the spontaneous mutation frequency subtracted.

The His^+ revertant values of the controls on Trp-P-2, IQ and DEGB was 799±11, 862±18, and 273±10/plate, respectively.

Therefore, the results propose a widespread disparity of antimutagenicity among the genotypes, and the extracts showed dose-dependent inhibitory activities. Similar trends were also found by several researchers [4], [9]. The antimutagenic effect of the extract at low doses is relatively minor compared with the one from higher doses. We also evaluated the antimutagenic activity of the extract using several mutagens, such as Trp-P-2, IQ, B[a]P, and DEGB (Table 3). The DEGB were utilized at a dose of 100 µL/plate without dilution. The s-9 mix was added for the assay using Trp-P-1, Trp-P-2, IQ, B[a]P, and DEGB to cause mutations in TA 98. The extract used in doses of 50, 10, and 5 µL/plate. The extract reserved Trp-P-2 induced mutation by 14%, IQ by 88%, b[a]P by 27%, and Trp-P-1 by 71% respectively at the concentration of 10 µL/plate. Thus, the sweetpotato leaf extract effectively decreased the reverse mutations induced by all purified mutagens tested.

C. Total polyphenol content and antioxidant capacity

The antioxidant capacity (µmole Trolox/mg dry leaf powder) and total polyphenol (µmol TE g^{-1} DW) in the leaves of three genotypes are presented in Table 4.

Table 4. Effect of sweetpotato leaf extract on the antioxidant capacity and total phenolic contents of the Ipomoea batatas L. leaves

Genotypes	Antioxidant activity (µmol Trolox.g^{-1} DW)	Total phenol contents (µmol Trolox.g^{-1} DW)
UAPB-1	4.01± 1.11	7.89 ± 1.52
UAPB-2	6.89± 1.49	17.63± 2.61
UAPB-3	4.98± 0.97	9.13 ± 2.78

Data are the mean of three replication ± standard error

The genotypes fluctuated extensively in their total polyphenolic contents. The highest total phenolics found was 17.63 µmol TE g^{-1} DW and the lowest was 7.89 µmol TE g^{-1} DW. The results showed that sweetpotato leaves had higher or similar content of total polyphenolics than other vegetables [9]-[10], [14], [24], [28]. The antioxidant capacity of the genotypes ranges from 4.01 to 6.89 µmol Trolox g^{-1} DW. The genotypes UAPB-2 had the highest contents of phenolic followed by UAPB-3. The phenolics are pervasive bioactive compounds found in plant foods and beverages. Since polyphenolic compounds show numerous physiological functions, sweetpotato leaves might also be expected to have physiologically active

possessions because they comprise higher contents of polyphenolic compounds with high antioxidant capacity. The antioxidative substances contained in plant parts have attracted much consideration all over the world. Several researchers [9], [20], [26] have reported the radical scavenging and antioxidant activities of sweetpotato leaves. The polyphenolics contents and antioxidant activity in sweetpotato leaves, other different plants and foods showed a high correlation [8]-[9], [12], [19]. Usually, the antioxidant capacity of various plants is influenced by the genetic factor. Therefore, an extent of the antioxidant capacity may be a critical tool for use in plant breeding programs intended to improve antioxidant components available for human consumption. This result will be valuable for some chemical breeding programs to develop needed organoleptic and nutritional quality characteristics of crop plants.

CONCLUSION

This study exhibited that Ipomoea batatas L. leaves could be an outstanding source of naturally active compounds with numerous physiological functions. We tested the antimutagenicity, antioxidant and antidiabetic activities of extracts, and based on these results; sweetpotato leaves can be considered as a potential source of functional food and pharmaceutical agent.

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