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Some Bioactive Constituents, Antioxidant, and Antimutagenic Activities in the Leaves of *Ipomoea batatas* Lam. Genotypes

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Abstract The sweetpotato leaves are rich in phytonutrients such as polyphenolics, vitamins, protein, iron, calcium, and zinc. The sweetpotato is also selected by the United States National Aeronautics and Space Administration to develop in a controlled ecological life provision system as a key food basis. Therefore to find out suitable genotypes with higher phytonutrients contents and the physiological functions were compared in leaves of sixty sweetpotato (*Ipomoea batatas* Lam.) genotypes. Total phenol, carotenoid, anthocyanin and flavonoids contents of the sweetpotato leaves ranged from 2.0 to 22.5 (g/100g DW), 0.9 to 23.4 (beta carotene equivalents/100g; BET/100g), 2.2 to 24.5 (color value/g DW) and 62.8 to 272.2 (catechin equivalents; µg/g), respectively. The coefficient of variation (CV) of the caffeoylquinic acid (CQA) namely caffeic acid (CA), chlorogenic acid (ChA), 4,5-diCQA, 3,5-diCQA, 3,4-diCQA and 3,4,5-triCQA of the genotypes were 124.9%, 83.9%, 76.1%, 83.2%, 104.4% and 96.5%, respectively. The fallouts demonstrate that radical scavenging activity (RSA) broadly fluctuate among the genotypes (0.21-2.3 µmole Trolox/mg DW). Antimutagenicity in the leaves was explored using the *Salmonella typhimurium* TA 98. The genotypes effectively decreased the reverse mutation induced by Trp-P-1, and the mutagenic activities were dose dependent. Furthermore, the caffeic acid derivatives also capable of reduced the reverse mutation persuaded by Trp-P-1, Trp-P-2, IQ, and DEGB extract of grilled beef. Among the CAD, the 3, 4, 5-tri-CQA significantly higher earmarked the reverse mutation followed by 4-5 di-CQA. 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.

Keywords: *anthocyanin, carotenoid, flavonoid, phenolic acids, physiological functions, sweetpotato leaves*

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1. Introduction

The intake of the sweetpotato [(*Ipomoea batatas* (L.) Lam.] leaf of vegetable or raw material of food ingredient is a critical focus when common food deficiencies are considered. Agriculturists and food scientists, faced with the problem of feeding the world's hungry, are becoming increasingly interested in formerly ignored tropical green leafy vegetables like sweetpotato tops. The ingesting of sweetpotato tops as a vegetable in many parts of the world [1,2,3] indicates that they are acceptable as comestible like other traditional leafy vegetables. They are rich in protein, vitamin B, iron, calcium, and zinc, and are further tolerant of diseases and pests than numerous other green vegetables are grown in the tropics [4,5,6,7]. The sweetpotato continues to be of remarkable economic value as the sixth most plentiful food crop in the earth [8,9,10,11] and is among the plants selected by the United States National Aeronautics and Space Administration to develop in a controlled biological life provision system as a key food basis [12]. Polyphenolic compounds such as anthocyanins, carotenoids, flavonoids, phenolic acids are omnipresent bioactive composites and a diverse group of secondary metabolites commonly present in higher plants [13-21]. They play important roles and contribute to the structure of the plants and complicated by a significant number of metabolic pathways [22,23]. The carotenoids present in the sweetpotato leaves can scavenge free radical agents as singlet oxygen quencher [24,25,26]. The sweetpotato leaves cv. Oren was found to cover the highest concentration of carotenoids when compared to the different sweetpotato genotypes [25]. The anthocyanins are the major group of visible polyphenols and comprise the red, purple and blue pigmentation of many plants. They signify a diverse group of secondary metabolites [15] among which there are significant natural antioxidants and food colorants. As a food additive, anthocyanins are rich in color likened to cochineal pigments. Additionally, anthocyanins have been shown to have some valuable therapeutic and physiologic belongings as antineoplastic agents [27], radiation-protective agents [28], chemoprotective agents in contradiction of platinum

toxicity in anticancer therapy [29], and likely other beneficial effects owing to their interface with various enzymes and metabolic developments [30,31]. Hence, the phenolic compounds have attracted particular care since they can defend human body from oxidative stress, which may source many diseases counting cancer, aging and other cardiovascular diseases [32-41]. Thus, the phenolic plant complexes, because of their diversity and widespread distribution, are the paramount group of natural antioxidants, and add to the organoleptic and nutritional qualities of fruit and vegetables.

The new expansion of screening approaches for environmental carcinogens by determining their mutagenicity has allowed detecting numerous types of mutagens and carcinogens in foods [42-50]. Some of these constituents in foods have been found to be produced throughout storage, cooking, and digestion [21,43,47,48]. 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 [50]. Several authors described that the nutritive constituents of sweetpotato tops were comparable to those of leafy commercial vegetables [16,25,26,45,51,52]. However, the foliar antimutagenic activity of sweetpotato genotypes has not been investigated extensively. The structural feature accountable for the antioxidative and free radical scavenging action of caffeic acid is the ortho-dihydroxyl functionality in the catechol [53]. Therefore, the physiological function of CQA derivatives with plural caffeoyl groups is extra efficient than with a mono-caffeoyl one. Several investigators have partly elucidated some physiological functions of CQA derivatives [40,53]. However, the physiological function of sweetpotato leaves and the CQA derivatives have 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 mutagenicity and radical scavenging activity are explored.

2. Materials and Methods

2.1. Materials

The leaves from sixty sweetpotato genotypes were used for this study. Features of the *Ipomoea batatas* L. genotypes were presented in Table 1. 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.

2.2. Extraction and Measurement of Total Anthocyanin

Determinations of total anthocyanin were made according to the previous paper [17] with a slight adjustment. Five hundred mg leaf powder was taken, added to 10 ml of 0.5% H2SO4, and soaked overnight at room temperature. The sample was centrifuged for 15 min and filtered through a 0.2 πm filter membrane. The resultant supernatant was diluted fourfold with Mcllvaine's buffer solution and the pH adjusted to 3.0 and was used for the measurement of optical densities at 530 nm with a dual-wavelength flying spot scanning densitometer (Shimadzu, Japan) with a microplate system. The color value (CV) for the pigment extract, which is a scalable indicator of total anthocyanin, was calculated using the following formula:

CV = 0.1 x OD_{530} x 4 x 20/g DW, where OD_{530} is a spectrophotometric reading at 530 nm, 4 and 20 are the levels of dilution, DW is dry leaf weight [54].

2.3. Total Flavonoids Determination

The vanillin-HCl assay was used to determine the number of total flavonoids in the sweetpotato leaf extract [55]. Diluted catechin standards (250µl) were added to vanillin-HCl (1 ml) reagent in a 2 ml microcentrifuge tube. Then, the reaction combination was incubated in a water bath for 20 min at 30°C. The absorbance was measured at 500 nm and blanked with 80% methanol. The stages above were recurrent by substituting catechin with the leaf extracts. The calculation for the total flavonoids was originated on Sun et al [55].

2.4. Total Carotenoid Determination

Total carotenoid contents were determined using the slightly adapted method as described by Thaipong [56]. Separate leaf extracts were diluted with n-hexane solvent. The absorbance of the leaf extracts (2 ml) was measured at 470 nm. Beta carotene was used as the standard. The total carotenoid contents were determined as the percentage of total beta-carotene equivalents/100g leaf extract (g BET/100g).

2.5. Extraction and Measurement of Total Phenolic

Determinations of total polyphenols were made according to the Folin-Ciocalteau method with slight modification [57]. 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 standards. The absorbance was measured at 600 nm, and the results were stated as g/100 g dry leaf powder.

2.6. Quantification of Phenolic Acids by RP-HPLC

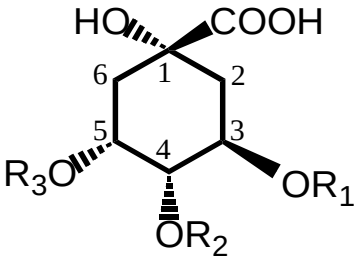
We identified and isolated the five biologically active caffeic acid derivatives from sweetpotato leaves [18]; Figure 1). The lyophilized sweetpotato leaf extracts (50 mg) was vigorously mixed with 4 mL of ethanol in a centrifuge tube with cap. The mixture was boiled for 5

min and centrifuged at 3000 x g for 10 min. The supernatant was filtered through a cellulose acetate membrane filter (0.20 mm) and used for analysis. A 5-mL portion of the filtrate was injected into the HPLC system and eluted as described below. The HPLC system consisted of two Model LC-10AT pumps, a Model SIL-10AXL auto-injector, a Model CTO-10AC column oven and a Model SPD-M10AVP UV-Vis photodiode array (Shimadzu, Japan). The column was a 150 mm x 4.6 mm i.d., 5 µm YMC-Pack ODS-AM AM-302 (YMC, Japan). The column oven temperature was fixed at 40°C. The mobile phase contained water containing 0.2% (v/v) formic acid (A) and methanol (B). Elution was achieved with the linear gradient as follows: 2% B from 0 to 15 min, 2% to 45% B from 15 to 50 min, and 45% B from 50 to 65 min. The flow rate was 1 mL/min. The polyphenols were detected at 326 nm. The retention times of the caffeic acid derivatives were compared with those of the authentic reagents.

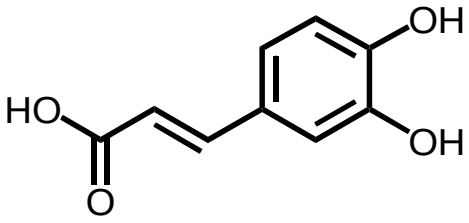
Table 1. Features of the sixty sweetpotato genotypes are studied

Sl. No.	Genotype	Parental information	Origin
1	Setoaka	Kyushu-12 (M)/Gokokuimo (F)	Japan
2	Kyushu -22	Gokokuimo (M)/Norin-7(F)	Japan
3	Kyushu-106	Kyukei-20 (M)/K-88 (F)	Japan
4	Kyushu-137	Kyushu-165 (M)/Tanegashima (F)	Japan
5	Chikei-3	Unknown	Japan
6	7-1043	Shichifuku (M)/2-2057(F)	Japan
7	FV-62-64	MA Kei 1789 (M)/O.P.	Japan
8	B 64-3	Unknown	Brazil
9	AIP 539	Unknown	Papua New Guinea
10	Okierabu-4	Unknown	Japan
11	Ninjin imo	Unknown	Japan
12	Damaciigaraimo-2	Unknown	Japan
13	Kyukei 116	Beniwase (M)/Satsumahikari (F)	Japan
14	Kyukei 86214-2	Kyukei -83398-2 (M)/P.C.	Japan
15	Yen 136	Unknown	Philippines
16	Yen 634	Unknown	Peru
17	89SB-38	Unknown	Malaysia
18	90SR EXT-4	Unknown	Malaysia
19	F 6913-1	F-59-12 (M)/Kyukei 17-30 (F)	Japan
20	Kyukei 36	Kanto-85 (M)/Minamiyutaka (F)	Japan
21	Kyushu-140	Kyukei 82124-1 (M)/Shiroyutaka (F)	Japan
22	Shimobukure matsuda	Kumamoto Matsuda Farm	Japan
23	JW Keishokuheni	JW mutation	Japan
24	Shekishinshi	Unknown	China
25	Yen 626	Unknown	Peru
26	Bitambi	Unknown	Uganda
27	Gina	Unknown	Papua New Guinea
28	Santa Cruz	Unknown	Papua New Guinea
29	Hokunou Josho 18	Unknown	China
30	Nankin 51-92	Unknown	China
31	AIP 092	Unknown	Papua New Guinea
32	Toku-10	Unknown	Japan
33	Chirugaimo	Unknown	Japan
34	Annouimo-1	Unknown	Japan
35	Kyukei 7021-6	Kyushu-55 (M)/F-53-6 (F)	Japan
36	IB-722-17	IB-64316-10 (M)/Kyushu-65 (F)	Japan
37	Toku-27	Unknown	Japan
38	F59-1	Unknown	Japan
39	90IDN-27	Unknown	Indonesia
40	Yen 116	Unknown	Philippines
41	Tainou-56	Unknown	Taiwan
42	89SB-19	Unknown	Malaysia
43	89SB-50	Unknown	Malaysia
44	90SB-90	Unknown	Malaysia
45	Iwate-9	Unknown	Japan
46	F697-11	Unknown	Japan
47	90SR EXT-1	Unknown	Malaysia
48	90IDN-65	Unknown	Indonesia
49	No. 232	Unknown	USA
50	90SR-16	Unknown	Malaysia
51	Oki 100 hosozuru	Okinawa-100 mutation	Japan
52	Benihayato	Centennial (M)/Kyushu-66 (F)	Japan
53	K-114W	K-114 mutation	Japan
54	Suigen	Unknown	Japan
55	F56-20	Unknown	Japan
56	C217-17	Unknown	Japan
57	B 64-8	Unknown	Brazil
58	L 4-5	Unknown	USA
59	Suioh	Tsurusengan mutation	Japan
60	Abana	Unknown	Japan

M= Mother; F= Father.



Quinic acid



Caffeic acid

	R_1	R_2	R_3
Chlorogenic acid:	caffeic	H	H
4,5-diCQA:	H	caffeic	caffeic
3,5-diCQA:	caffeic	H	caffeic
3,4-diCQA:	caffeic	caffeic	H
3,4,5-triCQA:	caffeic	caffeic	caffeic

Figure 1. Chemical structures of polyphenolic compounds in sweetpotato leaves

2.7. Determination of Radical Scavenging Activity

Radical scavenging activity was determined using a stable radical, DPPH, adaptation to the method defined in a previous paper [19] with a minor modification. All the reactions were in a 96-well microplate with an entire volume of 300µL. A sample solution (75µL) including the test compound at varying concentrations in 0.1M MES buffer (pH 6.0) with 50% ethanol was added to 150µL of the same buffer. 75µL of 0.4 mM DPPH solution in 50% ethanol was added to the mixture, which was then shaken and held for 2 min at room temperature. The decrease in DPPH absorbance at 520 nm was measured in triplicate. The radical scavenging activity of the leaf extract was expressed regarding IC_{50} (concentration in µmole Trolox/g of dry powder, required for a 50% decrease in the absorbance of DPPH radicals). The IC_{50} of purified compounds was stated on a molar base. A plot of the absorbance vs. the concentration was made to calculate IC_{50}.

2.8. Assay of Antimutagenicity

The antimutagenicity assay was performed as described in earlier papers [19,53]. 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°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°C.

2.9. Chemicals, Mutagen, and Bacteria

Trp-P-1 [3-amino-1,4-dimethyl-5H-pyrido-(4,3-b)indol], Trp-P-2 [3-amino-1-methyl-5H-pyrido-(4,3-b)indol], IQ (2-amino-3-methylimidazo [4,5-f]quinoline, B[a]P (benzo[a]pyrene, 4-NQO, DMSO (Dimethyl sulfoxide), DPPH and other chemicals used were the highest grade. Chlorogenic acid was the product of Sigma Chemical Co. (St. Louis, MO, USA). The S-9 fraction prepared from rat liver pretreated with Phenobarbital and 5, 6-benzoflavone and cofactors were the products of Oriental Yeast Co., Ltd. (Japan). Other chemicals used were standard grade. Strain TA 98 of Salmonella typhimurium was supplied by the Institute for Fermentation, Osaka, Japan. The bacterium was cultured in nutrient broth for 16 h at 37 OC before the mutagenicity assay. The purified (>97%) 3, 4-diCQA, 3, 5-diCQA, 4, 5-diCQA and 3, 4, 5-triCQA were used as standards for HPLC analysis. DMSO extracted grilled beef (DEGB) as a sample of mutagenic substances in daily foods was prepared [40,50].

2.10. 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.

2.11. Abbreviations Used

B[a]P, benzo [a]pyrene; ChA, chlorogenic acid; CA, Caffeic acid; BEGB, dimethyl sulfoxide extract of grilled beef; DMSO, dimethyl sulfoxide; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; Trp-P-1, N-hydroxy-3-amino-1,4-dimethyl-5H-pyrido-(4,3-b)indol; Trp-P-2, 3-amino-1-dimethyl-5H-pyrido-(4,3-b)indol; 4,5-diCQA, 4,5-di-O-caffeoylquinic acid; 3,5-diCQA, 3,5-di-caffeoylquinic acid; 3,4-diCQA, 3,4-di-O-caffeoylquinic acid; 3,4,5-triCQA, 3,4,5-tri-O-caffeoylquinic acid.

3. Results and Discussion

3.1. Anthocyanin, Flavonoid and Carotenoid Contents in Sweetpotato Leaves

The total anthocyanin (color value/g dry weight), flavonoid (catechin equivalents; µg/g) and carotenoid (g beta-carotene equivalents per 100 g) contents were presented in Figure 2. The results demonstrated substantial variation in all biochemical parameters of the leaves of sixty sweetpotato genotypes studied. Across all genotypes, the mean total anthocyanin content ranged from 2.2 to 24.5 (color value/g dry weight); flavonoid and carotenoid contents ranged 62.8 to 272.2 (catechin equivalents; µg/g), and 0.9 to 23.4 (g beta-carotene equivalents per 100g), respectively. Among the three quality traits, total carotenoid showed the highest level of coefficient of variation (65.6%), followed by total anthocyanin (58.7%), while the least difference was found for total flavonoid (37.9%). Targeted biochemical enhancement depends on identifying their change in biochemical traits. The anthocyanins are a major group of visible polyphenols and comprise red, purple and blue pigmentation of numerous plants that signify a diverse group of secondary metabolites [15]; they are biologically ubiquitous compounds found in plant and beverages and have received much attention because of their biological activities [7,17,21,53,58,59]. The flavonoids are small molecular weight compounds and possess different physiological functions [13,60,61,62,63]. The carotenoids were found highly concentrated in the color-fleshed sweetpotatoes, and many authors [25,26,64,65] stated that sweetpotatoes contain, in addition to beta-carotene in all-trans form, isomers of cis beta-carotenes and other minor carotenoids such as lutein, and zeaxanthin. The outcomes indicate the enormous distinction in total anthocyanin, flavonoid, and carotenoid contents among the genotypes, suggesting that there is potential for genetic improvements of sweetpototoes to influence anthocyanin, flavonoid, and carotenoid contents. The higher degree of observed genotypic variations in phytochemicals contents also indicates the potentiality for plant and chemical breeding to enhance quality criteria(s). These will facilitate the development of sweetpotato genotype(s) with higher contents of phytochemicals in their leaves that can be used for the preparation of different functional foods to fight against various human health conditions.

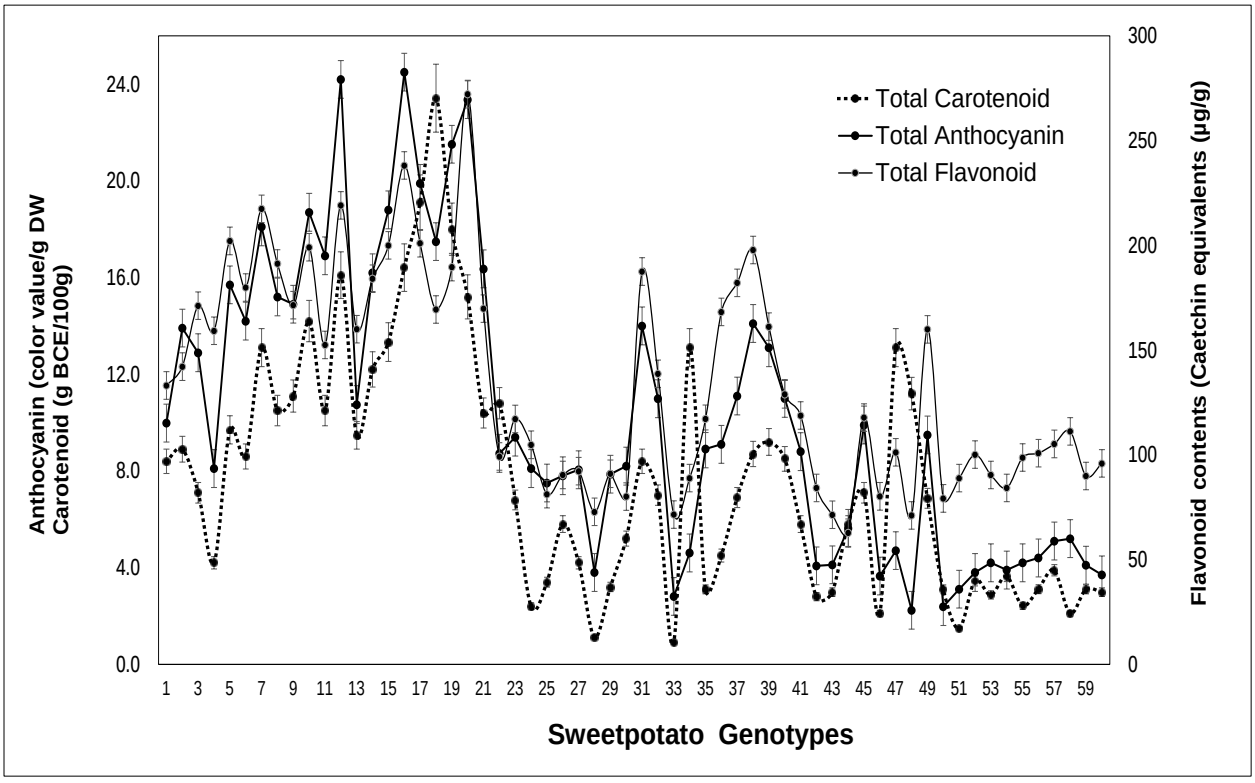


Figure 2. Radical scavenging activity (µ mole Trolox/mg dry leaf powder) and total polyphenol (g/100 g dry leaf powder) in the leaves of selected sixty sweetpotato genotypes. *Name of the genotypes is the same as in Table 1

3.2. Total Polyphenol Content and Radical Scavenging Activity of the Genotypes

The radical scavenging activity (µmole Trolox/mg dry leaf powder) and total polyphenol (g/100 g dry leaf powder) in the leaves of selected sixty sweetpotato genotypes are presented in Figure 3. The genotypes fluctuated extensively in their total polyphenolic contents. The highest total phenolics found was 15.2 g/100 g dry weight and the lowest was 4.1 g/100 g dry weight, and most of the genotypes (>95%) contained > 6.40 g/100 g dry weight total polyphenolics. The results showed that sweetpotato leaves had higher or similar content of total polyphenolics than other vegetables [7,8,18,23,24,51]. The RSA of the genotypes ranges from 0.21 to 2.3 µ mole Trolox/mg dry leaf powder. Figure 3 shows that RSA depends on their respective polyphenol content of sweetpotato leaves. 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 actual possessions because they comprise higher contents of polyphenolic compounds with

high RSA. The antioxidative substances contained in plant parts have attracted much consideration all over the world. Several researchers [19,46,66] 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 [18,19,21,43,46]. 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.

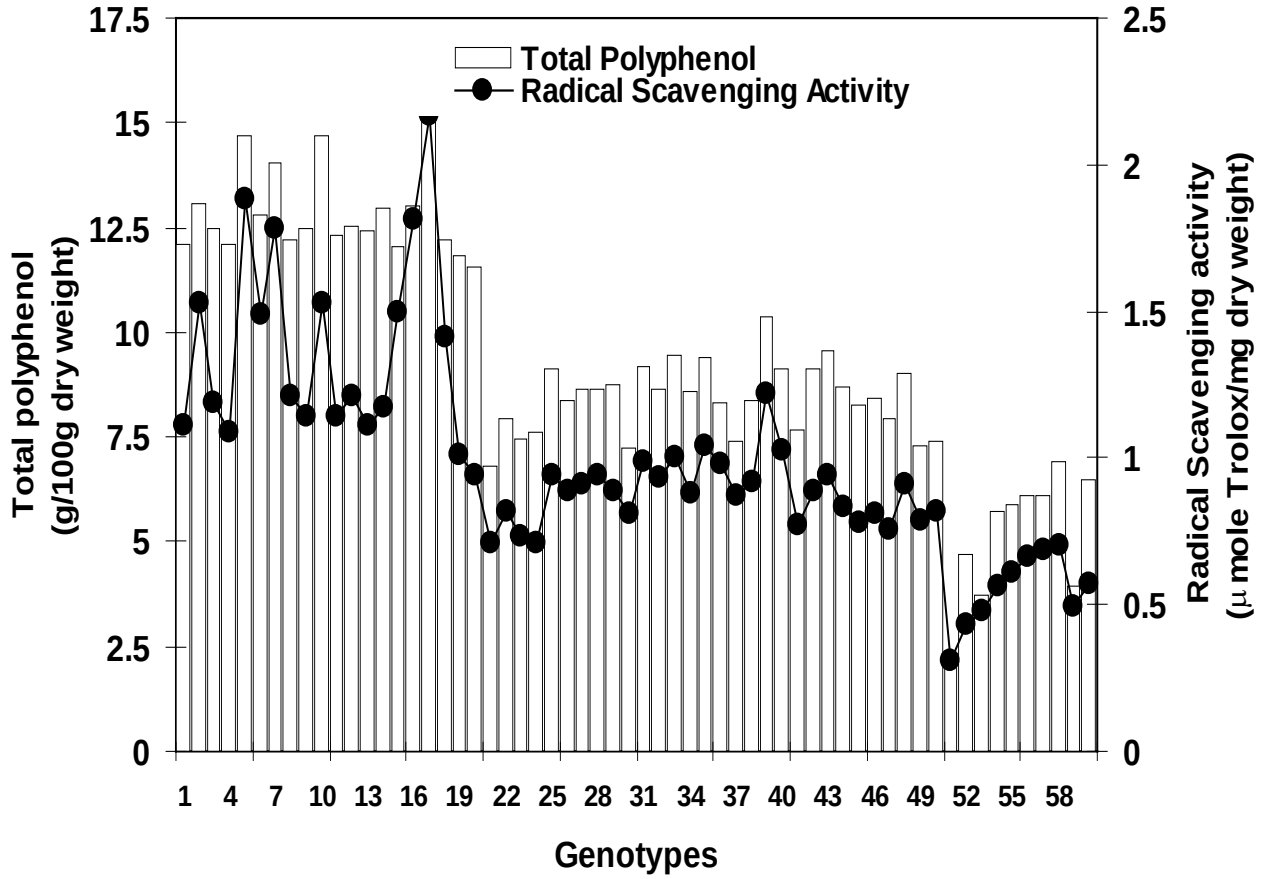


Figure 3. Anthocyanin (color value/g dry weight), carotenoid (g BCE/100g) and flavonoids (catechin equivalents; µg/g) contents in the leaves of the sweetpotato genotypes. *Name of the genotypes is the same as in Table 1

3.3. Effects of Water Extract of Leaves on the Mutagenicity

The antimutagenic impact of the water extracts from sweetpotato leaves of selected 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). The results found that inhibitory activity was higher at higher doses in all genotypes studies. The inhibitory activity (%) ranged from 87 to 97 at 100µg/plate, 70 to 94 at 50µg/plate and 48 to 70 at 10 µg/plate doses. The highest activity found in the genotypes Setoka, Kyushu-137 and Chikei-3 while Yen 634 had the lowest. Therefore, the results propose a widespread disparity of antimutagenicity among the genotypes, and the extracts showed dose-dependent inhibitory activities. Several researchers19,40,53 also found similar trends. 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-1, Trp-P-2, IQ, B[a]P, and DEGB. 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. Sweetpotato leaf extract effectively depressed the mutation caused by Trp-P-1 as shown in Table 2. The extract inhibited 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.

3.4. Distribution of Caffeoylquinic Acid Derivatives in Sweetpotato Leaves

The caffeoyl quinic acid derivatives (mg/g DW) in the leaves of sixty sweetpotato genotypes studied were quantified by using RP-HPLC (Table 3; Figure 1). The vast discrepancy was observed in about free caffeic acids in the sweetpotato leaves considered. Across all genotypes, the mean for CA was 2.2 to 179.6 mg/g DW with an average of 22.2 mg/g DW, for ChA, 4,5-diCQA, 3,5-diCQA, 3,4-diCQA and 3,4,5-triCQA contents ranged

from 26.5 to 701.5, 35.2 to 1183.3, 105.3 to 3503.6 and 13.5 to 750.6 mg/g DW, respectively. Among the caffeoylquinic acid derivatives, CA showed the highest level of coefficient of variation (124.9%), followed by 3,4-diCQA (104.4%), 3,4,5-triCQA (96.5%), ChA (83.9%) and 3.5-diCQA (83.2%), while the least variation was found for 4,5-diCQA (76.1%). The results of this study revealed that the sweetpotato leaf caffeoylquinic acid derivatives (CAD) did vary according to genotypes, but proportions were relatively similar in all the genotypes studied. The CAD are ubiquitous biologically active compounds found in plant foods and beverages and have received increased attention because of their potential physiological functions that may exert cardioprotective effects in humans [21,32,43,67-72]. It has been shown that intake of these compounds was inversely related to coronary heart disease mortality [73,74]. Since the CAD shows various physiological functions, sweetpotato leaves might also be expected to have physiologically active properties because they contain higher contents of bioactive compounds.

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

Genotypes	Added volum (μL)	His^+ revertants (per plate[b])	Inhibition (%)
Setoaka	100	23 ± 3	97
	50	51 ± 5	93
	10	159 ± 8	77
Kyushu-22	100	27± 4	96
	50	55 ± 5	92
	10	162 ± 7	77
Kyushu-106	100	31 ± 3	96
	50	57 ± 24	92
	10	176 ± 8	74
Kyushu-137	100	20 ± 2	97
	50	48 ± 4	93
	10	143 ± 7	79
Chikei-3	100	19 ± 2	97
	50	41 ± 5	94
	10	142 ± 6	79
7-1043	100	29 ± 3	96
	50	66 ± 6	90
	10	194 ± 9	72
Fv-62-64	100	33 ± 3	95
	50	69 ± 5	90
	10	199 ± 8	71
B 64-3	100	30 ± 4	95
	50	75 ± 6	89
	10	203 ± 9	70
AIP-539	100	37 ± 4	95
	50	79 ± 7	89
	10	219 ± 11	68
Okierabu-4	100	29 ± 4	95
	50	84 ± 8	88
	10	222 ± 10	68
Ninjin imo	100	49 ± 5	93
	50	99 ± 8	85
	10	242 ± 12	65
Damaciigaraimo-2	100	52 ± 5	92
	50	115 ± 7	83
	10	267 ± 11	61
Kyukei-116	100	74 ± 6	89
	50	169 ± 11	75
	10	321 ± 17	53
Yen 136	100	79 ± 6	88
	50	195 ± 12	71
	10	339 ± 16	50
Yen 634	100	91 ± 8	87
	50	201 ± 14	70
	10	359 ± 18	48

[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.

Table 3. Distribution of caffeoyl quinic acid derivatives (mg/g DW) in the leaves of sixty sweetpotato genotypes studied

Genotypes	CA	ChA	4,5-diCQA	3,5-diCQA	3,4-diCQA	3,4,5-TriCQA
1	16.1	411.6	965.2	1062.3	143.0	79.3
2	11.4	427.2	916.9	2328.8	495.5	79.4
3	7.3	643.1	1037.2	2101.6	524.4	149.3
4	23.6	410.8	551.9	952.9	332.9	139.1
5	88.4	232.4	533.6	1677.5	744.9	15.8
6	80.5	365.0	1154.0	1814.8	286.5	142.0
7	179.6	265.3	735.7	3503.6	750.6	59.0
8	29.1	327.3	881.7	1652.4	316.6	110.4
9	23.1	392.5	670.1	1040.8	467.8	182.6
10	9.9	602.2	1046.5	2381.6	207.6	56.3
11	60.6	350.2	1047.4	1412.5	222.2	86.9
12	67.1	276.4	741.6	1876.4	112.7	23.7
13	10.7	485.4	775.6	1260.8	365.0	60.7
14	26.3	454.6	813.5	1349.1	366.7	143.0
15	17.8	510.7	666.9	1002.4	235.9	60.2
16	11.9	637.6	914.8	1497.2	359.1	65.9
17	16.8	701.5	1011.5	1280.7	369.7	50.0
18	17.5	437.4	883.8	1619.3	247.3	64.8
19	21.5	334.1	1183.3	1514.0	230.7	221.0
20	23.4	657.1	315.4	2278.0	238.7	63.4
21	16.4	104.6	412.1	498.8	123.2	37.6
22	8.7	162.3	270.0	334.2	98.0	30.2
23	5.7	59.5	157.9	246.4	52.3	21.2
24	33.9	53.6	221.6	542.8	91.5	13.0
25	19.8	59.3	174.2	268.7	51.3	9.1
26	36.3	61.7	262.6	352.4	44.1	40.9
27	8.0	143.8	286.7	591.7	147.1	36.5
28	3.8	144.4	406.0	622.2	83.8	49.6
29	7.9	212.3	378.9	698.1	64.5	67.4
30	35.5	100.5	144.3	468.6	49.4	13.1
31	20.1	116.5	293.7	452.6	79.6	37.3
32	11.0	91.4	182.8	257.6	73.2	23.4
33	2.8	93.3	195.9	299.4	40.0	15.4
34	4.6	115.2	244.3	467.9	87.9	26.1
35	8.9	146.2	311.4	750.0	102.0	67.8
36	19.4	175.6	352.5	726.5	175.1	52.8
37	11.2	65.3	119.7	404.8	35.6	8.5
38	15.0	103.3	187.0	365.0	72.8	12.0
39	13.5	130.7	213.1	522.2	60.3	11.3
40	31.6	138.9	246.7	591.1	185.4	116.6
41	9.6	177.9	172.7	805.4	150.9	56.6
42	4.1	152.3	242.0	469.7	44.0	20.4
43	4.1	145.7	251.9	332.6	52.1	29.1
44	5.6	115.7	190.7	259.1	44.2	10.8
45	29.9	126.9	369.3	722.9	159.7	12.3
46	3.7	89.1	338.1	412.4	33.1	11.6
47	4.7	165.9	460.2	524.8	76.6	52.4
48	2.2	175.3	268.6	374.7	62.4	18.3
49	9.5	113.9	246.7	440.3	65.1	42.5
50	2.4	126.6	257.5	280.8	34.6	16.4
51	55.3	32.6	35.2	130.7	24.1	13.6
52	29.3	34.1	69.9	105.3	13.5	6.8
53	7.0	26.5	118.6	152.6	25.1	17.2
54	16.2	62.3	99.4	338.8	34.9	5.9
55	8.8	85.0	121.8	356.0	30.2	16.4
56	4.3	75.9	263.9	315.7	33.8	18.9
57	8.1	74.9	169.4	373.7	25.6	17.6
58	41.5	36.8	144.9	448.9	69.4	14.9
59	20.6	46.4	65.5	294.6	18.6	6.4
60	9.5	93.5	142.1	531.2	49.5	9.4
Average	22.2	219.3	432.3	845.6	163.0	49.0
SD	27.8	184.1	328.8	703.9	170.2	47.3
SE	3.6	23.8	42.4	90.9	22.0	6.1
CV	124.9	83.9	76.1	83.2	104.4	96.5

CA= caffeic acid , ChA= Chlorogenic acid; 3,4-diCQA= 3-4-di-*O*-caffeoyl quinic acid;
3,4,5-TriCQA =3,5-diCQA = 3-5-di-*O*-caffeoyl quinic acid; 4,5-diCQA= 4-5-di-*O*-caffeoyl quinic acid;
3-4-5-tri-*O*-caffeoyl quinic acid. SD= Standard deviation; SE= Standard error; CV= Coefficient of variation
[Y]Name of the genotypes is as shown in Table 1.

3.5. Antimutagenicity of Caffeoylquinic Acid Derivatives

The present report revealed that sweetpotato leaf extract effectively depressed the reverse mutation persuaded by several mutagens. We have demonstrated that sweetpotato tops comprise a very high content of polyphenolics and have further identified caffeic acid and five kinds of derivatives, ChA, 3-4-diCQA, 3, 5-diCQA, 4-5-diCQA and 3, 4.5-triCQA (18). The effects of caffeoylquinic acid derivatives on the reverse mutation tempted by Trp-P-1 are shown in Table 4. ChA inhibited the reverse mutation by 29 to 41% in a dose range of 0.14 to 0.57 mM, while 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA, respectively inhibited the reverse mutation by 39 to 59%, 25 to 59%, and 32 to 61%. The 3, 4, 5 - triCQA suppressed the reverse mutation by 46 to 84% in the same dose range of 0.14 to 0.57 mM. Table 5 demonstrations the antimutagenicity of caffeic acid derivatives at a dose of 0.5 mg/plant against the reverse mutations induced by Trp-P-2, IQ, and DEGB. The 3, 4.5-triCQA appears to have stronger antimutagenicity against the mutants tested followed by 4-5-diCQA. From the Table 4, it was apparent that all compounds tested showed a dose-dependent antimutagenicity. Three di-caffeoylquinic acid derivatives exhibited almost similar antimutagenic activity in a dose of 0.57 mM. Antimutagenicity of three di-caffeoylquinic acid derivatives and 3,4,5-triCQA was about 1.5 and 2.0 times higher than ChA, respectively. Those values settle with a previous report [53] that compounds analogous to CA or ChA efficiently reduction the mutagenic activity of the mutagens as heterocyclic amines. Caffeoylquinic acid derivatives in sweetpotato leaves may exhibit many kinds of physiological functions other than radical scavenging activity. The 3,4,5-triCQA and 4,5-diCQA have been noted to inhibit HIV replication [75]. The 3,5-diCQA inhibits the histamine secretion induced by concanavalin A plus phosphatidylserine from rat peritoneal mast cells [76]. Kwon et al. [77] found that 3,5-dicaffeoyl-muco-quinic acid more efficiently inhibited HIV-1 integrase than 3,5-diCQA, 4,5-diCQA, and ChA in Aster scaber. Yagasaki et al. [78] indicated that ChA, CA, and QA suppress hepatoma cell invasion without altering the cell proliferation. Murayama et al... [79] identified ChA, 3,5-diCQA, and 4,5-diCQA as the primary antioxidants in edible chrysanthemums. ChA and diCQA derivatives were isolated from various plants including sweetpotato leaves [80,81], as described above, but there are very few reports on 3,4,5-triCQA. Isolation of 3,4,5-triCQA was reported in *Securidaka longipedunculata* (*Polygalaceae*) [75], *Tessaria integrifolia*, and *Mikania cordifolia* (*Asteraceae*) [82]. These studies indicate that the antimutagenicity of 3,4,5-triCQA is more efficient than mono- or diCQA derivatives. These data also suggest that 3,4,5-triCQA might exceed mono- and diCQA derivatives in physiological function. Several varieties of sweetpotato contain a high content (>0.2%) of 3,4,5-triCQA [20,21,44], suggesting that the sweetpotato leaf is a source of not only mono and diCQA derivatives but also 3,4,5-triCQA. A contrast of the actions and structures of these compounds counseled that the number of caffeoyl groups bound to quinic acid played a part in the antimutagenicity of the caffeoylquinic acid derivatives. Thus, sweetpotato leaves confined distinct polyphenolic components with a high content of mono-, di-, and tri caffeoylquinic acid derivatives. The results obtained from this study showed that sweetpotato leaves possess high total phenolic, anthocyanin, carotenoid and flavonoid contents with the ability to protect against certain types of human diseases. Furthermore, sweetpotato genotypes with greater bioactive compounds used as a vegetable, tea, food ingredient, and as a nutritional supplement, can be claimed to have the positive impact on the advancement of health. The results also revealed that sweetpotato leaves had a very high concentration of naturally active caffeoylquinic acid derivatives that show improved physiological functions, which might have values in the prevention of certain human conditions like cancer, HIV infection, hepatotoxicity, allergies, aging, coronary heart disease, and cardiovascular disease. Therefore, sweetpotato leaves could be an outstanding source of naturally active compounds with numerous physiological functions.

Table 4. Effect of caffeoylquinic acid derivatives from sweetpotato leaves on the mutagenicity of Trp-P-1 against *Salmonella typhimurium* TA 98[a]

CAD	Dose (mM)	His^+revertants (per plate[b])	Inhibition (%)
Chlorogenic acid	0.14	369 ± 5	29
	0.29	335 ± 23	35
	0.57	307 ± 15	41
3-4-di-*O*-caffeoyl quinic acid	0.14	317 ± 6	39
	0.29	267 ± 6	48
	0.57	211 ± 13	59
3-5-di-*O*-caffeoyl quinic acid	0.14	391 ± 19	25
	0.29	283 ± 13	45
	0.57	213 ± 20	59
4-5-di-*O*-caffeoyl quinic acid	0.14	350 ± 14	32
	0.29	241 ± 27	54
	0.57	200 ± 9	61
3-4-5-tri-*O*-caffeoyl quinic acid	0.14	281 ± 27	46
	0.29	137 ± 22	74
	0.57	85 ± 6	84

[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 for the caffeoylquinic acid derivatives (CAD) were 518±49 per plate.

Table 5. Effects 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]				
	ChA	3-4-diCQA	3-5-diCQA	4-5-diCQA	3-4-5- triCQA
Trp-P-2(0.20 µg)	44±5.03	49±4.50	47±4.55	58±4.04	80±5.03
IQ (0.20 µg)	33±3.66	39±5.35	33±3.10	61±5.46	62±4.23
DEGB (0.20 µl)	58±3.51	50±4.30	58±4.61	72±5.51	83±5.57

[a]Mutagenicity was tested with S-9 mix. [b]The caffeoylquinic acid derivatives 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.

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