Antimutagenicity and Antioxidant Activity in the Ipomoea batatas L. Genotypes in Relation to Polyphenolics

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***Abstract*—** Antimutagenicity of the water extracts and radical scavenging activity (RSA) of the water extracts prepared from the leaves of sixty genotypes of sweetpotatoes (*Ipomoea batatas* L) were investigated using the *Salmonella typhimurium* TA 98. The extract from the high polyphenol accumulator genotypes effectively decreased the reverse mutation induced not only by Trp-P-1 and Trp-P-2, IQ, B(a)P, and 4-NQO, but also by dimethyl sulphoxide extract of grilled beef. Comparison of the inhibitory activity of the extracts from the medium and low polyphenol accumulator genotypes suggested that the polyphenol content in the leaves decreases the mutagenic activity of the mutagens as heterocyclic amines. Three constituents of leaves polyphenols, 4,5 di-CQA (caffeoylquinic acid) and 3,4,5-tri-CQA, effectively inhibited the reverse mutation induced by Trp-P-1, Trp-P-2, and IQ. Furthermore, determination of the inhibitory activity of sectional portions using the FV-85 genotypes demonstrated that the inhibitory components are abundant in the leaves, suggesting the involvement of phenolics in the antimutagenicity of the extract from the sweetpotato leaves. The results also suggested that RSA is positively correlated with total phenolics of sweetpotato leaves.

***Keywords*—**Antimutagenicity, antioxidant, sweetpotato leaves, phenolics, caffeic acid and derivatives.

I. Introduction

THE sweetpotato [(*Ipomoea batatas* (L.) Lam.] is the seventh most important food crop in the world (FAO 1997), and is among the crops selected by the U. S. National Aeronautics and Space Administration to be grown in a controlled ecological life support system as a primary food source (Hoff et al., 1982). Recent development of screening methods for environmental carcinogens by determining their mutagenicity has enabled to detect various types of mutagens and carcinogens in foods (Ames et al., 1975, Islam, 2006; 2008; 2009; 2014; Islam et al., 2009). Some of these substances in foods have been found to be generated during storage, cooking, and digestion. (Nagao et al., 1977; Kasai et al., 1979; Yamaizumi et al., 1980; Islam, 2006; 2008). On the other hand, it is now known that various types of inhibitors that act against mutagens and carcinogens in food. They play an important role in reducing the risks of mutagenesis and carcinogenesis (Shinohara et al., 1988). The nutritive components of sweetpotato leaves are comparable to those of commercial leafy vegetables (Villareal et al., 1979; Woolfe 1992; Yoshimoto et al., 2001; Islam and Jalaluddin, 2004; Islam, 2006; 2008; 2014). However, foliar antimutagenic activity of sweetpotato genotypes has not been investigated. The structural feature responsible for the antioxidative and free radical scavenging activity of caffeic acid is the *ortho*-dihydroxyl functionality in the catechol (Mahmood et al., 1993). Therefore, the physiological function of these CQA derivatives with plural caffeoyl groups is more effective than with a mono-caffeoyl one. Several investigators have partially clarified some physiological functions of CQA derivatives (Kimura et al., 1985; Murayama et al., 2002; Son and Lewis 2002; Islam et al., 2009). However, the physiological function of sweetpotato leaves and the CQA derivatives have not yet been studied synthetically. In the present paper, the effects of the water extracts of the sixty selected genotypes of sweetpotato with different polyphenol level on the mutagenicity and radical scavenging activity are investigated.

II. Materials and Methods

Materials

The leaves from sixty sweetpotato genotypes were used for this study. Features of the *Ipomoea batatas* L. genotypes used in the study was presented in Table 1. After harvest, the leaves were washed gently, transferred into pre-labeled individual vinyl bags and immediately frozen at –85 ^{O}C. The following day all the frozen samples were freeze-dried for 48 h in freeze dryer. The freeze-dried samples were powdered by a blender and used for laboratory analysis. The extract was made from the lyophilized flour (1 g) using 20 mL of ice-cold water for 1 h. 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.

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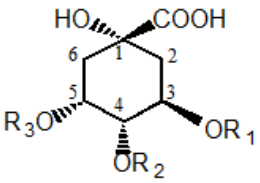
Table 1. Features of the genotypes used in the study.

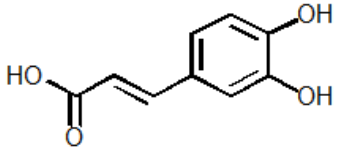
Sl. No.	Genotype	Parental information	Origin
1	Setoaka	Kyushu-12 (M)/Gokokuimo (F)	Japan
2	Kyushu -22	Gokokuimo (M)/Norin-7(F)	Japan
3	Kyushu-106	Kyukei-20 (M)/K-88 (F)	Japan
4	Kyushu-137	Kyushu-165 (M)/Tanegashima (F)	Japan
5	Chikei-3	Unknown	Japan
6	7-1043	Shichifuku (M)/2-2057(F)	Japan
7	FV-62-64	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

Extraction and Measurement of Total Phenolics. Determinations of total polyphenols were made according to the Folin-Ciocalteau method with slight modification (Islam et al., 2002). The lyophilized sweetpotato leaf flour was vigorously mixed with 10 times its equivalent volume of 80% ethanol. The mixture was boiled for 5 min and centrifuged at 5000*g* for 10 min, and the supernatant was collected. The residue was mixed with additional 80% ethanol and boiled for 10 min to re-extract the phenolics, and centrifuged under the same conditions. The extracts were combined and made up to 10 mL and used for the measurement of total phenolics. The alcohol extract was diluted to obtain an absorbance reading within the range of the standards (800-40 *íg* 3-*O*caffeoylquinic acid/mL). The absorbance was measured at 600 nm with a dual-wavelength flying spot scanning densitometer (Shimadzu Co., Japan) with a microplate system. The results were expressed as g/100 g dry leaf powder.



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

Fig. 1 Chemical structures of polyphenolic compounds in sweetpotato leaves

Determination of radical scavenging activity. Radical scavenging activity was determined using a stable radical, DPPH, according to the method reported by Brand-Williams et al. (1995) with a slight modification. All the reaction was in a 96-well microplate with a total volume of 300 µL. A sample solution (75 µL) containing the test compound at different concentrations in 0.1M MES buffer (pH 6.0) with 50% ethanol was added to 150 µL of the same buffer. 75 µL 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 by a dual wavelength flying spot scanning densitometer (Shimadzu Co., Japan) fitted with a microplate system. All tests were performed in triplicate. The radical scavenging activity of the samples extracted from the leaves was expressed in terms of IC_{50} (concentration in µmole Trolox/g of dry powder, required for a 50% decrease in the absorbance of DPPH radicals). However, IC_{50} of purified compounds was expressed on a molar base. A plot of the absorbance vs. the concentration was made to calculate IC_{50}.

Assay of antimutagenicity. The antimutagenicity assay was performed as described in previous papers (Islam et al., 2003a; 2003b). The antimutagenic activity was evaluated for *Salmonella typhimurium* TA 98 using a mutagen, Trp-P-1. These mutagens require metabolic activation to induce mutation in TA 98. S-9 mix contained 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 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 simultaneously incubated with 0.1 mL of bacterial suspension at 37 ^{O}C for 20 min, and then poured on 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.

Chemicals, Mutagen and bacteria. Trp-P-1 [3-amino-1,4-dimethyl-5*H*-pyrido-(4,3-*b*)indol], Trp-P-2 [3-amino-1-methyl-5*H*-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 supplied by Wako Pure Chemicals Industries Ltd., Osaka, Japan. 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 ^{O}C prior to 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 according to Islam (2006).

Statistics. A randomized complete block design with five replications was adopted. Data for the different parameters were analysed by analysis of variance (ANOVA) procedure, and the level of significance was calculated from the F value of ANOVA. The relation between total polyphenols and RSA were described with linear correlation analysis.

Abbreviation. 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; 4-NQO, 4-nitroquinoline-*N*-oxide; N-OH-Trp-P-1, *N*-hydroxy-3-amino-1,4-dimethyl-5H-pyrido-(4,3-*b*)indol; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido-(4,3-*b*)indol; Trp-P-2, 3-amino-1-dimethyl-5*H*-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

III. Results and Discussion

Radical scavenging activity and total polyphenol of the genotypes

The 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 was presented in Figure 1. A strong relationship was found between the polyphenol contents and RSA. The genotypes differed widely in their polyphenolic contents. The highest found was 17.1 g/100 g dry weight and the lowest was 2.62 g/100 g dry weight and most of the genotypes (>95%) contained > 6.00 g/100 g dry weight total polyphenolics, which is a very high concentration compared to other commercial vegetables (Walter et al., 1979; Lugasi et al., 1999; Yoshimoto 2001). The genotypes were classified into 3 groups according to polyphenolic content, namely, (A) high polyphenol accumulator (>12.0 g/100 g dry weight); (B) medium polyphenol accumulator (>9.0-12.0 g 100/g dry weight); and (C) low polyphenol accumulator (542) (<9.0 g/100 g dry weight) (Islam et al., 2002). The RSA ranges from 0.21 to 2.3 µ mole Trolox/mg dry leaf powder. The results exhibited that RSA is depended on their respective polyphenol content of sweetpotato leaves. The phenolic acids are ubiquitous bioactive compounds found in plant foods and beverages. Since polyphenol compounds show various physiological functions, sweetpotato leaves might also be expected to have physiologically active properties because they contain higher contents of polyphenolic compounds with high RSA. This result may be useful for various chemical breeding programs to improve desirable organoleptic and nutritional quality characteristics of crop plants.

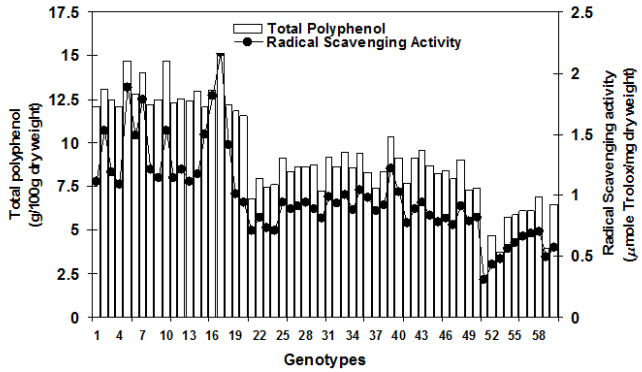


Fig. 2. Radical scavenging activity (µ mole Trolox/mg dry leaf powder) and total polyphenol (g/100 g dry leaf powder) in the leaves of selected sixty sweetpotato genotypes. *Please check Table 1 for the name of individual genotype

Effects of water extracts from leaves on the mutagenicity of Trp-P-1

The antimutagenic effect of the water extracts from sweetpotato leaves of sixty genotypes with different levels of polyphenolics was examined using Trp-P-1 at a dose of 0.075 µg/plate. The extract was used at doses of 1, 5, and 10 mg/plate. Table 1 shows the results at a dose of 1.0 mg/plate. The inhibitory activity was 41% at a dose of 1.0 mg/plate of the extract from high polyphenol accumulator genotypes (average of 5 genotypes) and the extract showed a dose-dependent antimutagenicity (data not shown). The extracts from medium and low polyphenol accumulator genotypes showed the inhibitory activities of 0, 0, and 5% at a dose of 1.0 mg/plate, respectively. The extracts from low polyphenol accumulator genotypes rarely inhibited the reverse mutation even at doses of 5 or 10 mg/plate (data not shown). These results suggest that the antimutagenic effect of the extract from low and medium polyphenol accumulator sweetpotato genotypes is minor compared with the one from high polyphenol accumulator.

Effect of sweetpotato leaf extract on the mutagenicity of various mutagens

The antimutagenic activity of the extract was evaluated using several mutagens, such as Trp-P-1, Trp-P-2, IQ, B[a]P, and DEGB (Table 2). DEGB was used at a dose of 100 µL/plate without dilution. 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 was used at doses of 50, 10, and 5 µL/plate, since sweetpotato leaf extract effectively depressed the mutation induced 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. The sweetpotato leaf extract also inhibited DEGB-induced mutation by 40%-68% at a dose of 5-50 µL/plate. These results showed the dose dependent antimutagenicity of sweetpotato leaf extract against the reverse mutation induced by DEGB, as well as Trp-P-1, Trp-P-2, B[a]P and IQ.

TABLE II
EFFECT OF SWEETPOTATO LEAF EXTRACT ON THE MUTAGENICITY OF TRP-P-1 AGAINST *SALMONELLA TYPHIMURIUM* TA 98[A]

Sample No.	Added volume (μL)	His^+ revertants (per plate[b])	Inhibition (%)
1	100	23 ± 3	97
	50	51 ± 5	93
	10	159 ± 8	77
2	100	27± 4	96
	50	55 ± 5	92
	10	162 ± 7	77
3	100	31 ± 3	96
	50	57 ± 24	92
	10	176 ± 8	74
4	100	20 ± 2	97
	50	48 ± 4	93
	10	143 ± 7	79
5	100	19 ± 2	97
	50	41 ± 5	94
	10	142 ± 6	79
6	100	29 ± 3	96
	50	66 ± 6	90
	10	194 ± 9	72
7	100	33 ± 3	95
	50	69 ± 5	90
	10	199 ± 8	71
8	100	30 ± 4	95
	50	75 ± 6	89
	10	203 ± 9	70
9	100	37 ± 4	95
	50	79 ± 7	89
	10	219 ± 11	68
10	100	29 ± 4	95
	50	84 ± 8	88
	10	222 ± 10	68
11	100	49 ± 5	93
	50	99 ± 8	85
	10	242 ± 12	65
12	100	52 ± 5	92
	50	115 ± 7	83
	10	267 ± 11	61
13	100	74 ± 6	89
	50	169 ± 11	75
	10	321 ± 17	53
	100	79 ± 6	88
	50	195 ± 12	71
	10	339 ± 16	50
15	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.

Antimutagenicity of caffeoylquinic acid derivatives

Present report revealed that sweetpotato leaf extract effectively depressed the reverse mutation induced by several mutagens. Recently, we have reported that sweetpotato leaves contain 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 (Islam et al., 2002). The effects of caffeoylquinic acid derivatives on the reverse mutation induced by Trp-P-1 are shown in Table 3. 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 inhibited the reverse mutation by 46 to 84% in the same dose range of 0.14 to 0.57 mM. All compounds tested showed a dose dependent antimutagenicity. No killing effect was apparent, at least with the doses tested (data not shown). 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.

TABLE III
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.

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 (Mahmood et al., 1993). The 3,5-diCQA inhibits the histamine secretion induced by concanavalin A plus phosphatidylserine from rat peritoneal mast cells (Kimura et al., 1985). Kwon et al. (2000) 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. (2000) indicated that ChA, CA, and QA suppress hepatoma cell invasion without altering the cell proliferation. Murayama et al. (2002) recently 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 (Shimozono et al., 1996; Walter et al., 1979), 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) (Mahmood et al., 1993), *Tessaria integrifolia*, and *Mikania cordifolia* (Asteraceae) (Peluso et al., 1995). These study indicates that the antimutagenicity of 3,4,5-triCQA is more effective 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 (Islam et al., 2002), suggesting that the sweetpotato leaf is a source of not only mono and diCQA derivatives but also 3,4,5-triCQA.

In conclusion, sweetpotato leaves contain a much higher polyphenolic content than commercial vegetables with the ability to protect against certain types of human diseases. Furthermore, sweetpotato genotypes with high polyphenolic content used as vegetable, tea, food ingredient, and as a nutritional supplement, could be claimed to have positive impact for the promotion of health. Our results showed that sweetpotato leaves contain a very high concentration of biologically active caffeoylquinic acid derivatives that exhibited enhanced antimutagenic and antioxidative properties, 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, these reports and our data indicate that sweetpotato leaves could be excellent source of biologically active compounds with multiple physiological functions.

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