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Antimutagenicity of the Water Extracts, Radical Scavenging Activity, and Phenolic Acids in the Tops of Diverse *Ipomoea batatas* (L.) Lam

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Abstract

Radical scavenging activity (RSA) and antimutagenicity of the water extracts were investigated using diverse sweetpotatoes [*Ipomoea batatas* (L.) Lam] accessions. The results revealed that RSA is depended on their respective polyphenol content of sweetpotato leaves. The extract from the enhanced polyphenol contents accessions successfully diminished 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. The physiological function of caffeoylquinic acid (CQA) derivatives with plural caffeoyl group are more effective than with a monocaffeoyl one. Furthermore, determination of the inhibitory activity of sectional portions using the samples A-35, A-09, and A-28 were demonstrated that the inhibitory components are abundant in the leaves, suggesting the involvement of phenolics in the antimutagenicity of the extract from the sweetpotato tops. This result may be useful for various chemical breeding programs to improve desirable organoleptic and nutritional quality characteristics and health benefits of peoples.

Keywords

Antimutagenicity, Radical Scavenging Activity, Phenolics, Caffeic Acids, Sweetpotato

1. Introduction

Sweetpotato [*Ipomoea batatas* (L.) Lam] is the seventh most important food crop in the world, and possibilities of utilizing this crop for new purposes have been identified [1], 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 [2]. Recent studies have indicated that sweet potato contains such functional components as polyphenols, anthocyanins, dietary fiber, etc., which are essential for the protection of human health. In addition to its food value as a good source of carbohydrates in the roots, sweetpotato tops (leaves and stems) are found to contain unique nutritional components. Some of these dietary components are higher in sweetpotato than many other commercial vegetables. Sweetpotato leaves are used as a cooked vegetable in many parts of the world [4]. They are rich in vitamin B, iron, calcium, zinc, and protein [14]. Sweetpotato tops can be harvested several times a year, and the annual yield is, therefore, much higher than many other green vegetables. The recent development of screening methods for environmental carcinogens by determining their mutagenicity has enabled to detect various types of mutagens and carcinogens in foods [3-8]. Some of these substances in foods have been found to be generated during storage, cooking, and digestion. [4, 5, 9-11]. 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 [12]. The nutritive components of sweetpotato leaves are comparable to those of commercial leafy vegetables [4, 5, 7, 13-16]. Phenolic compounds are a diverse group of secondary metabolites present in higher plants that play important roles in the structure of plants and are involved in a number of metabolic pathways (Harborne 1980). Plant phenolics, because of their diversity and extensive distribution, can be argued to be an important group of natural antioxidants, and contribute to organoleptic and nutritional qualities of fruit and vegetables. Functional food products are aimed at introducing human dietary ingredients that aid specific bodily functions in addition to being nutritious. Several authors report that sweetpotato leaves are an excellent source of antioxidative polyphenolics, among them anthocyanins and phenolic acids such as caffeic, monocafeoyl quinic (chlorogenic), dicaffeoylquinic, and tricaffeoylquinic acids [5-7, 16, 21, 23-24]. The sweetpotato leaves nutritional attributes are increasingly recognized as a better understanding emerges in the relationship between diet and human health. However, the 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 [17]. 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 [8, 18-20]. 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.

2. Materials and Methods

2.1. Plant Materials

The tops from 12 sweetpotato accessions were used for this study. After harvest, the leaves were washed gently, transferred into pre-labeled individual vinyl bags and immediately frozen at –80°C. The following day all the frozen samples were freeze-dried for 48 h in a freeze dryer. The freeze-dried samples were powered 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 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 laboratory analysis.

2.2. Measurement of Phenolics

Determinations of total polyphenols were made according to the Folin-Ciocalteau method with slight modification [21]. 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 an 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 *í*g 3-*O*caffeoylquinic acid/mL). The absorbance was measured at 600 nm with a dual-wavelength flying spot scanning densitometer with a microplate system. The results were expressed as g/100 g dry leaf powder.

2.3. Measurement of Radical Scavenging Activity

Radical scavenging activity was determined using a stable radical, DPPH, according to the method reported by Brand-Williams et al. [22] 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. 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 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}.

2.4. Assay of Antimutagenicity

The antimutagenicity assay was performed as described in previous papers [23-24]. 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 $MgCl_2$, 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°C for 20 min and then poured on minimal-glucose-agar plates with 2 mL of soft agar. The colony number of each dish was accounted after 48 h cultivation at 37°C.

2.5. 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. Other chemicals used were standard grade. Strain. The bacterium was cultured in nutrient broth for 16 h at 37°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.

2.6. Statistical Analysis

Randomized complete block design with five 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. The relation between total polyphenols and RSA were described with linear correlation analysis.

2.7. 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.

3. Results and Discussion

3.1. Radical Scavenging Activity (RSA) and Polyphenol of the Genotypes

The total polyphenol (g/100 g dry leaf powder) and the radical scavenging activity (µ mole Trolox/mg dry leaf powder) and in the leaves of selected accessions was presented in Figure 1 and Figure 2. A strong relationship was found between the polyphenol contents and RSA. The genotypes differed widely in their polyphenolic substances. The highest found was 11.1 g/100 g dry weight, and the lowest was 5.27g/100 g dry weight and most of the accessions contained > 6.00 g/100 g dry weight total polyphenolics, which is a very high concentration compared to other commercial vegetables [15, 25-26]. The RSA ranges from 0.81 to 1.70 µ mole Trolox/mg dry leaf powder (Figure 2). The results revealed that RSA is depended on their respective polyphenol content of sweetpotato leaves.

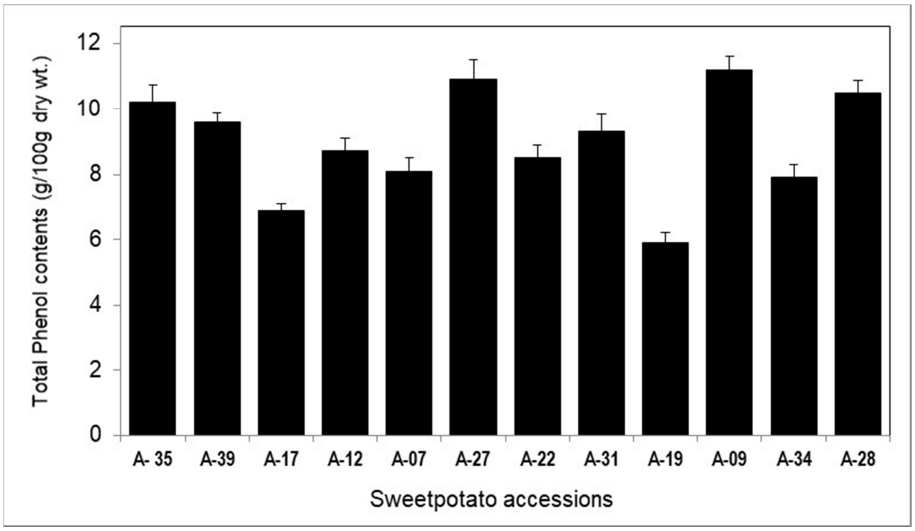

Figure 1. *Total phenol (g/100 g dry leaf powder) in the leaves of selected sweetpotato accessions. The bar indicates the standard error of the five replications.*

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.

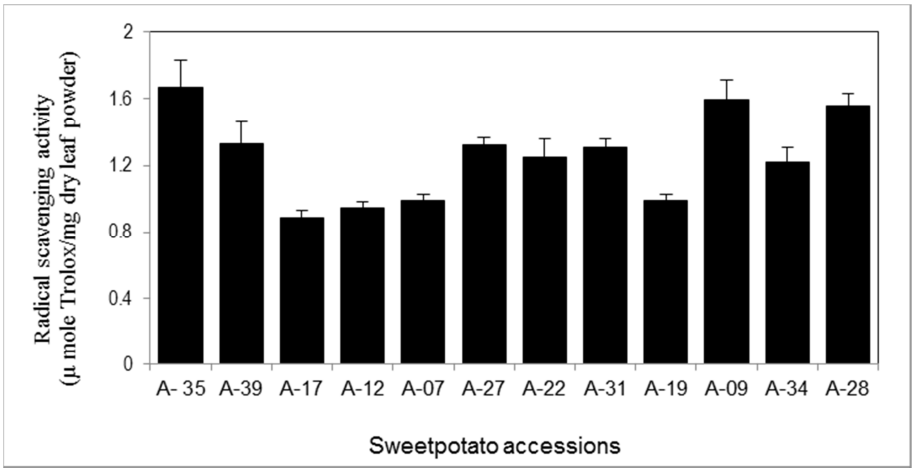

Figure 2. *Radical scavenging activity (µ mole Trolox/mg dry leaf powder) in the leaves of selected sixty sweetpotato accessions. The bar indicates the standard error of the five replications.*

3.2. Effects of Water Extracts from Leaves on the Mutagenicity of Trp-P-1

The antimutagenic effect of the water extracts from sweetpotato leaves 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 the treatment 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. 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. These results suggest that the antimutagenic effect of the extract from low and medium polyphenol accumulator sweetpotato accessions is negligible compared with the one from high polyphenol accumulator.

Table 1. *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	33 ± 3	97
	50	62 ± 5	93
	10	159 ± 8	71
2	100	27± 4	95
	50	55 ± 5	91
	10	162 ± 7	76
3	100	31 ± 3	97
	50	57 ± 24	92
	10	176 ± 8	75
4	100	20 ± 2	98
	50	48 ± 4	94
	10	143 ± 7	80
5	100	19 ± 2	98
	50	41 ± 5	94
	10	142 ± 6	79
6	100	29 ± 3	95
	50	66 ± 6	89
	10	194 ± 9	71
7	100	33 ± 3	96
	50	69 ± 5	88
	10	199 ± 8	70
8	100	30 ± 4	94
	50	75 ± 6	88
	10	203 ± 9	69
9	100	29 ± 2	99
	50	51 ± 5	92
	10	132 ± 6	72
10	100	31 ± 3	96
	50	77± 6	90
	10	187± 9	70
11	100	45 ± 3	95
	50	79 ± 5	87
	10	199 ± 8	70
12	100	38 ± 4	96
	50	82 ± 6	87
	10	201 ± 9	70

[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 679 ±16 per plate.

3.3. 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. 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 1. 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.

3.4. Antimutagenicity of Caffeoylquinic Acid Derivatives

The present report revealed that sweetpotato leaf extracts effectively depressed the reverse mutation induced by several mutagens. Recently, reported that sweetpotato leaves contain 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 [21]. The effects of caffeoylquinic acid derivatives on the reverse mutation induced by Trp-P-1 are shown in Table 2. 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 treatments proved (data not shown). Three di-caffeoylquinic acid derivatives exhibited almost similar antimutagenic activity in the therapy 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 2. *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 (%)
	0.14	369 ± 5	29
Chlorogenic acid	0.29	335 ± 23	35
	0.57	307 ± 15	41
	0.14	317 ± 6	39
3-4-di-*O*-caffeoyl quinic acid	0.29	267 ± 6	48
	0.57	211 ± 13	59
	0.14	391 ± 19	25
3-5-di-*O*-caffeoyl quinic acid	0.29	283 ± 13	45
	0.57	213 ± 20	59
	0.14	350 ± 14	32
4-5-di-*O*-caffeoyl quinic acid	0.29	241 ± 27	54
	0.57	200 ± 9	61
	0.14	281 ± 27	46
3-4-5-tri-*O*-caffeoyl quinic acid	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 [17]. The 3,5-diCQA inhibits the histamine secretion induced by concanavalin A plus phosphatidylserine from rat peritoneal mast cells [18]. Kwon et al. [27] 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. [28] indicated that ChA, CA, and QA suppress hepatoma cell invasion without altering cell proliferation. Murayama et al. [19] 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 [25, 29], 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*) [17], *Tessaria integrifolia*, and *Mikania cordifolia* (Asteraceae) [30]. This 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 [21], suggesting that the sweetpotato leaf is a source of not only mono and diCQA derivatives but also 3,4,5-triCQA [31].

4. Conclusion

Hence, sweetpotato tops comprise higher polyphenolic content with the aptitude to defend in contradiction of certain sorts of human illnesses. Furthermore, sweetpotatoes with high polyphenolic content used as a herb, tea, food ingredient, and as a nutritional supplement, could be demanded to have a positive impact on the promotion of health. The high concentration of biologically active caffeoylquinic acid derivatives that exhibited enhanced antimutagenic and antioxidative properties in the leaves extract, which might have values in the prevention of specific human conditions like cancer, HIV infection, hepatotoxicity, allergies, aging, coronary heart disease, and cardiovascular disease.

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