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ARTICLE

Antiproliferative Activity and Cytotoxicity of *Solanum jamesii* Tuber Extracts on Human Colon and Prostate Cancer Cells *in Vitro*

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Some tuber-bearing wild potato species are reportedly higher in potential health-promoting traits, such as antioxidant activity (AOA) and total phenolic content (TP), than commercial cultivars; therefore, they could be used as parental material in breeding for high AOA and TP. However, using wild species might result in progenies that are toxic for human consumption because of the presence of high total glycoalkaloids (TGAs) and other unknown compounds. Therefore, wild potato accessions should be screened for cytotoxicity before their introduction into breeding programs. The objective of this study was to investigate antiproliferative activity and cytotoxicity of tuber extracts from 15 *Solanum jamesii* accessions on human HT-29 colon and LNCaP prostate cancer cell lines *in vitro*. Also, correlations among AOA, TP, TGA, and antiproliferative activity were determined. The tuber extracts significantly inhibited proliferation of HT-29 and LNCaP cell lines and were not cytotoxic to the cells compared to the control (DMSO). The antiproliferative activity exhibited by tuber extracts was not due to necrosis, because the amount of lactate dehydrogenase (LDH) released from cells incubated with the extracts was not significantly different from that released from cells incubated without extracts (control). Colon cancer cells were more responsive to tuber extract treatment than prostate cancer cells. In both HT-29 and LNCaP cells, there were no observable significant correlations between antioxidant activity (DPPH and ABTS) and inhibition of cell proliferation or between TP and cell proliferation inhibition. Also, glycoalkaloids did not exhibit significant correlations with the inhibition of cancer cell proliferation. Findings of this study show that *S. jamesii* accessions probably pose no cytotoxic effects when used as parental material in improving the nutritional value of potato cultivars. Correlation results, along with cell proliferation data, suggest that not only the compounds measured in this study but also other bioactive compounds present in the matrix acting additively or synergistically may be more responsible for the antiproliferative effects of potato tuber extracts than higher concentrations of a single or group of compounds.

KEYWORDS: *Solanum* species; antioxidants; glycoalkaloids; wild potato

INTRODUCTION

Several studies have reported that bioactive compounds present in fruits and vegetables are important in the prevention of chronic diseases, such as cancer, cardiovascular disease, and diabetes (*1*, *2*). The health benefits derived from these bioactive compounds could be attributed to their antioxidant capabilities (*3*), modulation of potential carcinogenic xenobiotics (*4*), and influence on digestive and metabolizing enzymes (*5*). Several anti-inflammatory, antinecrotic, and neuroprotective drugs have an antioxidant and/or radical-scavenging mechanism as part of their activity (*6*, *7*).

Juan et al. (*8*) reported that olive fruit extract inhibited proliferation of HT-29 human colon cancer cells by inducing apoptosis. Crude extracts from sweet potato (*Ipomoea batatas*) inhibited proliferation of the human leukemia NB4 cell line *in vitro* (*9*). Several studies have demonstrated that tea extracts exhibit anticancer properties on breast (MCF-7), liver (HepG2), colon (HT-29) (*10*), lung (*11*), stomach (*12*), prostate (PC-3) (*10*, *13*), and skin (*14*) cancer cells.

Potato (*Solanum tuberosum*) tuber extracts have also been tested on several cancer types. Chu et al. (*1*) observed minor antiproliferative activity of potato tuber extract on $HepG_2$ human liver cancer cells *in vitro*. Reddivari et al. (*15*) reported that whole potato tuber extract and anthocyanin fractions inhibited proliferation and induced apoptosis in both LNCaP and PC-3 prostate cancer cells. Glycoalkaloids from commercial potato

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cultivars have also been reported to inhibit growth of human colon (HT-29), liver (HepG2), cervical (HeLa), lymphoma (U937), stomach (AGS and KATO III) (*16*), and both LNCaP and PC-3 human prostate (*15*) cancer cell lines.

Recent research is focusing mostly upon increasing the amount of beneficial phytochemicals in food crops and also increasing the variety of plant products consumed by introducing exotic and wild fruits and vegetables, spices, and herbs (*17*). However, some of the wild and exotic products may contain natural toxicants at levels that might cause health problems. For example, development of pest-resistant varieties or changes in methods of cultivation, storage, and preparation can change the balance between beneficial and toxic compounds in staple foods, with significant consequences on human health (*18*). This has been especially observed in the potato of commerce with regard to glycoalkaoids (*19*−*21*).

Wild potato species have been reported to contain higher amounts of beneficial phytochemicals, such as chlorogenic acid and caffeic acid, than the potato of commerce (*22*) and are therefore potential sources of parental material in breeding for these phytochemicals. Some of the species identified as containing high antioxidant activity were *Solanum jamesii*, *Solanum pinnatisectum*, *Solanum megistacrolobum*, and *Solanum microdontum* (*22*, *23*).

However, wild potato species are also known to contain higher amounts of toxic compounds, such as glycoalkaloids (*20*), that are considered a health hazard for human consumption at higher concentrations. Tuber extracts from wild potato species may also contain other unknown compounds that may be toxic to humans; thus, in addition to identifying and quantifying toxic compounds present in wild potato accessions, they should be screened for potential cytotoxicity before their introduction in breeding programs.

Therefore, the objective of this study was to investigate antiproliferative activity and cytotoxicity potential of tuber extracts from *S. jamesii* accessions with different antioxidant activity, phenolic and glycoalkaloid contents on human prostate (LNCaP) and colon (HT-29) cancer cell lines *in vitro*. Colorectal cancer is the third most common cancer in both men and women in the U.S., and prostate cancer is the most frequently diagnosed cancer and the second leading cause of cancer death in men (*24*). Cancer is characterized by uncontrolled growth of abnormal cells resulting in tumor formation. Tumor promotion is the only reversible event during cancer development (*10*); therefore, early intervention targets inhibition of cancerous cell proliferation (*25*). Also, the present study sought to determine correlations among antioxidant activity, phenolics, glycoalkaloid content, and both antiproliferative activity and cytotoxicity. *S. jamesii* was selected because of the lack of previous use in breeding programs and because it was recently found to contain high antioxidant activity.

MATERIALS AND METHODS

Plant Material. A total of 15 accessions of *S. jamesii* (PI_564049, PI_564056, PI_592398, PI_592411, PI_595775, PI_595784, PI_603051, PI_603054, PI_605364, PI_605368, PI_605372, PI_612450, PI_612453, PI_620870, and PI_632325) with a wide range of antioxidant activity (419−958 μg of trolox equivalent/g of fresh weight) and total glycoalkaloids (8.5−20.0 mg/100 g) were selected from 92 accessions obtained from the U.S. Potato Genebank, Sturgeon Bay, WI. The selected accessions were evaluated for antioxidant activity, phenolic compounds, total glycoalkaloids, and antiproliferative effects and cytotoxicity using human prostate (HT-29) and colon (LNCaP) cancer cell lines *in vitro*.

Chemicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin−Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, sodium phosphate dibasic, sodium phosphate monobasic, sodium chloride, ammonium phosphate, and sodium carbonate were purchased from Fisher Scientific (Pittsburgh, PA). Methanol, dimethyl sulfoxide (DMSO), and acetonitrile were obtained from VWR International (Suwanee, GA). α-Chaconine, ammonium hydroxide, 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate) (chlorogenic acid), quercetin-3-rutinoside hydrate (rutin hydrate), 3,4-dimethoxycinnamic acid (caffeic acid), 3,5-dimethoxy-4-hydroxycinnamic acid (sinapic acid), and 3,3′,4′,5,5′,7-hexahydroxyflavone (myricetin) were purchased from Sigma-Aldrich (St. Louis, MO). α-Solanine and tomatine were obtained from MP Biomedicals (Solon, OH). The cell proliferation reagent WST-1 {4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate} and cytotoxicity detection kit were obtained from Roche Applied Sciences (Indianapolis, IN).

Cell Lines. Human prostate cancer LNCaP (androgen-dependent) cells and HT-29 colon cancer cells were obtained from the American Type Culture Collection (Manassas, VA). The LNCaP cells were maintained at 37 °C in a 5% CO_2 jacketed incubator in RPMI 1640 (Sigma-Aldrich) supplemented with 2.38 g/L *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 2.0 g/L sodium bicarbonate, 0.11 g/L sodium pyruvate, 4.5 g/L glucose, 100 mL/L fetal bovine serum (FBS), and 10 mL/L antibiotic antimycotic solution (Sigma-Aldrich). HT-29 cells were maintained under similar conditions in Dulbecco's modified Eagle medium (DMEM) containing 10% FBS.

Antioxidants and Total Phenolics Extraction. A set of five tubers from each accession was washed and diced into 0.5 cm cubes, and a 5 g sample of chopped tubers was weighed and extracted with 20 mL of high-performance liquid chromatography (HPLC)-grade methanol. The samples were homogenized with an IKA Ultra-turrax tissuemizer for 3 min. The tuber extract was centrifuged at 31000*g* for 20 min with a Beckman model J2-21 refrigerated centrifuge. A total of 5 mL of supernatant was collected in glass vials and dried to completion using a Speed Vac. The dried extract was redissolved in DMSO and filtered through 0.45 μm syringe filters. Sample extracts were stored at −20 °C until analysis of antioxidant activity (AOA), phenolics and glycoalkaloids in DMSO-soluble phytochemicals.

Total Glycoalkaloid Extraction. Extraction of glycoalkaloids followed the method of Rodriguez-Saona et al. (*26*). A total of 5 g of fresh tubers was homogenized with 10 mL of acetone to a uniform consistency. The extract was centrifuged at 13000*g* for 15 min, and the clear supernatant was collected into a falcon tube. The residue was re-extracted with 10 mL of aqueous acetone [30:70 acetone/water (v/v)]. The extract was centrifuged, and the supernatant was combined with the first extract. Chloroform was added to the acetone extract (two volumes of chloroform for each volume of acetone extract), thoroughly mixed by shaking the tubes, and stored overnight at 1 °C. The top aqueous portion was collected into glass vials and concentrated in a rotovapor SpeedVac at 40 °C until all residual acetone was evaporated. The extract was brought to a known volume with nanopure water and analyzed for glycoalkaloids.

Antioxidant Activity Analysis. Total antioxidant activity was estimated using both the DPPH (*27*) and ABTS (*28*) assays.

DPPH Assay. A 150 μL aliquot was placed into scintillation vials; 2850 μL of DPPH methanol solution was added; and the mixture was placed on a shaker for 15 min. The mixture was transferred to UV cuvettes, and its absorbance was recorded using a Shimadzu BioSpec-1601 spectrophotometer at a wavelength $\lambda = 515$ nm. Trolox, a synthetic antioxidant, was used as a standard to generate a standard curve, and total AOA in tuber extracts was expressed as micrograms of trolox equivalents per gram of potato tuber fresh weight (μg of TE/g of fw).

ABTS Assay. The $ABTS^{\bullet+}$ radical was generated by reacting potassium persulfate with ABTS salt [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt]. A working solution composed of a mixture of 5 mL of stock solution and 145 mL of phosphate buffer was prepared. The stock solution contained equal volumes of 8 mM ABTS and 3 mM potassium persulfate solutions, and the phosphate buffer solution at pH 7.4 was composed of 40.5 mL of 0.2 M Na_2HPO_4 dibasic, 9.5 mL of 0.2 M $NaHPO_4$ monobasic, and 150 mM NaCl. A total of 100 μL of tuber extract was used for analysis. A total of 2900 μL of the working solution was added to tuber extracts and reacted for 30 min on a shaker. Absorbance of the solution was measured at a wavelength $\lambda = 734$ nm with a Shimadzu BioSpec-1601 spectrophotometer. Trolox was used as a

standard, and total AOA was expressed as micrograms of trolox equivalents per gram of tuber fresh weight (μg of TE/g of fw).

Total Phenolic Analysis. The total phenolic content was determined following the method of Singleton et al. (*29*). A total of 150 μL of tuber extract was pipetted into scintillation vials, and 2.4 mL of nanopure water was added. A total of 150 μL of 0.25 N Folin−Ciocalteu reagent was added, and after 3 min of reaction, 0.3 mL of 1 N Na_2CO_3 reagent was added and allowed to react for 2 h. The spectrophotometer (Shimadzu BioSpec-1601) was zeroed with a blank (150 μL of methanol, 2.4 mL of H_2O, 150 μL of 0.25 N Folin−Ciocalteu, and 0.3 mL 1 N Na_2CO_3) before sample analysis. Absorbance of tuber extracts was read at 725 nm. Chlorogenic acid was used as a standard, and total phenolic content was expressed as milligrams of chlorogenic acid equivalents per 100 g of potato tuber fresh weight (mg of CGA equiv/100 g of fw).

Phenolic Composition. Composition of phenolics in tuber extracts was determined using a HPLC system. A total of 5 mL of the extract was concentrated with a speed vac concentrator and then redissolved in 1 mL of methanol. The concentrated extracts were filtered through 0.45 μm syringe filters. The HPLC system consisted of a binary pump system (Waters 515), autoinjector (Waters 717 plus), photodiode array (PDA) detector (Waters 996), column heater (SpectraPhysics SP8792), and an Atlantis C-18 reverse-phase column (4.6 × 150 mm, 5 μm) from Waters (Milford, MA), maintained at 40 °C. Phenolic acids were separated using a linear-gradient elution with mobile-phase solvents A (acetonitrile) and B (water/acetic acid at pH 2.3). The solvent flow rate was set at 1 mL/min and a gradient of 0/85, 5/85, 30/0, and 35/0 (min/% A) in 35 min. Pure phenolic compounds used as standards for HPLC analysis were chlorogenic acid, rutin hydrate, caffeic acid, myricetin, and sinapic acid. Identification and quantification of phenolic acids present in the extracts was accomplished by comparing retention time and area of peaks in the extracts to those of the standard compounds. Method validation was performed by spiking tuber extracts with pure phenolic compounds of known concentration and then comparing retention times, peak spetra, and concentrations of spiked samples, nonspiked samples, and pure compounds. Reproducibility was checked by analyzing the same sample 3 times. Quantities of phenolic acids were expressed as μg/g of fresh weight.

Determination of Glycoalkaloids. Glycoalkaloids were analyzed with a HPLC system following the method of Sotelo and Serrano (*30*). A HPLC system (Waters) and Atlantis C-18 reverse-phase columns (4.6 × 150 mm, 5 μm) were used for glycoalkaloid analysis. The mobile phase used for separating glycoalkaloids was (35:65, v/v) acetonitrile/0.05 M monobasic ammonium phosphate buffer $(NH_4)H_2PO_4$, adjusted to pH 6.5 with NH_4OH. The solvent flow was isocratic at a rate of 1 mL/min, with the UV absorbance detector set at 200 nm with 5% AUFS sensitivity. The amount of extract sample injected was 20 μL. Again, the method was validated by spiking tuber extracts with pure α-solanine, α-chaconine, and tomatine and comparing retention times and peak spectra of spiked samples, nonspiked samples and pure glycoalkaloid standards. Different concentrations of pure α-solanine, α-chaconine, and tomatine were used to prepare standard curves by regressing known concentrations of the glycoalkaloid standards to their corresponding peak areas, and these curves were used to quantify amounts of glycoalkaloids in the tuber extracts.

Cell Proliferation. Cells were plated at a density of 1×10^4/well in 96-well plates. They were allowed to attach to the plate for 24 h. After 24 h, media was replaced with DMEM F-12 media containing 2.5% charcoal-stripped serum and tuber extracts. Two concentrations (5 and 10 μg/mL) of tuber extract deemed potent (*15*) were used. After every 24 h, cell proliferation was measured using the WST-1 assay. The WST-1 assay has a good interlaboratory reproducibility, which is a prerequisite for acceptance of an *in vitro* test (*31*). The assay required preincubation of cells in media with the tetrazolium salt WST-1 {4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate} (10 μL/well) for 4 h, followed by measuring absorbance at 450 nm with the enzyme-linked immunosorbent assay (ELISA) plate reader. The cell proliferation assay was repeated at 48 and 72 h of incubation with potato extracts. Percent cell proliferation due to each tuber extract treatment was calculated as a percentage of the control (DMSO) according to the following equation: percent cell proliferation = [(cell proliferation with extract treatment/cell proliferation in control) × 100]. All extracts were tested in triplicate.

Cytotoxicity Analysis. Cytotoxicity of tuber extracts to cancer cells was determined by measuring the amount of lactate dehydrogenase (LDH) enzyme leaked from the cytosol of damaged cells into the medium (*32*) after exposure of cells to the extracts for 24 h. The LDH release represents necrosis as opposed to apoptosis. LDH in the supernatant was measured using the cytotoxicity detection kit (LDH) (Roche Applied Science, Mannheim, Germany) following the protocol of the manufacturer. Triton X-100 was used as a positive control. A total of 100 μL of the supernatant from the cells was placed in a 96-well plate, and 100 μL of LDH assay solution [mixture of catalyst lyophilizate (catalyst, diaphorase/NAD^+, and lyophilizate) and dye solution (iodotetrazolium chloride and sodium lactate)] was added to each well and incubated for 30 min in the dark. Absorbance of the mixture was read with an ELISA plate reader at 490 nm. Two concentrations (5 and 10 μg/mL) of the extracts were used, and extract cytotoxicity was calculated as a percentage of the control (DMSO) following the equation: percent LDH released = [(LDH released with extract treatment/LDH in control) × 100]. All samples were analyzed in triplicate.

Statistical Analysis. Results of each treatment were expressed as mean ± standard error for three treatment replicates. Analysis of variance (ANOVA) was performed to determine the variability of antiproliferative activity and cytotoxicity of the tuber extracts of the accessions. Treatment means for each variable were compared to the unrestricted least significant difference (LSD) multiple comparison procedure. Correlations among antioxidant activity, total phenolics, and glycoalkaloid content were computed following Pearson's correlation method. All statistical analyses were performed using SAS version 9.1 software (*33*).

RESULTS AND DISCUSSION

Antioxidants, Phenolic Compounds, and Glycoalkaloids in Tuber Extracts. Tuber extracts of the 15 *S. jamesii* accessions were significantly different in antioxidant activity, total phenolic content, and individual phenolic compounds. Also, levels of α-solanine and α-chaconine and total glycoalkaloids in the tuber extracts were significantly different (**Table 1**).

Effect of Tuber Extract on Cell Proliferation. ANOVA results for antiproliferative activity exhibited by tuber extracts from the 15 accessions on human colon (HT-29) and prostate (LNCaP) cancer cells *in vitro* are presented in **Figures 1** and **2**, respectively. Significant reductions in proliferation of HT-29 colon and LNCaP prostate cancer cells by the tuber extracts were observed. The tuber extracts decreased proliferation of HT-29 colon cancer and LNCaP prostate cancer cells in a dose- and time-dependent manner. Proliferation of both HT-29 colon cancer cells and LNCaP prostate cancer cells decreased with increased time of incubation with the extracts. The maximum cell proliferation inhibition was observed after 72 h of incubation. Also, accessions exhibited varying degrees of cell proliferation inhibition at each incubation period, and all accessions showed more antiproliferative activity with longer times of incubation.

All extracts at concentrations of 5 and 10 μg/mL significantly reduced proliferation of HT-29 cells compared to the DMSO control (**Figure 1**). Cell proliferation was less than 60% of the control (DMSO) after 24 h of cell incubation with tuber extracts of either 5 or 10 μg/mL concentration (panels **A** and **D** of **Figure 1**). After 48 and 72 h of incubation with either of the extracts (5 and 10 μg/mL), HT-29 cell proliferation was less than 40% of the DMSO control (panels **B**, **C**, **E**, and **F** of **Figure 1**).

HT-29 colon cancer cells were more responsive to tuber extract treatment than prostate (LNCaP) cancer cells. LNCaP cancer cell proliferation was variably inhibited by the tuber extracts. At the 5 μg/mL extract concentration, only three accessions (PI_595784, PI_592411, and PI_620870) significantly reduced LNCaP cell proliferation compared to that of the control (DMSO) after 24 and 48 h of incubation (panels **A** and **B** of **Figure 2**). However, after 72 h of incubation, seven accessions in the following order: PI_620870 > PI_595784 > PI_592411 > PI_603054 > PI_605372 >

Table 1. Mean Values of Antioxidant Activity in μg of TE/g of fw (DPPH and ABTS Assays), Total Phenolic Content in mg of CGA equiv/100 g of fw (TP), α-Solanine in mg/100 g of fw (SOL), α-Chaconine in mg/100 g of fw (CHA), Total Glycoalkaloids in mg/100 g of fw (TGA), Chlorogenic Acid in μg/g of fw (CGA), Caffeic Acid in μg/g of fw (CA), Sinapic Acid in μg/g of fw (SA), Rutin Hydrate in μg/g of fw (RH), and Myricetin in μg/g of fw (MYC) in 15 *S. jamesii* Accessions (ACNO)

ACNO	DPPH	ABTS	TP	SOL	CHA	TGA	CGA	CA	SA	RH	MYC
PI_564049	647 ± 29	2618 ± 175	128 ± 1.1	9.3 ± 1.2	8.3 ± 0.4	17.5 ± 1.4	270 ± 9.6	132 ± 16.5	2.4 ± 0.0	28.9 ± 1.8	3.2 ± 0.3
PI_564056	820 ± 23	2279 ± 61	139 ± 1.7	7.9 ± 0.3	6.3 ± 0.4	14.2 ± 0.7	409 ± 6.1	223 ± 11.5	2.5 ± 0.2	29.7 ± 0.7	4.1 ± 0.1
PI_592398	589 ± 21	2051 ± 63	118 ± 2.5	12.9 ± 0.5	6.5 ± 0.1	19.4 ± 0.6	222 ± 8.5	254 ± 13.3	4.2 ± 0.1	23.7 ± 1.2	5.3 ± 0.4
PI_592411	607 ± 8	2121 ± 61	124 ± 1.0	5.5 ± 0.3	6.0 ± 0.2	11.4 ± 0.3	339 ± 5.9	76 ± 8.3	5.2 ± 0.5	43.7 ± 5.1	6.0 ± 0.8
PI_595775	879 ± 28	2607 ± 135	161 ± 2.7	5.0 ± 1.3	6.4 ± 0.4	11.4 ± 1.6	368 ± 12.9	136 ± 18.0	5.0 ± 0.6	42.9 ± 3.2	8.9 ± 1.4
PI_595784	789 ± 20	2641 ± 111	137 ± 1.8	11.0 ± 0.5	5.6 ± 0.3	16.7 ± 0.9	503 ± 3.2	117 ± 9.4	2.4 ± 0.3	40.0 ± 2.7	2.5 ± 0.7
PI_603051	758 ± 30	2904 ± 141	129 ± 1.9	4.9 ± 0.4	6.5 ± 0.0	11.4 ± 0.3	282 ± 0.9	209 ± 2.1	2.0 ± 0.3	32.4 ± 1.2	5.3 ± 0.1
PI_603054	783 ± 28	2206 ± 120	140 ± 2.2	13.6 ± 0.8	6.4 ± 0.1	20.0 ± 0.9	466 ± 16.0	205 ± 1.2	2.0 ± 0.3	31.2 ± 2.5	3.0 ± 0.3
PI_605364	958 ± 6	2947 ± 84	140 ± 0.4	4.1 ± 1.4	4.5 ± 0.3	8.6 ± 1.7	413 ± 16.3	154 ± 3.2	2.2 ± 0.4	42.1 ± 2.9	2.6 ± 0.3
PI_605368	560 ± 18	1857 ± 88	99 ± 2.6	4.8 ± 0.7	5.1 ± 0.4	9.9 ± 1.1	217 ± 1.9	157 ± 13.6	2.0 ± 0.1	22.9 ± 1.2	3.4 ± 0.5
PI_605372	687 ± 14	2687 ± 62	128 ± 1.8	4.3 ± 0.5	5.1 ± 0.3	9.4 ± 0.8	363 ± 16.2	254 ± 8.7	1.7 ± 0.1	33.9 ± 1.0	2.5 ± 0.3
PI_612450	648 ± 21	2237 ± 103	112 ± 1.3	8.9 ± 0.4	6.2 ± 0.0	15.0 ± 0.4	306 ± 6.5	213 ± 10.7	2.4 ± 0.3	20.9 ± 0.8	4.6 ± 0.4
PI_612453	814 ± 24	1998 ± 84	127 ± 2.5	4.1 ± 0.4	4.6 ± 0.2	8.7 ± 0.6	370 ± 6.7	211 ± 5.3	1.9 ± 0.3	31.0 ± 2.4	3.2 ± 0.2
PI_620870	895 ± 19	2727 ± 79	141 ± 1.2	13.3 ± 0.3	6.6 ± 0.3	19.9 ± 0.6	351 ± 6.7	221 ± 17.4	3.9 ± 0.4	36.3 ± 2.6	7.4 ± 0.4
PI_632325	416 ± 19	1906 ± 197	92 ± 1.7	4.1 ± 0.1	4.7 ± 0.4	8.9 ± 0.4	164 ± 2.4	44 ± 4.1	1.9 ± 0.3	16.4 ± 1.3	2.3 ± 0.1
$LSD_{0.05}$	33	206	3	1.3	0.7	1.7	28	28	0.9	5.7	1.3

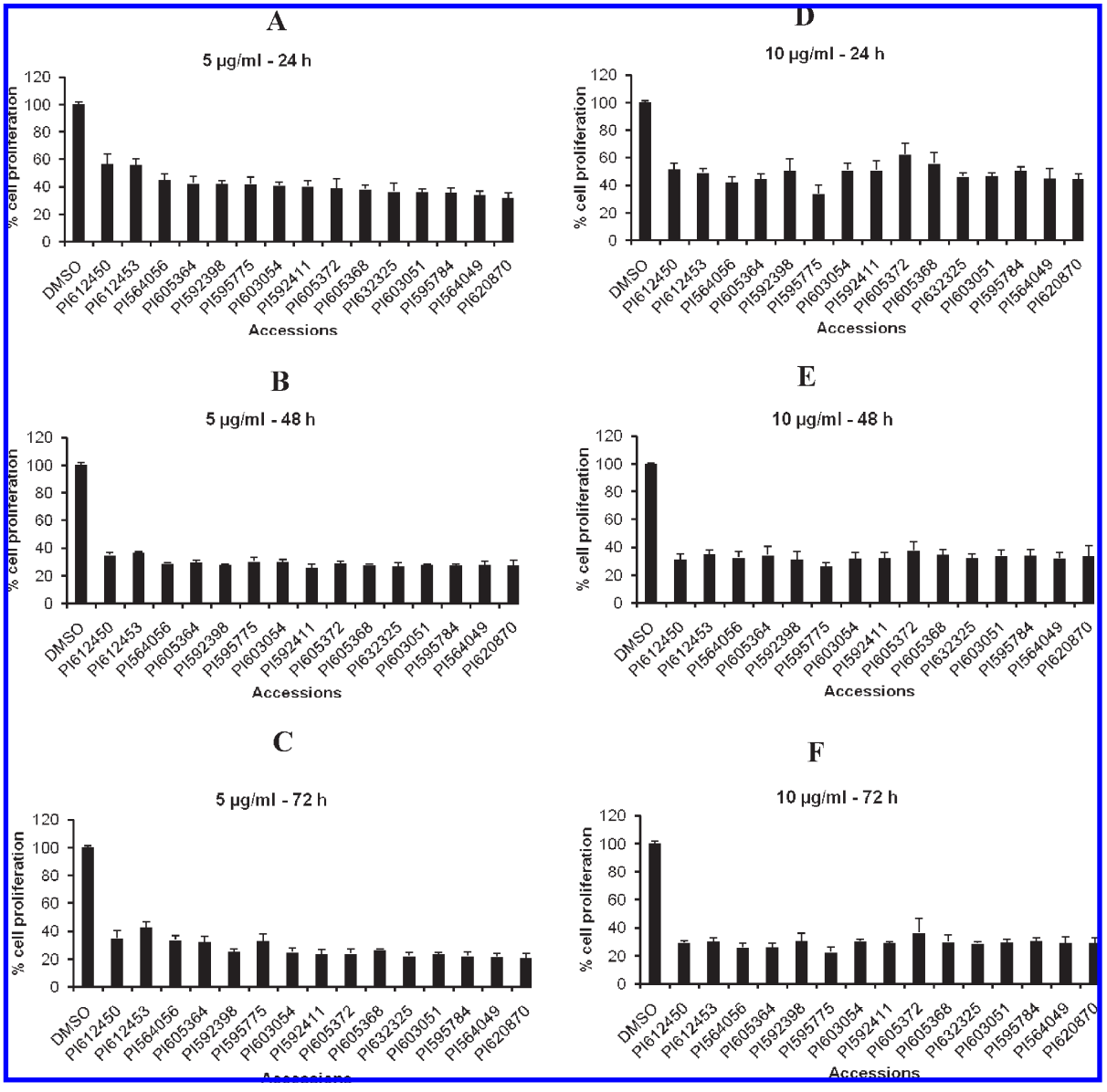

Figure 1. Cell proliferation of HT-29 colon cancer cells determined after 24, 48, and 72 h of incubation with 5 and 10 μg/mL of tuber extracts from 15 *S. jamesii* accessions. Results are presented as means ± standard error (SE) of three experiments.

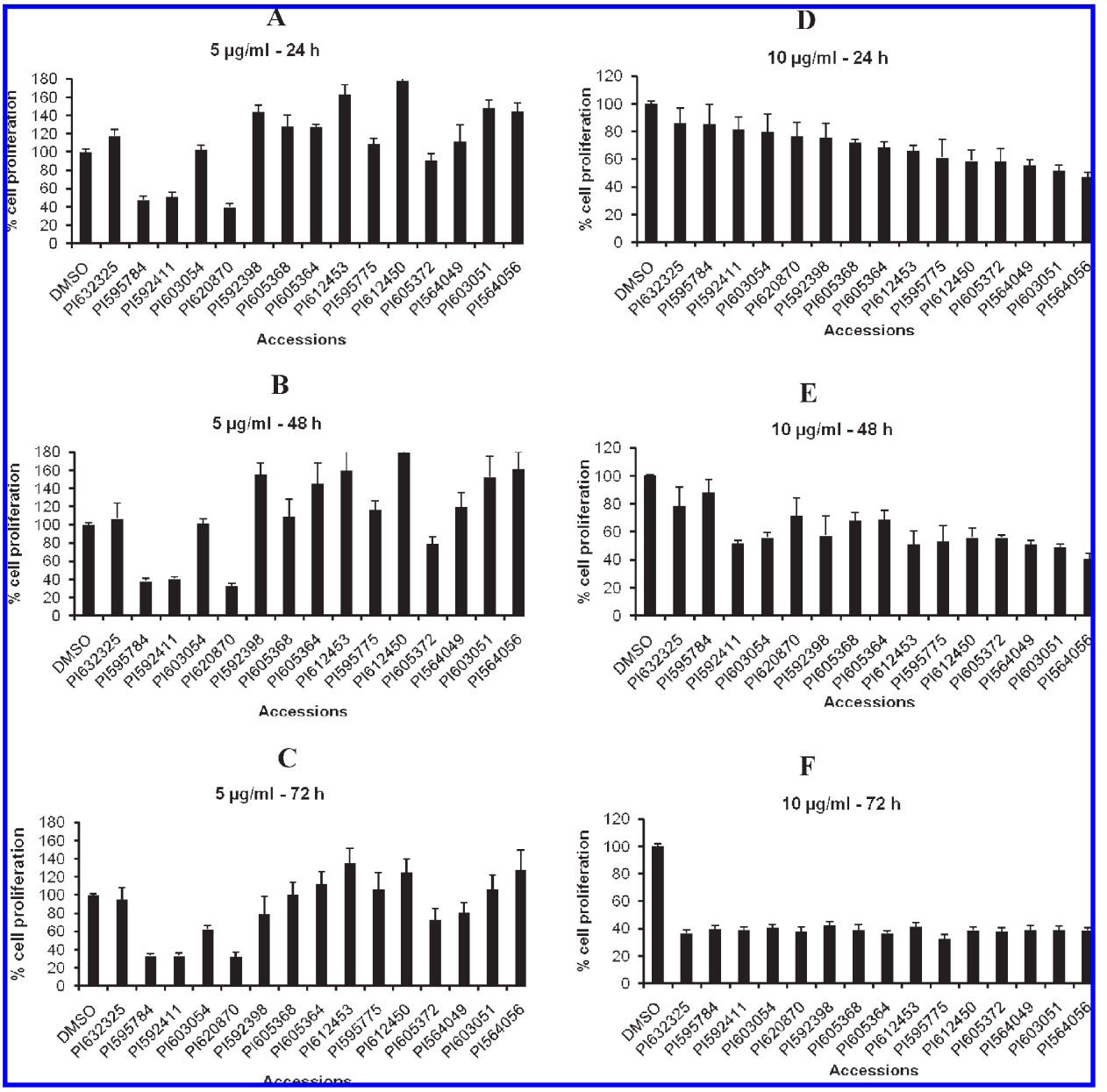

Figure 2. Cell proliferation of LNCaP prostate cancer cells evaluated after 24, 48, and 72 h of incubation with 5 and 10 μg/mL of tuber extracts from 15 *S. jamesii* accessions. Results are presented as means ± SE of three experiments.

PI_592398 > PI_564049 significantly inhibited LNCaP cell proliferation compared to the DMSO control (**Figure 2C**). With a higher extract concentration (10 μg/mL), all accessions exhibited significant inhibition of LNCaP cell proliferation compared to the DMSO control after 24, 48, and 72 h of incubation (panels **D**, **E**, and **F** of **Figure 2**). After 72 h of incubation with 10 μg/mL extracts, cell proliferation was reduced by all accessions by about 60% of the DMSO control.

Colon and prostate cancer cells responded differently to tuber extract treatments. Colon (HT-29) cancer cell lines seemed more responsive to tuber extract treatment than prostate (LNCaP) cancer cell lines. Proliferation of colon (HT-29) cancer cells was significantly reduced by all extracts at 5 μg/mL (**Figure 1**), yet a higher concentration (10 μg/mL) of extract was required for all accessions to inhibit proliferation of LNCaP prostate cancer cells (**Figure 2**). These results agree with Kim et al. (*34*), who reported that polyphenol concentrations required for anticancer effects depend upon the type of cancer cell line. Friedman et al. (*16*) came to a similar conclusion while investigating anticarcinogenic effects of glycoalkaloids against cervical, liver, lymphoma, and stomach cancer cells.

Cytotoxicity of Tuber Extracts. Accessions PI_595784 and PI_620870 at a concentration of 5 μg/mL caused slightly more but not significant LDH leakage from HT-29 cells than the DMSO control. Even at higher concentrations, none of the accessions were cytotoxic to HT-29 cells. LDH released by LNCaP prostate cells after treatment with 5 μg/mL of tuber extracts of PI_564056, PI_595775, PI_595784, PI_605368, and PI_605372 accessions was significantly lower than that of the control (DMSO) (**Figure 4A**). A total of 5 μg/mL of tuber extracts of PI_592398, PI_612450, and PI_620870 caused slightly higher LDH leakage than the control, but they were not significantly different from the control. Even at twice the concentration (10 μg/mL), most of the accessions were not significantly cytotoxic, except for two (PI_595784 and PI_620870) (**Figure 4B**).

Accessions tested were not cytotoxic in HT-29 at both 5 and 10 μg/mL concentrations (**Figure 3**) and in LNCaP cells at the low concentration (**Figure 4A**). Only two accessions (PI_595784 and

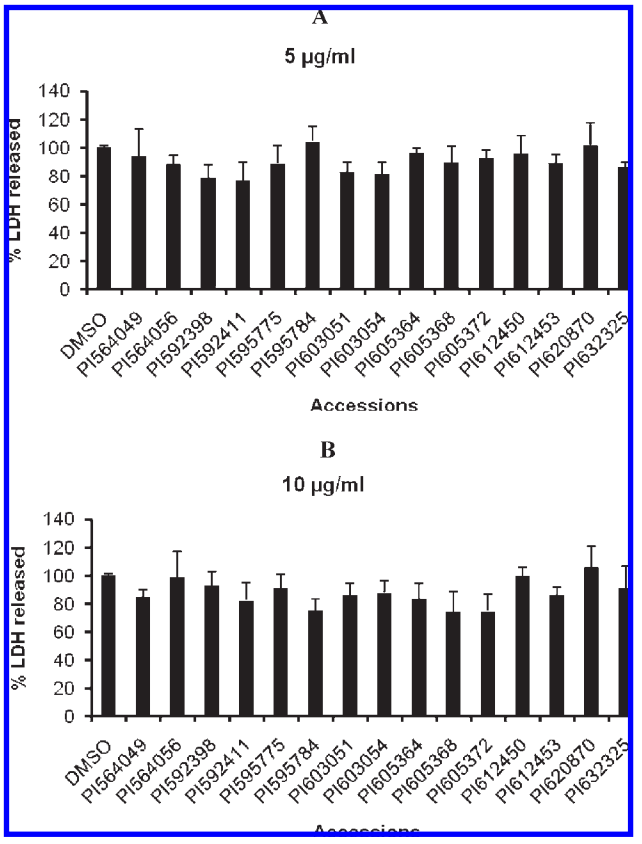

Figure 3. Cytotoxicity of tuber extracts from 15 *S. jamesii* accessions (5 and 10 μg/mL) to HT-29 human colon cancer cells expressed as a percentage of LDH released from the cells after 24 h of incubation. Results are presented as means ± SE of three experiments.

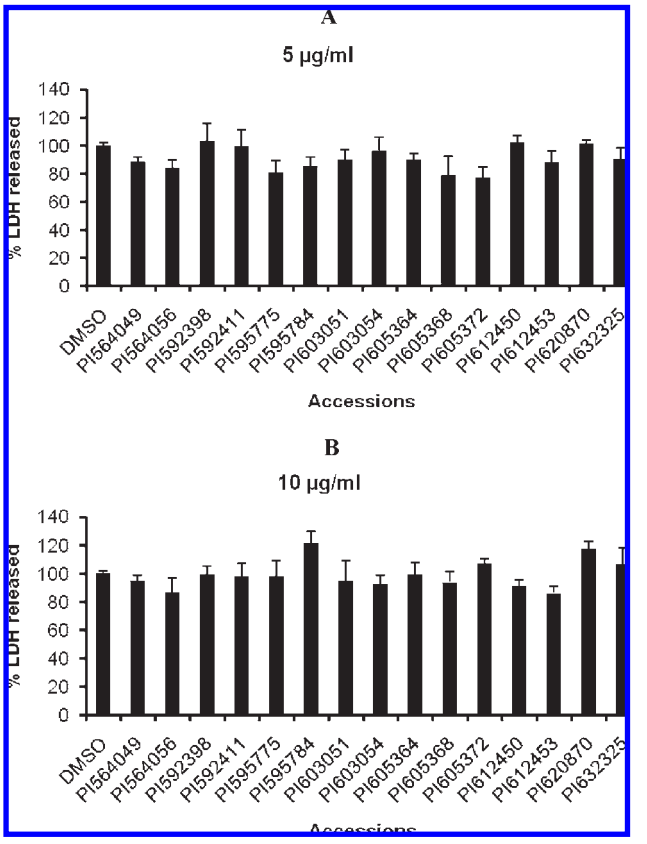

Figure 4. Cytotoxicity of tuber extracts from 15 *S. jamesii* accessions (5 and 10 μg/mL) to LNCaP human prostate cancer cells expressed as a percentage of LDH released from the cells after 24 h of incubation. Results are presented as means ± SE of three experiments.

PI_620870) at a high concentration (10 μg/mL) showed significantly higher LDH leakage than the control in LNCaP cells (**Figure 4B**). The observed reduction in proliferation of HT-29 and LNCaP cancer cells after incubation with tuber extracts of *S. jamesii* accessions was not due to necrosis but probably enhanced apoptosis. A previous study (*15*) reported that tuber extracts from specialty potato cultivars contain phytochemicals that can inhibit LNCaP and PC-3 cell growth and induce apoptosis. Hence, most of these accessions should not pose a health problem if used as parental material in improving the nutritional value of potato cultivars. For confirmatory purposes, *in vivo* experiments using animal models would be useful.

Findings of this study support the use of *S. jamesii* accessions in breeding for high health-promoting phytochemicals in the potato of commerce. Dietary constituents in foods must be sufficient to attain the cellular concentrations that display effective bioactivity and chemopreventive capacity (*35*). The presence of high amounts of chemopreventive compounds in plant foods, such as the potato of commerce, would increase bioavailability of the bioactive phytochemicals.

Correlations among Antioxidants, Phenolics, Glycoalkaloids, and Antiproliferative Activity. Relationships among antioxidant activity, phenolic and glycoalkaloid content in tuber extracts, and their inhibition of HT-29 colon and LNCaP prostate cancer cell proliferation were investigated, because several studies have associated consumption of foods rich in antioxidants with a decrease in the prevalence of degenerative diseases, such as cancer (*36*). Other studies specifically investigated polyphenols extracted from plants for their potential effect in curing colon (*8*, *34*) and prostate (*13*, *15*) cancers. Glycoalkaloids have also been reported to play a role in reducing cancer cell proliferation (*15*, *16*) by upregulating apoptosis in these cells.

Results from correlation analysis among antioxidant activity, total phenolics, glycoalkaloids, and antiproliferative activity on HT-29 colon cancer cells showed no significant relationships. There were no significant correlations between antioxidant activity (DPPH and ABTS assays) and inhibition of HT-29 cell proliferation or between total phenolic content and inhibition of HT-29 cell proliferation. Similarly, individual phenolic acids (chlorogenic acid, caffeic acid, sinapic acid, rutin hydrate, and myricetin) measured from the tuber extracts exhibited no significant correlation with the inhibition of colon cancer cells. Also α-solanine, α-chaconine, and total glycoalkaloids showed no significant correlation with the inhibition of HT-29 cell proliferation after 24, 48, and 72 h of incubation (**Table 2**).

Antioxidant activity in tuber extracts measured by the DPPH and ABTS assays, total phenolic content, and total glycoalkaloids were not significantly correlated with the inhibition of LNCaP prostate cancer cell proliferation after 24, 48, or 72 h of incubation with 5 or 10 μg/mL of tuber extract. Also, individual phenolic acids (chlorogenic acid, caffeic acid, sinapic acid, rutin hydrate, and myricetin) and individual glycoalkaloids (α-solanine and α-chaconine) measured from the tuber extracts exhibited no significant correlation with the inhibition of prostate LNCaP cell proliferation (**Table 3**).

Actual proliferation values (**Figures 1** and **2**) show that proliferation of HT-29 and LNCaP cells was significantly inhibited by treatment with tuber extracts. Therefore, correlation results, together with cell proliferation data, suggest that not a single or group of compounds at a higher concentration but a combination

Table 2. Correlation Analysis of Antioxidant Activity (DPPH and ABTS), Total Phenolic Content (TP), Chlorogenic Acid, Caffeic Acid, Sinapic Acid, Rutin Hydrate, Myricetin, α-Solanine, α-Chaconine, and Total Glycoalkaloids (TGA) in *S. jamesii* Accessions and Percentage Inhibition of HT-29 Colon Cancer Cell Proliferation

	percent inhibition of HT-29 colon cancer cell proliferation[a]					
	tuber extract (5 μg/mL)			tuber extract (10 μg/mL)		
parameter	24 h	48 h	72 h	24 h	48 h	72 h
DPPH	−0.097	−0.279	−0.360	0.441	0.058	0.363
ABTS	0.361	0.148	0.207	0.262	−0.081	0.081
TP	0.059	−0.086	−0.165	0.528[b]	0.318	0.393
α-solanine	0.193	0.137	0.345	0.017	0.219	−0.109
α-chaconine	0.289	0.223	0.351	0.321	0.468	0.155
TGA	0.236	0.172	0.378	0.091	0.294	−0.057
chlorogenic acid	−0.115	−0.198	−0.207	0.089	−0.073	0.069
caffeic acid	−0.306	−0.382	−0.262	−0.261	−0.245	−0.354
sinapic acid	0.103	0.291	0.093	0.426	0.326	0.439
rutin hydrate	0.190	0.154	−0.001	0.282	0.063	0.255
myricetin	0.052	0.087	−0.028	0.257	0.358	0.412

[a] Values are correlation coefficients. [b] Indicates significant values at $p < 0.05$.

Table 3. Correlation Analysis of Antioxidant Activity (DPPH and ABTS), Total Phenolic Content (TP), Chlorogenic Acid, Caffeic Acid, Sinapic Acid, Rutin Hydrate, Myricetin, α-Solanine, α-Chaconine, and Total Glycoalkaloids (TGA) in *S. jamesii* Accessions and Percentage Inhibition of LNCaP Prostate Cancer Cell Proliferation

	percent inhibition of LNCaP prostate cancer cell proliferation[a]					
	tuber extract (5 μg/mL)			tuber extract (10 μg/mL)		
parameter	24 h	48 h	72 h	24 h	48 h	72 h
DPPH	0.126	−0.001	−0.061	0.278	0.120	0.244
ABTS	0.273	0.149	0.159	0.357	−0.034	0.374
TP	0.294	0.145	0.132	0.262	0.247	0.329
α-solanine	0.287	0.220	0.409	−0.271	−0.135	−0.458
α-chaconine	0.105	0.013	0.239	0.329	0.398	−0.092
TGA	0.271	0.193	0.489	−0.159	−0.030	−0.409
chlorogenic acid	0.347	0.262	0.223	−0.007	−0.017	0.001
caffeic acid	−0.349	−0.373	−0.224	0.485	0.432	−0.386
sinapic acid	0.389	0.314	0.380	−0.201	0.142	0.253
rutin hydrate	0.470	0.465	0.383	−0.029	0.026	0.329
myricetin	0.175	0.116	0.125	0.127	0.279	0.409

[a] Values are correlation coefficients.

of several bioactive compounds in the matrix (acting together) were responsible for inhibiting HT-29 and LNCaP cell proliferation.

In summary, proliferation of colon and prostate cancer cells was inhibited by *S. jamesii* tuber extracts and, also, the extracts were not cytotoxic to the cells. However, correlations between antiproliferation and antioxidant activity, phenolics, and glycoalkaloids in the tuber extracts were not significant in both HT-29 and LNCaP cell lines, yet actual proliferation data showed significant differences among extract treatments and the control (**Figures 1** and **2**). This study suggests that combinations of diverse phytochemicals, i.e., those that were analyzed (chlorogenic acid, caffeic acid, sinapic acid, rutin hydrate, myricetin, α-solanine, and α-chaconine) as well as other compounds, such as ascorbic acid and carotenoids, that may be present in tuber extract are important in inhibiting cell proliferation. A similar idea that combinations of different phytochemicals synergistically confer more health benefits than individual chemicals was proposed by Liu (*2*) and Milde et al. (*37*). Likewise, Chu et al. (*1*) observed no significant correlations between phenolic compounds of vegetables and inhibition of $HepG_2$ human liver cancer cell proliferation. They suggested that inhibition of human liver cancer cells by vegetables does not solely depend upon their phenolic content, but that other chemicals in the vegetables were also responsible for antiproliferative activities. Therefore, a network of phytochemicals is necessary in promoting health, and compounds required in high amounts should be selected for and those required in low amounts, such as glycoalkaloids, selected against during crop development. *S. jamesii* was reported to contain higher antioxidant activity and glycoalkaloids than common cultivars; therefore, its potential cytotoxicity was addressed in this study, and results indicated that *S. jamesii* accessions would probably pose no health problems when used as parental material in improving the nutritional value of potato cultivars.

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