

# Radical Scavenging Capacity and Antimutagenic Properties of Purified Proteins from *Solanum betaceum* Fruits and *Solanum tuberosum* Tubers

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**ABSTRACT:** In this study, antioxidant activities in free-radical-mediated oxidative systems and the genotoxic/antigenotoxic effects of two proteins with molecular mass around 17 kDa, purified from *Solanum betaceum* fruits (cyphomine) and *Solanum tuberosum* tubers (solamarine), were investigated. Both proteins inhibited uric acid formation with IC<sub>50</sub> values between 55 and 60 μg/mL, and both proteins were able to reduce oxidative damage by scavenging hydroxyl radicals and superoxide anion in a dose-dependent manner. Furthermore, the DPPH• reduction assay showed SC<sub>50</sub> values of 55–73 μg/mL. Cyphomine and solamarine were able to retain their antioxidant activity after heat treatment at 80 °C for 15 min. *Allium cepa* and *Salmonella*/microsome assays showed no genotoxic and mutagenic effects. Solamarine showed an antimutagenic effect against a direct mutagen (4-nitro-*o*-phenylenediamine). Consequently, the present study showed that the investigated proteins are promising ingredients for the development of functional foods with a beneficial impact on human health and an important source for the production of bioactive peptides.

**KEYWORDS:** Antioxidant activity, antimutagenic activity, genotoxicity, bioactive proteins, *Solanum betaceum* fruits, *Solanum tuberosum* tubers

## 1. INTRODUCTION

Proteins are fundamental food components, both nutritionally and functionally. They may play bioactive roles by themselves and/or can be the precursors of biologically active peptides by *in vivo* or *in vitro* hydrolysis.<sup>1,2</sup> From this point of view, the best known examples are casein-derived peptides, which have proven to possess immunomodulating, antihypertensive, antithrombotic, and opioid activities.<sup>3,4</sup> Moreover, some proteins and peptides are multifunctional and can exert more than one of the mentioned effects.<sup>5</sup>

Under conditions of severe oxidative stress, cellular defenses do not provide complete protection from the attack of reactive oxidants, which could lead to disease-related oxidative damage. This situation calls for an external supply of antioxidants to counter this trend. The use of natural protein extracts or purified proteins as antioxidants has attracted particular interest. Many food proteins, including milk proteins, such as lactoferrin, β-lactoglobulin, and casein, soy, mushroom, and egg albumen proteins, egg yolk phosvitin, maize zein, potato patatin, and yam dioscorin, were reported to have antioxidant activity.<sup>6–11</sup> Proteins owe their antioxidant activity to their constituent amino acids. For example, the antioxidant activity of aromatic amino acids, such as tyrosine, phenylalanine, and tryptophan, and that of the sulfur-containing amino acid, cysteine, are due to their ability to donate protons to free radicals,<sup>12–14</sup> whereas basic amino acids, such as lysine and arginine, and acidic amino acids, such as aspartate and glutamate, are antioxidants because of the chelating capacity of metallic ions.<sup>14</sup> *Solanum betaceum* and *Solanum*

*tuberosum* are two plant species whose fruits and tubers have an important consumption in America. In previous studies, we characterized bioactive proteins isolated from *S. betaceum* fruits and *S. tuberosum* tubers, which we called cyphomine and solamarine, respectively.<sup>15–18</sup> These proteins showed some *in vitro* biological properties, such as inhibitory action, on hydrolases isolated from phytopathogenic bacteria and fungi (pectinase, pectate lyase, and polygalacturonase enzymes). They also proved to have an inhibitory effect on the growth of phytopathogenic bacteria. Solamarine and cyphomine were able to retain their biological activity after warming at 80 °C for 10 min.<sup>16,18,19</sup> Thus, these proteins could be employed in agriculture as postharvesting control agents.<sup>18</sup>

The purpose of this study was to analyze the antioxidant properties (free radical scavenging activity) and evaluate the genotoxicity/mutagenicity/antimutagenicity of cyphomine and solamarine proteins isolated from ripe fruits of *S. betaceum* and tubers of *S. tuberosum*, respectively.

## 2. MATERIALS AND METHODS

**2.1. Plant Material.** *S. tuberosum* L. var. Kennebec mature tubers were obtained from the Estación Experimental Agroindustrial Obispo

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Colombres, Tucumán, Argentina, and maintained at 4 °C until use. *S. betaceum* Sendt. was grown in San Miguel de Tucumán in a greenhouse, and the ripe fruits (95 days after anthesis) were collected.

**2.2. Reagents.** The reagents employed are listed as follows: 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethylenediaminetetraacetic acid (EDTA), dimethylsulfoxide (DMSO), butylated hydroxytoluene (BHT), phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), superoxide dismutase (SOD), xanthine oxidase (XOD), 2-thio-barbituric acid (TBA), trichloroacetic (TCA), Folin–Ciocalteu reagent, and sodium dodecyl sulfate (SDS). All reagents used in this work were of analytical or microbiological grade and were purchased from Sigma (St. Louis, MO) or Merck (Darmstadt, Germany).

**2.3. Protein Extraction and Purification.** *S. tuberosum* tuber protein was extracted, isolated, and purified according to Isla et al.<sup>16</sup> and was called “solamarine”. Briefly, the juice of potato tubers (500 g) was extracted with a juicer. The mixture was centrifuged at 26000g for 10 min. Then, the supernatant was adjusted to pH 4, and the mixture was recentrifuged at 26000g for 10 min. The pellet was suspended in 0.2 M NaCl and adjusted to pH 6. The preparation was stirred for 1 h at 4 °C and centrifuged at 26000g for 10 min, and the supernatant was then adjusted to pH 5.5. Solid ammonium sulfate added up to 25% saturation. After 10 min at 4 °C and recentrifugation, the supernatant was adjusted to pH 5 and solid ammonium sulfate added up to 35% saturation. The precipitate was collected by centrifugation, dissolved in 0.2 M NaCl, and adjusted to pH 6. The preparation was dialyzed against 0.2 M NaCl for 90 min and clarified by centrifugation. Further solamarine purification was made by gel filtration on a Sephadex G-100 column (90 × 1.2 cm) equilibrated and eluted with 0.2 M NaCl. Fractions of 3 mL were collected. Proteins were detected by absorbance at 280 nm. The inhibitory effect on glycosidase activity was assayed in each purification step and in all tubes.

The protein from *S. betaceum* Sendt. fruit was purified according to Ordóñez et al.<sup>17</sup> and was called “cyphomine”. Briefly, ripe fruits (1 kg) were homogenized in 1 L of cold water. The preparation was adjusted to pH 3 by the addition of 2 M HCl, stirred at 4 °C for 15 min, and centrifuged at 15000g for 15 min. The insoluble fraction (cell wall) was washed with 1.5 L of cold water at pH 3. Then, it was suspended in 200 mL of 0.25 M NaCl. The pH was adjusted to 1.7, and then the mixture was centrifuged at 15000g for 15 min. The supernatant was adjusted to pH 4.75 and concentrated to 97 mL by tangential ultra-filtration. Then, cyphomine protein was purified in two steps: ionic exchange chromatography (DEAE-Sepharose CL-4B) and gel filtration (Sephadex G-75), until electrophoretic homogeneity. The inhibitory effect on glycosidase activity was assayed in each purification step.

The protein isolation was repeated several times in separate experiments.

**2.3.1. Protein Determination.** The protein concentration was determined by the method of Lowry et al.<sup>20</sup> using bovine serum albumin as the standard.

**2.4. Antioxidant Activity Determination.** **2.4.1. DPPH Free Radical Scavenging Activity.** The hydrogen-donor activity of purified protein was measured by the DPPH method.<sup>21</sup> A DPPH solution (1.5 mL of 300 μM in 96% ethanol) was incubated with different dilutions of protein solutions (5–100 μg/mL). The reaction mixture was incubated for 20 min at room temperature. Absorbance was measured at 514 nm. The free radical scavenging activity was calculated in relation to the control without proteins. SC<sub>50</sub> values denote the protein concentration required to scavenge 50% DPPH free radicals. Quercetin was used as a positive control.

**2.4.2. Superoxide Radical Scavenging Activity: Enzymatic Assay.** The superoxide radical scavenging activity was determined spectrophotometrically by monitoring the protein effect on NBT reduction by O<sub>2</sub><sup>•−</sup>. Superoxide radicals were generated by the xanthine/XOD system as described previously.<sup>22</sup> Briefly, 0.1 mL of protein (10–200 μg) was

added to a 1.0 mL reaction mixture (0.4 mM xanthine and 0.24 mM NBT in 0.1 M phosphate buffer (pH 7.8) containing 0.1 mM EDTA). A total of 0.05 unit of XOD was added. The mixture was incubated at 37 °C for 20 min. The reaction was terminated by adding 1.0 mL of 69 mM SDS solution, and absorbance was measured at 560 nm. Superoxide scavenging activity was calculated as a percentage of scavenging.

**2.4.3. XOD Inhibitory Activity Assay.** The XOD inhibitory effect was measured spectrophotometrically at 295 nm under aerobic conditions.<sup>23</sup> The reaction mixture consisted of 400 μL of 200 mM sodium phosphate buffer (pH 7.5), 20 μL of protein solutions (10–200 μg), 180 μL of distilled water, 200 μL of enzyme, and 200 μL of 0.6 mM xanthine. Uric acid formation was recorded at 295 nm, and XOD inhibitory activity was calculated. IC<sub>50</sub> values denote the protein concentration required to inhibit at 50% level of the enzyme activity.

**2.4.4. Superoxide Radical Scavenging Activity: Non-enzymatic Assay.** Superoxide radicals were generated by the NADH/PMS system following a method as described by Valentaõ et al.<sup>24</sup> A fixed volume of 50 μL of each tested protein (final concentration of 10–200 μg/mL) was mixed with 40 μL of NADH (996 μM), 60 μL of NBT (250 μM), and 150 μL of PMS (5.4 μM). All reagents were dissolved in a phosphate buffer (19 mM, pH 7.4). The reaction mixture was incubated for 30 min at 37 °C, and absorbance was measured at 560 nm.

SC<sub>50</sub> values denote the protein concentration required to scavenge 50% of the superoxide radical.

**2.4.5. Hydroxyl Radical Scavenging Activity.** Hydroxyl radical scavenging was carried out by measuring the competition between deoxyribose and each protein for hydroxyl radicals generated from the Fe<sup>3+</sup>/ascorbate/EDTA/H<sub>2</sub>O<sub>2</sub> system. The attack of the hydroxyl radical on deoxyribose lead to the formation of thiobarbituric acid reactive substances (TBARS).<sup>25</sup> Each protein (5–200 μg/mL) was added to the reaction mixture containing 3 mM deoxyribose, 0.1 mM FeCl<sub>3</sub>, 0.1 mM EDTA, and 20 mM phosphate buffer (pH 7.4), making up a final volume of 3 mL. Controls without EDTA were realized to determine the capacity of the protein reaction with iron ions.<sup>26,27</sup> To start the Fenton reaction, 0.1 mM aqueous solution of H<sub>2</sub>O<sub>2</sub> and 0.1 mM ascorbic acid in buffer were added.

The reaction mixture was incubated at 37 °C for 1 h. Formed TBARS were measured by the method given elsewhere. A total of 1 mL of thiobarbituric acid (TBA, 1%) and 1 mL of trichloroacetic acid (TCA, 2.8%) were added to test tubes and incubated at 100 °C for 20 min. After cooling, absorbance was measured at 530 nm.<sup>26</sup> SC<sub>50</sub> values were determined.

A modification was carried out without the addition of ascorbic acid, which was replaced by the same volume of the buffer. The negative control contained the full reaction mixture without 2-deoxy-D-ribose. The positive control was the H<sub>2</sub>O<sub>2</sub>/Fe<sup>3+</sup>/ascorbic acid system mixture without proteins (100% MDA).

Percent inhibition (*I*) of deoxyribose degradation was calculated according to the equation

$$I (\%) = (A_0 - A_1/A_0) \times 100$$

where A<sub>0</sub> is the absorbance of the positive control (100% MDA) reaction and A<sub>1</sub> is the absorbance of proteins tested. IC<sub>50</sub> values denote the protein concentration required to inhibit the degradation of 50% 2-deoxy-D-ribose present.

**2.5. Toxicity Biotest.** **2.5.1. General Toxicity Assay Using *Artemia salina* (Brine Shrimp).** Brine shrimp eggs were hatched in a shallow rectangular dish filled with artificial seawater. The phototropic nauplii were collected after 24 h. A microwell toxicity assay was carried out according to Solis et al.<sup>28</sup> Triplicate serial dilutions of each protein (until 1000 μg/mL) were made in microplate wells in 100 μL of seawater. A 10 nauplii suspension (100 μL) was added to each well, and the covered plate was incubated at 22–29 °C for 24 h. Plates were then examined under a microscope, and dead (nonmotile) nauplii in each well were

counted. Methanol (100  $\mu\text{L}$ ) was added to each well, and after 15 min, the total number of shrimp in each well was counted.  $\text{LC}_{50}$  values were calculated by Probit analysis.<sup>29</sup>

$\text{LC}_{50}$  values were confirmed using 3-(4,5-dimethyl-2-yl)-2,5-dimethyltetrazolium bromide (MTT). The amount of formazan formed 4 h after adding a solution of MTT in each well was measured at 590 nm in a Beckman DU 650 spectrophotometer.<sup>30</sup>

**2.5.2. Plant Genotoxicity Test.** The *Allium cepa* test was performed according to Nieva Moreno et al.<sup>31</sup> Young bulbs of common onions were kept in commercial mineral water for 48 h until root emergency. Six onion bulbs were exposed to 0.25, 0.5, and 2.5  $\mu\text{g}/\text{mL}$  of each protein for 24 h in the dark. Then, the roots of three bulbs were cut up and fixed for 24 h in a 1:3 acetic acid/ethanol solution. The remaining three bulbs were kept in tap water for another 24 h (recovery time), and then the roots were fixed, as indicated previously. Finally, all roots were stored in 70% ethanol. In the negative control experiment, three onion bulbs were exposed only to a water solution and they were submitted to the procedures previously described for treated bulbs. Three onion bulbs were exposed to a  $10^{-4}$  M aqueous solution of methyl methanesulfonate in a positive control experiment. Macroscopic parameters (root length and root modifications in consistency and form) were recorded. The mitotic index (MI, 1000 cells per slide) was used to evaluate the cellular division rate. Anaphasic aberrations (bridges, laggard chromosomes, and fragments; 800 anaphasic cells per sample) and micronuclei formation (5 slides, 1000 cells per slide) were the microscopic parameters assessed as indicators of DNA damage. For  $40\times$  microscopic observations, chromosomes were stained by the following procedure. Tips were cut from roots to be further hydrolyzed in 1 M HCl at 60  $^{\circ}\text{C}$  for 10 min before staining in Schiff's reagent for 15 min. A portion of the stained tip was immersed in a drop of 45% acetic acid, placed on a clean slide, and covered with a cover glass to have a single layer of cells.<sup>32</sup>

**2.5.3. Ames Test.** The mutagenicity of cyphomine and solamarine was evaluated by measuring their ability to induce reverse mutations in *Salmonella typhimurium* strains TA98 and TA100 with and without activation by Aroclor-1254-induced rat liver S9.<sup>33</sup> The protein samples and S9 mix (0.5 mL) or 0.1 M phosphate buffer at pH 7 (0.5 mL) instead of the S9 mix were added to overnight-cultured *S. typhimurium* strains (30–480  $\mu\text{g}/\text{plate}$ ). The mixture was diluted with phosphate buffer and poured onto nutrient agar plates. The plates were incubated at 37  $^{\circ}\text{C}$  for 48 h, and the number of His<sup>+</sup> revertant colonies was counted.

Mutagenicity relation (MR) was determined as

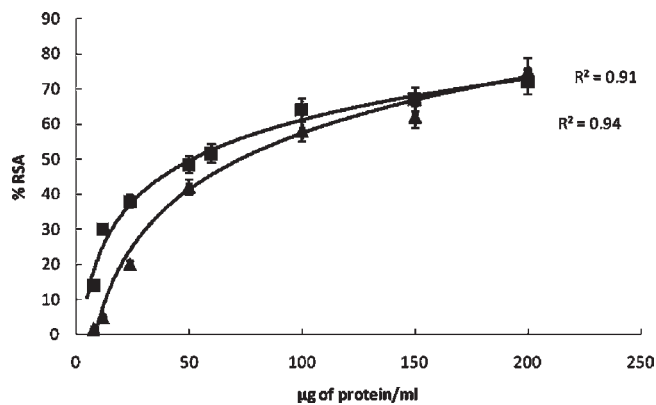
$$\text{MR} = \text{His}^+ \text{ revertant per plate} / \text{His}^+ \text{ spontaneous revertant}$$

All experiments were performed in triplicate with at least two replicates. The criterion of positive results was that defined by Maron and Ames,<sup>33</sup> a 2-fold or greater increase in the number of revertants exposed to the test material over spontaneous reversion rates. Negative and positive controls were included in each assay. The mutagens used as positive controls were 4-nitro-*o*-phenylenediamine (NPD, 5  $\mu\text{g}/\text{plate}$ ), which is a direct-acting mutagen, and isoquinoline (IQ, 0.1  $\mu\text{g}/\text{plate}$  for TA98 and 0.5  $\mu\text{g}/\text{plate}$  for TA100), which required the S9 mix for metabolic activation.

**2.6. Antimutagenicity Test.** The antimutagenic effects of proteins were assayed using the Ames *Salmonella*/mammalian microsome mutagenicity test, but the mutagen was added before preincubation. The NPD mutagen (0.1 mL) was added to the mixture of protein dilutions in phosphate-buffered saline (PBS) buffer and bacterial culture (0.1 mL). The mutagenicity of each mutagen in the absence of samples was defined as 100%.

**2.7. Protein Heat Treatment.** The proteins in buffer acetate (0.2 M, pH 5.5) were heated at 80  $^{\circ}\text{C}$  for 5, 10, 15, or 20 min. Antioxidant activity by DPPH methods was assayed in each sample.

**2.8. Statistical Analysis.** The analyses were performed in triplicate, and the data are presented as the mean  $\pm$  standard deviation (SD).



**Figure 1.** Scavenging activity of superoxide anion scavenging generated by the xanthine/XOD system. The assay was carried out in triplicate, and the values were expressed as the mean  $\pm$  SD: (■) cyphomine and (▲) solamarine.  $R^2$  values were included.

The correlation between two variants was analyzed by the Pearson test using GraphPad Prism 5.0 software, with the level of significance set at  $p < 0.05$ .

### 3. RESULTS AND DISCUSSION

Both proteins were purified until electrophoretic homogeneity.

Cyphomine and solamarine were effective to inhibit uric acid formation by xanthine oxidase inhibition (enzymatic system), with  $\text{IC}_{50}$  values of 55 and 60  $\mu\text{g}/\text{mL}$ , respectively. The total xanthine oxidase inhibition was reached at 105 and 120  $\mu\text{g}/\text{mL}$  for solamarine and cyphomine, respectively.

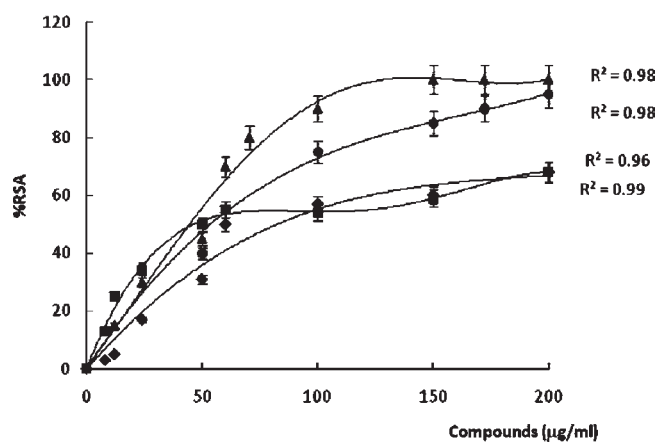
In a non-enzymatic system, both proteins were effective  $\text{O}_2^{\bullet-}$  scavengers, with  $\text{SC}_{50}$  values of 50 and 60  $\mu\text{g}/\text{mL}$  for cyphomine and solamarine, respectively (Figure 1). A significant positive correlation between the scavenging activity and protein concentration was found ( $R^2 = 0.91$  and  $0.94$  for cyphomine and solamarine, respectively).

Hydroxyl radicals are known to be the most reactive of all the reduced forms of oxygen and are thought to initiate *in vivo* cell damage.<sup>34</sup> These proteins were also evaluated for their ability to scavenge hydroxyl radicals and/or chelate iron using the deoxyribose degradation assay. The results showed that both proteins were able to reduce oxidative damage by scavenging hydroxyl radicals dose dependently (Figure 2). The effective concentrations to achieve 50% inhibition were 50 and 60  $\mu\text{g}/\text{mL}$  (2.7–3.3  $\mu\text{M}$ ) for cyphomine and solamarine, respectively. These values were lower than those reported for synthetic antioxidants ( $\alpha$ -tocopherol and ascorbic acid), with  $\text{SC}_{100}$  values around 400  $\mu\text{M}$ .<sup>35,36</sup> The iron ion chelating ability and pro-oxidant effect were not seen in the range of protein concentrations that was used in the experiment.

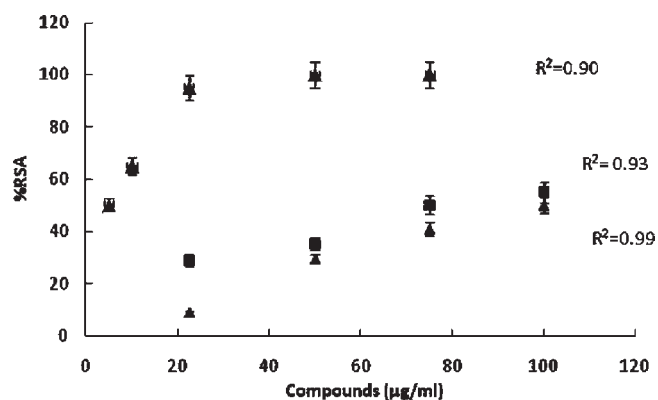
Experimental results indicate that both proteins were effective as hydroxyl and superoxide anion scavengers. According to our results, both proteins has similar antioxidant potency (significant differences between  $\text{SC}_{50}$  values were not observed).

The DPPH $\bullet$  scavenging activities of cyphomine and solamarine are shown in Figure 3. The electron-donor effect increased ( $p < 0.05$ ) with the protein concentration. The  $\text{SC}_{50}$  values were similar in both cases (around 100  $\mu\text{g}/\text{mL}$ ), but the scavenging kinetic was different. Cyphomine was more active than solamarine at lower concentrations of proteins, with a scavenging activity of 30% DPPH $\bullet$  at 20  $\mu\text{g}/\text{mL}$ , while solamarine showed this scavenging percentage at 50  $\mu\text{g}/\text{mL}$ . Previous research has also





**Figure 2.** Dose–response graphs of hydroxyl radical scavenging activity in the presence of EDTA by (▲) cyphomine, (●) solamarine, (■) ascorbic acid, and (◆)  $\alpha$ -tocopherol against different concentrations. The assay was made in triplicate, and the values are expressed as the mean  $\pm$  SD.  $R^2$  values were included.



**Figure 3.** Antioxidant activities of solamarine and cyphomine using the DPPH assay (dose–response curve): (■) cyphomine, (▲) solamarine, and (●) quercetine as the positive control. The assay was made in triplicate, and the values are expressed as the mean  $\pm$  SD.  $R^2$  values were included.

shown free radical scavenging activity of the proteic extract obtained by potato tubers.<sup>37</sup>

Heat is also used to modify the functional properties of protein ingredients. It causes protein denaturation and may be used, for example, to improve the water-binding ability and emulsification. On the other hand, heating usually decreases protein solubility because of aggregation or coagulation. Heat denaturation temperatures of different dietary proteins vary from 60 to 90 °C. No significant statistically change occurred in the free radical scavenging capacity after solamarine and cyphomine were subjected to a thermal treatment at 80 °C for 15 min.

The occurrence of many natural bioactive proteins in *S. betaceum* fruits and *S. tuberosum* tubers is now well-established. There are, however, some issues to be solved, such as toxicity, for example, before these substances can be optimally exploited for human nutrition and health.

A general toxicity assay (lethality test of *A. salina*) was performed prior to genotoxicity/antigenotoxicity assays to select non-toxic concentrations of cyphomine and solamarine. Cyphomine and solamarine LC<sub>50</sub> values were around 450 and 650  $\mu\text{g}/\text{mL}$ , respectively.

**Table 1.** Cyphomine and Solamarine Genotoxic Effects (Root Elongations, MI, and Anaphasic Aberration) against *A. cepa* Roots<sup>a</sup>

concentration	root elongation (cm)	MI (%)	anaphasic aberration
negative control <sup>b</sup>	2.7 $\pm$ 0.1	59 $\pm$ 9	NO <sup>c</sup>
positive control <sup>d</sup>	1.2 $\pm$ 0.1	35 $\pm$ 2	18 $\pm$ 1
Cyphomine ( $\mu\text{g}/\text{mL}$ )			
50	2.5 $\pm$ 0.1	58 $\pm$ 4	NO <sup>c</sup>
100	2.4 $\pm$ 0.1	50 $\pm$ 3	NO <sup>c</sup>
150	1.9 $\pm$ 0.1	43 $\pm$ 5	2 $\pm$ 1
Solamarine ( $\mu\text{g}/\text{mL}$ )			
50	2.3 $\pm$ 0.1	55 $\pm$ 5	NO <sup>c</sup>
100	1.8 $\pm$ 0.1	50 $\pm$ 7	NO <sup>c</sup>
150	1.7 $\pm$ 0.1	45 $\pm$ 3	NO <sup>c</sup>

<sup>a</sup> Mean  $\pm$  SD. <sup>b</sup> Negative control experiment; the onion bulbs were exposed only to a water solution. <sup>c</sup> Not observed. <sup>d</sup> Methyl methanesulfonate ( $10^{-4}$  M) was used in the positive control experiments.

**Table 2.** Toxicity Assay of Solamarine and Cyphomine against *S. typhimurium* TA98 and TA100<sup>a</sup>

protein ( $\mu\text{g}/\text{plate}$ )	solamarine		cyphomine	
	TA98	TA100	TA98	TA100
colony number				
30	1590 $\pm$ 60	1763 $\pm$ 30	1004 $\pm$ 10	1407 $\pm$ 40
60	1793 $\pm$ 60	1623 $\pm$ 10	1043 $\pm$ 50	1536 $\pm$ 50
120	1729 $\pm$ 40	1628 $\pm$ 50	1081 $\pm$ 50	1445 $\pm$ 20
240	1628 $\pm$ 40	1674 $\pm$ 50	989 $\pm$ 20	1470 $\pm$ 20
480	1768 $\pm$ 20	1546 $\pm$ 20	1140 $\pm$ 20	1381 $\pm$ 20
negative control <sup>b</sup>	1670 $\pm$ 20	1680 $\pm$ 40	992 $\pm$ 10	1330 $\pm$ 10

<sup>a</sup> Results are expressed as histidine revertants/plate and represent the mean  $\pm$  SD of triplicates. <sup>b</sup> Negative control experiments were carried out with distilled water.

The analysis of the effect of both proteins on the *Allium* root shows an inhibition of root elongation around 30% and a slight decrease of the MI in the exposed roots. The root macroscopic analysis revealed no modifications in consistency and shape. Tumors and hooked or twisted roots were not observed. Cyphomine and solamarine did not induce chromosomal damage (anaphasic aberrations and micronuclei formation) at a concentration similar to the one used with the *A. salina* test (Table 1).

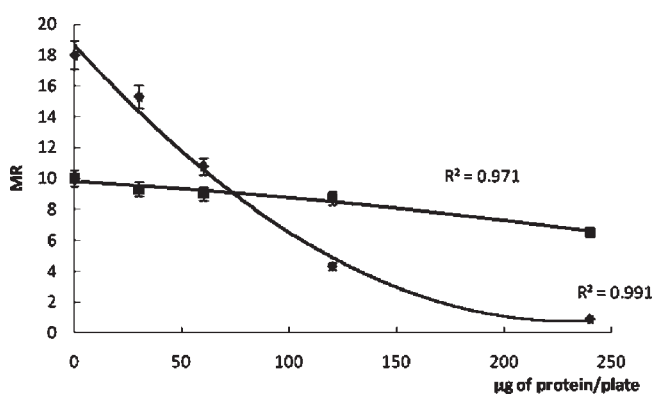
Both proteins showed no toxicity to assayed strains (*S. typhimurium* TA98 and TA100) at concentrations that have biological activities (30–480  $\mu\text{g}/\text{plate}$ ) (Table 2) and showed MR values below 2 that indicate that the proteins do not exert mutagenic effects (Table 3).

Anticarcinogenic and antimutagenic activity of phytochemicals may be due to a variety of mechanisms, such as inhibition of genotoxic effects, inhibition of cell proliferation, signal transduction modulation, scavenging of free radicals, induction of detoxification enzymes, induction of cell-cycle arrest and apoptosis, modulation of cytoskeletal proteins that play a key role in mitosis, and the inhibition of topoisomerase I or II activity.<sup>38</sup> On the basis of antioxidant potential, the proteins were screened for their antimutagenic potential against *S. typhimurium* tester strains.

**Table 3. Mutagenicity Effects of Cyphomine and Solamarine toward *S. typhimurium* TA98 and TA100 in the Absence of the S9 Mixture<sup>a</sup>**

protein ( $\mu\text{g}/\text{plate}$ )	solamarine		cyphomine	
	TA98	TA100	TA98	TA100
	MR			
30	0.94 $\pm$ 0.10	0.99 $\pm$ 0.05	0.81 $\pm$ 0.10	1.07 $\pm$ 0.10
60	0.78 $\pm$ 0.05	0.65 $\pm$ 0.05	0.78 $\pm$ 0.05	0.70 $\pm$ 0.05
120	0.66 $\pm$ 0.05	1.08 $\pm$ 0.10	0.74 $\pm$ 0.05	1.01 $\pm$ 0.10
240	0.80 $\pm$ 0.10	0.95 $\pm$ 0.05	0.88 $\pm$ 0.05	1.05 $\pm$ 0.10

<sup>a</sup>The results were expressed as MR, which was calculated as follows: MR = His<sup>+</sup> revertant per plate/His<sup>+</sup> spontaneous revertant. Positive controls were used.



**Figure 4.** Inhibition of the mutagenicity of direct-acting mutagens by solamarine protein. The study was carried out using *S. typhimurium* strains (■) TA98 and (◆) TA100. Results for MR calculations are presented as the mean  $\pm$  SD of triplicates.

Using the Ames test, only solamarine showed antimutagenicity ( $p < 0.05$ ) against NPD, with and without metabolic activation (Figure 4). The linear regression analysis between protein dose and antimutagenic response against the respective test mutagen showed a strong correlation with respect to the dose-dependent response in TA100 ( $R^2 = 0.99$ ). In conclusion, we have shown for the first time that a protein isolate from potato tubers is a promising antimutagenic compound and this activity may be determined by its antioxidant properties.

Our results suggest that both proteins might be used as natural antioxidants and that they might be considered for the preparation of harmless functional foods from *S. betaceum* fruits and *S. tuberosum* tubers.

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## ■ ERRATUM STATEMENT

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