



## Mutagenic Activity of Glycoalkaloids from *Solanum palinacanthum* Dunal (Solanaceae) found in the Brazilian cerrado

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**SUMMARY.** Solanaceous plants are widely distributed around the world and they are traditionally used as drugs for the treatment of cancer and herpes, and include familiar foods such as potato, tomato and egg-plant and some berries popular in Brazil. As part of a program of research on pharmacologically active new molecules, the aim in this study was to assess the mutagenic effects of *Solanum palinacanthum*, known popularly as joá. The crude 95% ethanol extract and purified solamargine obtained from the fruits of *S. palinacanthum* Dunal were investigated by the Ames test, using the *Salmonella typhimurium* strains TA98, TA97a, TA100 and TA102 as test organisms, with and without metabolic activation. The concentrations tested ranged from 0.07 to 15.0 mg/plate for the crude ethanolic extract and from 1.25 to 5.0 mg/plate for the solamargine. The results showed a mutagenic effect of both the extract and the solamargine in the TA98 strain (without metabolic activation). The present study showed the potential mutagenicity and suggests confirming this effect in other models, before recommending their indiscriminate consumption by the population.

### INTRODUCTION

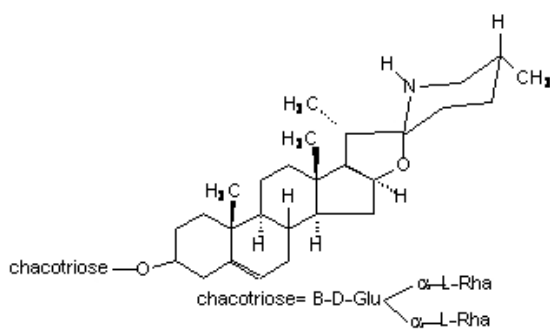
The use of medicinal plants has increased in recent decades in Brazil, but there is as yet little information about their potential risk to health. As they contain chemical substances well known as mutagens and/or carcinogens<sup>1,2</sup>, many of these plants are correlated with a raised frequency of disease and tumors since they contain chemical substances well known as mutagens and/or carcinogens. In developed countries, tests on herbal medicines follow similar rules and criteria to those established for synthetic drugs. Even so, there is little information about the genotoxicity of natural drugs. Therefore, the evaluation of the mutagenic effects of compounds extracted from plants is, without any doubt, a crucial step towards reducing the risk due to exposure to these agents. Among the approaches used to assess the mutagenic activity of a substance and consequently

its carcinogenic potential, the AMES test or *Salmonella* microsome assay<sup>3</sup> is widely accepted.

*Solanum palinacanthum* Dunal is a member of the family Solanaceae that grows in tropical and temperate zones, including the savannah-like cerrado biome of Brazil, where it has been used to treat skin diseases<sup>4</sup>. Like many members of the Solanaceae family of plants, it synthesizes secondary metabolites, such as polyphenols and glycoalkaloids, which protect it against animals and insects by their toxicity<sup>5-7</sup>. The principal glycoalkaloids in *Solanum palinacanthum* Dunal are solamargine and solasonine. The solamargine (Fig. 1) is a major chemical constituent, it has been shown to inhibit the growth of human tumor cells, e.g., colon, prostate, breast, and human hepatoma cells<sup>8,9</sup>. Moreover, it has been reported that some of the glycoalkaloids are used in anticancer medica-

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**Figure 1.** Solamargine structure.

tions, for their medicinal or bioactive properties, as precursors for the synthesis of useful pharmaceuticals and also for their antifungal properties<sup>10,11</sup>. However, the mutagenic effects of the glycoalkaloids and extract obtained from the fruits of this plant have not been described yet. The main aim of the present investigation was to assess the possible mutagenic activity of solamargine and crude 95% ethanol extract of the fruits of *S. palinacanthum*.

## MATERIALS AND METHODS

### Plant material

*Solanum palinacanthum* Dunal (Solanaceae) used in this study was collected in March 2007 from Itápolis, São Paulo state. A voucher specimen has been deposited at the herbarium of the Institute of Biosciences of São Paulo State University at Rio Claro, SP, Brazil.

### Extraction and Isolation

Air-dried and powdered fruits of *S. palinacanthum* (500 g) were extracted exhaustively for 24h with 95% (v/v) ethanol at 60 °C, 4 times in succession, with a 1:5 (w/v) plant: solvent ratio. Subsequent exhaustive extraction with hot EtOH and evaporation of the solvent under reduced pressure afforded an extract (100.2 g). The extract was redissolved with 5% (v/v) aqueous acetic acid and, after 24 h, it was subjected to preliminary purification involving defatting by successive benzene extraction. The pH of the aqueous solution was then increased to pH 10 with ammonium hydroxide. After 24 h at room temperature, the precipitate was filtered and dried (24.2 g). This glycoalkaloid mixture, isolated from *S. palinacanthum*, was subjected to column chromatography (aluminum oxide, neutral). Elution with 40% aqueous ethanol (v/v) yielded solamargine (0.92 g) (Fig. 1). The separation was monitored by Thin Layer Chromatog-

raphy (TLC) in the solvent system *n*-butanol: glacial acetic acid: water (6:3:1), the plate being sprayed with 10% sulfuric acid (v/v) and then heated at 150 °C for 5 min. Partially purified solamargine was obtained and rechromatographed on alumina and repeatedly recrystallised from methanol, for further purification, and its identity ascertained by comparing physical and spectral data (mp, IR)<sup>7</sup>.

### Ames test

*Salmonella typhimurium*/microsome assay: The following chemicals: dimethylsulfoxide (DMSO), nicotinamide adenine dinucleotide phosphate sodium salt (NADP), D-glucose-6-phosphate disodium salt, L-histidine monohydrate and D-biotin were purchased from Sigma Chemical Co. (St. Louis, MO). Standard mutagens: sodium azide, 2-anthramine, mitomycin C and 4-nitro-*o*-phenylenediamine were also obtained from Sigma. Oxoid Nutrient Broth n° 2 (Oxoid, England) and Difco Bacto Agar (Difco, USA) were used for the preparation of bacterial growth media. All the other reagents used to prepare buffers and media were from Merck (Whitehouse Station, NJ) and Sigma. The *Salmonella* mutagenicity assay was performed with *S. typhimurium* strains TA100, TA98, TA97a and TA102, incubation method, with and without metabolic activation, for 20-30 min<sup>3</sup>. The S9-mix was freshly prepared before each test with an Aroclor-1254-induced rat liver fraction purchased (lyophilized) from Molttox?Molecular Toxicology Inc. *S. typhimurium* strains were kindly provided by Dr. B. Ames, University of California, Berkeley, CA, USA. Various concentrations of the extract (0.07, 1.25, 2.5, 3.75, 5.0, 7.5, and 15.0 mg/plate) and of solamargine (1.25, 2.5, 3.5, 4.5, and 5.0 mg/plate) dissolved in DMSO were tested. These concentrations were based on the bacterial toxicity, established in a preliminary test. In all subsequent assays, the upper limit of the dose range tested was either the highest non-toxic dose or the lowest toxic dose determined in this preliminary assay. Toxicity was apparent either as a reduction in the number of His<sup>+</sup> revertants, or as an alteration in the auxotrophic background (*i.e.* background lawn). The test solutions were added to 500  $\mu$ L of buffer pH 7.4 and 100  $\mu$ L of bacterial culture and incubated at 37 °C for 20-30 min. After this time, 2 mL of top agar was added to the mixture and poured on to a plate containing minimal agar. The plates were incubated at 37 °C for 48 h and the His<sup>+</sup> revertant colonies were

manually counted. The influence of metabolic activation was tested by adding S9 mixture (4%) to the buffer before incubation. All experiments were analyzed in triplicate.

The standard mutagens used as positive controls in experiments without S9 mix were 4-nitro-*o*-phenylenediamine (10 µg/plate) for TA98 and TA97a, sodium azide (1.25 µg/plate) for TA100 and mitomycin (0.5 µg/plate) for TA102. 2-anthramine (0.125 µg/plate) was used in the experiments with metabolic activation for all strains. DMSO served as the negative (solvent) control.

### Statistics

The results were performed with the statistical software Salanal, adopting the Bernstein model<sup>12</sup>. The data were assessed by analysis of variance (ANOVA) followed by a linear regression. The mutagenic index (MI) was also calculated for each dose; that is, the average number of revertants per plate divided by the average number of revertants per plate for the negative control (solvent). A sample was considered positive when the mutagenic index was equal to or greater than 2 for at least one of the tested doses and if it had a reproducible dose-response curve<sup>13</sup>.

### RESULTS AND DISCUSSION

The present study was carried out to investigate the mutagenic properties of a crude 95% ethanol extract and the compound solamargine obtained from the fruit of *S. palinacanthum*.

The results obtained are shown in Table 1, indicating the average and standard deviation of the numbers of His<sup>+</sup> revertants per plate, both for the negative control group and the various tested concentrations, in all the *S. typhimurium* strains, with and without metabolic activation. The mutagenic index (MI) values are also shown in Table 1. The extract and solamargine were both mutagenic to strain TA98 (-S9), which detects frameshift mutations. Substitution of bases of DNA on TA100 strain or oxidative DNA damage detected in the strain TA102 were not observed. The results were also negative in the TA97a strain which detect frameshift mutations but is more sensitive to heavy metal mutagens<sup>3,14</sup>.

Mutational events are involved in several degenerative diseases, such as cancer and arteriosclerosis<sup>15,16</sup>. The literature describes many plants containing mutagenic compounds, such as furocoumarins<sup>17</sup>, tannins, anthraquinones<sup>18</sup>,

alkaloids<sup>19</sup> and flavonoids<sup>20</sup>. Therefore, research on the mutagenicity of medicinal plants is fundamental, since the presence of mutagens in medicines can be dangerous to human health.

Even in the middle of the last century it was believed that the secondary metabolites of plants did not have function in the organisms that produced them, being considered molecular garbage. Now it is known that many of those substances possess several activities, as antibiotic, pesticide, repellent, controller of cellular division, among other<sup>21</sup>.

Hecker & Schmidt<sup>22</sup> and Weber & Hecker<sup>23</sup> described that the called phorbol ester diterpene, derivate of the secondary metabolism of several plants of the genus *Croton*, presents irritant and carcinogenic effects. On the other hand, Ames<sup>24</sup> affirms that many elements present in the human diet presents noxious substances.

This information reveals the importance of the studies about the genetic risks of plant compounds, mainly with plants used for the population in its diet or in medicinal treatments.

In Brazil, a large number of herbal extracts are used in folk medicine to treat various types of disease and *S. palinacanthum* has been used to treat skin diseases and as an antimicrobial<sup>4</sup> but little is known about the risks of the consumption of its preparations and chemical composition. In this study mutagenicity was detected by the Ames assay.

The obtained results, indicating some mutagenicity in the extract and its main compound, emphasize the importance of careful assessment and severe investigation before recommending their indiscriminate consumption by the population.

### CONCLUSION

Both the *S. palinacanthum* extract and solamargine exhibited a mutagenic potential, suggesting that the effect observed in the extract may be related to the presence of solamargine, the main substance isolated from the fruits of this plant. More chemical constituents need to be elucidated in future work and further studies are required to determine their possible mutagenic effects. The results of such a screen for mutagenic activity, allied to more specific biological assays, would contribute to the safe use of these extracts and substances obtained from *S. palinacanthum* fruits.

Treatment (mg/plate)	Revertants per plate for each <i>Salmonella typhimurium</i> test strain							
	TA98		TA97a		TA100		TA102	
	- S9 <sup>a</sup>	+S9 <sup>b</sup>	- S9 <sup>a</sup>	+S9 <sup>b</sup>	- S9 <sup>a</sup>	+S9 <sup>b</sup>	- S9 <sup>a</sup>	+S9 <sup>b</sup>
<b>Extract</b>								
0	25 ± 4.0	20 ± 2.5	228 ± 21.5	157 ± 9.6	174 ± 1.5	152 ± 5.6	264 ± 18.9	276 ± 6.1
0.07	26 ± 2.0 (1.0)	22 ± 2.0 (1.0)	210 ± 8.1 (0.9)	147 ± 4.2 (0.9)	175 ± 7.5 (1.0)	151 ± 7.6 (0.9)	243 ± 12.3 (0.9)	270 ± 9.0 (0.9)
1.25	27 ± 1.5 (1.1)	25 ± 1.2 (1.3)	204 ± 15.2 (0.9)	155 ± 4.2 (0.9)	174 ± 10.1 (1.0)	160 ± 7.1 (1.1)	252 ± 8.1** (0.9)	276 ± 5.1 (1.0)
2.50	34 ± 6.1 (1.4)	27 ± 4.2 (1.4)	208 ± 10.0* (0.9)	155 ± 4.3 (0.9)	191 ± 9.0 (1.1)	160 ± 7.0 (1.1)	283 ± 25.5** (1.1)	280 ± 6.2 (1.0)
3.75	37 ± 5.1 (1.5)	28 ± 2.0* (1.4)	230 ± 15.3* (1.0)	162 ± 10.1 (1.0)	189 ± 13.7 (1.1)	172 ± 7.5* (1.1)	287 ± 20.6 (1.1)	282 ± 6.8 (1.0)
5.00	37 ± 5.2 (1.5)	32 ± 0.6** (1.6)	249 ± 15.9* (1.1)	165 ± 6.4 (1.0)	243 ± 17.2* (1.4)	175 ± 5.6* (1.1)	293 ± 9.2 (1.1)	283 ± 8.5 (1.0)
7.50	54 ± 6.9* (2.2)	37 ± 4.2* (1.8)	237 ± 10.7** (1.1)	169 ± 5.6 (1.1)	244 ± 17.4* (1.4)	181 ± 3.6** (1.2)	287 ± 21.1 (1.1)	287 ± 8.7 (1.0)
15.0	62 ± 4.8** (2.5)	31 ± 2.7* (1.6)	234 ± 7.1** (1.0)	174 ± 4.0 (1.1)	250 ± 12.3* (1.4)	182 ± 7.9** (1.2)	302 ± 17.3* (1.1)	288 ± 7.5 (1.0)
<b>Control +</b>	1885 ± 126.5 <sup>a</sup>	748 ± 36 <sup>b</sup>	1251 ± 44.2 <sup>c</sup>	1209 ± 55 <sup>b</sup>	1157 ± 207.6 <sup>c</sup>	1225 ± 45 <sup>b</sup>	886 ± 122.6 <sup>d</sup>	1035 ± 167 <sup>b</sup>
<b>Solamargine</b>								
0	36 ± 4.0	30 ± 1.5	172 ± 5.0	166 ± 4.0	171 ± 3.1	152 ± 5.6	261 ± 10.1	276 ± 6.1
1.25	34 ± 1.0 (0.9)	31 ± 2.1 (1.0)	168 ± 6.0 (0.9)	162 ± 4.5 (0.9)	163 ± 5.4 (0.9)	158 ± 5.3 (1.0)	251 ± 8.1 (0.9)	278 ± 6.7 (1.0)
2.50	37 ± 2.7 (1.0)	30 ± 2.0 (1.0)	171 ± 4.0 (0.9)	167 ± 7.0 (1.0)	169 ± 2.5 (0.9)	170 ± 4.7* (1.1)	264 ± 6.0 (1.0)	287 ± 8.5 (1.0)
3.50	48 ± 3.5* (1.3)	36 ± 1.5* (1.2)	176 ± 4.9 (1.0)	171 ± 9.0 (1.0)	174 ± 4.5 (1.0)	176 ± 3.6* (1.2)	276 ± 5.3 (1.0)	302 ± 11.1 (1.1)
4.50	76 ± 3.1** (2.1)	38 ± 4.0 (1.3)	175 ± 5.0 (1.0)	177 ± 5.0 (1.1)	175 ± 5.5 (1.0)	178 ± 4.0** (1.2)	286 ± 6.0 (1.1)	305 ± 7.5* (1.1)
5.00	82 ± 2.5** (2.3)	41 ± 5.1 (1.4)	184 ± 2.7 (1.1)	176 ± 4.0 (1.1)	178 ± 4.0 (1.0)	176 ± 4.1* (1.2)	282 ± 5.9 (1.1)	292 ± 7.6 (1.0)
<b>Control +</b>	667 ± 15.0 <sup>a</sup>	691 ± 12.0 <sup>b</sup>	1042 ± 79 <sup>c</sup>	1125 ± 44 <sup>b</sup>	1145 ± 89 <sup>c</sup>	1225 ± 45 <sup>b</sup>	999 ± 53	1035 ± 167 <sup>b</sup>

**Table 1.** Mutagenic activity expressed as the mean ± standard deviation of total revertants per plate in *Salmonella typhimurium* strains TA98, TA100, TA97a and TA102 exposed to crude 95% ethanolic extract and solamargine obtained from *Solanum palinacanthum* Dunal fruits, at various concentrations, with (+S9) and without (-S9) metabolic activation. Extract: crude 95% ethanolic extract of the *S. palinacanthum* Dunal.; Solamargine: glycoalkaloid isolated from the extract. 0 = negative control (DMSO - 100 µL/plate); Control + : positive control: <sup>a</sup> NPD (4-nitro-*o*-phenylenediamine - 10.0 µg/plate), <sup>b</sup> 2-anthramine (1.25 µg/plate); <sup>c</sup> sodium azide (1.25 µg/plate); <sup>d</sup> mitomycin C (0.5 µg/plate). \*  $P < 0.05$ ; \*\*  $P < 0.01$  (ANOVA). The mutagenic index is given in brackets.

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