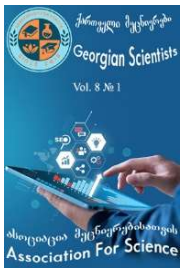
Georgian Scientists
ქართველი მეცნიერები
Vol. 8 Issue 1, 2026
https://doi.org/10.52340/gs.2026.08.01.14



The study of cytotoxic activity of some alkaloid-bearing plants growing in Georgia

Lali Kintsurashvili[1], Tamar Suladze[2], Nino Kachlishvili[3], Jean Legault[4], Vakhtang Mshvildadze[5], Nina Vachnadze[6]

[1]Lali Kintsurashvili, Doctor of Pharmacy, Senior Researcher, I. Kutateladze Institute of Pharmacochemistry,Tbilisi State Medical University, Tbilisi, Georgia, E-mail:l.kintsurashvili@ tsmu. edu; [2]Tamar Suladze, Doctor of Pharmacy, Researcher, I. Kutateladze Institute of Pharmacochemistry, Tbilisi State Medical University, Tbilisi, Georgia; [3]Nino Kachlishvili, Doctor of Biology, Researcher, I. Kutateladze Institute of Pharmacochemistry, Tbilisi State Medical University, Tbilisi, Georgia; [4]Jean Legault, Doctor of Biology; Professor at Fundamental Sciences Department, Université du Québec a Chicoutimi (Canada); [5]Vakhtang Mshvildadze, Doctor of Pharmacy, Senior Researcher, I. Kutateladze Institute of Pharmacochemistry, Tbilisi State Medical University, Tbilisi, Georgia; Professor at Fundamental Sciences Department, Université du Québec a Chicoutimi (Canada); [6]Nina Vachnadze, Doctor of Chemistry, Chief Researcher, I. Kutateladze Institute of Pharmacochemistry, Tbilisi State Medical University, Tbilisi, Georgia;

Abstract.

The aim of the study was to examine the aboveground parts of plants growing in Georgia: *Delphinium elisabethae N. Busch., Solanum nigrum L., Sophora japonica L., Vinca herbacea Waldst. et Kit.*, for their alkaloid content and to evaluate their biological activity. Purified total alkaloid fractions were obtained using liquid-liquid extraction from the aforementioned plants, in the composition of which, as Phytochemical analysis revealed the major alkaloid constituents correspond to diterpene (*D. elisabethae*), steroidal (*S. nigrum*), quinolizidine (*S. japonica*), and indole-type bases (*V. herbacea*). Cytotoxic activity in vitro was assessed using three cell cultures: A-549 (human lung carcinoma cell culture, ATCC#CCL-185); DLD-1 (cell culture of the rectum adenocarcinoma, ATCC#CCL-221); WS-1 (human dermal fibroblasts) with the Resazurin and Hoechst tests.

It was found that the total alkaloid fraction from *V. herbacea* demonstrated strong cytotoxic activity against A-549 and DLD-1 cells, with no toxicity toward WS-1. The *S. nigrum* fraction displayed moderate cytotoxicity against all tested cell cultures. The total alkaloid fractions from *D. elisabethae* and *S. japonica* showed only weak cytotoxic activity against all cell lines.

Introduction. Modern comprehensive approaches used in the treatment of oncological diseases represent a set of complementary strategies, including surgical intervention, radiation therapy, chemotherapy, targeted and immunotherapeutic methods [1,2]. These approaches target the tumour and the patient's body at the molecular, cellular and systemic levels. Despite the progress achieved, significant problems still remain in the treatment of malignant neoplasms due to the high toxicity of anticancer drugs, the development of drug resistance and insufficient selectivity of their action [3,4]. In this regard, the search for new, safer and biologically active natural compounds that can become the basis for alternative or auxiliary chemotherapeutic agents remains relevant [5].

One of the promising areas of cancer therapy is the use of chemotherapeutic drugs of plant origin. Secondary metabolites, especially alkaloids, play an important role in the treatment of malignant neoplasms [6, 7]. A number of widely used cytostatics were obtained from plant materials: vincristine and vinblastine from *Vinca rosea L.*, taxol from *Taxus baccata L.*, Ukrain -based on an alkaloid from *Chelidonium majus L.*, camptothecin from *Camptotheca acuminate Decne*. [8-11]. Alkaloids, due to their wide range of pharmacological activity, in particular their strong cytotoxic and antiproliferative effects, attract special attention of researchers. [12]. The biological activity of these compounds is realized through multiple molecular mechanisms. For instance, disturbances in microtubule dynamics are characteristic for indole alkaloids, inhibition of topoisomerases - for camptothecin, and induction of apoptosis - for berberine and soforidine. Such a diverse mechanisms of action on malignant neoplasms underscore the significant therapeutic potential of alkaloids and generate increasing interest in their further research [13].
The species examined—*Delphinium elisabethae* N. Busch. *(Helleboraceae), Solanum nigrum* L. *(Solanaceae), Sophora japonica* L.*(Leguminosae),* and *Vinca herbacea* Waldst. et Kit. *(Apocynaceae)*—were selected for their established biological and pharmacological propertiesand as the objects, that allow for a comprehensive analysis of representatives of diverse structural classes based on their cytotoxic action.
According to the literature data, the chemical composition *of D. elisabethae is* characterized by the presence of alkaloids with potential cytotoxic activity. However, the number of publications related to research in this area remains limited. In this regard, the development of an optimal method for isolating total alkaloid fractions enriched with biologically active bases exhibiting selective activity from *D. elisabethae*, growing in Georgia, represents an urgent and scientifically justified task [14].
Numerous studies of foreign scientists related to *S. nigrum* have demonstrated its high antiproliferative activity. However, there are no systematic studies of the alkaloid composition and cytotoxic activity of this species in Georgia. It is known that the chemical composition of the plant includes valuable steroidal glycoalkaloids (solasonine, solamargine) with established antitumor activity. In this regard, the study of *S. nigrum* growing in Georgia is of interest for

identifying biologically active components, expanding phytochemical data on the flora of Georgia and searching for promising natural compounds with cytotoxic potential [15].

The rich chemical composition of *S. japonica*—flavonoids, isoflavonoids, quinolizidine alkaloids, and phenolic acids—makes it a valuable source of biologically active metabolites. However, as in the previous case, information on cytotoxic activity is limited, which determines interest in assessing the potential antitumor activity of this species and identifying novel biologically active compounds [16, 17].

The genus *Vinca* plays a key role in oncopharmacology, being the source of a number of successful anticancer drugs such as vincristine and vinblastine. *V. herbacea* is considered a promising object for the search for cytotoxically active alkaloids. Studying the chemical composition and biological activity of the aboveground parts of *V. herbacea* will allow to approach the study of this plant from a novel perspective, creating the prerequisites for identifing new compounds and evaluating their biological activity, and determining their potential for the development of natural anticarcer agents [18].

An analysis of these species will enable the identification of the most promising plant sources of biologically active compounds and provide a scientific foundation for further phytochemical and pharmacological research

The aim of research. The aim of our research was to study the aboveground vegetative organs of the following plants: *Delphinium elisabethae* N. Busch. (*Helleboraceae*); *Solanum nigrum* L. (*Solanaceae*); *Sophora japonica* L. (*Leguminosae*), *Vinca herbacea* Waldst. et Kit. (*Apocynaceae*), growing in Georgia, for the content of alkaloids and to evaluate their physiological activity.

Materials and methods. The plant material was collected during the flowering phase, collected in Western Georgia. Air-dried raw materials were crushed. Extraction was performed by maceration in 70% ethanol at room temperature for 48 hours, followed by filtration and evaporation to a dry residue. The residue was treated with a 5% hydrochloric acid solution. The acidic solution was alkalized with 25% ammonia, after which extraction was performed with chloroform. The combined chloroform fractions were washed with distilled water to a neutral pH, dehydrated with anhydrous sodium sulphate, filtered and evaporated to dryness.

Qualitative analysis of the isolated total alkaloid fractions was performed by thin-layer chromatography (TLC) on plates (Silica gel 60 F_{254} (Merck, Germany) using the following solvent system: chloroform-methanol (4:1; 6:1; 9:1); chloroform-benzene-ethanol 95% - ammonia 25% (40:40:10:0.2); benzene-ethylacetate-methanol (2:2:1). For visualization, the following detectors were used: Dragendorff reagent, ammonium cerium sulfate solution in 85% orthophosphoric acid (for indole bases), in the presence of standards. Also, identification of alkaloids was realized using 7890B GC System and 5977A Single Quadrupole GC/MSD System (Agilent Technologies, USA).

Cytotoxic activity was evaluated on three cell lines: A549 (human lung carcinoma, ATCC#CCL-185); DLD-1 (human colorectal adenocarcinoma, ATCC#CCL-221); WS-1 (human dermal fibroblasts,) which were received from ATCC (American Type Culture Collection - Manasa, USA). Cancer cells were cultivated in Earle's salt and L-glutamine growth medium. (Earle's salts

content: KCl, NaCl, $NaH_2PO_4 \cdot H_2O$, D-Glucose, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, $NaHCO_3$, red phenol), then supplemented with 10% fetal calf serum (Hyklon, Logan, USA) vitamins (1X), penicillin (100 I.U/ml) and streptomycin (100 mkg/ml), amino acids (1X), sodium pyruvate (Mediaech Cellgro, VA). Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

Results and discussion. Spectral analysis (GC/MS) and thin-layer chromatography (TLC) revealed the presence of diterpene alkaloids in the chemical composition of the aboveground organs of *D. elisabethae*, steroidal alkaloids in *S. nigrum*, quinolizidine alkaloids in *S. japonica*, and indole alkaloids in *V. herbacea*.

Table 1 presents the dominant alkaloid bases identified in the total alkaloid fractions isolated from the aboveground vegetative organs of the studied species.

Table1. Alkaloids identified in the aboveground parts of *D. elisabethae, S. nigrum, S. japonica, and V. herbacea.*

Plant	Vegetative part of the plant	Total alkaloid content (%) in dry plant material	Identified alkaloids	Alkaloid class	Formula	Molecular weight (g/mol)	Melting point (°C)	Optical rotation ([α]D)
D. elisabethae	Aboveground	1, 21	Methyllycaconitine	diterpene	$C_{37}H_{50}N_2O_{10}$	666.81	157-158 (decomp.)	+49° (EtOH)
			Anthranoillicoctonine	diterpene	$C_{32}H_{46}N_2O_8$	586.72	-	-
			Lycoctonine	diterpene	$C_{25}H_{41}NO_7$	467.60	169-171	-
S. nigrum	Aboveground	1,0	Solasonine	steroidal	$C_{45}H_{73}NO_{16}$	868.04	290-295 (decomp.)	-
			Solamargine	steroidal	$C_{45}H_{73}NO_{15}$	852.04	295-300 (decomp.)	
S. japonica	Aboveground	0,2	Sophorine	quinolizidine	$C_{11}H_{14}N_2O$	190.24	146-147	-
V. herbacea	Aboveground	0.94	Vincarine	indole	$C_{21}H_{24}N_2O_3$	352.1787	263-264	+14 (MeOH)
			Herbamin	indole	$C_{22}H_{28}N_2O_4$	382.1893	176-179 (MeOH decomp.)	0±5° ($CHCl_3$)
			Vincamain	indole	$C_{22}H_{26}N_2O_3$	366.1943	226-227 (EtOH)	-54° (EtOH)
			Herbadine	indole	$C_{21}H_{24}N_2O_3$	368.1736	206-208 (decomp.)	-

As shown in Table 1, qualitative and quantitative analysis of the aboveground parts of these species revealed clear differences in alkaloid content. The highest total alkaloid fraction was observed in *D. elisabethae* -1.21%, whereas the lowest was found in *S. japonica* - 0.2%. Among

the steroidal glycoalkaloids, *S. nigrum* exhibited 1.0%, whereas *V. herbacea* - a moderate content of 0.94%.

Physicochemical properties, such as melting point and optical rotation ([α]D), reflect the features of the stereochemical configuration and the degree of purity of the alkaloids. Thus, methyllycaconitine exhibits a positive optical rotation (+49°), whereas *V. herbacea* alkaloids exhibit a wide range of [α]D values (from –54° to +14°), indicating the presence of various stereoisomers in the samples.

Based on the data in Table 1, it can be concluded that representatives of the genera *Delphinium* and *Vinca* are characterized by a high content and diverse array of biologically active alkaloids, whereas *Sophora* and *Solanum* exhibit a narrower but more pronounced profile, emphasizing their chemotaxonomic and pharmacological significance.

The cytotoxic activity of total alkaloid fractions isolated from the plant samples was assessed by the inhibitory concentration (IC_{50}), which suppresses cell growth by 50%. Daunorubicin served as a positive control. According to the results of cytotoxic activity in vitro using the Resazurin and Hoechst tests, the studied fractions revealed varying degrees of cytotoxic activity depending on the plant species, assay method, and cell line. The research results are presented in Table 2.

Table 2. In vitro cytotoxic activity of total alkaloid fractions from *D. elisabethae, S.* nigrum, *S. japónica, V. herbacea*, as determined by Resazurin and Hoechst tests (IC_{50}, μg/ml).

plant	Methods					
	Resazurin			Hoechst		
	A-549	DLD-1	WS-1	A-549	DLD-1	WS-1
D. elisabethae	159±12 μg/ml	>200 μg/ml	>200 μg/ml	92±14 μg/ml	>200 μg/ml	>200 μg/ml
S. nigrum	38±4 μg/ml	69±5μg/ml	39±3 μg/ml	37±3 μg/ml	49±6 μg/ml	46±5 μg/ml
S. japonica	182±7 μg/ml	147±8 μg/ml	110±3 μg/ml	125±6 μg/ml	113±8 μg/ml	107±2 μg/ml
V. herbacea	18±2 μg/ml	44±3 μg/ml	75±15 μg/ml	48±12 μg/ml	29±5 μg/ml№	>200 μg/ml
Daunorubicin	0,68± 0,08 μM	<0,078 μM	0,12±0,02μM	<0,078 μM	<0,078 μM	0,13± 0,03 μM

According to the data presented in Table 2, the cytotoxic activity of total alkaloid fractions from *D. elisabethae, S. nigrum, S. japonica, V. herbacea* varies significantly depending on the plant species, alkaloid class, and cell line used and also is consistent with the pharmacological characteristics described in the literature.

The total alkaloid fractions of *D. elisabethae* exhibited generally low cytotoxic activity, with the exception of line A-549 according to the Hoechst test (IC_{50} = 92 ± 14 μg/ml), IC_{50} values exceeded 200 μg/ml. This activity is probably due to the dominant alkaloids present in the composition of

the total alkaloid fractions. Modifying extraction conditions, fractionation, or using alternative purification methods may alter the chemical composition of the total fraction, including the ratio of minor and major alkaloids, which, in turn, may affect its biological activity profile.

The steroidal alkaloids of *S. nigrum* demonstrated moderate cytotoxic activity, with IC_{50} values for all tested lines ranging from 37–69 μg/ml. The similar levels of activity in different cell lines may suggest that these compounds act on cells through a common mechanism, without showing pronounced selectivity. Considering the known ability of glycoalkaloids to enhance the cytotoxicity of several chemotherapeutic agents, further investigation of the potential synergism of S. nigrum with anticancer drugs seems appropriate.

The total alkaloid fraction of *S. japonica* containing quinolizidine alkaloids exhibited low cytotoxic activity, particularly against A-549 cells (IC_{50} = 182 ± 7 μg/ml), which is consistent with literature data on the generally weak cytotoxicity of most quinolizidine bases. However, to identify the application potential of this fraction, it can be further evaluated in terms of the antibacterial, antifungal, antioxidant, and neurotropic activities, which, according to the literature, are characteristic of many quinolizidine alkaloids. In addition, changing the extraction method and fractionation may enable the isolation of components with more pronounced cytotoxic activity, which suggests continuation of the research in this direction.

The most pronounced cytotoxic effect was observed for the total alkaloid fraction of *V. herbacea*, in particular, against the A-549 cell line (Resazurin method) with the lowest value of IC_{50} = 18 ± 2 μg/ml, indicating the high sensitivity of cells to this class of alkaloids. According to the Hoechst test, strong activity was also noted against the DLD-1 cell line (IC_{50} = 29 ± 5 μg/ml), further supporting the potential of *V. herbacea* compounds against colorectal cells. Taken together, these findings highlight the pharmacological potential of *V. herbacea* as a promising source of cytotoxically active natural compounds.

Thus, among the studied objects, the diterpene (*D. elisabethae*) and quinolizidine (*S. japonica*) bases were less active, whereas steroidal alkaloids (*S. nigrum*) demonstrated moderate activity, and bases belonging to the indole class (*V. herbacea*) showed the highest cytotoxic potential. The observed differences correlate well with the chemical nature of the corresponding classes of alkaloids and can be used for a targeted search for biologically active compounds.

Conclusions. An analysis of the alkaloid composition of the aboveground parts of *D. elisabethae, S. nigrum, S. japonica,* and *V. herbacea*, introduced and growing in Georgia, was conducted in the study. The compounds belonging to various structural classes of alkaloids, including diterpene, steroidal, quinolizidine, and indole bases were identified, and their dominant components were determined using GC/MS and TLC methods.

The obtained total alkaloid fractions exhibited varying degrees of cytotoxic activity in vitro, depending on the plant species. Of particular interest is *V. herbacea*, the alkaloid complex of which demonstrated pronounced cytotoxic effects while showing no toxicity toward normal cells.

The results highlight the pharmacological potential of the studied species and indicate the prospects for further in-depth investigation of individual alkaloids, their mechanisms of action, and possible areas of application as prototypes of antitumor agents.

References:

1. A. Mondal, A. Gandhi, C. Fimognari, A.G. Atanasov, A. Bishayee. Alkaloids for cancer prevention and therapy: Current progress and future perspectives. Eur. J. Pharm., 2019, 858. Article 172472, 10.1016/j.ejphar.2019.172472
2. Hanahan, D. Hallmarks of cancer: new dimensions. 2022, 12(1), 31–46. DOI: 10.1158/2159-8290.CD-21-1059
3. Holohan, C., Van Schaeybroeck, S., Longley, D. B. & Johnston, P. G. Cancer drug resistance: an evolving paradigm. Nature Reviews Cancer 2013, 13, 714–726. DOI: 10.1038/nrc3599)
4. Longley, D. B. & Johnston, P. G. Molecular mechanisms of drug resistance. Journal of Pathology. 2005, 205, 275–292.
5. Cragg, G. M. & Newman, D. J. Natural products in cancer therapy. Journal of Natural Products. 2013, 76, 2024–2034.
6. Wink, M. Modes of action of herbal medicines and plant secondary metabolites. Medicines (Basel). 2015, 2(3), 251–286. DOI: 10.3390/medicines 2030251
7. Harvey, A. L., Edrada-Ebel, R. & Quinn, R. The re-emergence of natural products for drug discovery in the genomics era. Nature Reviews Drug Discovery 2015, 14, 111–129. DOI: 10.1038/nrd4510
8. Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P. & McPhail, A. T. Plant antitumor agents. Taxol. Journal of the American Chemical Society. 1971, 93(9), 2325–2327. DOI: 10.1021/ja00738a045
9. Wall, M. E., Wani, M. C., Cook, C. E., Palmer, K. H., McPhail, A. T. & Sim, G. A. Plant antitumor agents. Camptothecin. Journal of the American Chemical Society. 1966, 88(16), 3888–3890. DOI: 10.1021/ja00925a039
10. Nowicky, J. W. Ukrain — anticancer preparation from *Chelidonium majus* L. Drugs under Experimental and Clinical Research. 1992, 18(1), 1–11.
11. Cushnie, T. P. T., Cushnie, B. & Lamb, A. J.Alkaloids: general overview and relevance to cancer. Phytochemistry Reviews. 2020, 19, 815–842. DOI: 10.1007/s11101-019-09604-w
12. Newman, D. J. & Cragg, G. M. Natural products as sources of new drugs. Journal of Natural Products. 2020, 83, 770–803. DOI: 10.1021/acs.jnatprod.9b01285
13. Schmidt, T. J. Cytotoxic natural products. Natural Product Reports. 2017, 34,942–1017. DOI: 10.1039/c7np00031k
14. Chen S., Meng L., Fatma M., Demerdash E., Zhou L., Rizvi S., Cui L., Kang W. Review of Compounds and Pharmacological Effects of Delphinium. Journal of Chemistry. 2020, Article ID 9375619:23. doi.org/10.1155/2020/9375619].

15. Chen, Y., Li, S., Sun, F., Han, H., Zhang, X., Fan, Y., et al. In Vivo antimalarial Activities of Glycoalkaloids Isolated from Solanaceae Plants. Parm. Biol., 2010, 48(9), 1018-1024. doi: 10.3109/13880200903440211
16. Kang, N., et al. Sophoridine induces apoptosis. Oncology Reports. 2011, 25, 853–858. DOI: 10.3892/or.2010.1122
17. Farias A., Iturriaga P. A. systematic review on secondary metabolites of genus Sophora: chemical diversity. J Chil Chem Soc. 2022; 67(3), 5571-5581. doi:10.4067/S0717-97072022000305571
18. N. Gagua, A. Bakuridze, N. Vachnadze, D. Berashvili, V. Vachnadze. Extraction of pharmacologically active alkaloids from Vinca species. Georgian Medical News. 2011, 6 (195), 172-178.

საქართველოში გავრცელებული ზოგიერთი ალკალოიდშემცველი მცენარის ციტოტოქსიკური აქტივობის შესწავლა

ლალი კინწურაშვილი[1], თამარ სულაძე[1], ნინო ქაჩლიშვილი[1],ჟან ლეგო[2], ვახტანგ მშვილდაძე[1,2], ნინა ვაჩნაძე[1]

[1] *თსსუ იოველ ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი, 0159, თბილისი, საქართველო;*
[2] *კვებეკის უნივერსიტეტის ფუნდამენტურ მეცნიერებათა დეპარტამენტი, შიკუტიმი, კანადა*

აბსტრაქტი

კვლევის მიზანს წარმოადგენდა საქართველოში გავრცელებული მცენარეების *Delphinium elisabethae* N. Busch, *Solanum nigrum* L., *Sophora japonica* L. და *Vinca herbacea* Waldst. et Kit. მიწისზედა ნაწილების შესწავლა ალკალოიდების შემცველობისა და მათი ბიოლოგიური აქტივობის შეფასებისთვის.

სითხე-სითხოვანი ექსტრაქციის შედეგად მიღებულ ალკალოიდების ჯამებში ფიტოქიმიური კვლევებით იდენტიფიცირებულია დიტერპენული (*D. elisabethae*), სტეროიდული (*S. nigrum*), ქინოლიზიდინის (*S. japonica*) და ინდოლის ჯგუფის ფუძეები (*V. herbacea*).

In vitro ციტოტოქსიკური აქტივობა შეფასდა სამ უჯრედულ ხაზზე — A-549 (ადამიანის ფილტვის კარცინომა), DLD-1 (სწორი ნაწლავის ადენოკარცინომა) და WS-1 (ადამიანის ნორმალური ფიბრობლასტები) Resazurin-ისა და Hoechst-ის მეთოდების გამოყენებით.

V. herbacea ალკალოიდების ჯამი გამოირჩეოდა მკვეთრად გამოხატული ციტოტოქსიკური მოქმედებით A-549 და DLD-1 ხაზებზე, მაგრამ არ იყო ტოქსიკური WS-1 უჯრედების მიმართ. *S. nigrum* ჯამმა გამოამჟღავნა ზომიერი აქტივობა ყველა ხაზის მიმართ, ხოლო *D. elisabethae* და *S. japonica* ალკალოიდების ჯამებმა წარმოადგინეს სუსტი ციტოტოქსიკური ეფექტი.