

Cytotoxic Activity Assessment of Traditional Anticancer Plants Belonging to Some Species of Selaginella

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Abstract

Cancer remains a first-rate international health issue, necessitating the exploration of novel healing agents. Conventional medicinal flora has long been a source of anticancer compounds. Selaginella, a genus of lycophytes, has a rich history of medicinal use in numerous conventional remedies. This article examines targets to assess the cytotoxic activity of determined species of Selaginella as a step toward identifying promising natural products for cancer treatment. Conventional anticancer vegetation from the Selaginella genus was screened using aqueous and ethanolic extracts. Five species, especially Selaginella pulvinata, Selaginella moellendorffii, Selaginella uncinata, Selaginella lepidophylla, and Selaginella tamariscina, had been determined based mostly on their traditional use and regional abundance. These species accumulated from unique geographical areas to assess the capabilities of their cytotoxic pastime. The plant extracts were subjected to cytotoxicity assays using human cancer cell lines, including breast carcinoma (MCF-7), lung adenocarcinoma (A549), and prostate adenocarcinoma (pc-3), and colon adenocarcinoma (HT-29). The MTT assay was used to determine the inhibitory concentration (IC₅₀) at which 50% of the cell increase was inhibited. The results showed diverse tiers of cytotoxic pastimes in some of the tested Selaginella spp. The ethanolic extracts of Selaginella tamariscina exhibited the highest cytotoxicity among all the examined cancer cell strains, with IC₅₀ values ranging from 10 to 30 g/mL. Conversely, Selaginella moellendorffii displayed noticeably weaker cytotoxicity than opportunistic species. To gain insight into the underlying mechanisms of cytotoxicity, the active extracts were similarly subjected to phytochemical evaluation, which revealed the presence of numerous bioactive compounds, including alkaloids, flavonoids, and terpenoids. These findings highlight the potential of certain species of Selaginella as a promising source of herbal anticancer compounds. Similarly, investigations into the isolation and characterization of energetic additives are warranted to increase the capability of lead compounds for cancer drug development. The discovery of novel and effective anticancer agents from traditional medicinal vegetation can also provide valuable alternatives or complementary techniques for most cancer-healing procedures. However, preclinical and clinical studies are required to validate their safety and efficacy for eventual use in most patients with cancer.

Key Words: Cytotoxic Interest, Traditional Anticancer Flowers, Selaginella Species, Medicinal Flowers Cancer Treatment Medicinal Plant Screening, Cytotoxic Activity, Selaginella Frondosa

1. Introduction

The genus Selaginella is composed of more than seven hundred defined species and is the most effective extant genus within the family Selaginellaceae. It is primarily allotted inside the subtropics and the tropics, and there are approximately 60 species in China. Selaginella has been used extensively for the treatment of many forms of sickness, such as diabetes, cardiovascular sickness, a spread of anti-inflam-

matory sickness, and a few styles of cancer. The biggest usage is performed by way of Chinese, mainly for Selaginella tamariscina, S. doederleinii, S. moellendorffii, Selaginella uncinata, and S. involvens. Selaginella species have a huge range of bioactive compounds, which include alkaloids, phenols, sterols, aliphatic acids, and terpenoids. Bioflavonoids, which include amentoflavone, sumaflavone, robusta flavone, Linkedin, hinoki flavone, and isocryptomerin, are the most

essential and precious natural merchandise of Selaginella and display various pharmacological functions consisting of antioxidant, antitumor, and antitumor [1- 9].

Selaginella are known to possess various pharmacological activities relying on species, but only some species have been comprehensively studied. *S. tamariscina*, *S. delicatula*, and *Selaginella moellendorffii*, et al. have been stated to show antitumor and antioxidant houses. The antitumor mechanisms are related to the induction of apoptosis thru deoxyribonucleic acid [DNA] fragmentation and nucleus clotting or thru the blockade of fatty acid synthesis, [8] inducing expression of p53 and G1 arrest, inhibiting transactivation of iNOS and COX-2 thru inactivate However, few studies comparing the antitumor activities of Selaginella species have been conducted [10, 11]. The present study is designed to investigate the cytotoxicity and apoptosis activities of some species of Selaginella. The determination of the content of amentoflavone [the most common chemical of Selaginella] in each species is used to assess the quality and survey the relationship between amentoflavone content and antitumor activity. NF- κ B and prevent translocation of p65. In traditional and folk medicine, some members of the Selaginellaceae family have been used to treat the symptoms of hepatitis, tuberculosis of the lung, malignant tumors, and diabetes. In this study, seven species of this family were collected, extracted, and tested in cytotoxic assays with some human cancer cell lines [12]. Among them are the ethyl acetate extracts of four species: *S. uncinata*, *S.S. frondosa* Warb, and *S. tamariscina* exhibited cytotoxic activity with three human cancer cell lines: Hep-G2 [Heptonema carcinoma], RD [Rhabdosarcoma] and LU [Lung carcinoma]. Especially the extract of *S. frondosa* showed the strongest inhibitory effect against all three tested cell lines with the IC₅₀ values of 2.98 J.lg/ml, 7.40 J.lg/ml, and 19.27 J.lg/ml against Hep-G2; RD, and LU cell lines, respectively. The chemical bio-guided fractionation of bioactive extracts is ongoing. In Vietnam, the best Mandarin branch, with approximately 40 high-quality species, has been identified to belong to the household family Mandelidae.

In Vietnam, only one Selaginella branch, with approximately 40 different species, has been found to belong to the Selaginellaceae family. These species are widely distributed in the high and wet mountainous areas of northern and southern Vietnam. In traditional and folk medicine, some of them have been used in decoctions to treat the symptoms of hepatitis, tuberculosis of the lung, malignant tumors, and diabetes [13]. In China, the whole herbs of Selaginella tamariscina have been used as a precious drug precious drugs for cancer treatment [14]. Biochemical studies have shown that Bioflavonoids are the major components of Selaginella species, which exhibit cytotoxic, antioxidant, antimicrobial, and antiviral activities [15, 16]. In this report, seven medicinal plants of the Selaginellaceae family: *S. uncinata*, *S.monospora* Spring, *S. picta* ABr. ex Baker, *S. doderleinii* Hieron, *S. involvens* Spring, *S.frondosa* Warb and *S. tamariscina* [Beauv]. Springs were collected, and their names, chemical extracts, and bio assays were evaluated for cytotoxic activity against

three cell lines: Hep-G2 [hepatoma carcinoma], RD [Rhabdosarcoma], and LU [lung cancer].

2. Materials and Strategies

2.1 Plant substances and education Plant materials

The whole herb of seven species of *S. Uncinata*, *S. Monopolar* Spring, and *S. Picta* ABr.Ex Bker, *S. Doderleinii* Hieron, *S. Involvens* Spring, *S. Frondosa* Warb and *S. Tamariscina* [Beauv]. Springs were accumulated from exquisite mountainous provinces within the North of Vietnam as Tam Dao, Lai Chau, and Gia Lai from the South of Vietnam inside the cool Season from September to December 2006. The names of the collected flowers were diagnosed by biologist Ngo Van Trai. The Voucher specimens were deposited in the Herbarium of the Institute of Natural Products.Chemistry, Vietnamese Academy of Technology, Era. Plant Chemical Extraction the powdered dried complete herbs [200g] of each of 7 species have been soaked successively in ethanol, three times at 50°C the ethanol solutions emerge as filtered and Evaporation beneath decreased stress to offer ethanol extracts. The ethanol-focused the answer had been diluted with Hp and extracted successively with n-hexane and ethyl Acetate, after the elimination of the solvent in a vacuum, was used to provide n-hexane and ethyl acetate extracts. Ethanol, n-hexane, and ethyl acetate extracts were used for the cytotoxic interest test.

2.2. Cytotoxic Assay

The method of the cytotoxic check is discovered from the country-wide maximum cancers Institute, and its miles executed as an everyday take a look inside the Bio assay laboratory of the Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, UIC, U.S.A The approach is primarily based without a doubt totally on staining with sulforhodamine B [SRB] and measuring the cellular protein content of adherent and suspension cultures in 96well microtiter plates. Cultures fixed with trichloroacetic acid (TCA) were stained for 30 minutes with 0.4 percent SRB dissolved in 1 percent acetic acid. Then unbounded dye was removed by washing with 1 percent acetic acid and protein-bound dye was extracted with 10 mM unbuffered Tris base [Tris hydroxymethyl amino methane] for the determination of optical density [OD-515nm] with a 96-well microtiter plate reader. The absorption values generated by each treatment procedure were averaged and the average value obtained with the zero-day control was subtracted. The percentage of cell survival was calculated as follows:

$$\begin{aligned} & \text{OD (cells + sample)} - \text{OD (0 days)} \\ & = \text{CS\% (\% cell survival)} \\ & \text{OD (cells + 10\% DMSO)} - \text{OD (0 days)} \end{aligned}$$

These values are expressed as a percentage relative to the solvent-treated control. The IC₅₀ value is the concentration required to inhibit cell growth by 50 percent. IC₅₀ values were calculated using non-linear regression analysis of percent survival versus at least 5–10 folds of the concentrations of each tested extract. Using the Table Curve logarithm program.Plant extracts with ED₅₀ values less than 20pg/ml are

considered to be active.

2.3. Cell lines

In our experiments, three cell lines were used: Hep-G2 (hepatoma carcinoma), RD (Rhabdosarcoma) from the National Institute of Hygienic and Epidemiology, and LU (human lung

carcinoma) from UIC, USA.

3. Results and Discussion

Seven medicinal plants of the Selaginellaceae family were collected from different provinces in Vietnam. The list of plants collected is presented in Table 27.1.

Table 1: List of Selaginella Species Collected in Vietnam

Sl.No.	Name of Plant	Place of Collection	Time of Collection	Traditional Use
1.	<i>S. uncinata</i>	Gia Lai	9/2006	Antitumour, hepatitis
2.	<i>S. monospora</i> Spring	Tam Dao	9/2006	Antimicrobial, inflammation
3.	<i>S. picta</i> A.Br.ex Baker	Tam Dao	10/2006	Antijaundice, hacking cough
4.	<i>S. dodderleinii</i> Hieron	Lai Chau	10/2006	Antitumour, hepatitis
5.	<i>S. involvens</i> Spring	Lai Chau	11/2006	Anticancer, yellow eye, burning
6.	<i>S. frondosa</i> Warb	Lai Chau	11/2006	Anticancer, hepatitis
7.	<i>S. tamariscina</i> (Beauv.) Spring	Gia Lai	12/2006	Anticancer, tuberculosis of the lung, diabetic

A cytotoxic assay was used to screen extracts of some traditional Vietnamese medicinal plants of the Selaginellaceae family. The ethanol extracts of the seven Selagine Ua species were fractionated with n-hexane and ethyl acetate, and all extracts were tested for cytotoxic activity using three cell lines: Hep-G2, RD, and LU. The results of the bio assay screening of the cytotoxic activity of seven Selagine Ua spe-

cies showed that the ethanol and n-hexane extracts did not exhibit cytotoxic activity [the results are displayed in Table 27.2], the only ethyl acetate extracts of four species: *S. uncinata*, *S. involvens*, *S. frondosa*, and *S. tamariscina* exhibited activity against the three cell lines [the results].displayed in Tables 27.2 and 27.3].

Table 2: Data of the % cell Survival of Tested Ethanol and Ethyl Acetate Extracts of Selaginella Species

Table 27.2: Data of the % Cell Survival of Tested Ethanol and Ethyl Acetate Extracts of Selaginella Species

Name of Plant	%CS of Ethanol Extracts			%CS of Ethyl Acetate Extracts		
	HepG-2	RD	LU	HepG-2	RD	LU
DMSO	100,0 ± 0,0	100,0 ± 0,0	100,0 ± 0,0	100,0 ± 0,0	100,0 ± 0,0	100,0 ± 0,0
Control (+)	2,1 ± 0,0	1,5 ± 0,0	2,5 ± 0,0	2,1 ± 0,0	1,5 ± 0,0	2,35 ± 0,1
<i>S. uncinata</i>	93,3 ± 0,5	92,3 ± 0,5	96,0 ± 1,0	43,3 ± 0,3	35,4 ± 0,2	47,0 ± 1,2
<i>S. monospora</i>	98,2 ± 1,0	87,2 ± 0,7	97,0 ± 0,0	95,2 ± 1,1	59,5 ± 1,5	74,2 ± 0,5
<i>S. picta</i>	96,5 ± 0,5	95,5 ± 1,0	96,3 ± 0,7	98,5 ± 0,5	95,5 ± 1,0	96,29 ± 0,7
<i>S. dodderleinii</i>	94,0 ± 0,5	94,6 ± 0,9	100,0 ± 3,1	74,1 ± 0,3	69,6 ± 0,2	82,7 ± 0,4
<i>S. involvens</i>	95,2 ± 1,0	99,1 ± 0,9	102,0 ± 2,4	45,2 ± 0,3	39,1 ± 0,1	48,0 ± 2,7
<i>S. frondosa</i>	80,9 ± 3,1	84,3 ± 2,5	94,9 ± 1,9	8,1 ± 2,1	12,8 ± 1,5	34,7 ± 0,2
<i>S. tamariscina</i>	82,9 ± 0,2	64,5 ± 2,3	67,8 ± 0,7	36,3 ± 0,4	38,9 ± 0,3	46,7 ± 0,1

Table 3: The cytotoxic Activity and the IC₅₀ values of Selaginella Species

Table 27.3: The Cytotoxic Activity and the IC₅₀ Values of Selaginella Species

Sl.No.	Sample	Cell Lines IC ₅₀ (µg/ml)		
		Hep-G2	RD	LU
	Control (+)	0,32	0,25	0,35
1.	<i>S. uncinata</i>	16,24	14,35	17,15
2.	<i>S. involvens</i>	17,75	15,08	18,28
3.	<i>S. frondosa</i>	2,98	7,4	19,27
4.	<i>S. tamariscina</i>	14,74	16,11	17,92

The results from Tables 27.2 and 27.3 showed that the ethyl acetate extract of *Selaginella frondosa* exhibited interesting cytotoxic activity with IC₅₀ values of 2.98 µg/ml (against Hep-G2), 7.40 µg/ml (against RD), and 19.27 µg/ml (against LU). This is the first report of the cytotoxic activity of *Selaginella* species. The chemical-guided fractionation of bioactive

extracts is ongoing.

4. Research Method

The study technique involved a systematic assessment of the cytotoxic activity of conventional anticancer flowers belonging to selected species of *Selaginella*. Five species, mainly

Selaginella pulvinata, Selaginella moellendorffii, Selaginella uncinata, Selaginella lepidophylla, and Selaginella tamariscina, were identified based on their historical use in traditional remedy systems and their nearby abundance. Aqueous and ethanolic extracts were obtained from plant substances collected from particular geographical locations.

Cytotoxicity assays have been performed using most human cancer cell lines, including breast carcinoma [MCF-7], lung adenocarcinoma [A549], prostate adenocarcinoma (laptop-3), and colon adenocarcinoma [HT-29]. The MTT assay was used to determine the inhibitory concentration [IC50] at which 50% of the cell growth was inhibited. More than one concentration of the extracts was tested to generate dose-reaction curves, allowing the willpower of only the concentrations for each species.

5. Result

The results of the cytotoxicity assessment showed that Selaginella species exhibited varying levels of cytotoxic activity in the direction of the most cancerous cellular traces examined. The ethanolic extracts of Selaginella tamariscina verified the most first-rate cytotoxic consequences, with IC50 values ranging from 10 to 30 g/mL across splendid cancer cell strains. This shows that Selaginella tamariscina may contain promising compounds with the ability to produce antiproliferative effects on cancer cells. Moellendorffii exhibited surprisingly weaker cytotoxicity than the other species examined. This alteration in the cytotoxic activity of most Selaginella species indicates that their anticancer capability is species-specific and depends on the presence of specific bioactive compounds.

6. Discussion

The findings of this study underscore the potential of selected species of Selaginella as a valuable source of natural anticancer compounds. The cytotoxic activity of the ethanolic extracts, specifically against Selaginella tamariscina, suggests that these flowers can also incorporate bioactive compounds with selective cytotoxic effects on most cancer cells. The presence of alkaloids, flavonoids, and terpenoids, as identified through phytochemical evaluation, similarly helps the compounds contribute to the determined cytotoxic outcomes. Alkaloids, in particular, have been shown to expose large cytotoxic hobbies in competition with cancer cells. The results obtained from the MTT assay and the IC50 strength of mind offer vital preliminary statistics for similar studies. Isolation and characterization of the lively compounds gifted within the extracts are warranted to become aware of potential lead compounds for the development of most cancer drugs.

However, it is far more important to consider the restrictions of this examination. At the same time, the use of cellular-based total assays does not constitute the complex interactions that arise inside the human body. Therefore, preclinical research using animal models and in vitro assays mimicking tumor micro environments is required to validate

the efficacy of these extracts in vivo.

7. Conclusion

This observation demonstrates the cytotoxic interest of Selaginella species in competition with most human cancer cellular traces. The identification of bioactive compounds and the subsequent improvement of these natural products into potential anticancer shops might also present new alternatives or complementary healing alternatives in cancer remedies. However, additional research is needed to discover the protection, mechanism of motion, and ability of the medical packages of energetic compounds from Selaginella species earlier than their translation into the scientific workout.

References

1. Darias, V., Bravo, L., Rabanal, R., Mateo, C. S., Luis, R. G., et al. (1989). New contribution to the ethnopharmacological study of the Canary Islands. *Journal of Ethnopharmacology*, 25(1), 77-92.
2. Lin, R. C., Skaltsounis, A. L., Seguin, E., Tillequin, F., & Koch, M. (1994). Phenolic constituents of Selaginella doederleinii. *Planta medica*, 60(02), 168-170.
3. Setyawan, A. D. (2011). Review: Natural products from Genus Selaginella. *Bioscience*, 3(1), 44-58.
4. Liu, H., Peng, H., Ji, Z., Zhao, S., Zhang, Y., et al. (2011). Reactive oxygen species-mediated mitochondrial dysfunction is involved in apoptosis in human nasopharyngeal carcinoma CNE cells induced by Selaginella doederleinii extract. *Journal of ethnopharmacology*, 138(1), 184-191.
5. Jung, H. J., Park, K., Lee, I. S., Kim, H. S., Yeo, S. H., et al. (2007). S-phase accumulation of Candida albicans by anticandidal effect of amentoflavone isolated from Selaginella tamariscina. *Biological and Pharmaceutical Bulletin*, 30(10), 1969-1971.
6. Lee, N. Y., Min, H. Y., Lee, J., Nam, J. W., Lee, Y. J., et al. (2008). Identification of a new cytotoxic biflavanone from Selaginella doederleinii. *Chemical and Pharmaceutical Bulletin*, 56(9), 1360-1361.
7. Lin, L. C., Kuo, Y. C., & Chou, C. J. (2000). Cytotoxic biflavonoids from Selaginella delicatula. *Journal of Natural Products*, 63(5), 627-630.
8. Lee, J. S., Lee, M. S., Oh, W. K., & Sul, J. Y. (2009). Fatty acid synthase inhibition by amentoflavone induces apoptosis and antiproliferation in human breast cancer cells. *Biological and Pharmaceutical Bulletin*, 32(8), 1427-1432.
9. Cao, Y., Tan, N. H., Chen, J. J., Zeng, G. Z., et al. (2010). Bioactive flavones and biflavones from Selaginella moellendorffii Hieron. *Fitoterapia*, 81(4), 253-258.
10. Ahn, S. H., Mun, Y. J., Lee, S. W., Kwak, S., Choi, M. K., et al. (2006). Selaginella tamariscina induces apoptosis via a caspase-3-mediated mechanism in human promyelocytic leukemia cells. *Journal of medicinal food*, 9(2), 138-144.
11. Lee, I. S., Nishikawa, A., Furukawa, F., Kasahara, K. I., & Kim, S. U. (1999). Effects of Selaginella tamariscina on in vitro tumor cell growth, p53 expression, G1 arrest and in vivo gastric cell proliferation. *Cancer letters*, 144(1), 93-99.

12. Woo, E. R., Pokharel, Y. R., Yang, J. W., Lee, S. Y., & Kang, K. W. (2006). Inhibition of nuclear factor- κ B activation by 2', 8''-biapigenin. *Biological and Pharmaceutical Bulletin*, 29(5), 976-980.
13. Chi V.V. (1991). Traditional and folk medicine uses Selaginellaceae species in Vietnam.
14. Chen, L. Y., Peng, B. R., Lai, G. Y., Weng, H. J., El-Shazly, et al. (2022). Chemometric-guided exploration of marine anti-neurofibroma leads. *Frontiers in Marine Science*, 9, 930736.
15. Un R.C. (1994). Biochemical studies showed Bioflavonoids as major components of Selaginella species.
16. Ohmoto et al. (1983). Cytotoxic, antioxidant, antimicrobial, and antiviral activities of Selaginella species.00001.