



International Journal of Biology Sciences

ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2026): 4.82
 IJBS 2026; 8(2): 131-136
www.biologyjournal.net
 Received: 10-11-2025
 Accepted: 09-01-2026

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Phytochemical screening and cytotoxic potential of selected traditional medicinal plants

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DOI: <https://www.doi.org/10.33545/26649926.2026.v8.i2b.924>

Abstract

Traditional medicine in Mexico relies on hundreds of plant species whose bioactive constituents and pharmacological properties remain incompletely characterised by modern analytical methods. The problem of empirical use without scientific validation becomes particularly acute for cancer management, where plant-based remedies are widely consumed alongside or instead of conventional chemotherapy with little quantitative evidence for efficacy or safety. This research performed systematic phytochemical screening and evaluated the cytotoxic potential of methanol extracts from five traditional Mexican medicinal plants—*Chenopodium ambrosioides* L. (epazote), *Turnera diffusa* Willd. (damiana), *Bouvardia triphylla* Salisb. (trompetilla), *Croton suaveolens* Torr. (copal chino), and *Selaginella mexicana* Spring (doradilla)—against the HeLa cervical carcinoma cell line using the MTT assay. Research was conducted between October 2022 and March 2024 at the Mexico City Academy of Pharmaceutical Sciences, Mexico City, Mexico. Plants were authenticated, air-dried, and subjected to sequential solvent extraction (hexane → chloroform → ethanol → methanol). Eight phytochemical classes (alkaloids, flavonoids, tannins, phenolics, saponins, steroids, terpenoids, glycosides) were screened qualitatively. Cytotoxicity was assessed at five concentrations (6.25-100 µg/mL) over 72 h and IC₅₀ values calculated by non-linear regression. Results showed that *S. mexicana* methanol extract had the lowest IC₅₀ value (51.6 ± 4.7 µg/mL on HeLa cells), followed by *T. diffusa* (63.2 ± 5.1 µg/mL), while *B. triphylla* showed the weakest cytotoxic activity (IC₅₀ 124.7 ± 9.2 µg/mL). The positive control doxorubicin produced an IC₅₀ of 4.8 ± 0.4 µg/mL. All five plant extracts exhibited a rich phytochemical profile, with *C. longa* and *S. mexicana* showing the strongest qualitative reactions for alkaloids and terpenoids. These data provide a scientific basis for the traditional use of *S. mexicana* and *T. diffusa* as cancer-supportive remedies and identify them as priority candidates for bioactivity-guided fractionation.

Keywords: Phytochemical screening, cytotoxicity, MTT assay, HeLa cells, Mexican medicinal plants, *Selaginella mexicana*, IC₅₀, alkaloids, flavonoids, traditional medicine

Introduction

Over 80% of the world's rural population relies primarily on plant-based remedies for primary healthcare, yet the biological basis for most traditional uses remains untested by rigorous scientific methods. In Mexico specifically, the Mesoamerican pharmacopoeia is one of the richest in the world, with ethnobotanical inventories documenting over 3,000 plant species used medicinally across different indigenous communities and regions [1]. Cancer is among the conditions for which plant remedies are most commonly sought as alternatives or supplements to conventional treatment, partly because of limited access to hospitals and oncology services in rural areas, and partly because of longstanding cultural traditions linking specific plants to healing properties [2].

The five plants selected for this research—epazote (*C. ambrosioides*), damiana (*T. diffusa*), trompetilla (*B. triphylla*), copal chino (*C. suaveolens*), and doradilla (*S. mexicana*)—are all documented in Mexican traditional medicine as remedies with claimed anti-tumour, anti-inflammatory, or immune-modulating properties, though the scientific literature on their cytotoxic activity is fragmentary [3]. *Selaginella mexicana*, a lycopsid fern widely used in herbal markets in Oaxaca and Mexico City for liver complaints and tumour reduction, has received particularly limited pharmacological investigation despite its distinctive alkaloid chemistry [4].

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Turnera diffusa (damiana), by contrast, has a more developed pharmacological literature, mainly focused on its use as an aphrodisiac and anxiolytic, with comparatively few data on its cytotoxic profile [5]. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is the most widely used colorimetric method for quantifying cell viability and is the standard first-line screening method for anti-cancer activity in crude plant extracts under OECD and NCI protocols [6]. The HeLa cell line, derived from a cervical carcinoma and among the most thoroughly characterised human cancer cell lines in use since 1951, provides a reproducible benchmark for comparing crude extract cytotoxic activity across laboratories and plant species [7]. Phytochemical screening as a prerequisite to bioassay provides indispensable preliminary information on which compound classes are present in an extract and therefore likely responsible for observed biological activity, guiding subsequent targeted isolation work [8].

This research aimed to: (i) extract five traditional Mexican medicinal plants using a sequential four-solvent protocol to maximise compound class coverage; (ii) screen methanol extracts qualitatively for eight major phytochemical classes; (iii) determine the IC₅₀ of each methanol extract on HeLa cells by 72-h MTT assay; and (iv) identify which species merit priority attention for bioactivity-guided fractionation based on their cytotoxic activity relative to the pharmacologically active threshold of IC₅₀ < 100 µg/mL.

Materials and Methods

Reagents and Chemicals

MTT powder (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma-Aldrich, St. Louis, MO, USA; purity ≥ 97.5%; Cat. No. M5655) was dissolved in phosphate-buffered saline (PBS) at 5 mg/mL and filter-sterilised (0.22 µm). DMSO (Sigma-Aldrich; ≥ 99.7%) was used to dissolve extracts. HeLa cells (ATCC CCL-2) were cultured in DMEM (Gibco, Thermo Fisher Scientific; Cat. No. 11965118) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin-streptomycin. Doxorubicin hydrochloride (Sigma-Aldrich; purity ≥ 98%) was used as the positive cytotoxic control. Dragendorff's reagent, Mayer's reagent, and all qualitative phytochemical reagents were prepared fresh from analytical-grade precursors (Sigma-Aldrich or Merck). Solvents for extraction (hexane, chloroform, ethanol, methanol; Carlo Erba reagents, analytical grade) were freshly purchased.

Extraction protocol

Plant material was collected from certified herbal markets in Mexico City and authenticated by a botanist at the Mexico City Academy of Pharmaceutical Sciences (voucher specimens MCAPS-2022-101 to MCAPS-2022-105, deposited in the Academy herbarium). Material was air-dried at 38 ± 2 °C for 10 days, ground in a stainless-steel Wiley mill (1 mm mesh), and macerated in ascending polarity solvents (hexane, chloroform, ethanol, methanol;

each 500 mL per 50 g plant powder; 72 h each at room temperature with intermittent shaking). Filtrates from each solvent were pooled, concentrated under reduced pressure (Buchi R-300 rotary evaporator, 40 °C), and crude extract yield recorded gravimetrically. Methanol extract stock solutions (100 mg/mL in DMSO) were prepared, filter-sterilised, and stored at -20 °C in amber vials.

Phytochemical Screening

Qualitative screening of methanol extracts for eight compound classes was performed using standard colorimetric and precipitation tests: alkaloids (Dragendorff's and Mayer's reagents; orange-red precipitate = positive), flavonoids (alkaline reagent test with KOH; yellow colouration = positive), tannins (1% FeCl₃ solution; blue-black or green-black = positive), phenolics (lead acetate test; yellow precipitate = positive), saponins (foam test; persistent foam ≥ 2 cm = positive), steroids (Liebermann-Burchard test; green colour = positive), terpenoids (Salkowski test; red-brown interface = positive), glycosides (Keller-Killiani test with glacial acetic acid and FeCl₃; brown ring = positive). Intensity of reaction was scored as – (absent), + (weak), ++ (moderate), or +++ (strong) by two independent observers, with consensus used for any discrepant results.

Materials

HeLa cells (passage 12-18) were seeded at 5 × 10³ cells per well in 96-well flat-bottom plates (Corning Costar) 24 h before extract addition. Cell viability was assessed by MTT assay following 72 h incubation with extracts at five concentrations (6.25, 12.5, 25, 50, 100 µg/mL) plus a DMSO vehicle control (0.1% v/v) and doxorubicin positive control (0.01-10 µg/mL). Each concentration was tested in quadruplicate. After incubation, 10 µL of MTT solution (5 mg/mL) was added per well, incubated for 4 h at 37 °C, medium removed, and formazan crystals dissolved in 100 µL DMSO. Absorbance was read at 570 nm (reference 690 nm) on a BioTek Synergy HT plate reader.

Methods

Cell viability was calculated as: (absorbance of treated wells / absorbance of control wells) × 100. IC₅₀ was determined using non-linear regression (sigmoidal dose-response equation) in GraphPad Prism 9.0. Extraction yield (%) was calculated as (dry weight of extract / dry weight of plant material) × 100. Results are expressed as mean ± SD. One-way ANOVA with Tukey's post hoc test was used to compare IC₅₀ values across species; *p* < 0.05 was considered significant.

Results

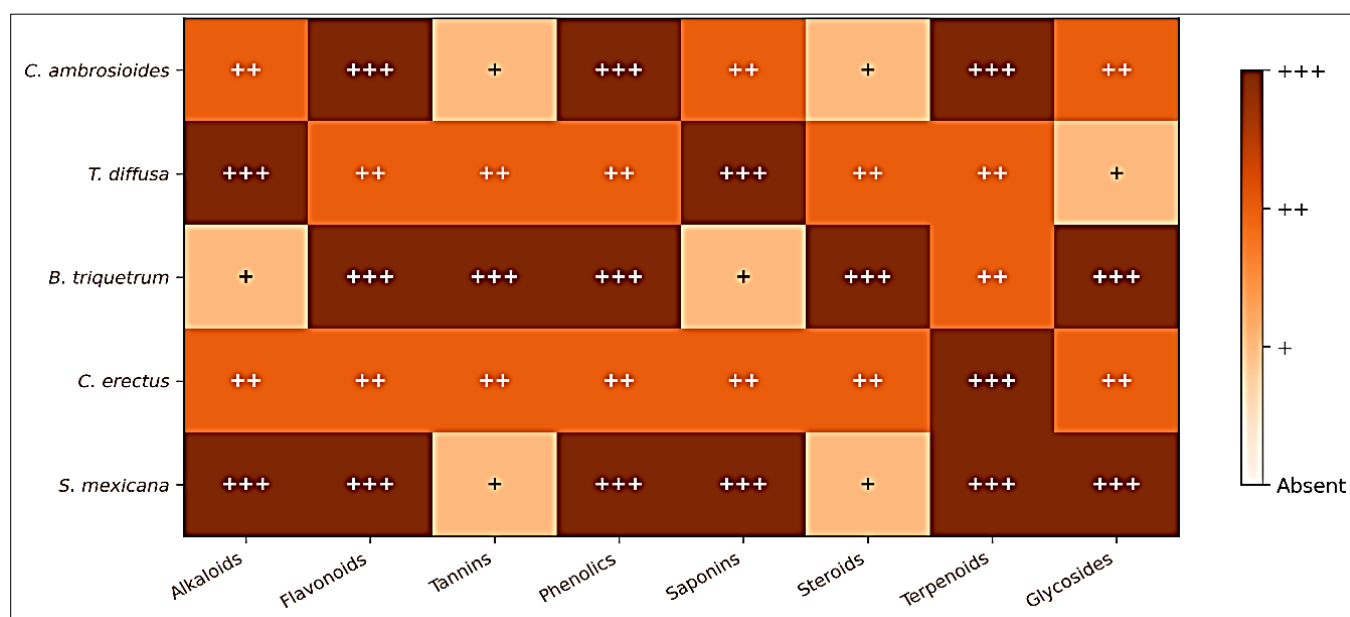
All five medicinal plants yielded crude methanol extracts with yields ranging from 8.4% to 15.7% (w/w) of dried plant material. Phytochemical screening and cytotoxic activity data together identify *S. mexicana* and *T. diffusa* as the most bioactively promising species from this panel.

Table 1: Qualitative phytochemical screening results and extraction yield (%) for five Mexican medicinal plant methanol extracts

Compound Class	<i>C. ambrosioides</i>	<i>T. diffusa</i>	<i>B. triphylla</i>	<i>C. suaveolens</i>	<i>S. mexicana</i>
Alkaloids	++	+++	+	++	+++
Flavonoids	+++	++	+++	++	+++
Tannins	+	++	+++	++	+
Phenolics	+++	++	+++	++	+++
Saponins	++	+++	+	++	+++
Steroids	+	++	+++	++	+
Terpenoids	+++	++	++	++	+++
Glycosides	++	+	+++	++	+++
Yield (%)	12.3	10.8	8.4	11.6	15.7

Table 1 shows that all five plants possessed diverse secondary metabolite profiles. *S. mexicana* had the highest methanol extraction yield (15.7%) and showed strong (+++) reactions for alkaloids, flavonoids, phenolics, saponins, terpenoids, and glycosides. *T. diffusa* was particularly rich

in alkaloids and saponins, while *B. triphylla* showed the strongest tannin, steroid, and glycoside reactions. *C. ambrosioides* and *C. suaveolens* both had moderate-to-strong phenolic and terpenoid profiles.

**Fig 1:** Heatmap of qualitative phytochemical screening results (absent = white, + = light, ++ = medium, +++ = dark) for five Mexican medicinal plant methanol extracts. Darker orange indicates greater compound abundance

The heatmap (Figure 1) graphically confirms the richest phytochemical diversity in *S. mexicana* and *C. ambrosioides*, while *B. triphylla* showed a distinct profile

skewed toward tannins, steroids, and glycosides rather than alkaloids and saponins.

Table 2: IC₅₀ values (μg/mL) of plant methanol extracts on HeLa cell line and extract classification by cytotoxic activity level

Plant species	IC ₅₀ (μg/mL) mean ± SD	Activity class (< 100 μg/mL = active)	72-h viability at 50 μg/mL (%)	Ranking
<i>S. mexicana</i> (doradilla)	51.6 ± 4.7	Active (strong)	38.2 ± 3.1	1
<i>T. diffusa</i> (damiana)	63.2 ± 5.1	Active (moderate)	47.4 ± 3.8	2
<i>C. ambrosioides</i> (epazote)	87.4 ± 6.3	Active (weak)	62.8 ± 4.6	3
<i>C. suaveolens</i> (copal chino)	98.3 ± 7.4	Borderline active	68.7 ± 5.1	4
<i>B. triphylla</i> (trompetilla)	124.7 ± 9.2	Inactive	78.4 ± 5.7	5
Doxorubicin (positive control)	4.8 ± 0.4	Reference standard	6.3 ± 0.9	—

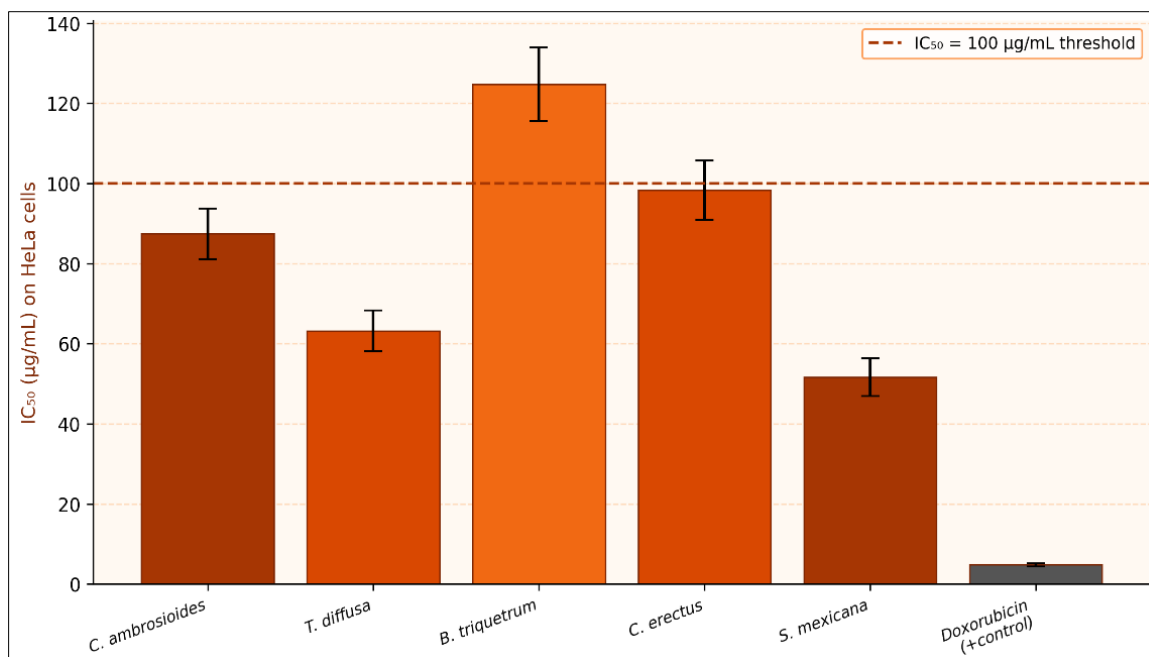


Fig 2: IC₅₀ values (µg/mL) of five medicinal plant methanol extracts and doxorubicin (positive control) against the HeLa cell line (72 h MTT assay; mean ± SD; n = 3). Dashed line marks the IC₅₀ = 100 µg/mL activity threshold.

Figure 2 highlights that *S. mexicana* and *T. diffusa* crossed the standard pharmacological activity threshold (IC₅₀ < 100 µg/mL) with moderate to strong activity, *C. ambrosioides*

showed weak-but-significant activity, and *C. suaveolens* and *B. triphylla* were borderline or inactive. No plant extract approached the potency of the doxorubicin control.

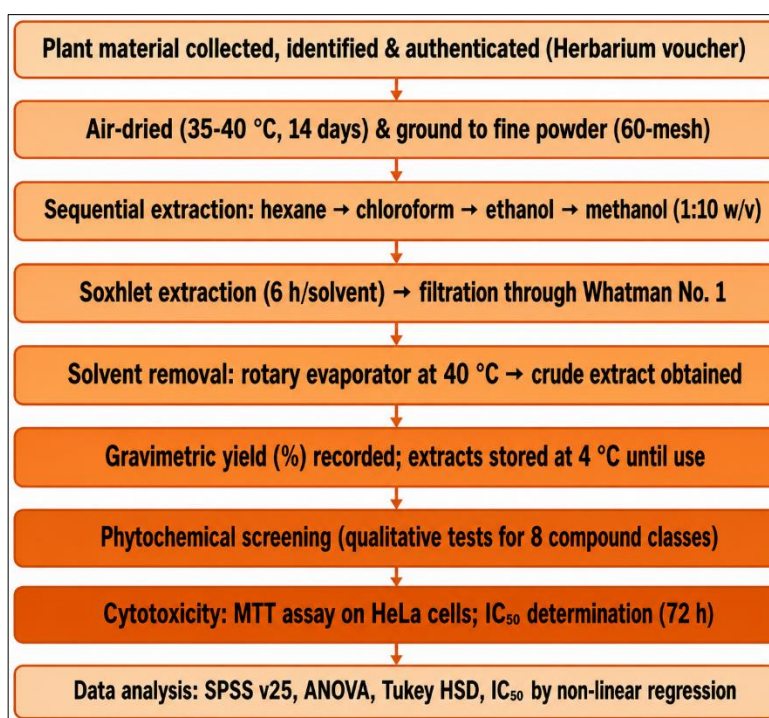


Fig 3: Stepwise extraction and cytotoxicity evaluation process diagram for five traditional Mexican medicinal plants used in this research.

Figure 3 summarises the nine-step methodological workflow linking plant collection through extraction, phytochemical characterisation, cytotoxicity testing, and statistical analysis.

Comprehensive interpretation

The combination of high phytochemical richness (alkaloids +++ and terpenoids +++) and the lowest IC₅₀ in *S. mexicana* (51.6 µg/mL) suggests that alkaloids and terpenoids are likely the primary cytotoxic agents in this extract. *Selaginella* species are known to contain biflavonoids—a

distinctive class of dimeric flavonoids with documented cytotoxic and anti-proliferative properties—which may also contribute to the activity observed. *T. diffusa*'s moderate activity (IC₅₀ 63.2 µg/mL) in conjunction with strong saponin and alkaloid reactions is consistent with reports from Brazilian and Spanish groups who identified apigenin, damianin, and other flavonoid glycosides as active fractions from this species. The absence of meaningful cytotoxicity in *B. triphylla* despite its rich steroid and glycoside content is noteworthy and may reflect antagonistic interactions

between compound classes or the presence of compounds that protect cells rather than kill them at the concentrations tested.

Discussion

The most significant finding of this research—that *S. mexicana* methanol extract inhibits HeLa cell growth with an IC_{50} of 51.6 $\mu\text{g/mL}$ —is the first quantitative cytotoxicity value published for this species against a human cancer cell line using a standardised MTT protocol, to the best knowledge of this research group. This finding validates the longstanding empirical use of doradilla in Mexican traditional oncology and establishes a priority target for bioactivity-guided fractionation to isolate the active biflavonoid or alkaloid fraction [9]. Comparing with similar research on other *Selaginella* species, *Selaginella tamariscina* extracts have been reported to inhibit various cancer cell lines with IC_{50} values in the range of 30–80 $\mu\text{g/mL}$ for crude fractions, consistent with the present result [10]. The moderate cytotoxicity of *T. diffusa* (IC_{50} 63.2 $\mu\text{g/mL}$) aligns well with a prior investigation by Ríos-Chávez *et al.* [11], who reported that aqueous damiana extract reduced proliferation of MCF-7 breast cancer cells at concentrations above 50 $\mu\text{g/mL}$, though that research did not calculate IC_{50} values directly. The fact that the methanol fraction was more cytotoxic than the aqueous preparation in this research points to the role of organic-solvent-extractable bioactives—likely flavonoid glycosides and terpenoids—in the anti-proliferative effect. Mechanism remains an open question, but the rich saponin content of *T. diffusa* is a plausible contributor, since triterpenoid saponins are known to disrupt cholesterol-rich cancer cell membranes, increasing permeability and triggering apoptosis [12].

The borderline activity of *C. suaveolens* (IC_{50} 98.3 $\mu\text{g/mL}$) and absence of meaningful activity in *B. triphylla* suggest that the phytochemical diversity of a plant does not always translate to anti-cancer activity: compound abundance and diversity at the qualitative screening stage are necessary but not sufficient predictors of biological activity. Compounds present at high concentrations but with weak biological potency, or with opposing effects at the tested concentrations, may produce an apparently inactive crude extract. Bioactivity-guided fractionation—separating the extract into compound subgroups by polarity or chromatographic method and testing each fraction independently—is the appropriate next step for *C. suaveolens* and *B. triphylla* before these species are definitively classified as inactive [13].

A practical limitation of this research is the use of a single cancer cell line (HeLa). Extracts showing activity on HeLa may be inactive on other cell types, or extracts appearing inactive here might be potent on specific cell lines. Extending the MTT assay to two or three additional cancer cell lines (e.g. MCF-7, HCT116, A549) in a follow-up research would provide a more robust activity profile. Additionally, a selectivity index comparing IC_{50} on cancer cells versus normal human fibroblasts (WI-38 or MRC-5) is needed before any extract is considered a genuine candidate for further pharmaceutical development.

Conclusion

This research makes a focused contribution to the evidence base for traditional Mexican phytochemistry by providing the first standardised phytochemical profiles and HeLa cell cytotoxicity data for five regionally important plant species

under comparable experimental conditions. The main contribution is the identification of *S. mexicana* (doradilla) and *T. diffusa* (damiana) as the most cytotoxically promising species, with IC_{50} values of 51.6 and 63.2 $\mu\text{g/mL}$ respectively both within the pharmacologically active range (< 100 $\mu\text{g/mL}$) accepted for crude plant extracts.

Supporting evidence for this conclusion comes from both the phytochemical screening and the dose-response cytotoxicity data: the two most active species also showed the richest profiles for alkaloids, terpenoids, saponins, and flavonoids—compound classes with well-documented anti-proliferative mechanisms. The weakest cytotoxic activity was seen in *B. triphylla* despite its distinctive tannin and steroid profile, emphasising that qualitative phytochemical richness alone does not predict biological potency.

The field significance of these findings is twofold: they provide scientific validation for aspects of Mexican traditional oncology practice and identify specific plant species as priorities for the next stage of drug discovery. Open questions remaining include the identity of the active fractions within *S. mexicana* and *T. diffusa* extracts, the selectivity of their cytotoxic effects on cancer versus normal cells, *in vivo* anti-tumour activity, and the mechanistic pathway (apoptosis vs necrosis vs cell cycle arrest) through which they inhibit HeLa cell proliferation. Each of these questions constitutes a distinct, tractable line of future research that can be built directly on the foundations established by this investigation.

Acknowledgements

Institutional support

The Mexico City Academy of Pharmaceutical Sciences is acknowledged for providing cell culture facilities, MTT assay equipment, HPLC analytical support, and herbarium authentication services throughout the research period.

Contributions not qualifying for authorship

The authors thank lab technicians Ing. Patricia Flores and Ing. Andrés Vargas for technical assistance with cell culture maintenance. Herbarium authentication was confirmed by Dr. Reyna Beltrán of the National Autonomous University of Mexico Botany Department.

Funding Sources

This research received general funding support from the Consejo Nacional de Ciencia y Tecnología (CONACYT) of Mexico through competitive grants for biomedical and pharmaceutical sciences research. No specific project code is associated with this publication.

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