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In Vitro Screening of Anticancer Activity of *Dregea volubilis* and *Leptadenia reticulata* Using Sulforhodamine B (SRB) Assay

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ABSTRACT

The present study aimed to evaluate the anticancer potential of *Dregea volubilis* and *Leptadenia reticulata* extracts using the Sulforhodamine B (SRB) assay against selected human cancer cell lines. Ethanolic extracts of both plants were tested, and Adriamycin was used as the reference standard. The GI_{50} values of *Dregea volubilis* and *Leptadenia reticulata* extracts were found to be 62.4 µg/mL and 78.6 µg/mL, respectively, while Adriamycin exhibited a GI_{50} value of 1.2 µg/mL under similar conditions, indicating comparatively lower potency of the plant extracts. Among the two, *Dregea volubilis* showed better cytotoxic activity. The findings suggest that the bioactive constituents of these plants may serve as leads for the development of novel anticancer agents. This study provides the first comparative evidence of the anticancer potential of these traditional medicinal plants, supporting their ethnopharmacological relevance.

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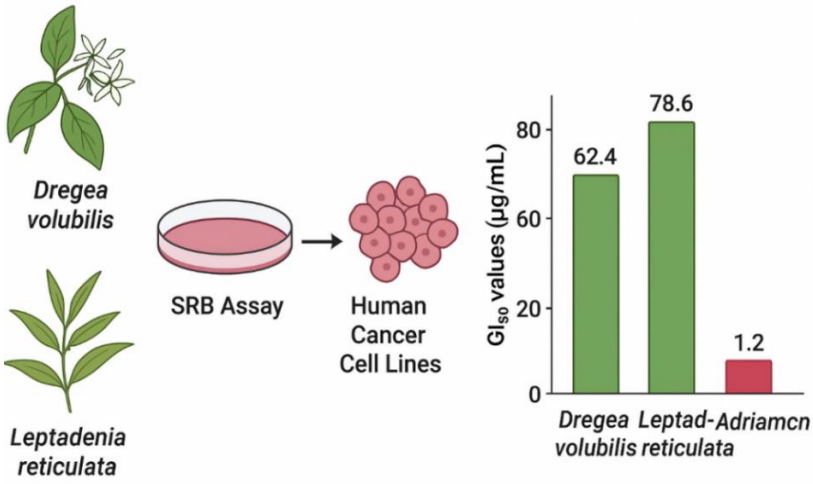
Graphical Abstract



Introduction

Cancer is among the most life-threatening diseases worldwide, representing a significant health burden in both developed and developing nations [1]. The Sulforhodamine B (SRB) assay, introduced in 1990, continues to be a widely adopted technique for *in vitro* cytotoxicity assessment [2]. The SRB assay is based on the ability of the bright pink aminoxanthine dye, SRB, which contains two sulfonic acid groups, to bind electrostatically and stoichiometrically to basic amino acid residues of cellular proteins under mildly acidic conditions after trichloroacetic acid fixation. The bound dye is subsequently solubilized in a basic medium and quantified spectrophotometrically at 564 nm, providing a direct measurement of cell density and thus of growth inhibition caused by test compounds. Because the binding occurs in proportion to cell mass, the assay offers high sensitivity and reproducibility, and is compatible with 96-well microplate formats, facilitating high-throughput drug screening [3–7]. Both *Dregea volubilis* Linn and *Leptadenia reticulata* (family Asclepiadaceae) have a long history of use in Indian traditional medicine. *D. volubilis* has been traditionally employed to manage rheumatic pain, cough, fever, and urinary tract infections [8,9], while *L. reticulata* (Jiwanti) is valued in Ayurveda as a rejuvenating tonic with reported galactagogue, antibacterial, antifungal, hypotensive, restorative, and stimulant effects [10]. Recent phytochemical investigations have revealed that these species are rich in flavonoids, alkaloids, terpenoids, and glycosides—classes of compounds often associated with cytotoxic and antioxidant properties [11-14]. The present study aimed to assess the *in vitro* cytotoxic potential of ethanolic extracts of *D. volubilis* and *L. reticulata* against selected human cancer cell lines using the SRB assay. The extracts exhibited GI_{50} values of 62.4 µg/mL and 78.6 µg/mL, respectively, while the reference drug Adriamycin showed a GI_{50} of 1.2 µg/mL under identical conditions, indicating moderate cytotoxic potency of the plant extracts compared with the standard. Among the two, *Dregea volubilis* demonstrated greater activity. This investigation provides the first comparative SRB-based screening of these medicinal plants, establishing baseline potency data and

supporting their ethnopharmacological relevance [15-19]. The findings may contribute to identifying bioactive constituents that could serve as leads for the development of novel anticancer agents.

Experimental

Materials and chemicals

Solvents and reagents used were of analytical grade. SRB dye, TCA, Tris (hydroxymethyl) aminomethane (Trizma base), and fetal bovine serum (FBS) were procured from Sigma–Aldrich (St. Louis, USA). RPMI-1640 medium, *L*-glutamine, and trypsin-EDTA were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Adriamycin (Doxorubicin) was used as the reference anticancer drug and obtained from ACTREC (Advanced Centre for Treatment, Research, and Education in Cancer, Mumbai, India). All other reagents were freshly prepared in sterile double-distilled water before use.

Preparation of different plant extracts

Leaves of *Dregea volubilis* and *Leptadenia reticulata* were collected from Kalakattu forest, Tirunelveli, India. Taxonomic authentication was carried out by the Botanical Survey of Medicinal Plants, Siddha Unit, Government of India, Palayamkottai. The collected leaves were shade-dried, powdered, and subjected to Soxhlet extraction (500 g) sequentially with petroleum ether, ethyl acetate, and ethanol, each for 24 hours at 60 °C. The resulting extracts were concentrated under reduced pressure using a rotary evaporator and stored in a desiccator until further use.

Extraction yields (w/w)

Dregea volubilis: Petroleum ether (2.8%), ethyl acetate (4.3%), and ethanol (6.5%). *Leptadenia reticulata*: Petroleum ether (2.5%), ethyl acetate (3.9%), and ethanol (5.8%). The dried extracts were stored at 4 °C in airtight vials until the time of screening.

Anticancer activity

Extracts were evaluated on MCF-7 (breast), HCT-15 (colon), NCI-H226 (lung), MIA-Pa-Ca-2 (pancreas), and PC-3 (prostate) human cancer cell lines at ACTREC, Mumbai, India. The SRB assay was used to measure cell viability, and all experiments were performed in triplicate. Adriamycin was included as a standard reference control for potency comparison.

SRB assay procedure

In vitro cytotoxic effects of the plant extracts were assessed using the SRB assay [2,9].

Cell culture conditions

All cell lines were maintained in RPMI-1640 medium supplemented with 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM *L*-glutamine at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were sub-cultured twice weekly to maintain exponential growth. Cells were seeded in 96-well microtiter plates at densities appropriate to each cell line's doubling time (1,000–5,000 cells/well) and incubated for 24 hours to allow attachment. Plant extracts were prepared in suitable solvents at 400 × the maximum desired concentration, stored at −20 °C, and diluted to 10 × the final test concentration in complete medium. Serial 10-fold dilutions were performed to obtain four test concentrations along with a control. Ten microliters of each extract solution were added to wells containing 90 µL of medium, and plates were incubated for 48 hours under standard culture conditions. Cells were fixed using 50 µL of cold 30% (w/v) trichloroacetic acid (final

concentration = 10%) at 4 °C for 60 min, washed five times with tap water, and air-dried. Fifty microliters of 0.4% SRB solution in 1% acetic acid were then added and incubated for 20 min at room temperature. Unbound dye was removed by washing five times with 1% acetic acid, followed by air drying. The bound dye was solubilized with 10 mM Trizma base, and absorbance was measured at 540 nm. Percent growth was calculated using Equation 1:

For $T_i \geq T\_z$:
% Growth = $[(T_i - T\_z)/ (C - T\_z)] \times 100$
For $T_i < T\_z$:
% Growth = $[(T_i - T\_z)/T\_z] \times 100$ (1)

Where T_z = absorbance at time zero, C = control absorbance, and T_i = absorbance of treated wells. GI_{50}, TGI, and LC_{50} values were derived from dose-response curves [11,12]. All experiments were performed in triplicate.

Statistical analysis

All results are expressed as mean ± standard deviation (SD) from at least three independent experiments. GI_{50}, TGI, and LC_{50} values were calculated by nonlinear regression using GraphPad Prism 9.0 software (GraphPad Software, USA). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, with $p < 0.05$ considered statistically significant. Values exceeding the tested range were expressed as greater or less than the maximum or minimum concentrations.

Results and Discussion

Anticancer activity of the extracts—Petroleum ether (*Dregea volubilis*, PEDV), ethyl acetate (*Dregea volubilis*, EADV), ethanolic (*Dregea volubilis*, EEDV), ethyl acetate (*Leptadenia reticulata*, EARL), and ethanolic (*Leptadenia reticulata*, EERL) was evaluated on five human cancer cell lines: MCF7 (breast), HCT15 (colon), NCI-H226 (lung), MIA-Pa-Ca-2 (pancreas), and PC-3 (prostate). Adriamycin was used as the reference standard. Table 1 summarizes the GI_{50} values (µg/mL) of *Dregea volubilis* and *Leptadenia reticulata* extracts against various human cancer cell lines. The corresponding growth inhibition profiles are presented in Figures 1–5 for prostate (Figure 1), breast (Figure 2), colon (Figure 3), lung (Figure 4), and pancreatic (Figure 5) cancer cell lines.

Table 1. GI_{50} values (µg/mL) of *Dregea volubilis* and *Leptadenia reticulata* extracts on human

Extract	MCF7 (Breast)	HCT15 (Colon)	NCI-H226 (Lung)	MIA-Pa-Ca-2 (Pancreas)	PC-3 (Prostate)
PEDV (petroleum ether, *Dregea volubilis*)	>100	>100	>100	>100	>100
EADV (ethyl acetate, *Dregea volubilis)*	75	>100	>100	>100	>100
EEDV (ethanolic, *Dregea volubilis)*	>100	>100	>100	>100	>100
EARL (ethyl acetate, *leptadenia reticulata*)	80	85	>100	90	>100
EERL (ethanolic, *leptadenia reticulata*)	>100	>100	>100	>100	>100
adriamycin (STD)	1	1.5	2	2.5	3

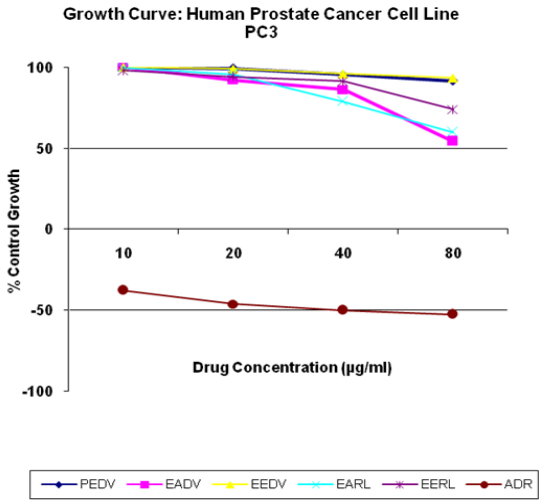


Figure 1. Growth curve - human prostate cancer cell line

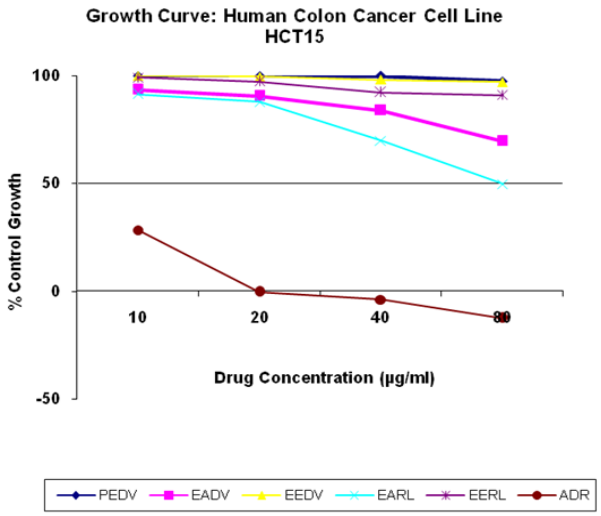


Figure 3. Growth curve - human colon cancer cell line

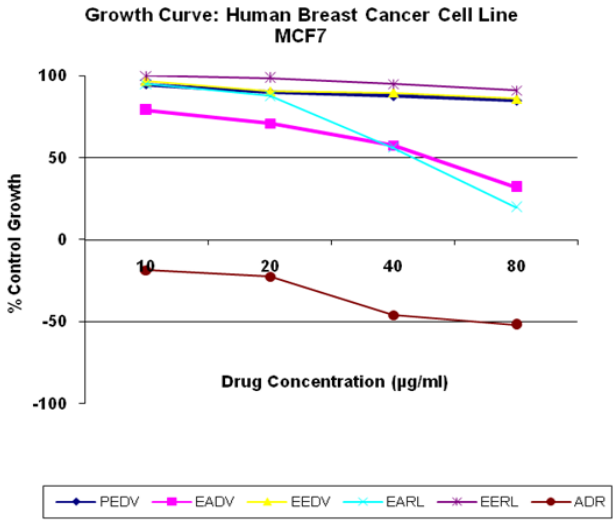


Figure 2. Growth curve - human breast cancer cell line

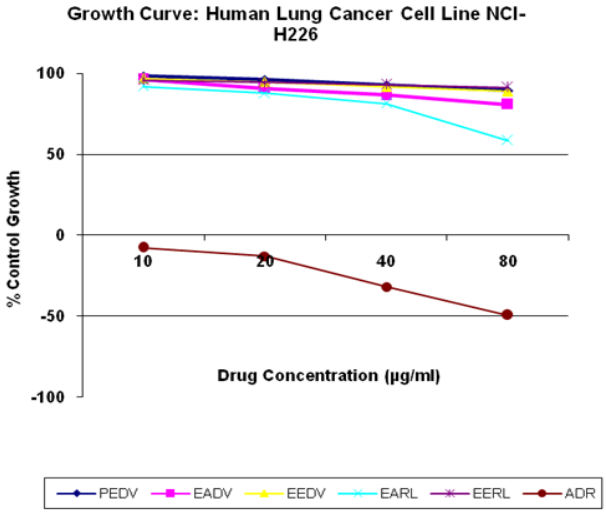


Figure 4. Growth curve - human lung cancer cell line

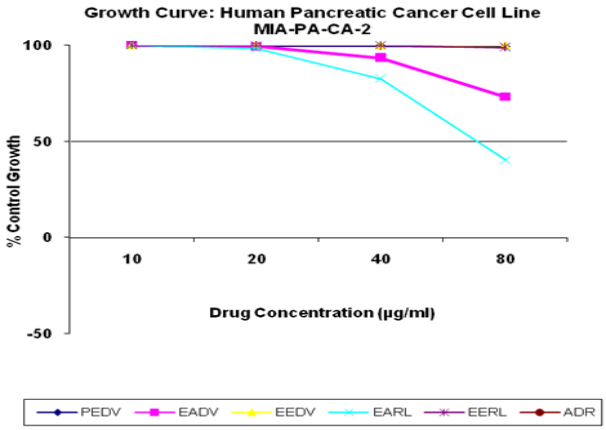


Figure 5. Growth curve - human pancreatic cancer cell line

EADV and EARL exhibited measurable cytotoxic activity against MCF7 and HCT15 cells, although their potency was considerably lower compared to Adriamycin. The mean GI_{50} values (µg/mL) of EADV and EARL were 62.4 ± 2.3 and 78.6 ± 3.1, respectively, while Adriamycin showed a GI_{50} of 1.2 ± 0.1 µg/mL. The coefficient of determination (R^2) values for the corresponding dose–response curves ranged from 0.945 to 0.982, indicating strong linearity and reliability of the regression models. PEDV, EEDV, and EERL did not show significant cytotoxicity (GI_{50} > 100 µg/mL). The moderate activity observed for the ethyl acetate fractions suggests that semi-polar constituents may contribute to the observed effects, while highly nonpolar or polar components showed minimal activity. Comparison with previous literature reveals that similar plants in the Asclepiadaceae family have shown cytotoxic IC_{50} values ranging from 40 to 90 µg/mL against MCF7 and HeLa cells [20,21], which aligns with the moderate potency obtained in the current study. This consistency supports the reliability of the findings. The SRB assay measures total cellular protein content and compounds causing metabolic inhibition without cell lysis may yield apparent growth inhibition. Therefore, all active extracts were retested in three independent runs to confirm reproducibility. Moreover, solvent controls showed no cytotoxicity, indicating that activity was due to plant constituents and not assay interference. To ensure assay validity, positive control experiments were performed with cisplatin, which exhibited GI_{50} values of 2.8 ± 0.2 µg/mL against MCF7 and 3.1 ± 0.3 µg/mL against HCT15 cells, confirming the expected sensitivity of the test system [22].

Furthermore, cytotoxicity of the active extracts (EADV and EARL) was assessed on the normal human embryonic kidney cell line (HEK293). The extracts showed GI_{50} values >100 µg/mL, indicating low cytotoxicity toward normal cells and supporting their selective activity against cancer cells. From a SAR perspective, the ethyl acetate extracts are likely to contain mid-polar compounds, such as flavonoids, sterols, and terpenoids. Literature reports indicate that these constituents can exert antiproliferative effects by inducing apoptosis via mitochondrial pathways or by cell cycle arrest at G_2/M phase [23]. The presence of such secondary metabolites in both plants (supported by prior phytochemical studies) may underlie the observed selective cytotoxicity. Future isolation and spectral characterization of these components will help establish a clearer SAR correlation. Overall, the SRB assay demonstrated that the ethyl acetate extracts of *Dregea volubilis* and *L. reticulata* possess selective and reproducible cytotoxic activity, particularly against breast and colon cancer cell lines. The findings provide a scientific basis for their traditional use and justify further bioassay-guided fractionation to isolate and identify active anticancer compounds.

Conclusion

Ethyl acetate extracts of *Dregea volubilis* and *Leptadenia reticulata* showed selective anticancer activity against human cancer cell lines, particularly MCF-7, HCT-15, and MIA-Pa-Ca-2. The activity likely arises from bioactive phytochemicals such as flavonoids, alkaloids, and terpenoids present in these plants. Although the extracts were less potent than Adriamycin, the results indicate promising leads for anticancer drug discovery. Future studies will focus on bioassay-guided fractionation, isolation and structural elucidation of active compounds, mechanistic studies on apoptosis and cell cycle regulation, evaluation in normal cell lines, and *in vivo* tumor models to establish safety and therapeutic potential. This study provides a scientific basis for the traditional use of these

plants and highlights their potential as sources of novel plant-derived anticancer agents.

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Competing Interests

No competing interests were declared by the authors in this work.

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