International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 03-12-2025, Accepted: 02-01-2026, Published: 17-01-2026
Volume 11, Issue 1, 2026, Page No. 100-106

Phytochemical profiling and anticancer activity of *Heliotropium marifolium* (J. Koenig ex Retz.) leaf and stem extracts against the A549 human lung cancer cell line

Srinivasan Nithya[1], Edward Gnanaraj Wesely[1*], Karunakaran Thangadurai[2], Michael Helan Soundra Rani[3], Mohammed Nazeena Bobby[4]

[1] Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India
[2] Department of Siddha, National Institute of Siddha, Chennai, Tamil Nadu, India
[3] Department of Biotechnology, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India
[4] Department of Biotechnology, Vignan's Foundation for Science, Technology and Research, Vadlamudi, Andhra Pradesh, India

Abstract

The present study investigates the effects of phytochemicals derived from the stem and leaves of *Heliotropium marifolium* on lung cancer. Phytochemicals were isolated using simple solvent extraction through conventional methods, and the extracts were analyzed by gas chromatography–mass spectrometry (GC–MS). Dried plant materials were subjected to qualitative phytochemical screening, and the most effective solvent extracts were further analyzed using GC–MS. Cytotoxic effects against lung cancer cells were evaluated using the MTT assay, nuclear condensation assay, and mitochondrial membrane potential assay for both stem and leaf extracts. Qualitative phytochemical analysis revealed that ethanol and isopropanol extracts were more effective than nonpolar solvent extracts. Quantitative assays demonstrated that leaves contained higher levels of phenols, alkaloids, and flavonoids compared to stems. GC–MS analysis showed that the leaf extract mainly contained bioactive compounds such as exadecenoic acid, phytol, 9,12-octadecadienoic acid (Z, Z), and 17-octadecynoic acid. In contrast, the stem extract primarily contained cholest-5-en-3-ol (3β), isocholesterylmethyl ether, and ergosta-5,22-dien-3-ol (3β,22E). At concentrations up to 100 µg/mL, neither extract exhibited cytotoxic effects on A549 cells; however, at 200 µg/mL, both extracts significantly inhibited cell proliferation in a concentration-dependent manner. The leaf extract exhibits a notably lower IC_{50} value, indicating stronger cytotoxic activity. Overall, the aerial parts of *H. marifolium* demonstrated significant anticancer activity against the A549 human lung cancer cell line. This study suggests that *H. marifolium* may serve as a potential, safe candidate for the development of novel therapeutic agents for early-stage cancer treatment without causing toxicity to healthy cells.

Keywords: *Heliotropium marifolium*, anticancer, A549 lung cancer cell line, phytol, nonadecanol, nuclear disintegration

Introduction

Globally, the incidence of cancer occurrences has surged, resulting in a rise in tolerance to chemotherapeutic agents and increasing awareness of the potential applications of phytochemicals in disease prevention and treatment. Substances found in medicinally significant plants have broad prospects for biosynthesis [1]. Nowadays, medicinal plants constitute a substantial and advantageous part of the advised course of care and hold great promise for many nations. Despite ample evidence supporting the numerous medical benefits of *Heliotropium* plants in the treatment of a wide range of illnesses, *Heliotropium*, a suffruticose perennial and annual herb, belongs to the Family Boraginaceae (Order Boraginales) and is commonly known as Seaforth Heliotrope. Their scorpioid cyme partial inflorescences and the significantly altered stigmatic head structure in the flower serve as the only distinguishing characteristics [2].

Although some *Heliotropium* species, such as *H. strigosum*, *H. europaeum*, and *H. zeylanicum*, has antifungal and antibacterial effects, others are significant in localized herbal remedies or traditional practices [3]. Phytochemicals found in *Heliotropium* species are bioactive and possess potent medicinal qualities. The *Heliotropium* genus contains large amounts of several kinds of chemical compounds, including flavonoids, pyrrolizidine alkaloids, terpenoids, and quinones [4]. There have also been discovered pyrrolizidine lactone alkaloids, namely lycopsamine and helindicine [5]. Hepatotoxic pyrrolizidine alkaloids, which have been linked to numerous liver illnesses, are known to produce toxic effects in a small number of species. The genus *Heliotropium* comprises approximately 250 species, which are found throughout the tropical, subtropical, and mild-temperate zones of all continents [6]. However, only a small number of these species have been thoroughly studied. It was discovered that *H. indicum* leaves have anti-inflammatory and wound-healing properties [7, 8]. The antiviral and antineoplastic properties of pyrrolizidine alkaloids derived from *H. marifolium*. Given that *Heliotropium* species secondary metabolites contain cytotoxic, phytotoxic, antibacterial, anticancer, and antiviral properties, as well as anti-inflammatory and wound-healing properties.

Materials and Methods

Processing of Plant and Extraction

The entire plant was cleaned with tap water, then rinsed with distilled water to reduce contamination from processing and transit. The plant was then shade-dried for 1 month. Petroleum ether, chloroform, methanol, ethanol, isopropanol, ethyl acetate, and distilled water were used for extraction. The extract was prepared by weighing out 20 g of the milled leaves and stems, then soaking them in 150 mL of the respective solvent in a conical flask and stirring vigorously with a glass rod to ensure complete extraction. The mixture was allowed to settle for 24 hours at room temperature. The extracts were then filtered using Whatman No. 1 filter paper (125 mm × 100 circles) and used for further experiments. The phytochemical properties of the extract were analyzed using conventional qualitative procedures, and standard quantitative analyses were performed for phenols, flavonoids, alkaloids, and glycosides.

Chemicals and Reagents

Dulbecco's Modified Eagle's Medium, Streptomycin, Penicillin-G, L-Glutamine, Phosphate-Buffered Saline, 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 2′,7′-dichlorofluorescein diacetate, Sodium Dodecyl Sulfate, Trypan Blue, Trypsin-EDTA, (Ethylenediaminetetraacetic acid), Acridine Orange, Ethidium Bromide, Rhodamine-123, Triton X-100, Ethanol, Dimethyl Sulfoxide (DMSO), and Bovine Serum Albumin were purchased from Sigma Aldrich Chemicals Pvt. Ltd. (India). All other chemicals used were of analytical grade and were purchased from HiMedia Laboratories Pvt. Ltd., India.

Cell Culture Maintenance

Human lung cancer A549 cell lines were procured from the Cell Repository of the National Centre for Cell Science (NCCS), Pune, India. Dulbecco's Modified Eagle Medium (DMEM) was used for maintaining the cell line, supplemented with 10% Fetal Bovine Serum (FBS). Penicillin (100 U/mL) and Streptomycin (100 µg/mL) were added to the medium to prevent bacterial contamination. The medium with cell lines was maintained in a humidified environment with 5% CO_2 at 37°C.

MTT Assay

The cytotoxicity of *Heliotropium marifolium* leaves and stem extracts on A549 cells was determined by MTT assay. A549 viable cells were harvested and counted using a hemocytometer and diluted in DMEM medium to a density of 1×10^4 cells/mL. The cells were seeded into 96-well plates and incubated for 24 h to allow attachment. After treatment with control and varying concentrations of extracts (50 to 300 µg/mL), the A549 cells were incubated at 37°C in a humidified incubator with 95% air and 5% CO_2 for 24 h. Following incubation, the cells were washed with fresh culture medium, and MTT (5 mg/mL in PBS) dye was added to each well, followed by incubation for another four hours at 37°C. The purple precipitated formazan formed was dissolved in 100 µL of concentrated DMSO, and cell viability was measured at 540 nm using a multi-well plate reader. The results were expressed as the percentage of viable cells with respect to the control. The half-maximal inhibitory concentration (IC_{50}) values were calculated, and the optimum doses were analyzed at different time periods.

$$\text{Inhibitory of cell proliferation (\%)} = \frac{\text{Mean absorbance of the control} - \text{Mean absorbance of the sample}}{\text{Mean absorbance of the control}} \text{ X } 100$$

The IC_{50} values were determined from the dose-response curves of *H. marifolium* leaf and stem extracts, where 50% cytotoxicity was observed relative to control cells. All experiments were performed at least three times in triplicate.

Propidium Iodide (PI) Staining

Apoptotic cell death in the control and *H. marifolium* leaves and stem extract-treated A549 cells was determined by PI staining. The A549 cells were seeded into 24-well plates at 1×10^6 cells per well in DMEM and incubated at 37°C for 24 h. Afterward, 150 and 200 µg of leaf and stem extracts were added to the A549 cells, and the plates were incubated for an additional 24 h at 37°C. After incubation, PI stain (5 µL) was added to each well, and the plates were kept in the dark for 20 min to allow the cells to stain. Apoptotic cell death was then analysed using a fluorescence microscope.

Evaluation of Apoptosis using DAPI Nuclear Staining

The induction of apoptosis by *H. marifolium* extracts in lung cancer cells was assessed by examining nuclear morphology *via* DAPI staining. DAPI staining is used to visualize atomic changes and assess apoptosis, as dying cells exhibit a more brightly stained nucleus than live cells. The A549 cells were seeded at a density of 2×10^5 cells per well in 24-well plates and incubated for 24 hrs in a CO_2 incubator. Cells were treated with 150 and 200 µg of *H. marifolium* leaves and stem extracts for 24 h, followed by fixation with 4% paraformaldehyde and permeabilization with 0.1% Triton X-100. The cells were rinsed with PBS and then stained with DAPI for 20 min at room temperature in the dark. The DAPI-stained cells were observed under a fluorescence microscope, and the images were later analyzed with ImageJ software.

Results and Discussion

Phytochemical Analysis of Leaves

Table 1 shows that ethanol and methanol are better solvents than others for extraction. Among the different solvents, ethanol and isopropanol extraction showed the presence of carbohydrates, glycosides, amino acids, alkaloids, flavonoids, phenols, tannins, and saponins. However, phytosterols, oil, and fat were absent. Similarly, the methanol extract showed positive results for all parameters except alkaloids, saponins, and fats. Chloroform extraction showed positive results for amino acids and alkaloids only. Water and ether extractions of leaves showed negative results for all tested parameters.

Table 1: Preliminary phytochemical screening of the leaf extract of *H. marifolium*

S. No	Name of the test	*H. marifolium* leaf extracts						
		Pet. ether	Chloroform	Methanol	Ethanol	Isopropanol	Ethyl acetate	Dist. water
1	Carbohydrates							
	a. Barfoed's test	-	-	+	++	+	+	-
2	Detection of Glycosides							
	b. Borntrager's test	-	-	+	+	+	+	-
3	Proteins and Amino Acids							
	c. Million's test	-	+	+	++	+	-	-
4	Fixed Oils and Fats							
	d. Saponification test	-	-	-	-	-	-	-

5	Detection of Alkaloids							
	e. Mayer's test	-	+	-	++	+	+	-
6	Detection of Flavonoids							
	f. Ferric chloride test	-	-	+	+	+	-	-
	g. Anthocyanin test	-	-	+	+	+	-	-
7	Detection of Phytosterols							
	h. Salkowski test	-	-	-	-	-	-	-
8	Tannins and phenolic compounds							
	i. Ferric chloride test	-	-	+	+	+	-	-
9	Detection of Saponins							
	j. Foam test	-	-	-	+	+	-	-

Likewise, the phytochemistry (Table 2) of the stem reveals that ethanol and isopropanol showed negative results only for fats, oils, and phytosterols. Methanol stem extraction showed negative results for fats, oils, saponins, and phytosterols. Petroleum ether and water extracts exhibited 100% negative results for phytochemicals.

Chloroform extraction was positive only for alkaloids. Phytochemical tests showed that all the plant extracts mentioned contained alkaloids, flavonoids, and tannins. The phytochemical screening of the aqueous leaf extract revealed negative results for all tested constituents in both the stem and leaves.

Table 2: Preliminary phytochemical screening of the stem extract of *H. marifolium*

S. No	Name of the test	***H. marifolium* stem extracts**						
		Pet. ether	**Chloroform**	**Methanol**	**Ethanol**	**Isopropanol**	**Ethyl acetate**	**Dist. water**
1	Carbohydrates							
	a. Barfoed's test	-	-	+	++	+	+	-
2	Detection of Glycosides							
	b. Ferric chloride test	-	-	+	++	+	+	-
3	Proteins and Amino Acids							
	c. Million's test	-	+	+	++	+	-	-
4	Fixed Oils and Fats							
	d. Saponification test	-	-	-	-	-	-	-
5	Detection of Alkaloids							
	e. Mayer's test	-	+	-	++	+	+	-
6	Detection of Flavonoids							
	f. Ferric chloride test	-	-	+	+	+	-	-
	g. Test for Anthocyanin	-	-	+	+	+	-	-
7	Detection of Phytosterols							
	h. Salkowski test	-	-	-	-	-	-	-
8	Tannins and Phenolic Compounds							
	i. Ferric chloride test	-	-	+	+	+	-	-
9	Detection of Saponins							
	j. Foam test	-	-	-	+	+	-	-

A variety of constituents have been identified and isolated from different species of the genus *Heliotropium* [9, 10]. However, the report on nonpolar solvents is the findings of Savadi *et al* [11], who reported the presence of some phytochemicals in nonpolar extractions. Additionally, it has been reported that plant flavonoids, alkaloids, and phenols possess several medicinal benefits, including anticancer properties [12]. Traditionally, medicinal plants have been used to cure fungal infections, inflammation, wound healing, and carminative symptoms. The concentration of total alkaloids, flavonoids, phenolics, and glycosides (Table 3) among leaves was 10.6±0.07, 8.33±0.16, 2.72±0.05, and 3.42±0.22 mg/g, respectively, whereas the stem showed 7.32±0.03, 5.21±0.42, 1.71±0.21, and 2.17±0.19 mg/g, respectively. Further GC-MS analysis of the leaf and stem extract of selected plants revealed the presence of identical compounds such as Heptasiloxane, Pentadecanal, 2-pentadecanone, Octasiloxane, Cyclononasiloxane, Hexadecanol, 9,12-octadecadienoic acid, 1H-purin-6-amine, and 2-acetylbenzoic acid. The leaf extract showed 25 peaks with different retention times (Figure 1). (Table 4) represents the list of identified compounds in the leaf extract. The major compounds are 9,12-octadecadienoic acid (26.6%) and 17-octadecynoic acid (16.6%). Similarly, the stem extract GC-MS (Figure 2) reveals 25 peaks, predominantly Cholest-5-en-3-ol (37%), Isocholesterylmethyl ether (36%), and Ergosta-5,22-dien-3-ol (3. beta.,22E) (5.13%), identified by NIST match (Table 5).

Table 3: Quantitative Phytochemicals present in the ethanol extract of the leaves and stems of *H. marifolium*

S. No.	Phytochemical	***H. marifolium* extracts**	
		Leaves (mg/g)	**Stem (mg/g)**
1.	Total alkaloids	10.6±0.07	7.32±0.03
2.	Flavonoids	8.33±0.16	5.21±0.42
3.	Total phenolics	2.72±0.05	1.71±0.21
4.	Glycosides	3.42±0.22	2.17±0.19

Table 4: NIST matched compound identified from the leaf extract

Peak#	R. Time	Area%	Name
1	10.050	0.45	1, 2-benzoldicarbonsaeure, di- (he
2	11.833	0.72	Octadecanoic acid (cas), stearic acid
3	12.173	2.14	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13
4	12.374	0.32	Pentadecanal-
5	12.527	1.55	Neophytadiene
6	12.649	0.39	2-Pentadecanone, 6,10,14-trimethyl-
7	12.792	0.93	1-Oxacyclopentadecan-2-one, 15-ethenyl-15-m
8	13.003	0.67	3-Eicosyne
9	13.069	1.15	1-Hexadecanol (cas) cetal
10	13.792	1.07	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,
11	13.935	12.60	N-Hexadecanoic acid
12	14.092	1.72	1, 2-benzenedicarboxylic acid, dibutyl ester (c
13	14.169	3.43	Hexadecanoic acid, ethyl ester (cas) ethyl pa
14	14.340	1.58	Bis (heptamethylcyclotetrasiloxy) hexamethyltr
15	14.503	0.83	Tetradecanal
16	15.130	1.16	N-Nonadecanol-1
17	15.283	1.81	2-Acetylbenzoic acid
18	15.451	13.61	Phytol
19	15.774	26.67	9,12-octadecadienoic acid (z, z)-
20	15.964	16.51	17-octadecynoic acid
21	16.243	1.05	Octadecanoic acid, ethyl ester (CAS) ethyl
22	16.913	1.95	Cyclononasiloxane, octadecamethyl-
23	18.993	2.99	Eicosamethylcyclodecasiloxan
24	19.108	1.86	1H-purin-6-amine, ((2-fluorophenyl) methyl)-
25	21.834	2.81	Iron, monocarbonyl-(1,3-butadiene-1,4-dicarb
		100.00	

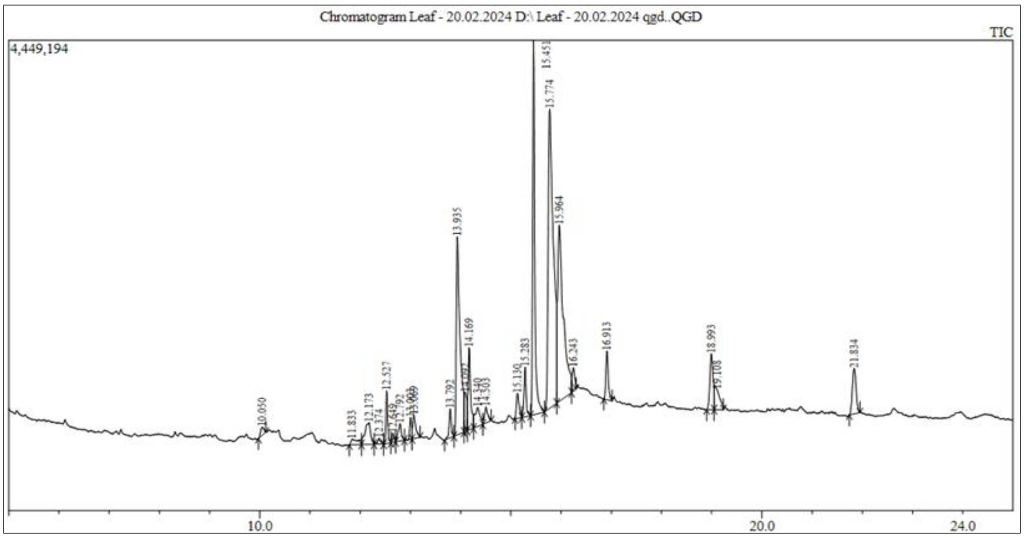


Fig 1: GC-MS spectrum of the leaf extract of *H. marifolium*

Table 5: NIST matched compound identified from the stem extract

Peak	R. Time	Area%	Name
1	10.058	0.20	Phthalicacid, di-(1-hexen-5-yl) ester
2	10.317	0.12	Phenethylamine, N-methyl-. beta.,3,4-tris (trime
3	12.092	0.16	Pentanoic acid,4-methyl-, ethylester
4	12.179	0.46	Cyclohexasiloxane, dodecamethyl-
5	12.531	0.25	2,6,10-Trimethyl,14-ethylene-14-pe
6	12.658	0.12	2-Pentadecanone,6,10,14-trimethyl-
7	12.796	0.19	6-octen-3-ol,3,7-dimethyl-
8	13.008	0.17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
9	13.079	0.13	1-Hexadecanol
10	13.802	0.16	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,
11	13.961	2.87	n-Hexadecanoicacid
12	14.176	1.50	Hexadecanoicacid, ethylester
13	14.349	0.45	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13

14	14.515	0.14	Pentadecanal-
15	15.329	37.08	Cholest-5-en-3-ol (3. BETA.)-
16	15.457	36.38	Isocholesterylmethylether
17	15.817	4.22	9,12-octadecadienoic acid (z, z)-
18	15.967	5.37	ethyl(9z,12z)-9,12-octadecadienoa
19	16.250	1.56	Octadecanoic acid, ethyl ester
20	16.558	0.35	Bicyclo (2.2.1) heptan-2-ol,4-chloro-1,7,7-trim
21	16.917	0.35	Cyclononasiloxane, octadecamethyl-
22	19.001	0.58	1H-purin-6-amine, ((2-fluoropheny))
23	19.088	1.63	2-Acetylbenzoicacid
24	19.786	5.13	Ergosta-5,22-dien-3-ol,(3.beta.,22E)-
25	21.842	0.42	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18
		100.00	

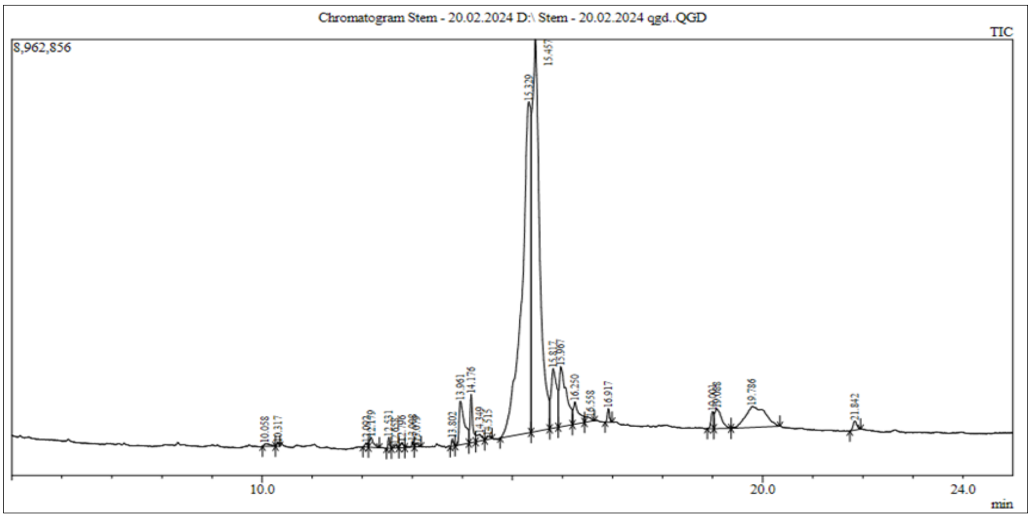


Fig 2: GC-MS spectrum of the stem extract of *H. marifolium*

The MTT assay was used to evaluate the *in vitro* cytotoxicity against A549 cells. It was discovered that live cells converted yellow MTT to dark-colored formazan at low concentrations, indicating high viability, whereas at high concentrations, low viability was observed [13]. The concentration-dependent viability of the leaf extract is shown in Figure 3, and the stem extract is represented in Figure 4. Cell viability and morphology of treated cells at 20x magnification are shown in Figure 5, which illustrates morphological changes in A549 cells at 250 µg of leaf and stem extracts. The cytotoxic potential of the leaf extract against the cancer cell line A549 was 33.8% with an IC_{50} value of 150.98 µg/mL, and the stem extract showed 25% viability with an IC_{50} of 429 µg/mL. Maximum cytotoxicity was 72.2% for the leaf extract and 82.1% for the stem extract at 300 µg/mL. Control showed normal intact cell morphology, and their images were captured using light microscopy [14]. The photomicrographic images revealed that the leaf extract affected the morphology of the cancer cells, showing better cell viability compared to the stem extract [15]. It is reported that apoptosis occurs through a series of unique morphological and cellular changes in the apoptotic cell, including nuclear condensation and fragmentation, chromatin suspension, and cell membrane alterations [16]. Morphological changes in A549 cells, such as shrinkage, detachment, membrane blebbing, and distorted shape, were induced at 250 µg/mL for 24 h compared with the control. The control showed normal intact cell morphology, and its images were captured by light microscopy.

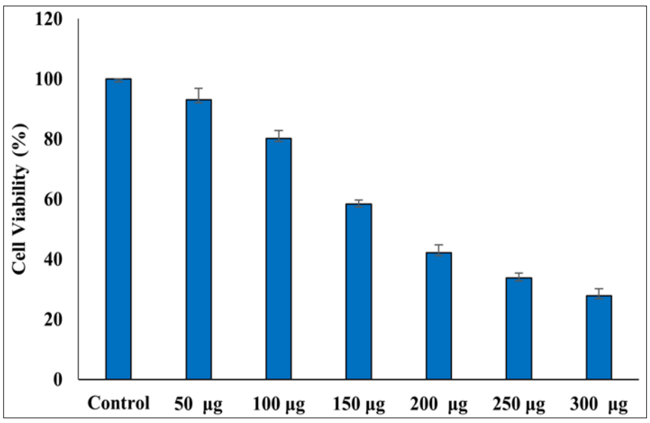


Fig 3: Percentage of cell viability of the leaf extract

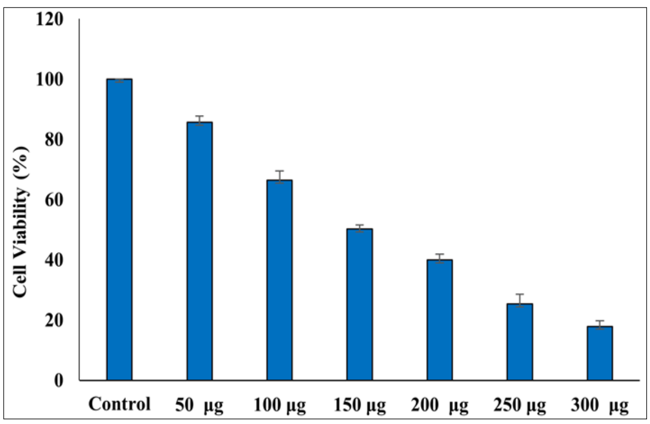
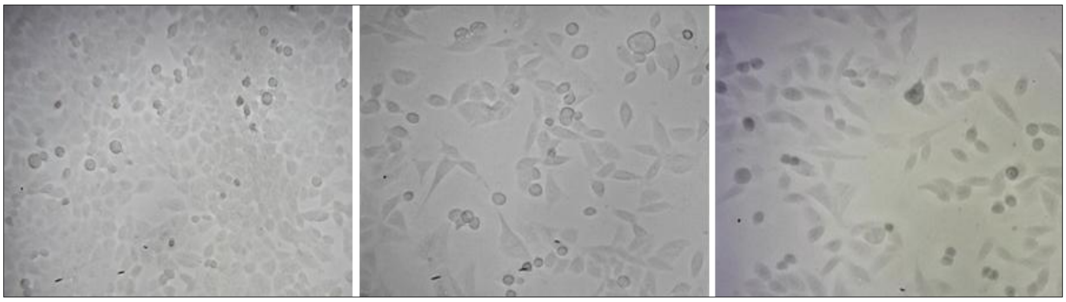


Fig 4: Percentage of cell viability of the stem extract



Control **Leaf extract** **Stem extract**

Fig 5: Morphological changes in the leaf and stem of lung cancer A549

The nuclear morphology of A549 cells was examined using DAPI, a nuclear stain. The outcome demonstrated that, compared with stem extract-exposed cells, leaf extract-exposed cells showed greater atomic condensation and fragmentation. While the leaf extract had a greater effect, the stem extract remained significant compared to untreated cells. The results demonstrate that both extracts have the potential to damage cancer cells at the nuclear level. DAPI-stained cells analyzed by fluorescence microscopy of control cells showed minimal fluorescence, indicating reduced apoptosis. However, the stem- and leaf-treated cells showed increased fluorescence, confirming the presence of fragmented nuclei and apoptotic cell death in A549 cells (Figure 6).

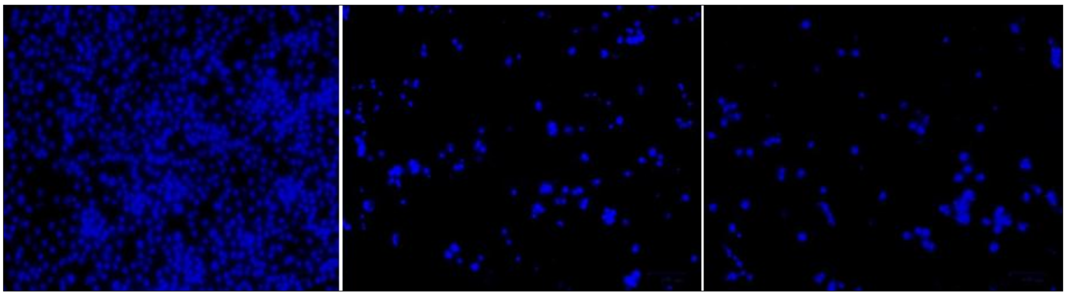


Fig 6: Effects of Leaf on the apoptosis in the A549 cells by DAPI stain

Furthermore, the use of propidium iodide helped clarify the concepts of nuclear and cell destruction. In contrast to untreated cells (control), A549 cells showed increased red fluorescence upon exposure to leaf and stem extracts (Figure 7). These findings support the cytotoxicity of the extracts of *H. marifolium*. The cytotoxic effects of *H. indicum* and *H. strigosum* was reported in the HeLa cell line, which showed good activity [17, 18]. In research on the toxicity of several *Heliotropium* species, it was discovered that the ethanolic extract of aerial parts showed cytotoxic potential at 3 to 50 mg/mL in the n-hexane fraction [19, 20, 21]. These findings suggest that the stem and leaf extracts of *H. marifolium* are a viable option for treating this particular type of cancer.

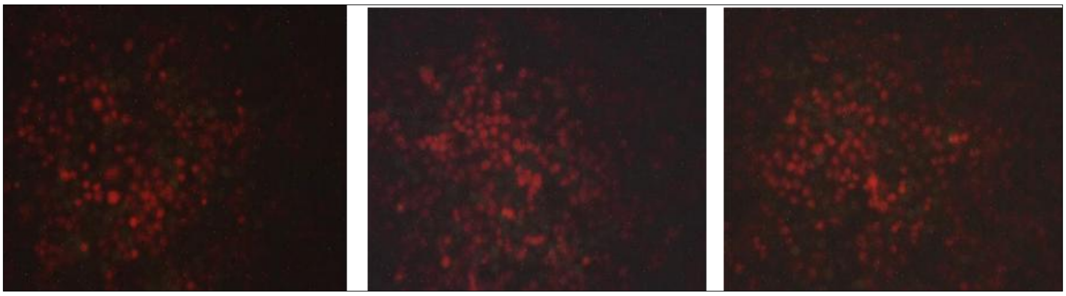


Fig 7: Effects of Leaf on the apoptosis in the A549 cells by PI stain

Conclusion

The present study demonstrates that phytochemicals extracted from the aerial parts of *Heliotropium marifolium*, particularly the leaves, exhibit significant cytotoxic activity against the A549 human lung cancer cell line. Among the solvents tested, ethanol and isopropanol proved to be the most effective for extracting bioactive compounds. Quantitative phytochemical analysis revealed higher concentrations of phenols, alkaloids, and flavonoids in leaf extracts compared to stem extracts, which correlated with enhanced anticancer activity. GC–MS analysis identified several biologically active compounds in the leaf extract that are known for their potential anticancer properties, while the stem extract contained fewer and less potent bioactive constituents. Cytotoxicity assays confirmed that both extracts inhibited cancer cell proliferation in a concentration-dependent manner, with significant effects observed at higher concentrations. Notably, the leaf extract exhibited a lower IC_{50} value, indicating superior cytotoxic efficacy. Overall, the findings suggest that *H. marifolium*, especially its leaf extract, possesses promising anticancer potential with minimal toxicity at lower concentrations. This study provides a scientific basis for further investigation of *H. marifolium* as a potential source of novel, plant-derived anticancer agents and supports its possible application in the development of safe therapeutic strategies for early-stage cancer treatment.

References

1. Zengin G, Mahomoodally MF, Aktumsek A, Jeko J, Cziaky Z, Rodrigues MJ, *et al*. Chemical profiling and biological evaluation of *Nepeta baytopii* extracts and essential oil: An endemic plant from Turkey. Plants,2021:10:1176.
2. Marwa AA, Fayed H. *Heliotropium*; a genus rich in pyrrolizidine alkaloids: A systematic review following its phytochemistry and pharmacology. Phytomedicine Plus,2021:1(2):100036.
3. Paulraj J, Govindarajan R, Palpu P. The genus *Spilanthes*: ethnopharmacology, phytochemistry, and pharmacological properties. Advances in Pharmacological Sciences, 2013, 22.
4. Fayed MA. *Heliotropium*; a genus rich in pyrrolizidine alkaloids: A systematic review following its phytochemistry and pharmacology. Phytomedicine Plus,2021:1:100036.
5. Souza JSN, Machado LL, Pessoa ODL. Pyrrolizidine alkaloids from *Heliotropium indicum*. Journal of the Brazilian Chemical Society,2005:16(6):1410–1414.
6. Dash KG, Abdullah SM. A review on *Heliotropium indicum* L. (Boraginaceae). International Journal of Pharmaceutical Sciences and Research,2013:4(4):1253–1258.
7. Reddy JS. Wound healing effects of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* in rats. Journal of Ethnopharmacology,2002:79:249–251.
8. Singh B, Sharma RA. Antineoplastic and antiviral activities of pyrrolizidine alkaloids from *Heliotropium marifolium* Koen. ex Retz. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences,2007:77(2):197–205.
9. Shoge MO, Ndukwe GI, Amupitan J. Phytochemical and antimicrobial studies on the aerial parts of *Heliotropium indicum*. Annals of Biological Research,2011:2(2):129–136.
10. Modak B, Salina M, Rodilla J, Torres R. Chemical composition of the resinous exudate isolated from *Heliotropium sclerocarpum* and antioxidant properties of the phenolic compounds and the resin. Molecules,2009:14(11):4625–4633.
11. Savadi R, Alagawadi K, Darade S. Antifertility activity of ethanolic extract and its n-hexane and benzene fractions of *Heliotropium indicum* leaves on albino rats. Journal of Pharmaceutical Research,2009:2(5):927–930.
12. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, *et al*. Important flavonoids and their role as therapeutic agents. Molecules,2020:25(22):5243.
13. Hanif A, Ibrahim AH, Ismail S, Al-Rawi SS, Ahmad JN, Hameed M, *et al*. Cytotoxicity against A549 human lung cancer cell line via mitochondrial membrane potential and nuclear condensation effects of *Nepeta paulsenii* Briq. Molecules,2023:28(6):2812.
14. Orabi MAA, Alqahtani OS, Alyami BA, Al Awadh AA, Abdel-Sattar ES, Matsunami K, *et al*. Human lung cancer (A549) cell line cytotoxicity and anti-Leishmania major activity of *Carissa macrocarpa* leaves. Pharmaceuticals,2022:15(12):1561.
15. Al Fayi MS. Anti-cancer effects of *Nepeta deflersiana* extract (NDE) in estrogen-positive and negative breast cancer. Pharmacognosy Magazine,2021:17:287–293.
16. Shaito AA, Omairi I, Al-Thani N, Seglab F, Ad-Darwish E, Kobeissy F, *et al*. Antioxidant, antihemolytic, and anti-cancer activities of *Medicago orbicularis* and its augmentation of cisplatin cytotoxicity in A549 cells. Plants,2024:13(3):442.
17. Sivajothi V. Cytotoxic effect of *Heliotropium indicum* extracts on HeLa cell line. International Journal of Pharmaceutical Sciences,2015:7:412–414.
18. Shah SM, Hussain S, Khan AU, Khan H, Ullah F. Cytotoxic and phytotoxic actions of *Heliotropium strigosum*. Toxicology and Industrial Health,2015:31(5):429–432.
19. Singh B, Sahu P, Jain S, Singh S. Antineoplastic and antiviral screening of pyrrolizidine alkaloids from *Heliotropium subulatum*. Pharmaceutical Biology,2002:40:581–586.
20. Abdel-Sattar E, Harraz FM, Al-Ansari SM, El-Mekkawy S, Ichino C, Kiyohara H, *et al*. Antiplasmodial and antitrypanosomal activity of plants from Saudi Arabia. Journal of Natural Medicines,2009:63(2):232–239.
21. Rahman M, Mia M, Shahid I. Pharmacological and phytochemical screening of roots of *Heliotropium indicum* Linn. Pharmacologyonline,2011:1:185–192.