ORIGINAL ARTICLE

Gas chromatography–mass spectrometry analysis and *in vitro* anticancer activity of *Plecospermum spinosum Trecul* leaf extract against A549 lung cancer cell line

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Abstract

Objective: This study investigates the cytotoxic effects and phytochemical composition of ethanolic leaf extract of *Plecospermum spinosum Trecul* against the A549 lung cancer cell line. **Methods:** The plant material was extracted using a Soxhlet apparatus with ethanol. Cytotoxicity was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and bioactive compounds were identified through gas chromatography–mass spectrometry (GC-MS) analysis. Physicochemical parameters and phytochemical screening were performed following standard protocols. **Results:** The ethanolic extract of *P. spinosum* demonstrated moderate cytotoxic activity against A549 cells with IC_{50} value of 110 µg/mL. GC-MS analysis revealed 30 bioactive compounds, with Ethyl (9Z,12Z)-9,12-Octadecadienoate (15.57%), 2-hexadecen-1-ol, 3,7,11,15-tetramethyl (13.9%), and hexadecanoic acid ethyl ester (12.03%) as major components. Qualitative phytochemical analysis confirmed the presence of alkaloids, terpenoids, phenols, tannins, flavonoids, and glycosides. **Conclusion:** *P. spinosum* leaf extract contains numerous phytochemicals with potential anticancer activity, warranting further investigation for drug development.

Key words: Phytochemical analysis, gas chromatography–mass spectrometry, *in vitro* anticancer, *Plecospermum spinosum*, A549 cell line, MTT assay

INTRODUCTION

Cancer is a complex and multifactorial disease characterized by the uncontrolled proliferation of abnormal cells, which can infiltrate adjacent tissues and metastasize to distant organs through the blood and lymphatic systems.[1] Once metastasis occurs, patient prognosis declines sharply, as disseminated tumors are often resistant to conventional therapies and difficult to eradicate completely. Lung cancer remains one of the leading causes of global cancer mortality,[1,2] and in India, it accounts for around 5.9% of all cancers and 8.1% of cancer-related deaths.[3]

The two main histological classes are small cell lung cancer (SCLC) and non-SCLC (NSCLC), with adenocarcinoma being the most common subtype of NSCLC, representing ~40% of NSCLC cases and nearly 30% of total lung cancers.[4] Lung adenocarcinoma typically develops in the peripheral lung tissue, often in response to carcinogen exposure, chronic inflammation, and genetic mutations in driver genes such as estimated glomerular filtration rate, KRAS, or ALK. Tumor progression involves activation of signaling pathways such as PI3K/Akt/mTOR, Ras/Raf/MEK/ERK, and dysregulation of apoptosis regulators such as Bcl 2 and p53.[5,6] Drug resistance

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in lung cancer may arise through multiple mechanisms: secondary mutations in target oncogenes, overexpression of drug efflux pumps, epithelial– mesenchymal transition, and tumor microenvironment-mediated survival signals. Such resistance, coupled with late-stage diagnosis and high metastatic potential, contributes to the persistently low survival rate.

Although chemotherapy, radiotherapy, targeted molecular therapy, and immunotherapy have brought incremental survival gains, they are frequently associated with significant systemic toxicity, adverse effects, and tumor recurrence.[7,8] These limitations have fuelled interest in plant-derived compounds as safer, more selective antineoplastic agents. Historically, natural products have played a pivotal role in cancer drug discovery: nearly 80% of the world's population relies on medicinal plants for primary health care.[9] and several hallmark anticancer drugs – vincristine, paclitaxel, irinotecan, etoposide – trace their origins to plant secondary metabolites.[10]

Phytochemicals are a chemically diverse group of bioactive plant constituents, with polyphenols, flavonoids, terpenoids, and alkaloids among the most studied for their anticancer properties.[8,11] Polyphenols and flavonoids scavenge reactive oxygen species (ROS), modulate inflammatory mediators, and influence gene expression by targeting key transcription factors such as NF-κB and AP-1. Terpenoids have been shown to inhibit tumor angiogenesis and induce cell cycle arrest, while alkaloids often interfere with DNA replication or microtubule formation, leading to apoptosis in malignant cells.

Plecospermum spinosum Trecul, a lesser-known member of the *Moraceae* family, is traditionally used in India and Southeast Asia for ailments including diabetes, cough, cold, syphilis, cholera, wounds, and infections.[12,13] Phytochemical analyses have confirmed that its leaves contain phenols, flavonoids, terpenoids, tannins, and glycosides.[12,14] Compounds from these classes are reported to display antioxidant, anti-inflammatory, analgesic, antimicrobial, and putative anticancer activities.[15,16] The potential anticancer effect may stem from their combined ability to neutralize ROS, modulate pro and anti-apoptotic protein expression, suppress angiogenesis, and arrest the proliferation of transformed cells. Despite this promising phytochemical profile, there is limited published evidence on the anticancer properties of *P. spinosum* extracts, particularly against human lung cancer cells.

Gas chromatography–mass spectrometry (GC–MS) is an indispensable analytical tool in phytochemistry for qualitative and semi-quantitative identification of volatile and semi-volatile constituents in complex plant mixtures.[17] The gas chromatography component separates compounds based on volatility and polarity, while mass spectrometry enables structural identification through fragmentation patterns. This dual capability allows researchers to generate phytochemical fingerprints, correlate specific compounds with biological activities, and prioritize molecules for further pharmacological testing. When used alongside *in vitro* cytotoxicity assays such as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) assay – which measures mitochondrial dehydrogenase activity as an indicator of live, metabolically active cells – GC–MS facilitates a targeted approach to discovering novel anticancer agents from botanical sources.[8,18]

The present study was therefore designed to evaluate the *in vitro* anticancer potential of the ethanolic extract of *P. spinosum* (*EEPS*) leaves against human lung adenocarcinoma A549 cells. The objectives were to: (i) Qualitatively assess the phytochemical composition of *EEPS*, (ii) determine its cytotoxic activity and IC_{50} value using the MTT assay, and (iii) identify and characterize its bioactive constituents through GC-MS analysis.

To the best of our knowledge, this is the first integrated phytochemical and cytotoxicity report on *P. spinosum*. By combining chemical profiling with biological evaluation, this research aims to provide a scientific foundation for the development of affordable, plant-based chemotherapeutic agents targeting lung cancer.

MATERIALS AND METHODS

Plant Material and Extraction

Fresh leaves of *P. spinosum Trecul* were collected from their natural habitat in region, identified and authenticated by a botanist at institution, and a voucher specimen deposited for future reference.[12] Leaves were washed with distilled water, shade dried at room temperature, and pulverized using a mechanical grinder. The powdered leaves were extracted with ethanol using a Soxhlet apparatus for 72 h, following phytopharmacological extraction protocols.[19] The ethanolic extract (EEPS) was concentrated under reduced pressure using a rotary evaporator and stored at 4°C until further use.[20]

Preliminary Phytochemical Screening

Qualitative phytochemical screening of EEPS was carried out to detect major secondary metabolite classes, including alkaloids (Mayer's and Dragendorff's tests), flavonoids (alkaline reagent and lead acetate tests), phenols (ferric chloride test), tannins (gelatin test), terpenoids (Salkowski test), glycosides, and saponins, using standard plant chemical analysis methods.[21,22]

Cell Culture

The human lung adenocarcinoma cell line A549 was obtained from the National Centre for Cell Science, Pune, India.[23] Cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin, and maintained at 37°C in a humidified incubator with 5% CO_2.[7,8]

Cytotoxicity Assay (MTT)

Cytotoxic activity of EEPS was assessed using the MTT assay as described by Mosmann, with modifications.[24] Briefly, A549 cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 h for attachment. EEPS was dissolved in dimethyl sulfoxide (DMSO) (final concentration ≤0.1% v/v in medium) and tested at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL for 24–48 h.[25] After treatment, 20 µL of 5 mg/mL MTT solution was added to each well and incubated for 4 h. The medium was removed, and formazan crystals were solubilized in 150 µL DMSO. Absorbance was recorded at 570 nm using a microplate reader. Percentage cell viability was calculated relative to untreated controls, and IC_{50} values were determined using nonlinear regression analysis.[24,25]

GC–MS Analysis

GC–MS analysis of EEPS was performed to identify volatile and semi-volatile constituents, using a Perkin Elmer Clarus 680 GC coupled with an Elite 5MS fused silica capillary column (30 m × 0.25 mm × 0.25 µm).[14,17] Helium was used as the carrier gas at 1 mL/min, with an injection volume of 1 µL in split mode (10:1), injector temperature 250°C, and oven program: initial 50°C (3 min), ramped to 280°C at 10°C/min, held for 10 min. Mass spectra were recorded under electron ionization (70 eV) over m/z 40–600. Compound identification was achieved through comparison of mass spectra and retention indices with the NIST library database.[17,18]

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± SD. Statistical analyses and IC_{50} values were calculated using GraphPad Prism software. A $P < 0.05$ was considered statistically significant.[26]

RESULTS AND DISCUSSION

Cancer remains a leading cause of mortality worldwide, with lung cancer accounting for approximately 1.8 million deaths annually.[1,2] Natural products contribute significantly to cancer chemotherapy, with 60% of current anticancer drugs being plant-derived.[10] *P. spinosum Trecul* (*Moraceae*) presents a promising candidate for anticancer drug discovery due to its rich phytochemical profile containing alkaloids, terpenoids, phenolic compounds, flavonoids, and tannins.[12]

Physicochemical Properties

Physicochemical analysis of *P. spinosum* extract revealed total ash content of 16%, acid-insoluble ash of 6.5%, water-soluble ash of 9.95%, and moisture content (LOD) of 2% (Table 1).

Standardization through physicochemical parameters is essential for maintaining quality, safety, and efficacy of herbal formulations.[27,28] Total ash indicates inorganic salts presence, acid- insoluble ash indicates siliceous matter, while water-soluble ash determines water-soluble salts.[21] Moisture content affects stability, as excess moisture leads to enzyme activation and microbial growth.[20] The ash values were within standard limits, indicating the leaves are suitable for drug preparation, while the low moisture content (2%) demonstrates good stability and quality.

Phytochemical Analysis

The results of qualitative phytochemical analysis revealed the presence of diverse secondary metabolites. Table 2 summarizes the results of the preliminary phytochemical screening of the ethanolic extract of *P. spinosum*. The extract was found to contain major bioactive compounds such as

Table 1: Physicochemical parameters of *Plecospermum spinosum* extract

S. No.	Parameters	Results (W/W) (%)
1	Total ash	16
2	Acid Insoluble ash	6.5
3	Water soluble ash	9.95
4	Moisture content (LOD)	2

LOD: Limit of detection

Table 2: Preliminary phytochemical screening of EEPS

S. No.	Constituents	EEPS
1.	Alkaloids	+
2.	Carbohydrates	-
3.	Protein	-
4.	Terpinoids	+
5.	Phenols	+
6.	Tannins	+
7.	Flavanoids	+
9.	Glycosides	+
10.	Saponins	-

EEPS: Ethanolic extract of *Plecospermum spinosum*

tannins, saponins, flavonoids, terpenoids, alkaloids, and polyphenols, while carbohydrates and steroids were absent.

The qualitative phytochemical analysis serves as an essential tool in discovering new chemical compounds from plants, laying the foundation for quantitative analyses and pharmaceutical applications.[19] In the current study, preliminary phytochemical screening of the *EEPS* leaves revealed the presence of key bioactive constituents, including alkaloids, terpenoids, phenols, tannins, flavonoids, and glycosides. These findings align well with previous phytochemical investigations of *P. spinosum*, confirming the consistency and reliability of the extract's biochemical profile.[12]

From a pharmacological perspective, the identified phytochemical groups are known for their significant anticancer potential through multiple mechanisms. Alkaloids, for example, possess antimetastatic and antiproliferative properties in various cancer types. Well-established anticancer drugs such as vinblastine and camptothecin belong to this group, underscoring the therapeutic relevance of alkaloid compounds.[10] Glycosides contribute by reducing cancer cell adhesion and migration, in addition to exerting anti-angiogenic and anti-invasive effects, which play critical roles in cancer progression and metastasis suppression.[29] Tannins exhibit a remarkable ability to inhibit cancer cell growth and have demonstrated potent anticancer activities across several studies.[30] Similarly, terpenoids have been widely recognized for their involvement in preventing and treating diverse diseases, including cancer, where they impact cell cycle regulation, apoptosis, and angiogenesis.[31] Phenols and flavonoids, abundant in the EEPS, are well-documented for their strong antioxidant and anticarcinogenic activities.[11,32]

The results obtained from quantitative analysis of total flavonoid content (TFC) and total phenolic content (TPC) are summarized in Table 3. Based on the absorbance, phenolic content was calculated to be 83.32±0.38 mg gallic acid equivalents (GAE)/g of dried extract, and the flavonoid content was found to be 123.06 ± 0.46 mg quercetin equivalents (QE)/g of dried extract.

The results obtained from a quantitative preliminary analysis of TFC and TPC were summarized in Table 3. Based on the absorbance phenolic content was calculated to be 83.32 ± 0.38 mg GAE/g of dried extract, and the flavonoid content was found to be 123.06 ± 0.46 mg QE/g of dried extract.

Phenolic compounds are closely associated with antioxidant properties as they act as powerful reducing agents, proton donors, and free radical scavengers, effectively protecting cells from oxidative damage that can lead to carcinogenesis.[33] The Folin–Ciocalteu method, used in this study to estimate TPC, reliably quantifies these antioxidants in plant extracts.[32] The considerable phenolic content observed in EEPS can be attributed to the efficient extraction by ethanol, a highly polar solvent approved by the Food and Drug Administration for pharmaceutical use. Ethanol's polarity favors the solubilization of polar antioxidant metabolites, including phenols.[34]

Flavonoids are a proactive group of polyphenolic compounds not synthesized by humans but obtained exclusively through diet, playing crucial roles in free radical scavenging and cellular protection.[35] The major class of polyphenols—flavonoids and phenolic acids—serve as primary antioxidants, mitigating oxidative stress implicated in cancer development.[36] Quantification of TFC via the $AlCl_3$ colorimetric method allows accurate measurement of flavones and flavonols—key subclasses of flavonoids.[37] The results from this study highlight *EEPS* as a rich source of flavonoids, further supporting its potential health benefits.

Taken together, the levels of total phenols and flavonoids in *EEPS* are considerable, pointing toward a strong antioxidant capacity likely contributing to its observed anticancer effects. This suggests that use of *P. spinosum* extract could enhance health benefits by scavenging free radicals and mitigating oxidative damage, thus offering protective effects against cancer development.

MTT Anticancer Assay

The cytotoxic potential of the *EEPS* was assessed in A549 lung cancer using the MTT assay. The extract exhibited a dose-dependent cytotoxic effect. As the concentration increased from 6.25 to 100 µg/mL, a progressive decline in cell viability was observed, with the highest concentration (100 µg/mL) reducing viability to 55.42%, compared to the control (100%).

The IC_{50} value of the extract was determined to be 110.54 µg/mL, indicating moderate antiproliferative activity.

Table 3: Summary of quantitative phytochemical contents

1	Total phenolic content	83.32±0.38 mg/g	Gallic acid
2	Total flavonoid content	123.06±0.46 mg/g	Quercetin

Table 4: Cell viability of A549 cells treated with *EEPS* (MTT assay)

S. No.	Concentration of HAEIC (µg/mL)	% Cell viability	IC_{50}
1	*EEPS* (6.25)	98.8137931	110.54
2	*EEPS* (12.5)	90.7862069	
3	*EEPS* (25)	81.73793103	
4	*EEPS* (50)	75.83448276	
5	*EEPS* (100)	55.42068966	

EEPS: Ethanolic extract of *Plecospermum spinosum*

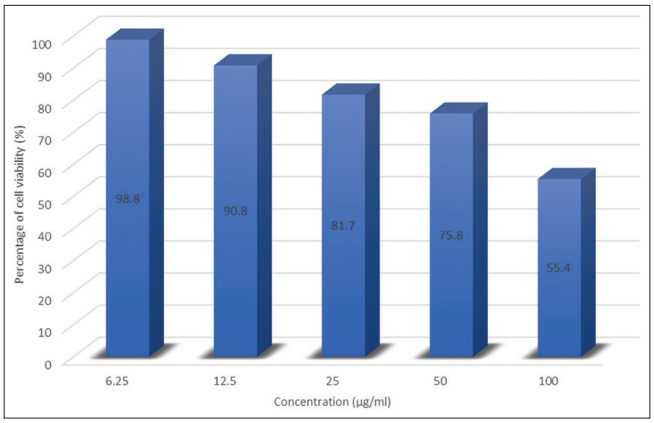

Figure 1: Cell viability of A549 cells treated with ethanolic extract of *Plecospermum spinosum* (MTT assay)

The cell viability percentages at various concentrations are presented in Table 4 and Figure 1.

The photomicrographs Figure 2 also reveal morphological changes including membrane shrinkage, rounding, and cellular fragmentation, supporting the occurrence of apoptosis.

Apoptosis is a critical mode of programmed cell death essential for eliminating genetically damaged cells under both normal physiological and pathological conditions. Dysregulation of apoptosis contributes to multiple disorders including cancer, viral infections, AIDS, autoimmune diseases, neurodegenerative conditions, stroke, and anemia.[38] Recently, induction of apoptosis and its related pathways have become key therapeutic strategies in cancer treatment.[39] Screening medicinal plants for their ability to induce apoptosis represents a major anticancer research approach, with the MTT reduction assay serving as a widely accepted antiproliferative method.[40] The MTT assay is recognized for its rapidity and accuracy in quantifying viable cells.[41]

In this study, the *EEPS* leaves demonstrated a concentration-dependent inhibition of A549 lung adenocarcinoma cell growth, with viability decreasing from approximately 99% at lower concentrations to 55% at 100 µg/mL after 24 h of exposure. The IC_{50} value of EEPS against A549 cells was determined to be 110 µg/mL. According to the National Cancer Institute and Geran protocol, cytotoxicity is classified based on IC_{50} values as: no cytotoxic effect (IC_{50} < 21 µg/mL), moderate cytotoxicity (21–200 µg/mL), weak cytotoxicity (201–500 µg/mL), and noncytotoxic (IC_{50} > 501 µg/mL).[42] Thus, EEPS demonstrates moderate cytotoxic activity.

Morphological examination revealed that untreated A549 cells exhibit smooth morphology and normal nuclei, whereas EEPS-treated cells showed reduced size, characteristic of cell shrinkage, membrane blebbing, and detachment from

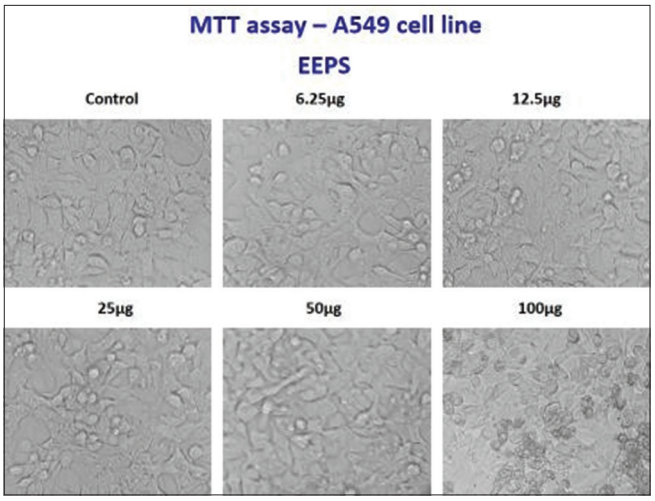

Figure 2: Morphological changes in MCF-7 cells treated with HAEPP at varying concentrations Phase-contrast microscopy images showing concentration-dependent morphological alterations in MCF-7 cells after 24-h treatment with ethanolic extract of *Plecospermum spinosum*

the culture surface. Such morphological changes are classical features of apoptosis. These observations concur with reports by Esra *et al.*[43] and Eswaraiah *et al.*,[44] who documented similar apoptotic morphological alterations in lung cancer cell lines following treatment with plant extracts.

Based on this study, *EEPS* holds promise as a potential source for the development of anticancer therapeutics. The observed cell shrinkage and membrane blebbing—typical apoptotic hallmarks[45-47]—indicate that *EEPS* exerts its antiproliferative effects on A549 cells potentially through apoptosis induction.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

GC-MS profiling confirmed the presence of 30 bioactive phytoconstituents as evident from the chromatogram Figure 3 and Table 5. Among these, Ethyl (9Z,12Z)-9,12-octadecadienoate was the major component, showing the highest peak area percentage (15.57%). This was followed by 2- Hexadecen-1-ol, 3,7,11,15- tetramethyl-, [R- [R, R-(E)]]- (13.9%), Hexadecanoic acid ethyl ester (12.03%), and Ethyl α-D- glucopyranoside (10.26%).

Ethyl (9Z,12Z)-9,12-Octadecadienoate

Ethyl (9Z,12Z)-9,12-octadecadienoate belongs to the linoleic acid derivatives. It is a polyunsaturated omega-6 fatty acid consisting of 18 carbon atoms with two cis double bonds at the 9- and 12-positions. The compound has a molecular weight of 308 g/mol and is known to exhibit anti-inflammatory and anticancer properties. This phytoconstituent has also been reported as a major metabolite in medicinal plants such

Table 5: Chemical composition and biological activities of identified compounds

S. No	RI	Compound Class	Peak %	Compound Class	Biological activity	References
1	4.136	N, N-Dimethyl-1,3- butadien-1-amine	3.22	Conjugated diene	Not available	
2	4.53	Cyclopent-4-ene-1,3- dione 1-Butanol, 3-methyl-, formate	0.32	Dione	GSK-3 inhibito anticancer,[48] antifungal,[49] effective against gram-positive bacteria[50]	[48,49,50]
3	8.982	1-Butanol, 3-methyl-, formate	7.3	Ester	Flavoring agent	[51]
4.	11.369	N-t-Butyldioxymethyl-N-methylcyclohexylamine	0.7	Cyclohexylamine	Antimalarial activity	[52]
5	11.736	Decanal	1.13	Aldehyde	Antifungal, flavoring agent,[53] antioxidant, antimicrobial[54]	[53,54]
6	11.953	Benzofuran, 2,3-dihydro- (cumaran)	0.59	Heterocyclic compound	Microsomal cytochrome P450 1A2 inhibitor, mPGES-1 Inhibitor.[55] acetylcholinesterase inhibitor,[56] antitubercular, anti- HIV, anticancer, cytotoxic, antiprotozoal[57]	[55,56,57]
7	14.818	Benzenemethanol, 4- hydroxy- (p-hydroxybenzyl alcohol)	1.33	Phenolic compound	*In vivo* and *in vitro* neurological activities,[58] anti-inflammatory, antioxidant, anti- nociceptive activity, neuroprotective effect, inhibitor of tumorangiogenesis and growth[59]	[58.59]
8	15.196	Benzenemethanol, 4-hydroxy-/ (4 formyl phenol)	1.13	Organic aromatic/ aromatic monocyclic compounds	Antineoplastic agent[60]	[60]
9	13.315	Phenol, 4-(ethoxymethyl)-	7.42	Benzyl ethers/phenol	Agonist for human estrogen receptors,[61] biomarker to differentiate bladder cancer from renal cell carcinoma[62]	[61,62]
10	16.87	2,6-Cresotaldehyde	0.53	Aromatic aldehyde	Antimicrobial[63]	[63]
11	20.382	Ethyl α-D-glucopyranoside	10.26	Sugar moiety	Preservative,[64] anticancer,[65] skin homeostasis and moisturizing functions[66]	[64,65,66]
12	20.882	β-D-Glucopyranose, 4-O- β-D-galactopyranosyl-	1.26	Sugar moiety	Treating lactose intolerance and improving gut health[67]	[67]
13	23.359	2 (4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a- trimethyl-, (6S-cis)-	0.59	Heterocyclic/ monoterpenoid lactones	Synonym of Loliolide, antitumor activities and antimicrobial activities	[16]
14	24.562	Neophytadiene	2.8	Alkene and diterpene	Anti-inflammatory, antioxidant, cardioprotective,[68,70] antimicrobial, antibacterial, treatment for headache, rheumatism and skin diseases[69,70]	[68-70]
15	25.293	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol)	0.89	Terpene alcohol	Antimicrobial, anti-inflammatory,[66] anticancer[67]	[71,72]
16	27.154	Hexadecanoic acid, ethyl ester	12.03	Fatty acids and esters	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic flavor, hemolytic, alpha-reductase inhibitor,[14] antimicrobial, reduces risk of coronary heart disease,[18] anticancer[73]	[14,18,73]

(Contd...)

Table 5: *(Continued)*

S. No	RI	Compound Class	Peak %	Compound Class	Biological activity	References
17	28.98	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]- (phytol)	13.9	Terpenoid	Antimicrobial, anti- inflammatory,[71] antidiabetes,[74] anticancer, antiproliferative, apoptosis,[75] immune enhancer[76]	[71,74-76]
18	29.728	Linoleic acid ethyl ester	3.04	Linoleic acid ethyl ester	Hypocholesterolemic, nematicide, antiarthritic, hepatoprotective, antiandrogenic, 5- alpha reductase inhibitor, antihistaminic, anticoronary,	[14]
11	20.382	Ethyl α-D-glucopyranoside	10.26	Sugar moiety	Preservative,[64] anticancer,[65] skin homeostasis and moisturizing functions[66] Insectifuge, antieczemic, antiacne	[64,65,66]
19	29.82	Ethyl (9Z,12Z)-9,12-octadecadienoate	15.57	Linoleic acid	Anti-inflammatory,[77] Anticancer[78]	[77,78]
20	30.232	Octadecanoic acid, ethyl ester	2.67	Stearic acid ester	Antioxidant, anti- inflammatory,[79] antimicrobial,[80] anticancer[73]	[73,79,80]
21	34.579	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	0.88	Amino compound	Hemolytic, pesticide, flavor, antioxidant,[81] anticancer, anti-inflammatory, antimicrobial, diuretic, hepatoprotective, anti-arthritic, Antiasthma[82]	[81,82]
22	35.89	Cyclononasiloxane, octadecamethyl-	0.96	Siloxane	Antifungal[61]	[83]
23	37.985	Benzeneacetaldehyde, α-(phenylmethylene)-	0.6	Aldehyde	Not available	-
24	38.01	Trimethyl [2-(tetramethyl-1,3,2-dioxaborolan-2-yl) ethynyl] silane	0.75	Silane/Boronic acid	Not available	-
25	38.327	Squalene	5.01	Vitamin E compound	Anti-aging, analgesic, antidiabetic, anti- inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, Anticoronary	[84]
26	39.035	(22E)-Stigmasta-4,22-dien-3-ol	2.63	Steroid	Antimicrobial and cytotoxic	[85]
27	39.36	2,2,3,5,6,6,7-heptamethyl [1,4,2,3,5,6,7] dioxapentasilepane	0.23	Long chain fatty acids	Cytotoxicity activity, antimicrobial activity, antioxidant activity	[18]
28	39.405	Eicosyl isopropyl ether	0.85	Long chain fatty acids	Antimicrobial activity	[86]
29	39.47	2-Naphthalenol, 1,2,3,4,5,6,7,8-octahydro-2,5,5-trimethyl-4- methylene-, (±)-	1.01	Naphthalene	Not available	-

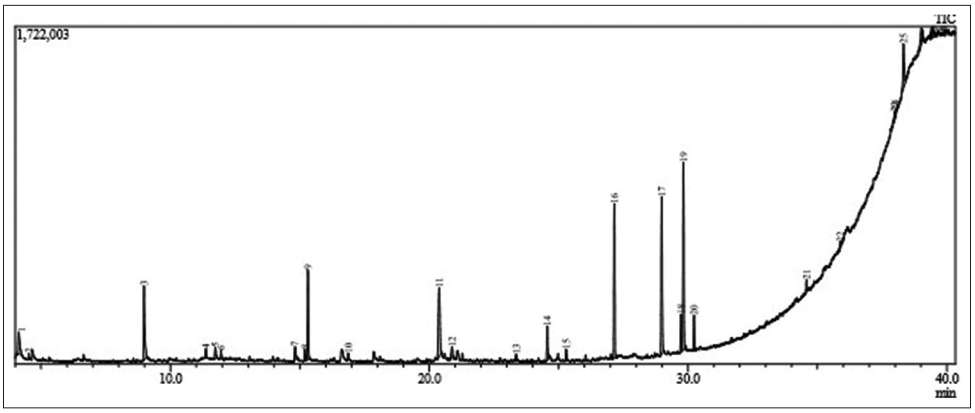

Figure 3: Gas chromatography–mass spectrometry spectrum of ethanolic extract of *Plecospermum spinosum*

as *Typhonium flagelliforme*,[18,78] *Artemisia argyi*,[87] and *Dioscorea* species.[88]

2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl-, [R-[R, R-(E)]]-(Phytol)

Phytol belongs to the terpenoid class. It is an acyclic hydrogenated diterpene alcohol (molecular weight: 296 g/mol). Phytol exhibits multiple pharmacological activities, including antimicrobial, anti-inflammatory, antidiabetic, anticancer (antiproliferative and apoptosis-inducing), and immune- enhancing properties. It has been detected abundantly in medicinal plants such as *Annona reticulata* (anticancer),[89] *Solanum xanthocarpum* (antimicrobial),[71] *Sargassum hystrix* (antidiabetic),[74] *Chlorella vulgaris* (anticancer),[18] and has also been reported in *Juniperus* and *Monteverdia truncate.*[90]

Hexadecanoic Acid Ethyl Ester (Ethyl Palmitate)

Hexadecanoic acid ethyl ester (synonym: Ethyl palmitate) belongs to the group of long-chain fatty acid ethyl esters. It is formed by the condensation of the carboxyl group of palmitic acid with the hydroxyl group of ethanol. This compound has a molecular weight of 284 g/mol and exhibits diverse biological activities, including antioxidant, hypocholesterolemic, nematicidal, pesticidal, antiandrogenic, flavoring, hemolytic, α-reductase inhibitory, and anticancer effects. It has been reported in several medicinal plants, including *T. flagelliforme* (anticancer),[18,78] *Fluggea leucopyrus* Willd. (bioactive constituents),[14] *Cyperus esculentus*, and *Psidium guajava.*[91]

Ethyl α-D-Glucopyranoside

Ethyl α-D-glucopyranoside (synonym: Ethyl hexopyranoside) is a sugar derivative with a molecular weight of 208.21 g/mol. It has been reported to exhibit anticancer activity and functions as a preservative, while also contributing to skin homeostasis and moisturization. Among the identified bioactive compounds, Ethyl α-D-glucopyranoside is notable for its antioxidant and anticancer potential. It is abundantly present in medicinal plants such as *Macrotyloma uniflorum*, *Myxopyrum serratulum* (preservative activity).[65] and *Rumex hastatus* (anticancer activity).[92]

CONCLUSION

The present study demonstrates that the EEPS leaves possesses significant anticancer potential against A549 lung cancer cells. GC-MS analysis revealed 30 bioactive compounds, with major components being ethyl (9Z, 12Z)-9,12-octadecadienoate (15.57%), 2-hexadecen-1-ol, 3,7,11,15-tetramethyl (13.9%), and Hexadecanoic acid ethyl ester (12.03%). The extract exhibited moderate cytotoxic activity with an IC_{50} value of 110 µg/mL and induced apoptotic morphological changes in treated cells.

The presence of diverse phytochemicals including alkaloids, terpenoids, phenols, tannins, flavonoids, and glycosides, along with substantial TPC (83.32 mg GAE/g) and TFC (123.06 mg QE/g), supports the anticancer activity observed. These findings warrant further investigation into the molecular mechanisms of action and *in vivo* studies to develop *P. spinosum* as a potential source of anticancer therapeutics.

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