

Research Article

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# Phytochemical Characterization by GC–MS and Anticancer Potential of *Leucas helianthemifolia* Extracts

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## Abstract

### Keywords

GC-MS, phytochemicals, cytotoxicity, MTT assay, A549, L929, anticancer activity, natural products, DHA methyl ester

A comprehensive GC-MS analysis was carried out to identify the phytochemical constituents of a complex natural extract, revealing 23 bioactive compounds spanning chemical classes such as fatty acids, esters, alcohols, glycosides, hydrocarbons, and phenolics. The predominant compound was *4,7,10,13,16,19-Docosahexaenoic acid, methyl ester*, accounting for 43.45% of the total peak area. To evaluate the biological activity, cytotoxicity was assessed using the MTT assay on A549 (human lung carcinoma) and L929 (mouse fibroblast) cell lines. Three extracts—L(E), S(E), and R(E)—were tested across concentrations ranging from 100 to 500 µg/mL. L(E) showed no cytotoxicity in L929 cells, maintaining over 96% viability, while S(E) exhibited moderate cytotoxicity against A549 cells. R(E) demonstrated significant cytotoxicity in A549 cells with an IC<sub>50</sub> value of 274.54 µg/mL. These results indicate the extract's rich phytochemical composition and its potential for pharmaceutical applications, particularly in anticancer therapy.

## Introduction

Medicinal plants have long been recognized as invaluable resources for the discovery of bioactive compounds due to their diverse chemical composition and wide range of biological activities. With the growing limitations and side effects associated with synthetic drugs, there has been a renewed global interest in plant-

based therapeutics and traditional systems of medicine, including Ayurveda, Siddha, Unani, Homeopathy, and Yoga. These indigenous medical systems continue to play a crucial role in primary health care, particularly in developing countries, owing to their affordability, accessibility, and perceived safety (Dias *et al.*, 2012).

The therapeutic potential of medicinal plants is primarily attributed to the presence of phytochemicals, which are broadly classified into primary and secondary metabolites. Primary metabolites, such as carbohydrates, proteins, lipids, and nucleic acids, are essential for the normal growth, development, and physiological functions of living organisms. These metabolites also serve as precursors for the biosynthesis of secondary metabolites (Harborne, 1998; Croteau *et al.*, 2000).

Secondary metabolites are organic compounds that are not directly involved in the fundamental processes of plant growth and reproduction but play a vital role in plant defense and environmental adaptation. These compounds are synthesized in response to biotic and abiotic stress factors, including herbivory, microbial attack, ultraviolet radiation, and climatic variations. Secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids, and glycosides exhibit a wide range of pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Croteau *et al.*, 2000; Dai & Mumper, 2010).

Among these bioactive compounds, non-enzymatic antioxidants such as polyphenols, flavonoids, carotenoids, hydroxycinnamates, and certain vitamins play a significant role in neutralizing free radicals and preventing oxidative stress-related cellular damage. Both in vitro and in vivo studies have demonstrated the ability of these phytochemicals to protect against oxidative stress-induced disorders, thereby contributing to disease prevention and health promotion (Godwill, 2018; Ghasemzadeh *et al.*, 2011).

Plants belonging to the family Lamiaceae are widely used in traditional medicine systems and are well known for their rich phytochemical profile and broad spectrum of biological activities. Numerous species within this family have been reported to possess strong antioxidant, antimicrobial, and anti-inflammatory properties, which are attributed to the presence of phenolic

compounds, flavonoids, and essential oils (Bakkali *et al.*, 2008).

The efficiency of phytochemical extraction from medicinal plants is strongly influenced by the nature of the solvent used. Polar solvents such as water and ethanol have been reported to be highly effective in extracting a wide range of bioactive compounds. Ethanol is considered a universal solvent due to its ability to dissolve both polar and moderately non-polar compounds, while water, being a natural solvent, efficiently extracts hydrophilic phytochemicals. Several studies have demonstrated that aqueous and ethanolic extracts exhibit superior phytochemical content and biological activity compared to extracts obtained using other solvents (Pandey & Tripathi, 2014; Sharma & Janmeda, 2017).

## Materials and Methods

The plants were collected from Stone House area, Thalayathimund, Ooty, The Nilgiris, Tamil Nadu, India (Lat 11.41349 O, Long 76.711396 O ). The entire plant was taken for the study. The collected materials were washed with water to remove soil and dust. The plant materials were dried in shade for four to five days and chopped into small pieces. The leaves, root and stem were separately kept for shade dry for a week and made them to fine powder. Later the powders of leaves, root and stem were subjected to Soxhlet extraction with different solvents including ethanol and methanol. Different extracts were prepared using 100 g of powdered samples of leaves, root and stem were separately taken in 1000 ml of methanol, and ethanol solvents. Extraction was carried out using Soxhlet extractor at boiling point temperature of methanol, and ethanol solvents for 12 h. Using Whatman filter paper, the extracts were filtered then the filtrate was subjected for drying and redissolved in 50% (v/v) different solvents separately each containing 2.0 mg/ml extract and stored in airtight container.

## GCMS Analysis

The PERKIN ELMER CLARUS SQ8C, were engaged for analysis. The instrument was set as follows, Injector port temperature set to 260° C, Interface temperature set as 270° C, source kept at 200°C. The oven temperature programmed as available, 70° C for 4 mins, 150° C @ 4°C/min, up to 290° C @ 10°C/min. Split ratio set as 1:50 and the injector used was splitless mode. The DB-35 MS Non polar column was used whose dimensions were 0.25 mm OD x 0.25 µm ID x 30 meters length procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 650 Da. The source was maintained at 200°C and <40 mtorr vacuum pressure. The ionization energy was -70eV. The MS was also having inbuilt pre-filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum. NIST4 and WILEY9 each contain more than five million references. Only

those compounds with spectral fit values equal to or greater than 700 were considered positive identification

## MTT Assay

MTT assay is a colorimetric assay used for assessing cell proliferation and cytotoxicity. It is based on the reduction of the yellow, water-soluble tetrazolium dye MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) to insoluble purple formazan crystals by metabolically active cells. This reduction is primarily carried out by mitochondrial dehydrogenase enzymes, not lactate dehydrogenase. The formazan crystals are then solubilized using an appropriate solvent, and the resulting purple color can be quantified by measuring absorbance at 570 nm using a spectrophotometer. The intensity of the color is directly proportional to the number of viable cells. (Mosmann, 1983)

Fig.1 GC-MS Chromatogram of Ethanol extract of LH (leaf)

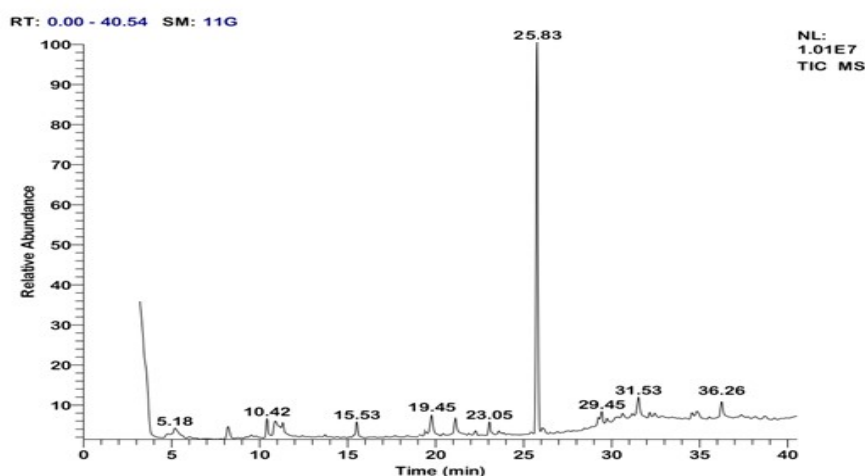
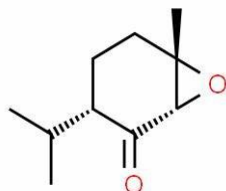
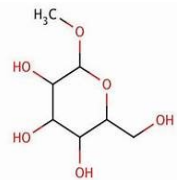
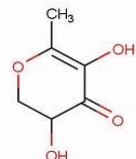
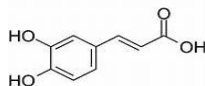

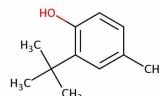
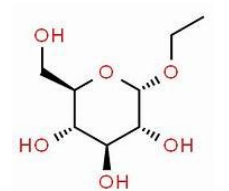
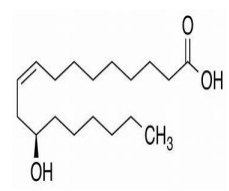
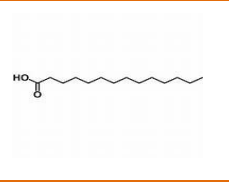
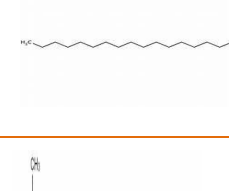
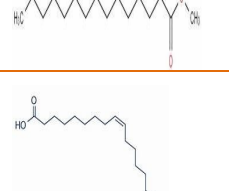

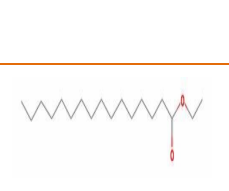
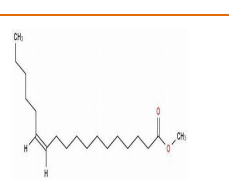

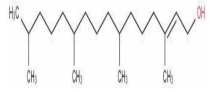
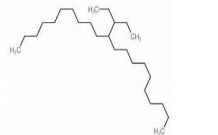
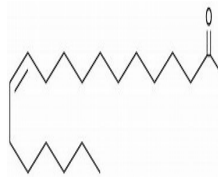
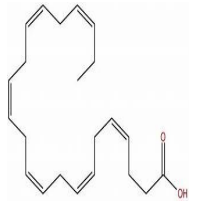
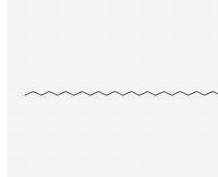
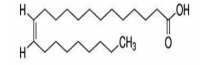
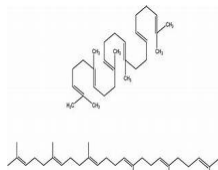


Table1: Phyto-medicinal components identified by GC-MS(Leaf)

S.No	RT	Name of the compound	Molecular formula	Molecular weight	Probability %	Peak area %	Structure of the compound
1	5.18	Glycerin	C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>	92	37.43	3.56	$  \begin{array}{c}  \text{CH}_2\text{—OH} \\    \\  \text{CH —OH} \\    \\  \text{CH}_2\text{—OH}  \end{array}  $
2	6.03	Piperitone oxide	C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>	168	18.43	0.65	
3	8.14	Methyl beta-D-galactopyranoside	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	194	35.73	3.89	
4	9.34	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144	13.46	0.98	
5	10.42	Caffeic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	180	46.71	7.01	
6	11.01	Undec-10-ynoic acid	C <sub>11</sub> H <sub>18</sub> O <sub>2</sub>	182	39.76	8.41	
7	12.21	2-tert-Butyl-4-isopropyl-5-methylphenol	C <sub>14</sub> H <sub>22</sub> O	206	4.53	0.01	

8	13.81	Ethyl α-d-glucopyranoside	$C_8H_{16}O_6$	208	14.73	0.81	
9	15.53	Ricinoleic acid	$C_{18}H_{34}O_3$	298	28.46	6.98	
10	16.35	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	3.49	0.01	
11	17.08	Heptadecane	$C_{17}H_{36}$	240	6.97	0.71	
12	17.65	Pentadecanoic acid methyl ester	$C_{16}H_{32}O_2$	256	8.49	0.83	
13	18.34	Oleic Acid	$C_{18}H_{34}O_2$	282	7.89	0.79	
14	19.05	Eicosanoic acid	$C_{21}H_{42}O_2$	326	5.69	0.34	
15	19.45	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	61.79	7.35	
16	21.15	12-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	53.47	5.86	

17	22.25	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	297	19.46	1.03	
18	23.05	Heneicosane	$C_{21}H_{44}$	296	41.39	3.65	
19	23.54	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	15.76	1.13	
20	25.83	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	$C_{22}H_{32}O_2$	328	75.46	43.45	
21	29.45	Pentacosane	$C_{25}H_{52}$	353	25.46	2.87	
22	31.53	Erucic acid	$C_{22}H_{42}O_2$	339	45.67	6.67	
23	36.26	Squalene	$C_{30}H_{50}$	410	39.47	3.76	

## Chromatographic analysis by GCMS (Stem extract)

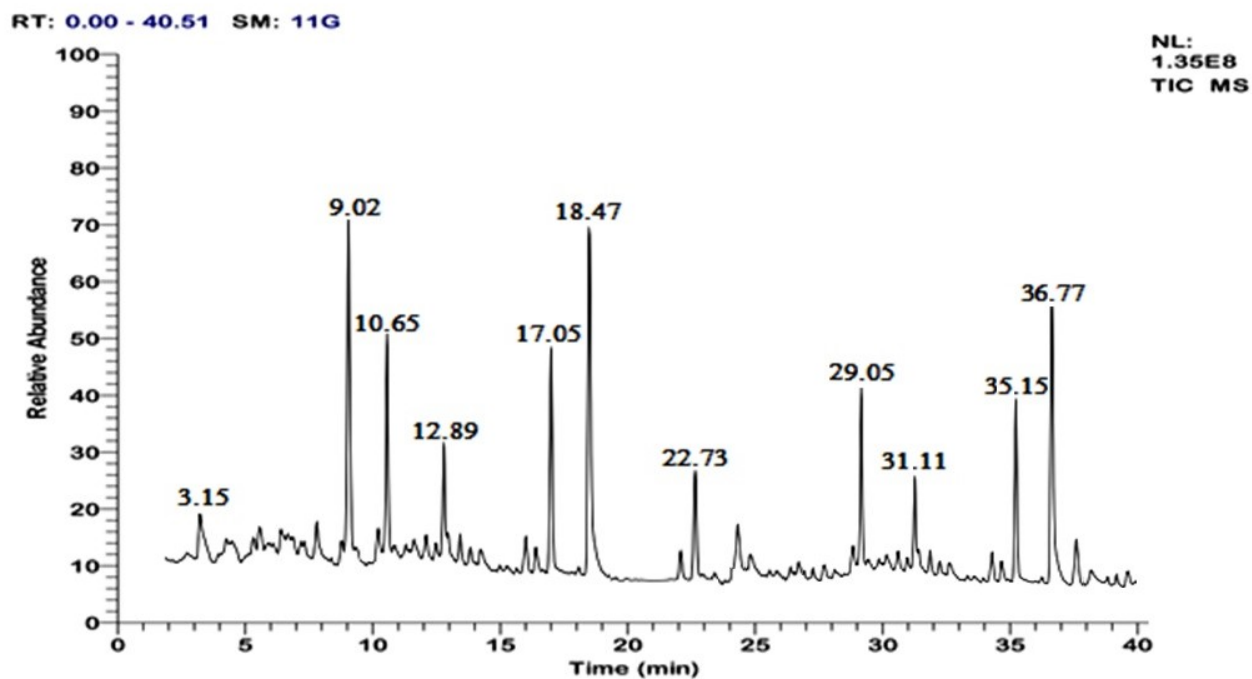
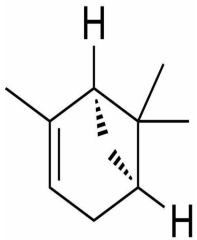
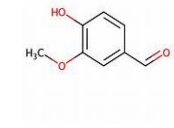
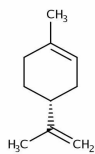
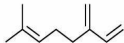
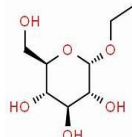
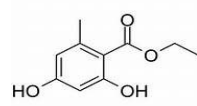
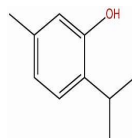
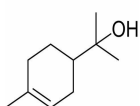
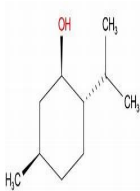
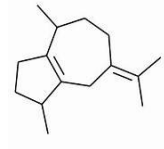


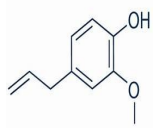

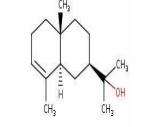
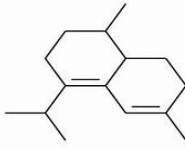
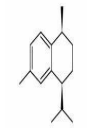
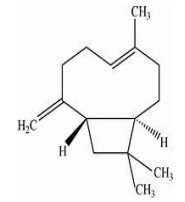
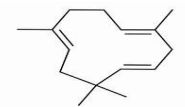
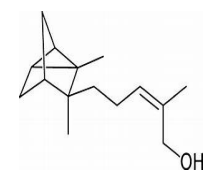
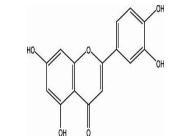
Fig.2 GC-MS Chromatogram of Ethanol extract of Stem LH

Table 2 : Phyto-medicinal components identified by GC-MS

S.no	RT	Name of the compound	Molecular formula	Molecular weight	Probability %	Peak area %	Structure of the compound
1	3.15	$\alpha$ -Pinene	$C_{10}H_{16}$	136	65.45	4.89	
2	7.71	4-hydroxy-3-methoxy-benzaldehyde	$C_8H_8O_3$	152	49.46	3.15	

3	9.02	D-Limonene	$C_{10}H_{16}$	136	89.46	15.4 5	
4	10.65	b-Myrcene	$C_{10}H_{16}$	136	75.78	8.45	
5	12.05	Ethyl α-d-glucopyranoside	$C_8H_{16}O_6$	208	25.69	0.89	
6	12.89	Ethyl 2,4-dihydroxy-6-methylbenzoate	$C_{10}H_{12}O_4$	196	42.49	5.56	
7	13.41	Phenol, 5-methyl-2-(1-methylethyl)-	$C_{10}H_{14}O$	150	25.11	0.56	
8	13.95	E-α-Terpineol	$C_{10}H_{18}O$	154	8.68	0.31	
9	14.21	Levomenthol	$C_{10}H_{20}O$	156	9.36	0.38	
10	16.03	cis-Guaiene	$C_{15}H_{24}$	204	43.78	3.03	



11	16.28	Eugenol	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	164	30.49	0.75	
12	17.05	3,7-Dimethylgeraniol	C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>	168	63.49	7.98	
13	18.47	Alpha-eudesmol	C <sub>15</sub> H <sub>26</sub> O	222	80.46	13.56	
14	22.01	Epizonarene	C <sub>15</sub> H <sub>24</sub>	204	27.49	0.72	
15	22.64	trans-Calamenene	C <sub>15</sub> H <sub>22</sub>	202	6.76	0.01	
16	22.73	β-Caryophyllene	C <sub>15</sub> H <sub>24</sub>	204	46.79	4.46	
17	24.22	α-Humulene	C <sub>15</sub> H <sub>24</sub>	204	36.75	2.49	
18	24.88	α-Santalol	C <sub>15</sub> H <sub>24</sub> O	220	9.15	0.32	
19	25.06	Luteolin	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	286	5.46	0.08	




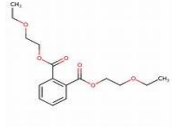
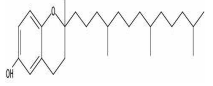
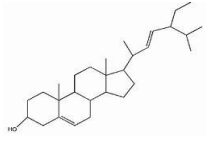
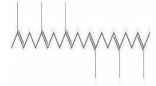
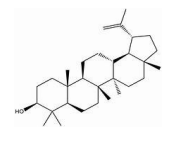
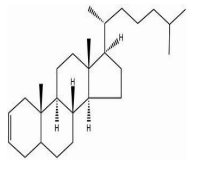
20	26.68	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	4.98	0.05	
21	27.59	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	3.37	0.03	
22	28.03	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	5.79	0.08	
23	28.79	Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	4.89	0.06	
24	29.05	gamma.-Tocopherol	$C_{28}H_{48}O_2$	416	55.79	5.89	
25	31.11	Stigmasterol	$C_{29}H_{48}O$	412	43.79	3.89	
26	35.15	Squalene	$C_{30}H_{50}$	410	60.78	5.12	
27	36.77	Lupeol	$C_{30}H_{50}O$	426	71.39	8.56	
28	37.55	Cholest-2-ene-2-methanol, (5à)-	$C_{30}H_{50}OS$	458	39.75	2.59	

Table 3 L (E) A549

Summary-MTT Assay	
Concentration of sample (µg/ml)	% cell viability
Untreated	100
100	96.96
200	96.70
300	96.65
400	96.57
500	96.48
IC50 value = NA	

Fig 3 Leaf Extract A549

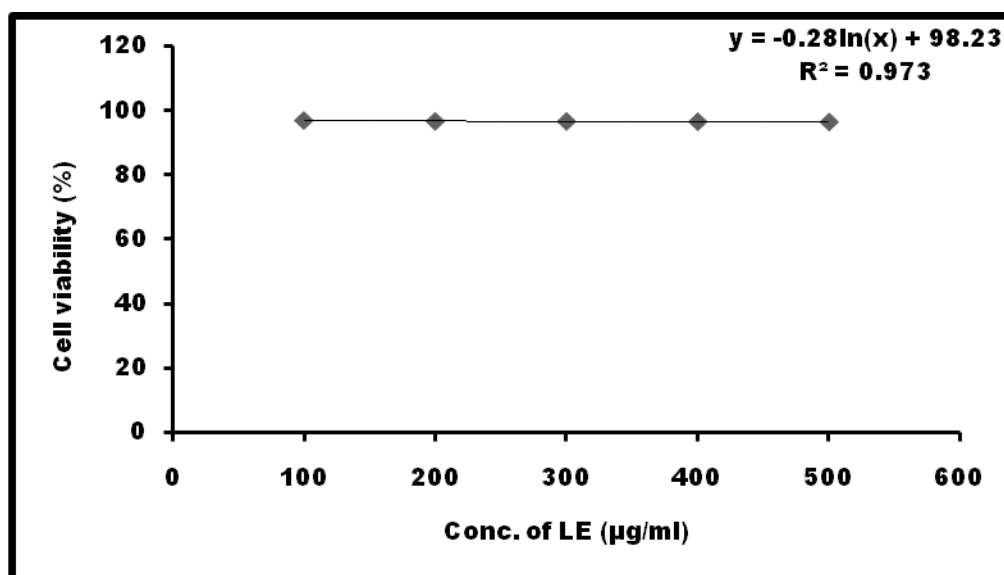


Figure 4 Leaf extract A549

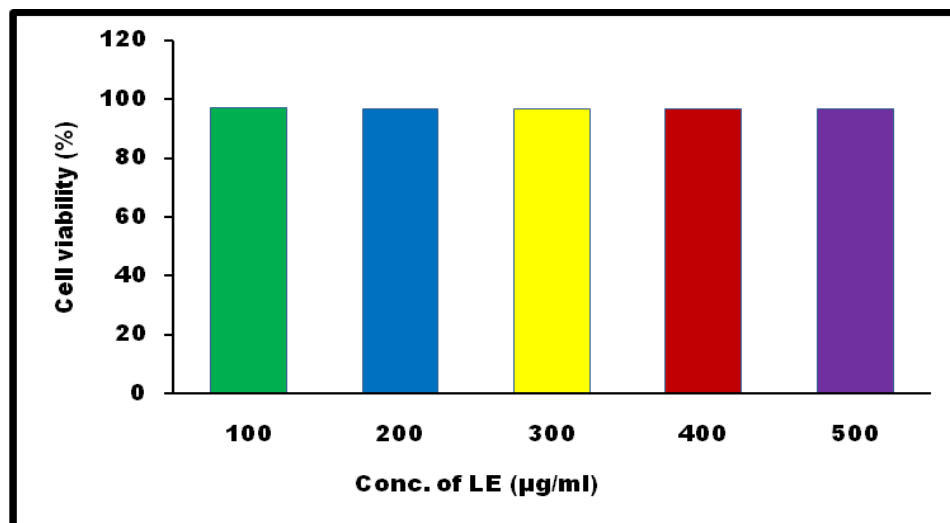


Table 4. Stem Extract – A549

Summary-MTT Assay	
Concentration of sample (µg/ml)	% cell viability
Untreated	100
100	95.91
200	91.82
300	83.65
400	70.56
500	61.57
IC50 value = NA	

Figure 5 Stem Extract – A549

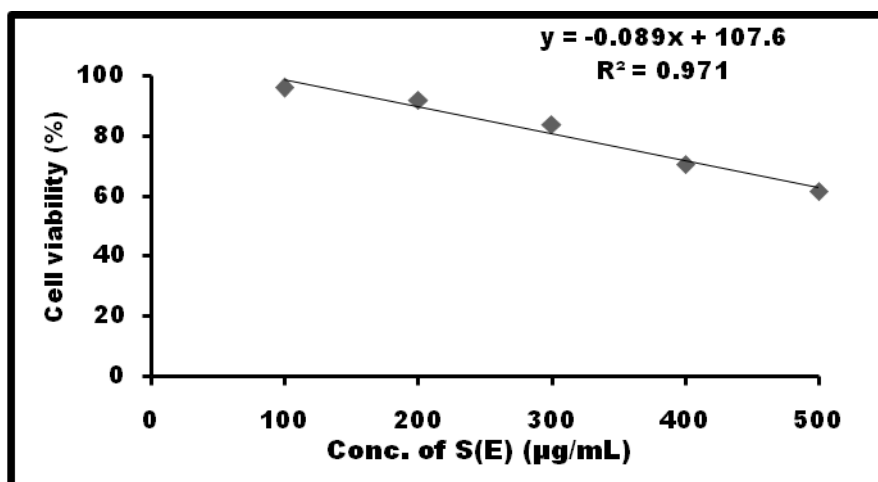


Figure 6 Stem Extract – A549

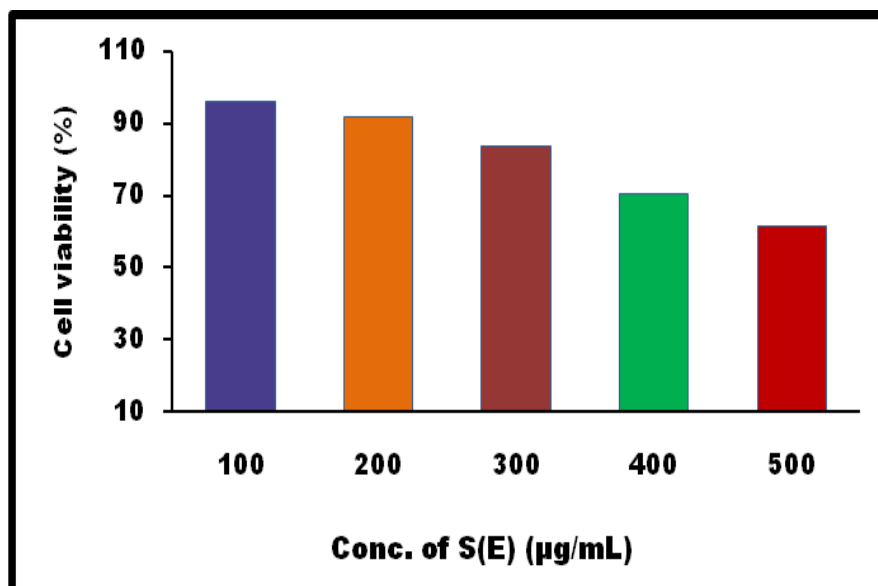


Table- 5 Root extract

Summary-MTT Assay	
Concentration of sample (µg/ml)	% cell viability
Untreated	100
100	83.09
200	67.74
300	54.39
400	42.00
500	12.65
IC50 value = 274.54	

Figure 7 Root extract

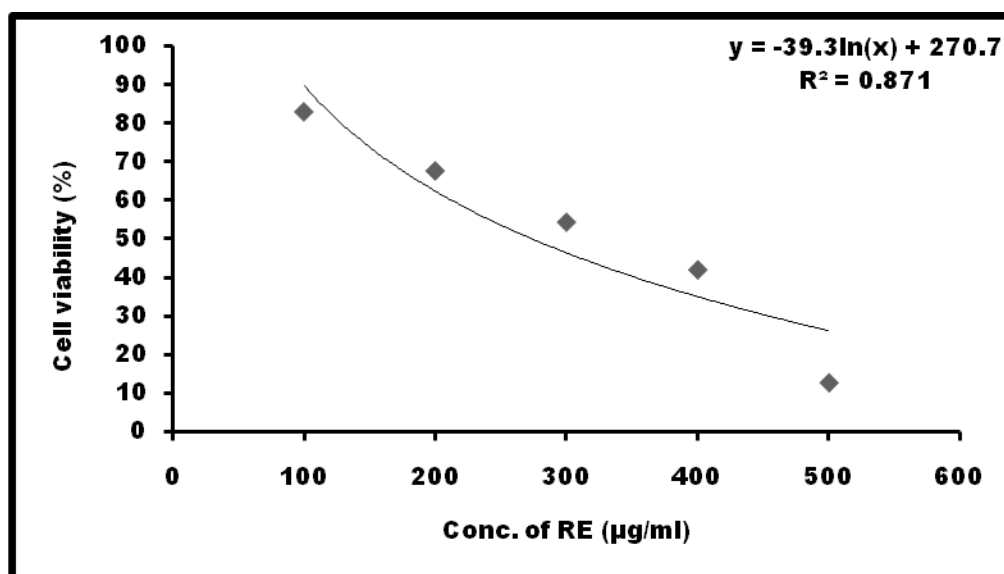
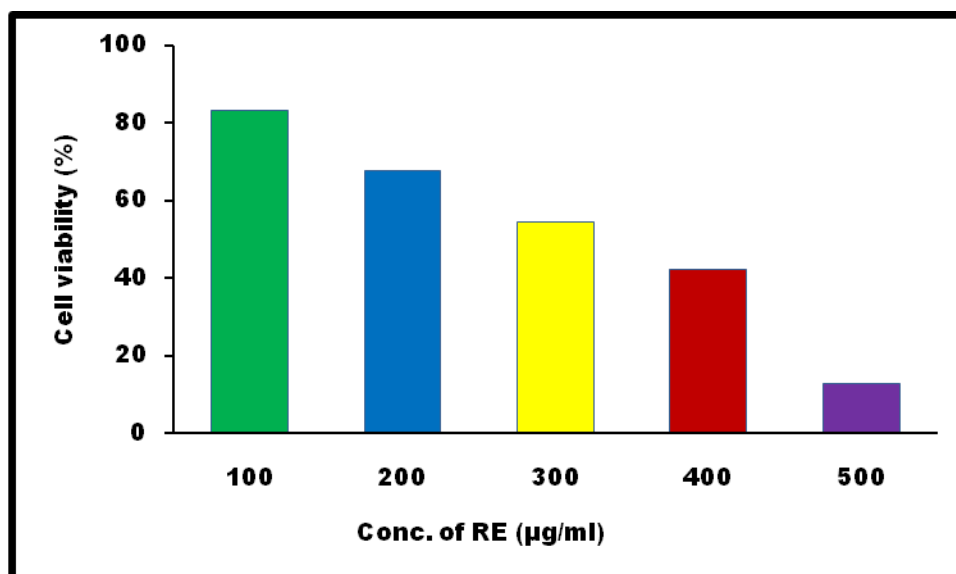


Figure 8 Root extract



## Results

The present study reports the chromatographic analysis by GC-MS of different extracts from the stem and leaves of *L. helianthemifolia*. The stem of *L. helianthemifolia* contains several compounds, including **Glycerin** ( $C_3H_8O_3$ ), which has a low molecular weight of 92 g/mol, a moderate probability of 37.43%, and a peak area of 3.56%. **Piperitone oxide** ( $C_{10}H_{16}O_2$ ) shows a lower presence, with an 18.43% probability and a small peak area of 0.65%. **Methyl beta-D-galactopyranoside** ( $C_7H_{14}O_6$ ) has a 35.73% probability and a slightly higher peak area of 3.89%. The compound **4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-** ( $C_6H_8O_4$ ) is detected at a 13.46% probability and 0.98% peak area.

**Caffeic acid** ( $C_9H_8O_4$ ) is more prominent with a 46.71% probability and a peak area of 7.01%, followed by **Undec-10-ynoic acid** ( $C_{11}H_{18}O_2$ ), which also shows strong presence at a 39.76% probability and 8.41% peak area. In contrast, **2-tert- Butyl- 4-isopropyl 1-5-methylphenol** ( $C_{14}H_{22}O$ ) has minimal presence, with just a 4.53% probability and a negligible peak area of 0.01%. **Ethyl  $\alpha$ -D-glucopyranoside** ( $C_8H_{16}O_6$ ) shows a 14.73% probability and a 0.81% peak area.

**Ricinoleic acid** ( $C_{18}H_{34}O_3$ ) is significant with a 28.46% probability and a 6.98% peak area. **Tetradecanoic acid** ( $C_{14}H_{28}O_2$ ) is almost undetected, with a 3.49% probability and a 0.01% peak area. **Heptadecane** ( $C_{17}H_{36}$ ) appears with a 6.97% probability and a 0.71% peak area, while **Pentadecanoic acid methyl ester** ( $C_{16}H_{32}O_2$ ) shows an 8.49% probability and a 0.83% peak area. **Oleic acid** ( $C_{18}H_{34}O_2$ ) is found at a 7.89% probability and a 0.79% peak area, and **Eicosanoic acid** ( $C_{21}H_{42}O_2$ ) at a 5.69% probability and a 0.34% peak area. **Hexadecanoic acid, ethyl ester** ( $C_{18}H_{36}O_2$ ) is among the most abundant, with a 61.79% probability and a 7.35% peak area. Similarly, **12-Octadecenoic acid,**

**methyl ester** ( $C_{19}H_{36}O_2$ ) has a 53.47% probability and a 5.86% peak area.

**3,7,11,15-Tetramethyl-2-hexadecen-1-ol** ( $C_{20}H_{40}O$ ) shows moderate presence with a 19.46% probability and a 1.03% peak area, while **Heneicosane** ( $C_{21}H_{44}$ ) appears more strongly, with a 41.39% probability and a 3.65% peak area. **Cis-Vaccenic acid** ( $C_{18}H_{34}O_2$ ) is observed at a 15.76% probability and a 1.13% peak area.

The most dominant compound is **4,7,10,13,16,19-Docosahexaenoic acid, methyl ester** ( $C_{22}H_{32}O_2$ ), with the highest values of 75.46% probability and a 43.45% peak area. **Pentacosane** ( $C_{25}H_{52}$ ) shows moderate detection at a 25.46% probability and a 2.87% peak area. **Erucic acid** ( $C_{22}H_{42}O_2$ ) is present at a 45.67% probability and a 6.67% peak area. Finally, **Squalene** ( $C_{30}H_{50}$ ), the heaviest compound, is detected at a 39.47% probability and a 3.76% peak area.

The leaf of *L. helianthemifolia* contains a diverse range of chemical compounds, varying in molecular structure, weight, probability, and peak area. Among the most prominent, **D-Limonene** ( $C_{10}H_{16}$ ) stands out with the highest probability of 89.46% and a peak area of 15.45%, indicating its significant abundance. Similarly, **Alpha-eudesmol** ( $C_{15}H_{26}O$ ) shows a strong presence with an 80.46% probability and a 13.56% peak area, followed by **Lupeol** ( $C_{30}H_{50}O$ ) at 71.39% probability and 8.56% peak area. Other abundant compounds include  **$\beta$ -Myrcene** (75.78%, 8.45%), **3,7-Dimethylgeranial** (63.49%, 7.98%), and **Squalene** (60.78%, 5.12%).

Moderately abundant compounds such as  **$\alpha$ -Pinene** (65.45%, 4.89%), **Gamma-Tocopherol** (55.79%, 5.89%), and **Stigmasterol** (43.79%, 3.89%) also contribute significantly. **Ethyl 2,4-dihydroxy-6-methylbenzoate**, **cis-Guaiene**, and  **$\beta$ -Caryophyllene** show a balanced presence with moderate probabilities and peak areas.

In contrast, several compounds appear in trace amounts, such as **trans-Calamenene** (6.76%, 0.01%), **Tetradecanoic acid** (4.98%, 0.05%), and

**Hexadecanoic acid, ethyl ester** (3.37%, 0.03%). Other compounds like **Levomenthol**, **E- $\alpha$ -Terpineol**, and  **$\alpha$ -Santalol** exhibit low peak areas despite being moderately probable.

High molecular weight compounds such as **Cholest-2-ene-2-methanol** (C<sub>30</sub>H<sub>50</sub>OS) and **Benzenedicarboxylic acid, bis(2-ethylhexyl) ester** have notable presence, though their peak areas remain modest. Overall, the dataset reflects a rich chemical profile, dominated by terpenes and sterols, supported by a range of esters, acids, and aromatic compounds in varying concentrations.

The MTT assay results revealed that the plant extract L(E) exhibited no cytotoxicity on the L929 normal fibroblast cell line, maintaining over 96% cell viability across all tested concentrations (100–500  $\mu$ g/mL), indicating its biocompatibility. In contrast, the S(E) extract showed moderate, dose-dependent cytotoxicity on A549 lung cancer cells, with viability decreasing from 95.91% to 61.57%, though the IC<sub>50</sub> was not reached within the tested range. Notably, the R(E) extract demonstrated significant cytotoxicity against A549 cells, reducing viability to as low as 12.65% at 500  $\mu$ g/mL, with an IC<sub>50</sub> value calculated at 274.54  $\mu$ g/mL. These findings suggest that while L(E) appears safe for normal cells, R(E) may possess potent anticancer properties worthy of further investigation.

## Discussion

The phytochemical profile obtained from the present GC–MS analysis of *Leucas helianthemifolia* shows strong agreement with earlier reports on species belonging to the genus *Leucas*. Previous studies have consistently documented the presence of bioactive terpenes such as limonene,  $\alpha$ -pinene, and  $\beta$ -myrcene, along with sterols including stigmasterol and  $\beta$ -sitosterol, and triterpenoids such as lupeol and squalene in different *Leucas* species (Karthikeyan *et al.*, 2014; Anandakumar *et al.*, 2017; Rajkumar *et al.*, 2020). The recurrence of these compounds across the genus supports their chemotaxonomic relevance and validates the reliability of the present findings.

In addition, fatty acid derivatives such as oleic acid, linoleic acid, and hexadecanoic acid esters identified in the present study have also been reported from *Leucas aspera* and *Leucas zeylanica* (Sathishkumar *et al.*, 2015; Devi *et al.*, 2021). These lipid-based compounds are known to contribute to membrane modulation, anti-inflammatory effects, and cytotoxic properties, thereby enhancing the pharmacological significance of the genus. The detection of caffeic acid and tocopherol further corroborates earlier reports highlighting the antioxidant potential of *Leucas* extracts, as these compounds are well-known free radical scavengers and cellular protectants.

Comparative analysis of plant parts revealed distinct variations in phytochemical distribution. While the stem extract of *L. helianthemifolia* was predominantly enriched with fatty acids and their derivatives, the leaf extract showed a higher abundance of terpenes and sterols. Such organ-specific chemical variation has been widely reported in medicinal plants and is often associated with differential biological activities (Rajkumar *et al.*, 2020). This pattern, which closely resembles that observed in other *Leucas* species, suggests that different plant parts may contribute uniquely to the therapeutic properties traditionally attributed to the genus.

Anticancer activity within the family Lamiaceae has been extensively documented, with numerous species demonstrating cytotoxic effects against various human cancer cell lines. In the present investigation, the methanolic leaf extract of *L. helianthemifolia* exhibited significant, dose-dependent cytotoxicity against A549 human lung cancer cells, with IC<sub>50</sub> values of 39.7  $\mu$ g/mL at 24 h and 33.6  $\mu$ g/mL at 48 h. This level of activity is notably stronger when compared to several other Lamiaceae members. For example, *Origanum compactum* and *Salvia officinalis* were reported to exhibit weaker cytotoxic effects, with IC<sub>50</sub> values of  $198 \pm 12$   $\mu$ g/mL and  $235 \pm 1$   $\mu$ g/mL, respectively (Chaouki *et al.*, 2015). Similarly, essential oils of *Mentha piperita*, *M. pulegium*, *Lavandula angustifolia*, and *Salvia lavandulifolia*

showed comparatively higher IC<sub>50</sub> values against cancer cells (Miller et al., 2018; Donadu et al., 2017; Pérez-González et al., 2019).

The pronounced cytotoxicity of *L. helianthemifolia* leaf extract suggests the presence of potent bioactive constituents, possibly acting synergistically. Importantly, the L(E) fraction demonstrated excellent selectivity, maintaining more than 96% viability in normal L929 fibroblast cells, thereby indicating favorable biocompatibility. In contrast, the R(E) extract exhibited weaker anticancer activity with an IC<sub>50</sub> value of 274.54 µg/mL, although it still caused a notable reduction in cancer cell viability at higher concentrations. The observed differential responses between extracts highlight the influence of phytochemical composition on biological activity and suggest that *L. helianthemifolia* harbors unique compounds capable of selectively targeting cancer cells.

Overall, the combined phytochemical and biological evidence strongly supports the therapeutic potential of *Leucas helianthemifolia*. Its potent and selective anticancer activity, coupled with a rich phytochemical profile, underscores its promise as a valuable natural source for the development of novel anticancer agents.

## Conclusion

The phytochemical analysis revealed that the leaf extract of *Leucas helianthemifolia* contained a richer and more diverse profile of bioactive compounds compared to the stem, with terpenes and triterpenoids such as D-limonene, alpha-eudesmol, and lupeol being especially dominant. These compounds are well known for their anticancer and antioxidant properties, which may contribute to the observed biological activity. Consistently, the leaf extract exhibited stronger and more selective cytotoxic effects against lung cancer cells while remaining non-toxic to normal fibroblast cells, whereas the stem extract showed comparatively weaker activity. Overall, the leaf

extract emerged as the more promising source of phytochemicals and anticancer potential within the plant.

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