Bioactive Compounds of *Schefflera stellata* (Geartn.) Baill. Leaf Methanolic Extract and their Cytotoxic Effect on Lung cancer Cell Line (A549)

Rajarajeshwari Ramakrishnan¹,², Selvankumar Thangaswamy¹,*, Mythili Gnanamangai Balasubramanian², Mohanraj Rajamanickam³, Naresh Srinivasan³, Dhana Sekaran Ganesan⁴

¹Department of Biotechnology, Mahendra Arts and Science College (Autonomous), Namakkal, Tamil Nadu, INDIA.
²Department of Biotechnology, Sengunthar Arts and Science College, Tiruchengode, Namakkal, Tamil Nadu, INDIA.
³Research Department of Biotechnology, K.S.Rangasamy College of Technology, Tiruchengode, Namakkal, Tamil Nadu, INDIA.
⁴Department of Botany, Sengunthar Arts and Science College, Tiruchengode, Namakkal, Tamil Nadu, INDIA.

**ABSTRACT**

**Objectives:** The present study was focused on the fractionation and isolation of biologically active plant secondary metabolites from methanol leaf extract of *Schefflera stellata* (Geartn.) Baill. and their cytotoxic effect were evaluated by performing an MTT assay over the Lung cancer cell line (A549). **Materials and Methods:** Bioactive molecules were fractioned and purified using Column and TLC. Purified constituents exhibit its antioxidant property by dot plot assay using DPPH method. The cytotoxic activity of the purified compound was evaluated by MTT assay. **Results:** Extraction, isolation, separation of bioactive molecules from methanol leaf extract of *Schefflera stellata* (Geartn.) Baill. Using silica column chromatography. 32 fractions were obtained in column and subjected to TLC and dot plot assay by DPPH method. Out of 32 fractions, 2 fractions (D4 and D5) revealed the presence of antioxidant property and were characterized by GC-MS. D5 exhibit bioactive compound, which were taken for cytotoxic studies against lung cancer cell line by MTT assay, the cell viability was found to decrease with increasing concentration (50, 100, 150, 200, 250 µg/ml) with an IC₅₀ concentration of 150 µg/ml. **Conclusion:** The present study proves that the purified D5 compound of *S. stellata* (Geartn.) Baill. has the natural source of antioxidant, which possess the strong cytotoxic activity against lung cancer line (A549). **Key words:** *Schefflera stellata*, GC-MS, Cytotoxic activity, Dot plot assay, DPPH, Lung Cancer.

**INTRODUCTION**

Cancer is abnormal cell growth, incursion of other tissues and dissemination to other sites in an unregulated way without regarding the body’s need. In recent days, one in six people is dying due to cancer all over the world. The risk factors, that depends upon genetic constitutions, lifestyle and environmental conditions, such as food habits, exposure to carcinogenic chemicals. According to a WHO report, premature death by non-communicable disease was estimated at 63% of all deaths and cancer was one of the prime causes (9%). Among males Lung, mouth, oesophagus and stomach were most regular and in the female breast, cervix uteri are the most regular sites of cancer. One in 68 males is affected by lung cancer, which is one of the common cancer in the year 2020 for males.² Lung cancer is mainly because of cigarette smoking, exhibit to toxins or inhaled chemicals can rapidly increase the risk. Current treatments encompass chemotherapy, radiotherapy and synthetic drugs. Treatments such as chemotherapy can put sufferer under a lot
of stress and further health damage on their long term use. Three so there is necessary in identifying the molecules for the treatment of cancer with low price, high efficient curing and with less side effects. Among natural products, plants have played a central role in treating several diseases including cancer. The complete variety and number of plants with medicinal properties around the world are entirely surprising. It's evaluated that around 70,000 plant species from lichens to tall trees have been used at one time or another for medicinal reason. The use of secondary metabolites from plant origin could be an benefit and the finest solution to narrow down the use of deleterious products. Schefflera is the largest and geographically most extensive genus in the Araliaceae. According to different estimates, it includes from 400 to 650-700 species. It is found in most tropical and subtropical regions but for the most part in Southeast Asia, Madagascar, New Caledonia, the Andes and the Guyana Highlands. Schefflera stellata (Geartn.) Baill. (=Heptapleurum stellatum Gaertn.) is broadly spread in peninsular India and Sri Lanka also in Kerala state of southern India, it is found in Silent valley and Agasthyamalai Hills. The previous study reveals the presence of secondary metabolites and bioactive compounds present in methanol leaf extract of Schefflera stellata, which also shows superior result in antioxidant studies which can be a promising source to inhibit cancer effects. Hence the present study is to emphasize the novel approach and unique properties of the purified compounds that were isolated from methanol leaf extract of Schefflera stellata (Geartn.) Baill. by using GC-MS. The cell viability and the cytotoxicity activity of the purified compounds were evaluated by performing an MTT assay with the Lung cancer cell line (A549) and proved that around 70,000 plant species from lichens to tall trees have been used at one time or another for medicinal reason. The use of secondary metabolites from plant origin could be an benefit and the finest solution to narrow down the use of deleterious products. Among natural products, plants have played a central role in treating several diseases including cancer. The complete variety and number of plants with medicinal properties around the world are entirely surprising. It's evaluated that around 70,000 plant species from lichens to tall trees have been used at one time or another for medicinal reason. The use of secondary metabolites from plant origin could be an benefit and the finest solution to narrow down the use of deleterious products. Schefflera is the largest and geographically most extensive genus in the Araliaceae. According to different estimates, it includes from 400 to 650-700 species. It is found in most tropical and subtropical regions but for the most part in Southeast Asia, Madagascar, New Caledonia, the Andes and the Guyana Highlands. Schefflera stellata (Geartn.) Baill. (=Heptapleurum stellatum Gaertn.) is broadly spread in peninsular India and Sri Lanka also in Kerala state of southern India, it is found in Silent valley and Agasthyamalai Hills. The previous study reveals the presence of secondary metabolites and bioactive compounds present in methanol leaf extract of Schefflera stellata, which also shows superior result in antioxidant studies which can be a promising source to inhibit cancer effects. Hence the present study is to emphasize the novel approach and unique properties of the purified compounds that were isolated from methanol leaf extract of Schefflera stellata (Geartn.) Baill. by using GC-MS. The cell viability and the cytotoxicity activity of the purified compounds were evaluated by performing an MTT assay with the Lung cancer cell line (A549) and proved that the compounds are suitable for pharmacological importance.

MATERIALS AND METHODS

Collection of Plant Materials

The plant samples were collected from Tiruchengode, Namakkal Dr. The specimens were preserved and authenticated by the Botanical Survey of India – Southern Circle, Coimbatore.

Preparation of Plant Extract

The plant leaf was shadow dried to remove water content from the plant resulting in a powder. As per the ratio, the powdered extract was extracted by taking 10 g of leaf powder in 100 ml of methanol. The mixture was kept in a rotary shaker for 24-48 hr. The cold extracted sample was poured into the petri dish and allowed for drying for about 2 weeks. After evaporation, the dried powder was scraped, weighed and purified by chromatographic techniques.

Purification of the Bioactive Molecule

Gradient solvent system

Gradient solvent system (non-polar to the high polar solvent system) provides the best elution and best separation of various organic compounds from any plant-based organic extract. Table 1 summarizes

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvent system</th>
<th>Ratio (ml)</th>
<th>Volume (ml)</th>
<th>Fraction</th>
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</tr>
<tr>
<td>2.</td>
<td>Hexane: Chloroform</td>
<td>95:5</td>
<td>40</td>
<td>B1</td>
</tr>
<tr>
<td>3.</td>
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<td>90:10</td>
<td>40</td>
<td>B2</td>
</tr>
<tr>
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<td>85:15</td>
<td>40</td>
<td>B3</td>
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<td>80:20</td>
<td>40</td>
<td>B4</td>
</tr>
<tr>
<td>6.</td>
<td>Hexane: Chloroform</td>
<td>75:25</td>
<td>40</td>
<td>B5</td>
</tr>
<tr>
<td>7.</td>
<td>Hexane: Chloroform</td>
<td>70:30</td>
<td>40</td>
<td>B6</td>
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<td>8.</td>
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<td>D7</td>
</tr>
<tr>
<td>29.</td>
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<td>40</td>
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</tr>
<tr>
<td>32.</td>
<td>Water</td>
<td>100</td>
<td>40</td>
<td>F1</td>
</tr>
</tbody>
</table>
the ratio of gradient solvent to be used in column chromatography.\textsuperscript{10}

**Column Chromatography**

A cylinder-shaped glass column containing stationary phase (silica gel 60-120 mesh) is encountered slowly from the top with a liquid solvent (mobile phase) that flows down the column with the help of gravity or external pressure applied. This technique is used for the purification of compounds from a mixture. Once the column is ready, the sample is loaded inside the top of the column. The mobile solvent is then allowed to flow down through the column. The compounds in the mixture have different interactions ability with the stationary phase, and mobile phase, thereby will flow along with the mobile phase at different time intervals or degrees. In this way, the separation of compounds from the mixture is achieved. The individual compounds are collected as fractions and analyzed further for structure elucidation.

A suitable size long cylindrical glass column (based on the amount of the sample) should be stand firm on a column-chromatography stand. Completely dried plant extract sample should be mixed with silica gel to make a fine powdered form for easy distribution of the sample in an already packed silica gel column. Sample powdered mass should be placed on the top of the pre-packed silica column and the sample should be covered with a layer of cotton. Then solvents of different polarities were passed through the column at the uniform rate under gravity to fractionate the sample extract. Each fraction was collected separately in a test tube and numbered consecutively for further analysis on thin-layer chromatography.

**Thin Layer Chromatography**

Thin layer chromatography provides partial separation of both organic and inorganic materials using thin-layered chromatographic plates especially useful for checking the purity of fractions. The plates were prepared with silica gel (Himedia). Each fraction is applied on activated TLC plates with the help of a capillary tube at a 1/2 inch apart from the lower edge of the TLC plate, and the plate is kept in a developing chamber containing a suitable solvent system for the specific time until the developing solvent reaches the top of the upper edge of TLC plate. The plate is taken out from the developing chamber, dried and the solvent front is marked by a lead pencil. Compound bands/spots visualized on TLC chromatogram can be detected by visual detection, under UV light (254 nm), in an iodine chamber for the presence of specific compounds. The visualized spots of the components in the chromatogram are marked and the Rf value (Rf-Retention factor), of each spot, is calculated by the formula:\textsuperscript{11}

\[ R_f = \frac{\text{Distance travel by solute (cm)}}{\text{Distance travel by solvent (cm)}} \]

**Determination of Antioxidant Activity**

**Dot-blot rapid screening assay**

The rapid screening assay was performed by the standard method. Aliquots of plant extracts were spotted carefully on TLC plates and dried for 3 min. The sheets bearing the dry spots were placed upside down for 10 sec in a 0.4 mM DPPH solution in methanol and the layer was dried. The stained silica layer revealed a purple background with yellow spots, which showed radical scavenging capacity. The ability of the plant extracts to scavenge the DPPH radical was tested in rapid dot-blot screening and quantified using a spectrophotometer.\textsuperscript{12}

**GCMS Analysis**

GC-MS is a technique where compounds can be identified by their mass spectral patterns and GC retention indices. The 2 purified compound of *S.stellata* (Geartn.) Baill. (D4 and D5) were performed on GC-MS equipment (PerkinElmerGC model: Clarus 680; Mass Spectrometer: Clarus 600). This method is used for identifying volatile compounds in complex mixtures. The extraneous mass spectral peaks commonly arise from co-eluting compounds, column bead, and ion-chamber contaminants. The height of the peak determines the compounds present in the sample. The high peak represents the highest amount of compound present in the given sample. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μmdf) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1μL of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min); followed by 300°C at the rate of 10°C min\(^{-1}\); and 300°C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of the spectrum of known compounds stored in the GC-MS NIST (2008) library.
Mass Condition (EI)
Solvent Delay: 2.00 min; Transfer Temp: 240°C; Source Temp: 240°C; Scan: 50 to 600 Da

Cytotoxicity Activity in Human Lung Cancer Cell Line

Cancer cell line and chemicals
Lung cancer line (A549) was obtained from NCCS, Pune, India. Dulbecco’s modified eagle medium (DMEM), Trypsin-EDTA, Fetal Bovine Serum (FBS), 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide (MTT), sodium bicarbonate, Dimethyl sulphoxide (DMSO) and antibiotic solution were purchased from Hi media laboratories, Mumbai, India. 96 well plates, 6 well plates, Tissue culture flasks (25 and 75 mm²), Centrifuge tubes (15 and 50 ml) were purchased from Tarsons products Pvt, Kolkata, India. Chemicals used in the present study were highest quality available locally.

Cell Viability Assay
The cell viability was assessed by MTT assay.\(^{13}\) Briefly, A549 cells (5×10³ cells/ml) were plated in 96 well plates with DMEM medium containing 10% FBS. The cells were incubated for 24 hr under 5% CO\(_2\), 95 % O\(_2\) at 37°C. The medium was removed and washed with PBS. The control cells received serum free medium and treatment cells received 50, 100, 150, 200, 250 μg/ml of the purified compound (D5) along with medium. Then the cultures plates were again incubated, after 24 hr, 10 μl of MTT stock solution was added to each well and the cultures were further incubated for 3 hr and the supernatant was decanted. 100 μl DMSO was added, the formed crystal was dissolved gently by pipetting 2 to 3 times. An absorbance at 570 nm was read at micro plate reader. Growth inhibition rate was calculated as follows:

\[
\text{Percentage of Growth inhibition} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100
\]

Ethical Statement
Not applicable.

Statistical Analysis
The data obtained in the present study were subjected to standard statistical analysis by using Microsoft excel data to find out the Standard deviations. Hypothesis testing method is one way analysis of variance (ANOVA) followed by least significant difference (LSD) test and p values of less than 0.01 were considered to indicate statistical importance. All these results were expressed as mean ± S.D.

RESULTS

Chromatographic Separation and Purification
The methanolic leaf extract of Schefflera stellata (Geartn.) Baill. were dried, powdered and the sample was subjected to chromatographic separation and purification. About 20 g of the extract was subjected to column chromatography on silica gel (100-200 mesh) pack shown in Figure 1. The selection of solvent in a systematic order proves the effect of polarity on the extraction. 32 fractions were obtained in the column chromatography were shown in Table 1. 32 fractions obtained from column chromatography were subjected to TLC. Out of 32 fractions, 2 fractions (D4 and D5) revealed the presence of bioactive compounds with the Rf value of D4 - 0.44, 0.54, 0.64, 0.71 and D5 - 0.76 were shown in Figure 2.

Cell morphological study
The general cell morphological changes of purified D5 compound of Schefflera stellata (Geartn.) Baill. treated with A549 cell line was observed under light microscopic visualization. The A549 (5×10⁴ cells/ml) were plated in 6 well plates with DMEM medium containing 10% FBS and was maintained as above. The medium was removed and the control dishes received again fresh medium and the treatment dishes received 50, 150 and 250μg/ml of D5 compound. The culture plates were incubated as above. After the incubation time the cells were visualized and photographed under inverted light microscope (Kyowa) at 20X magnification.

Figure 1: Column chromatography of Schefflera stellata (Geartn.) Baill. methanol leaf extract.
**Dot-blot Assay**

The main property of an antioxidant are, the ability to trap free radicals. The dot-blot assay was done for 32 fractions obtained from column chromatography, the samples were loaded (2µl) in TLC plate. The plate was dipped in DPPH and it was dried. The yellow colour zone indicates the presence of the anti-oxidant property. Out of 32 fractions, 2 fractions (D4 and D5) exhibit a yellow zone around the loaded sample, shown in Figure 3. The intensity of yellow colour depends upon the amount and rate of radical scavenger present in the fractioned sample. The remaining fractions exhibit light yellow shadow, which indicates the fractions don’t have radical scavenging effects. Free radicals play a vital role in the development of tissue damage in pathological events.

**GC-MS Analysis**

The purified fractions (D4 and D5) were subjected to GC-MS analysis and the chromatogram reveals the presence of bioactive constituents were shown in Figure 4 and 5. It is evident from Figure 6 and 9 that
two fractions have a complex chemical composition. Six compounds were identified in *S. stellata* (Geartn.) Baill. leaves by GC-MS analysis. The active principles with their Retention Time (Rt), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 2 and 3. The prevailing compounds are 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester 22.64 (100 %), 1,2-benzenedicarboxylic acid, ditridecyl ester 22.98 (6.728 %), Tetratriacontane 24.96 (12.975 %), Nonacosane 26.23 (24.895 %), 11-methyl-13-tetradecane-1-ol-acetate 27.59 (6.657 %), Silicic acid, diethyl bis (Trimethylsilyl) ester 31.18 (5.704 %). The investigation concluded that the stronger extraction capacity of methanol could have been produced the number of active constituents responsible for many biological activities. So these might be utilized for the development of traditional medicines which may create a new way to treat many untreatable diseases.

Plants extracts are an prime source of potentially useful compounds for the establishment of new anticancer drugs. The plant samples were given for lung cancer cell lines and their morphological and percentage cell inhibition were noticed. The result shows that the viability of the cells fall off based on the dosage level.

**Cytotoxic Activity in Human Lung Cancer Cell Line**

The cytotoxic effects of the purified D5 compound of *S. stellata* were studied using a lung cancer cell line. The results were identified for five different concentration such as 50, 100, 150, 200 and 250µg/ml. Among these concentrations, the IC$_{50}$ value is obtained at the concentration of 150µg/ml.

The test samples were given to the cell lines based on dosage level. There were decreased level of viable cells from the lower concentration to a higher concentration. The morphological results of the cell viability for different concentrations are depicted in Figure 8. This result shows that the viability of the cells decreases gradually in increasing concentrations. The percentage of cell viability for different concentrations of test sample were shown in Figure 8. In 250µg/ml of concentration, the viability of cells decreases higher than the concentration of 50µg/ml. The IC$_{50}$ value of 59.82±1.60 is determined at a concentration of 150µg/ml and the R$^2$ value was found to be as 0.829. Also the determination of percentage cell viability for purified D5 bioactive compounds isolated of *Schefflera stellata* (Geartn.) Baill. (D5) on A549 cell line, results are given as statistically significance at $p < 0.01^*$ between

![Figure 7: Morphological results for purified bioactive compound isolated from methanol leaf extract of *Schefflera stellata* (Geartn.) Baill. D5 on A549 cells. Scale bar 20X. (A) Control Cells; (B) Cells treated with 50µg/ml of D5 compound; (C) IC$_{50}$ at 150µg/ml of D5 compound (D) Cells treated with 250µg/ml of D5 compound.](image-url)
control cells Vs D5 compound treated cells. The current study reveals that the D5 purified compound exhibit its cytotoxic effect against lung cancer cell line, where the viability of the cancer cells had decreased depending on dosage level.

**Morphological Changes of A549**

The morphological observations of A549, lung cancer cell line were observed under light microscope. The control cells showed normal with epithelial irregular confluent aggregates seen with polygonal shape as well as clear nucleus at center of the cell (Figure 7A). The treatment of purified D5 compound shows the polygonal cells that tend to shrink and appeared spherical in shape, loss of colony formation and cytoplasmic blebbing were observed. The cell shrinkage were increased progressively due to increase in concentration (Figure 7 B, C and D), which shows the cytotoxic effect on A549 cell line.

**DISCUSSION**

Cancer is becoming a remarkable disease in a developed and other cancer treatments pre-exist. However, current methods such as chemotherapy have their constraint due to their toxic effects on non-targeted tissues develop human health problems. Therefore, there is a demand for alternative treatments with conventionally derived anticancer agents with plants being the preferred source. The secondary metabolites in the plant division have been studied for their inherent use as anticancer agents. Plant-derived anticancer agents are strong inhibitors of cancer cells lines, making them in high stipulation. The utilization of these agents needs to be managed to keep up with demands and be endurable.

In the present study, the plant samples were authenticated as Heptapleurum stellatum (Gaertn.) Baill. (Araliaceae) or Schefflera stellata (Geartn.) Baill. BSI/SRC/5/23/2016/Tech./850 by the Botanical Survey of India – Southern Circle, Coimbatore. Out of 32 fractions, 2 fractions (D4 and D5) revealed the presence of bioactive compounds effectively with its Rf value. Similarly, the chromatographic separation and purification of the cold methanol extracts of leaf of Dillenia indica provided a total of 4 compounds, such as 3,5,7-trihydroxy-3',4'-dimethoxy flavone, Betulinic acid, β-sitosterol, Stigmasterol.

Dot plot assay were performed for 32 fractions, in which D4 and D5 shows zone of inhibition which indicates its anti oxidant property. In previous report the methanol and water leaf extract of Capparis cartilaginea Decne (Capparaceae) revealed a yellow-coloured spot when stained with DPPH solution. All dots at concentration of 2.0 mg/ml, 1.0 mg/ml 0.5 mg/ml and 0.25 mg/ml showed higher scavenging activity, exception dots 0.25 mg/ml, 0.125 mg/ml (water extract) and 0.125 (methanol extract) showed weak scavenging activity in dot blot assay.

GC-MS were performed for purified D4 and D5 compounds which reveals the presence of bioactive compounds. In the previous report, the volatile oils from the roots, stem and leaves of Schefflera stellata (Gaertn.) Harms were isolated by hydrodistillation and distinguished by analytical gas chromatography and gas chromatography-mass spectroscopy. Sixty-nine (98.3%), seventy-eight (97.9%) and sixty-seven (98.0%) constituents were picked out from the root, stem and leaf oils, respectively. Sesquiterpene hydrocarbons were the most rich compounds in the root (73.8%), stem (68.8%) and leaf (63.4%) oils, followed by oxygenated sesquiterpenes, monoterpenoid hydrocarbons and oxygenated monoterpenes. β-Caryophyllene (11.1-19.2%), α-humulene (7.3-15.4%), germacrene D (3.3-14.4%), germacrene B (8.3-21.7%) and epi-α-cadinol (5.6-15.0%) were the considerable constituents in these oils.
The methanol extract of leaves of *Schefflera racemosa* contains fruitful compounds exhibiting therapeutic potential. The compound 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-1 is a flavonoid, n-hexadecanoic acid and tetradecanoic acids are fatty acids, 5-hydroxy methyl-2-furaldehyde, 2,3-dihydrobenzofuran and methyl salicylate exhibit antioxidant and anti-inflammatory properties.

In previous report twenty-seven volatile compounds were identified by GC-MS analysis from the essential oil acquired from the leaves of *S. heptaphylla* and 17 of them belonged to monoterpenes or sesquiterpenes. The main volatile constituent in *S. heptaphylla* was found to be a monoterpen, β-pinene, encompass about 22% of the total volatile components. The essential oil showed remarkable antiproliferative activity against 3 cancer cell lines, MCF-7, A375 and HepG2 cells, with IC₅₀ values of 7.3µg/mL, 7.5µg/mL and 6.9µg/mL, respectively. The result of the cytotoxicity assay indicates that (−)-β-pinene and (+)-β-pinene also have antiproliferative activity against the cancer cells MCF-7, A375 and HepG2 with IC₅₀ values ranging from 147.1 to 264.7µM.

The cytotoxic effect of purified compound (D5) of *S. stellata* shows inhibition against A549 cell lines, in which IC₅₀ were found at 150µg/mL. Similarly, the methanolic extract showed the cytotoxicity effect of *S. venulosa* on the MCF-7 cell line by inhibiting MCF-7 growth/proliferation. The compound were treated on the MCF-7 cell line also exhibit some cytotoxic activity. The cytotoxic activity was extremely minute in the first 3 (100,200,300µg/ml) concentration and the great activity was observed in the 4th and 5th (400,500µg/ml) concentration. The test drug revealed the cytotoxic effect and the cell viability was 87.517%, 78.581%. The estimated methanolic extract showed modest growth inhibition of MCF-7cells at 500µg/ml. The MTT assay in methanolic extract of *Tecoma stans* has excessive cytotoxic activity against the cancer cell line from 20µg/mL concentration i.e., 99.3%. The existence of phenolic compounds and their congeners have been shown to bring on a cascade based apoptosis in cancer cells, thus inducing cytotoxicity.

The cell viability of the lung cancer cell line decreased with an increase in the concentration of the plant extract and it was found to be the maximum effective in 100µg/mL concentration. The decrease in cell viability with increased concentration of the plant extract of *Tecoma stans*. The present investigation clearly indicates that the compound from methanolic leaf extract is effective against Human lung cancer cell line, which also paves a way in effective plant treatment with lesser side effects.

**CONCLUSION**

Medicinal plants are wealthy sources of herbal properties contributing to the findings of new drugs for various disorders, diseases including cancer without exhibiting any dangerous effects on the individuals treated. Treatment of cancer by the use of organic products and traditional medicine achieve a significant scope of cancer research. The present study demonstrates the isolation, identification and characterization of the purified D5 compound of *S. stellata* (Geartn.) Baill. which has been tested on lung cancer cell line by MTT assay for cell growth inhibition and its morphological studies. The viability of cell line decreased when the concentration increases and reveals the irregularity of aggregate, shrinkage and cytoplasmic blebbing proves its cytotoxic activity on lung cancer cell line (A549), the D5 compound may be used as strong anti-cancer agent in curing of lung cancer with lesser side effects.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**ABBREVIATIONS**

- GCMS: gas chromatography mass spectroscopy
- MTT: 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazolium bromide
- DPPH: 2,2-diphenyl-1-picrylhydrazyl
- Da: dalton
- °C: degree centigrade
- µg: microgram
- µM: micro molar
- Da: micron
- cm: centimeter
- nm: nanometer

**REFERENCES**


**PICTORIAL ABSTRACT**

**SUMMARY**

The identification, purification and separation of bioactive compounds from methanolic leaf extract of S. stellata (Geartn.) Baill. were performed by column chromatography, Dot blot assay and GCMS analysis. During column separation 32 fractions were received, out of them two compound (D4 and D5) shows their effective antioxidant property in Dot plot assay. GCMS analysis reveals the presence of bioactive compounds in purified compound D4 and D5. Compare to D4, the D5 compound exhibit more cytotoxic effect on Lung cancer cell line (A549). The results clearly indicates, there is decrease in cell viability when the concentration increases from the range of 50 to 250µg/ml. The IC_{50} were found at 150µg/ml. The cytotoxic effect on cell morphology were monitored, while increasing concentration the cancer cell lost their morphologial integrity. By which the study let out that the plant S. stellata (Geartn.) Baill. contains natural antioxidant activity that possess its cytotoxic effect on lung cancer cells with lesser side effects. This is the first report of this plant against cancer activity which paves a way for further studies in drug designing. The less expensive herbal drug treatment may highly be put forward to treat successfully the cancers of various type, is an perfect choice of anticancer treatment.
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About Authors

Dr. Selvankumar Thangaswamy, Professor and Head, PG & Research Department of Biotechnology, Mahendra Arts & Science College (Autonomous), Namakkal, Tamil Nadu, India. He obtained his PhD in Environmental Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. He has honored with several awards including Best Teacher Award -2019 of Periyar University. He has 23 years of teaching, research experience in Microbiology, Immunology, Bioprocess Technology and Nanotechnology Biotechnology. His current research work is concentrated on the identification of peptide drugs, Nanoparticle-based drug delivery and metagenomics. For his credit, he has published 108 research articles in National and International Journals and submitted 48 microbial nucleotide sequence data banks submitted in NCBI. He has carried out research projects from UGC, DST-FIST and DBT.

Ms. Rajarajeshwari Ramakrishnan, Assistant Professor, Research Department of Biotechnology, Sengunthar Arts & Science College, Tiruchengode, Namakkal, Tamil Nadu, India. Her field of specialization is Plant based medicine and Pharmacology in Drug Discovery and Drug designing for Cancer therapy.

Dr. B. Mythili Gnanamangai is Academician cum Researcher in the Multidisciplinary subjects of basic and advanced Sciences. And currently positioned as Assistant Professor in the Department of Biotechnology, K.S.Rangasamy College of Technology, Tiruchengode. The field of expertise includes Medical Biotechnology, Nanobiotechnology, Protein Biotechnology and Environmental Biotechnology. More specifically into deploying the bioinspired nanomaterials for Cancer treatment.

Mr. Mohanraj Rajamanickam is currently doing PhD in the field of Biotechnology in Department of Biotechnology, K.S.Rangasamy College of Technology, Tiruchengode, Namakkal, Tamil Nadu, India. He is specialized in the Environmental studies and Phytocompounds analysis with broad spectrum for environmental and biomedical applications.

Mr. Naresh Srinivasan is student of Department of Biotechnology, K.S.Rangasamy College of Technology, Tiruchengode, Namakkal, Tamil Nadu, India. He is skilled in plant Biotechnological techniques more specifically to extraction of bioactive compounds from plants.

Dr. G. Dhana Sekaran, Assistant Professor, Department of Botany, Sengunthar Arts and Science College, Tiruchengode, Namakkal Dt. Tamil Nadu, India. His field of research is toxinology of venomous animals and natural products with biomedical applications.

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