Original Article

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

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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, seperation 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 IC_{50} 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.

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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 is highest in India like cardiovascular, respiratory problems and Diabetics, cancer which is the vital public health concern.[1]

In India, non-communicable diseases were 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

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of stress and further health damage on their long term use.[3] So there is necessary in identifying the molecules for the treatment of cancer with low price, high efficient curing and with less side effects.[4]

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.[5] The use of secondary metabolites from plant origin could be an benefit and the finest solution to narrow down the use of deleterious products.[6] *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.[7] *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.[8] 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 Dt. 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.[9]

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 1: Gradient solvent system used in the column chromatography for the isolation of bioactive molecules from the test sample.

S.No.	Solvent system	Ratio (ml)	Volume (ml)	Fraction
1.	Hexane	100	40	A1
2.	Hexane: Chloroform	95:5	40	B1
3.	Hexane: Chloroform	90:10	40	B2
4.	Hexane: Chloroform	85:15	40	B3
5.	Hexane: Chloroform	80:20	40	B4
6.	Hexane: Chloroform	75:25	40	B5
7.	Hexane: Chloroform	70:30	40	B6
8.	Hexane: Chloroform	65:35	40	B7
9.	Hexane: Chloroform	60:40	40	B8
10.	Hexane: Chloroform	55:45	40	B9
11.	Hexane: Chloroform	50:50	40	B10
12.	Hexane: Chloroform	45:55	40	B11
13.	Hexane: Chloroform	40:60	40	B12
14.	Hexane: Chloroform	35:65	40	B13
15.	Hexane: Chloroform	30:70	40	B14
16.	Hexane: Chloroform	25:75	40	B15
17.	Hexane: Chloroform	20:80	40	B16
18.	Hexane: Chloroform	15:85	40	B17
19.	Hexane: Chloroform	10:90	40	B18
20.	Hexane: Chloroform	5:95	40	B19
21.	Chloroform	100	40	C1
22.	Chloroform: Acetone	90:10	40	D1
23.	Chloroform: Acetone	80:20	40	D2
24.	Chloroform: Acetone	70:30	40	D3
25.	Chloroform: Acetone	60:40	40	D4
26.	Chloroform: Acetone	50:50	40	D5
27.	Chloroform: Acetone	40:60	40	D6
28.	Chloroform: Acetone	30:70	40	D7
29.	Chloroform: Acetone	20:80	40	D8
30.	Chloroform: Acetone	10:90	40	D9
31.	Acetone	100	40	E1
32.	Water	100	40	F1

the ratio of gradient solvent to be used in column chromatography.[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 R*f* value (R_f-Retention factor), of each spot, is calculated by the formula:[11]

$$Rf = \frac{\text{Distance travel by solute (cm)}}{\text{Distance travel by solvent (cm)}}$$

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.[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 components 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 600Da

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-2yl)-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 mm^2), 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 (5×10^3 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, 250µg/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:

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

Cell morphological study

The general cell morphological changes of purified D5 compound of *S. stellata* (Geartn.) Baill. treated with A549 cell line was observed under light microscopic visualization. The A549 (5×10^4 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 compund. 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.

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 R*f* value of D4 - 0.44, 0.54, 0.64, 0.71 and D5 - 0.76 were shown in Figure 2.

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

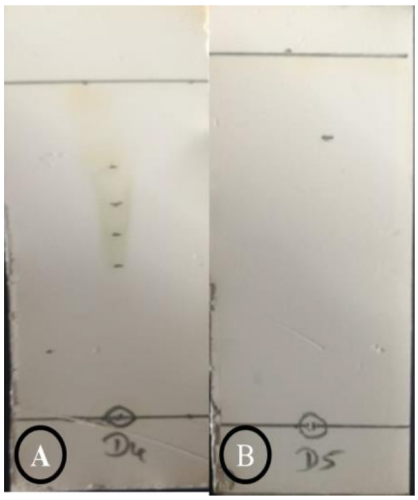


Figure 2: Isolation pattern of pure compounds on TLC plate using iodine (A) D4 (B) D5.

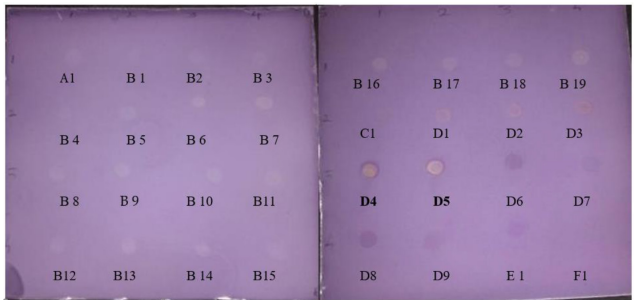


Figure 3: TLC dot-blot test by DPPH method for 32 fractions obtained from column chromatography.

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

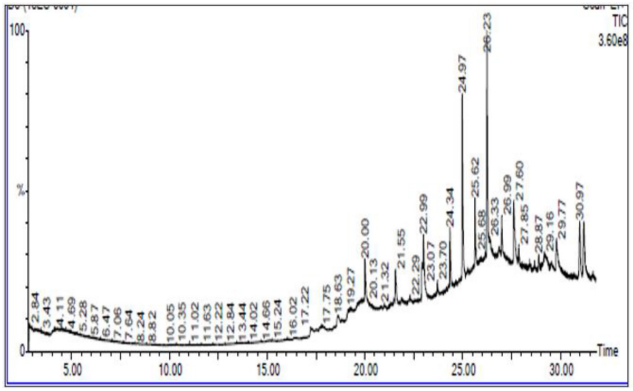


Figure 4: GC-MS chromatogram of *Schefflera stellata* (Geartn.) Baill. methanolic leaf extract (D4).

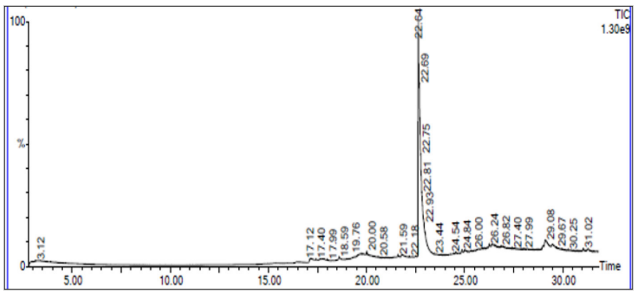


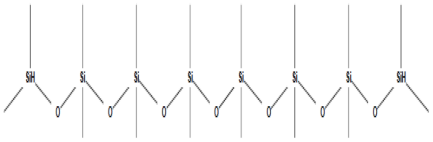
Figure 5: GC-MS chromatogram of *Schefflera stellata* (Geartn.) Baill. methanolic leaf extract (D5).

(i) 1,2-benzenedicarboxylic acid, ditridecyl ester

(ii) Tetratriacontane

(iii) Nonacosane

(iv) 11-methyl-13-tetradecen-ol acetate



(v) Silicic acid, diethyl bis(trimethylsilyl) ester

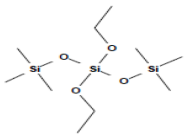


Figure 6: Molecular structures of purified bioactive compounds isolated from methanol extract of *Schefflera stellata* (Geartn.) Baill. (D4)

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 (R*t*), 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.

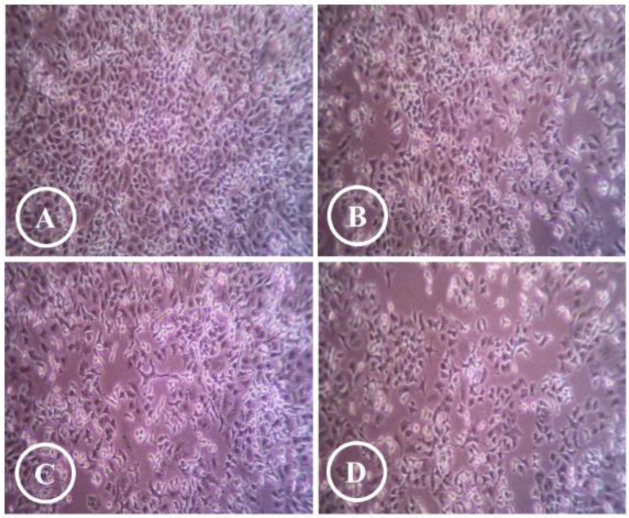


Figure 7: Morphological results for purified bioactive compound isolated from methanol leaf extract of *Schefflera stellata* (Geartn.) Baill. D5 on A549 cells. Scale bar 20X. (A) Control Cells; (B) Cells treated with 50µg/ml of D5 compound; (C) IC_{50} at 150µg/ml of D5 compound (D) Cells treated with 250µg/ml of D5 compound.

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

Table 2: Shows the components identified in methanolic extract of *Schefflera stellata* (Geartn.) Baill. leaf (D4).

S.No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1.	22.99	1,2-benzenedicarboxylic acid, ditridecyl ester	$C_{34}H_{58}O_4$	530	6.728
2.	24.98	Tetratriacontane	$C_{34}H_{70}$	478	12.975
3.	26.24	Nonacosane	$C_{29}H_{60}$	408	24.895
4.	27.61	11-methyl-13-tetradecen-ol acetate	$C_{17}H_{32}O_2$	268	6.657
5.	31.18	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296	5.704

Table 3: Shows the components identified in methanolic extract of *Schefflera stellata*(Geartn.) Baill. leaf (D5).

S.No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1.	22.64	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	100

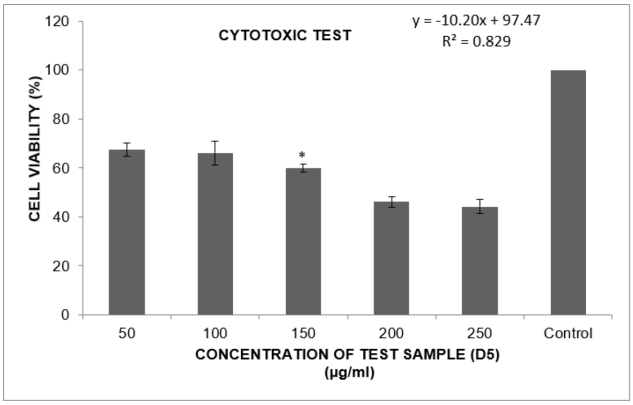


Figure 8: Determination of percentage cell viability for purified bioactive compounds isolated from methanol extract of *Schefflera stellata* (Geartn.) Baill.(D5) on A549 cell line, results are given as statistically significance at $p < 0.01$* between control cells Vs D5 compound treated cells.

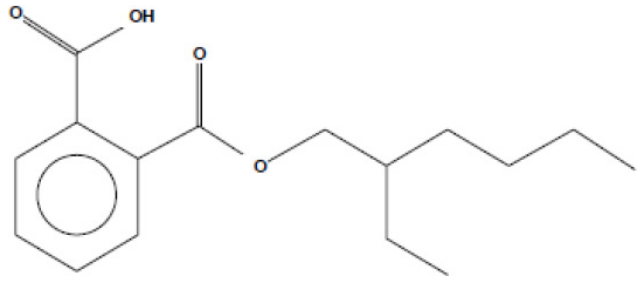


Figure 9: Molecular structures of purified bioactive compounds isolated from methanol extract of *Schefflera stellata* (Geartn.) Baill. (D5).

control cells Vs D5 compound treated cells. The current study reveals that the D5 purified compound exhibit its cytotoxic effecet 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 developing country. Synthetic drugs have been 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.[14] Dot plot assy 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.[15]

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, monoterpene 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.[16]

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-pyrane-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 antibacterial and antioxidant properties.[17]

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 monoterpene, 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 IC_{50} 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 IC_{50} values ranging from 147.1 to 264.7µM.[18]

The cytotoxic effect of purified compound (D5) of *S. stellata* shows inhibition against A549 cell lines, in which IC_{50} 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.[19] The MTT assay in methanolic extract of *Tecomastans* has excessive cytotoxic activity against the cancer cell line from 20µg/mL concentration i.e., 99.3%. The existence of phenolic compounds and their congers have shown to bring on a cascade based apoptosis in cancer cells, thus inducing cytotoxicity.[20] 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*.[21] 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 morphologial 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 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; µl: microlitre; **cm:** centimeter; **nm:** nanometer; µM: micro molar; **mM:** milli molar.

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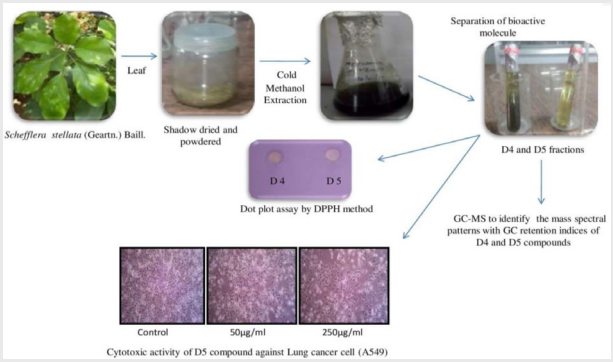
PICTORIAL ABSTRACT



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 assy. 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 morphological 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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