INTRODUCTION

From the ancient era to modern-day society, medicinal herbs and plants are playing a significant role in curing several diseases. Starting from ancient folk medicine to modern scientific medicine, plants have played a pivotal role. Natural products obtained from plant origin contain several bioactive compounds that are using as the base compounds of drugs. A large proportion of the drugs of modern medicine is either directly isolated from plants or in a synthetic form modified from a lead compound. The plant occurs in China in the southern and western regions and in the north-eastern region of India. This plant belongs to the Clusiaceae family. Locally the plant is known as Kau tree among the people of the north-eastern region of India. This plant belongs to the Clusiaceae family. Local tribes use this plant extensively for preparing adhesive from the latex. Also, ethnomedicinally the plant is very famous among the local ethnic people. Fruits are edible. The plant grows in the Northeast region and the Andaman Islands. It is cultivated in the Assam state of India for their acidic fruits. Dry fruit slices used for the culinary purpose as well as to treat dysentery. Previously G. cowa reported with good anti-bacterial activities, antiplatelet aggregation capacity, anticancer activity against human colorectal adenocarcinoma cells. However, the least information is present about the complete metabolomic profiling and phytoc hemothermal potential of G. cowa. This study has been done to estimate the anti-proliferative potential of the methanolic extract of G. cowa leaf. Methods: Anti-proliferative potential of ethyl acetate and methanol extract of G. cowa leaf assessed by MTT assay. Metabolomic profiling obtained by GC/MS analysis. Nuclear morphology visualized by DAPI staining. Caspase activation analysed through spectrophotometric assay. Results: The study reveals, that the methanolic extract is more potential in inducing anti-proliferative activity than ethyl acetate extract. Robust anti-proliferative activity of the methanolic extract evidenced in lung cancer cell line, A549 followed by MCF–7, HepG2, MOLT – 4, MDA-MB-468 cells. The anti-proliferative effect was negligible in normal PBMC. Further, a dose-dependent increase of nuclear fragmentation visualized in A549 cells treated with the methanolic extract. Post metabolic extract treatment upregulation of caspase-3 and caspase-9 also evidenced in A549 cells. GC/MS analysis revealed the presence of phytoconstituents of different phytochemical groups comprising of 3.45% diterpenoid, 5.45% triterpenoid, 11.24% steroid, 2.03% phytosterol, etc. in methanol extract, as well as 4.53% diterpenoid, 2.88% triterpenoid, 1.09% steroid, 2.11% phytosterol, etc. in ethyl acetate extract with considerable biological importance. Conclusion: This is the maiden report of the metabolomic profiling of leaf extracts of G. cowa which possess a good repository of potentially bioactive molecules that holds a great promise as a future therapeutic agent in combating lung cancer. Key words: G. cowa, Cancer, GC-MS, Metabolic profiling, Anti-proliferative.
and metabolomic profiling of different solvent extracts of G. cowa leaf. Here, for the first time, we are reporting the metabolomic profiling of G. cowa leaf and the anti-proliferative activity in a panel of human cancer cell lines.

MATERIALS AND METHODS

Test sample and chemical extraction

Fresh leaves of G. cowa Roxb. Ex DC. were collected in July 2017 from the mature plant located in the Madhupur locality, near Tripura University campus, Tripura, India (23°45'43.4" N 91°15'39.7" E). The plant specimen was identified by Prof. B. K. Dutta, Professor of Botany, Tripura University. A voucher specimen (Specimen number 2724) was deposited in the Tripura University Herbarium. The plant leaves then cut into small pieces. It was then properly air-dried and then ground into a fine powder and kept in dark. Powdered leaves of G. cowa (3.2 kg) was extracted with hexane (15 L) at room temperature for 24 hours and filtered to remove non-polar impurities. The filtrate was collected. This process of extraction was repeated thrice with ethyl acetate and methanol respectively to obtain ethyl acetate and methanol extract respectively. Under reduced pressure of a rotary evaporator at 45°C the extract was concentrated. Brown gummy ethyl acetate extract (133.08 g) and methanol extract (173.64 g) was obtained. In a sealed round bottom flask the dried extract was kept at 4°C for further assays.

Detection of Anti-proliferative potential by MTT assay

Cancer cell lines

Seven human cancer cell line; Jurkat (Human acute T cell leukemic cell), HCT 116 (Human colon cancer cell), A549 (Human epithelial lung carcinoma cell), MCF – 7 (Human breast cancer cell), MOLT – 4 (Human acute T lymphoblastic leukemia cell), HepG2 (Human liver cancer cell) and MDA-MB-468 (Human triple-negative breast cancer cell) were used for the anti-proliferative potential study. Dulbecco’s Modified Eagle Medium (DMEM) has been used for culturing HCT 116, A549, MCF – 7, HepG2, MDA-MB-468, whereas Roswell Park Memorial Institute medium (RPMI 1640) has been used for MOLT – 4, Jurkat cells respectively. Further, 10 % (v/v) heat-inactivated fetal bovine serum (FBS), 2mM L-glutamine, 10 U/ml penicillin and streptomycin were supplemented in the media. It was maintained in humidified 5% CO₂ at 37°C in a CO₂ incubator. 1x trypsin/EDTA was used for harvesting adherent cells in the confluent phase and further centrifuged at 120 x g for 5 min. The cell pellet was reconstituted in respective media.

Isolation of peripheral blood mononuclear cells (PBMC)

Peripheral blood mononuclear cells (PBMC) isolation from heparinized venous blood of healthy normal donors was done according to Gopal et al 2014 through Percoll density gradient centrifugation method. After the isolation, the cells were washed. After then, it was resuspended to each well. The percentage of the viable cells are represented by the formazan crystal. After removing the medium the formazan crystal was dissolved in DMSO and the absorbance was measured at 560 nm using a microplate reader. 100% cellular death was obtained by the lysis of cells in 5% SDS lysis buffer.

The cell viability percentage was calculated as mentioned below:

\[
\text{Cell viability} = \left( \frac{\text{Absorbance of Sample} - \text{Absorbance of 100 %Lysis}}{\text{Absorbance of 0% Lysis} - \text{Absorbance of 100% Lysis}} \right) \times 100
\]

Nuclear morphology analysis with DAPI staining

A549 cells (1x10⁵ cells/well) were seeded in 6 well plate and treated with different concentrations of leaf methanolic extract of G. cowa for 24h. After the treatment, collected cells were washed with phosphate buffer saline (PBS). Cells were fixed with 80% methanol. At room temperature, 1µg/mL DAPI in PBS was added. It was incubated for 15 min. The stained cells were observed under the fluorescence microscope (Dewinter, Germany).

Determination of caspase activity

The spectrophotometric method was applied for the detection of Caspase-3, 8 and 9 activity using the assay kit. Caspase activity was checked in the cell lysates (100µg protein containing in the 50µl lysis buffer) according to the instruction provided by the manufacturer. A549 cells (1x10⁵ cell/mL) were treated with methanolic extract (1µg/ml, 18h at 37°C). After the incubation period, with ice-cold PBS the cells were washed twice. After then the cell lysates were prepared and the estimation of the protein concentration was done. The cell lysates were combined with 50µl of 2X reaction buffer (containing 10 mM DTT), caspase 3 substrate DEVD- pNA (4 mM, 5ml) or the caspase 8 substrate IETD pNA (4 mM, 5ml) or the caspase 9 substrate LEHD – pNA (4 mM, 5ml). It was then incubated for 0-2 h at 37°C. By the activity of caspase parainitroanilide (pNA), the chromophore was released. The chromophore was quantified spectrophotometrically by measuring absorbance at 405 nm every 30 minutes for 2h. To examine the fact that the methanolic extract induced anti-proliferative activity was caspase-dependent or not, a pan-caspase inhibitor Z-VAD FMK (20 mM, 1 h) was pre-treated in A549 cells followed by 24h incubation with the methanolic extract. Finally, the cell viability was checked by the MTT assay as described above.

Statistical analysis

All the presented experimental data were presented as mean ± standard deviation obtained from three independent experiments. Difference between the groups has been assessed by one-way analysis of variance (ANOVA) followed by Turkey multiple comparison test (wherever applicable) using GraphPad Prism version 7.0 (GraphPad Software Inc, San Diego, CA, USA); p < 0.05 was considered statistically significant.

Detection of metabolites through Gas chromatography-mass spectrometry

Preparation of sample for GC/MS analysis

10mg dried Ethyl acetate and methanol extract were dissolved in 1 ml of GCMS grade Ethyl acetate and Methanol respectively. After then it was vortexed properly and then centrifuged. After centrifugation, the clear supernatant was collected. 0.22 µm syringe filter was used for the filtration of the supernatant. Finally, for the analysis, one microliter sample solution was injected into the GC/MS system.

Instrumentation and chromatographic conditions

The GC analysis was performed in an Agilent 7890A GC system, fitted to
with a fused silica Agilent HP-5MS capillary column (30 m x 0.25 mm i.d.; 0.25 μm film thickness), coupled to an Agilent triple quadrupole Mass Selective Detector MSD 7000; ionization voltage 70 eV; electron multiplier energy 2914 V; transfer line temperature, 280 °C. Helium was the carrier gas (1 mL min\(^{-1}\)). The initial temperature was 60 °C for 0 min, then gradually increased to 320 °C at 8 °C min\(^{-1}\) rate, held for 12 min. One 1 μL of samples was injected at 250 °C automatically and in the split mode (2:1).

**Identification of the individual phytoconstituents**

National Institute Standard and Technology, Mass Spectral Library version 2.2 (NIST MS Search 2.2) database was used to identify the individual compounds by analysing the mass spectrum of the detected compounds through GC/MS. Finally, name, molecular weight, CAS number of the individual compounds were determined further using NIST WebBook and PubChem.

**RESULTS**

**Evaluation of Anti-proliferative potential of the methanolic extract**

Initially, we have performed MTT assay against HCT 116 and Jurkat cell line for the comparison of the anti-proliferative potential of the methanolic (GcME) and ethyl acetate extract (GcEA) of the leaves of *Garcinia cowa*. We observed that after treating the cells with 5, 10, 50 and 100 μg/mL of GcME and GcEA for 24 hours methanolic extract shown to be more potent in anti-proliferative activity than ethyl acetate extract in both the cell lines tested (Figure 1). Further, we have extended the screening process by adding different human cancer cell lines viz. A549, MCF – 7, HepG2, MOLT – 4, and MDA-MB-468. The cell lines were cultured with the presence or absence of 5, 10, 50 and 100 μg/mL of GcME for 24 and 48-hour time point. DMSO used as a vehicle. As shown in Figure 2a and 2b, GcME shows a dose and time-dependent induction of anti-proliferative activity in all five cancer cell lines tested. The growth inhibition, induced by GcME treatment has found to be statistically significant (p<0.05). Anti-proliferative activity of the methanolic extract on normal cells was evaluated by performing MTT assay on peripheral blood mononuclear cells (PBMC) isolated from healthy donors. The anti-proliferative effect was negligible in the case of PBMC. A549 was the most sensitive among all the cancer cells. IC\(_{50}\) value was 0.58 ± 0.47 μg/mL in 24th treatment. IC\(_{50}\) value was recorded in case of MCF-7, HepG2 and MOLT-4 cell line as 5.775 ± 1.66 μg/mL, 5.86 ± 0.82 μg/mL and 8.51 ± 0.05 μg/mL respectively in 24th treatment (Table 1). During 48 hours of treatment, the number of viable cells was even lesser (Figure 2b). All the cell line tested showed a noticeable anti-proliferative response in very low concentration except MDA-MB-468 cells (Figure 2). MDA-MB-468 cells are triple-negative breast cancer cells and show poor prognosis. Despite the poor prognosis, leaf methanolic extract of *Garcinia cowa* has shown considerable good activity against MDA-MB-468 cells in MTT assay with an IC\(_{50}\) value of 72.64 ± 3.29 μg/mL in 24th treatment. On the other hand, the anti-proliferative activity of leaf methanolic extract of *Garcinia cowa* in normal PBMC was very negligible. Hence, it can be concluded that the anti-proliferative effect is cancer cell-specific.

**Nuclear morphology analysis with DAPI staining**

Robust anti-proliferative activity was recorded from A549 cells. Next, we wanted to measure the effect of GcME on nuclear morphology using DAPI staining. Treating the cells with methanolic extract for 24 hours in three different concentrations, fragmented nuclei was observed in a concentration-dependent manner under a fluorescent microscope in respect to untreated control (Figure 3).

**Determination of caspase activity**

A protease group of enzyme caspases plays an essential role in programmed cell death. Caspase activation eventually directs the degradation of cellular components in a controlled manner, leading to apoptosis. Further, caspase activity was determined in the methanol extract treated A549 cells. Treatment with methanolic extract shows upregulation of caspase-3 and caspase-9 in A549 cells. Caspase 8 showed no significant upregulation or downregulation (Figure 4c). The increase in the activity of caspase 3 and 9 was observed exponentially up to 90 minutes after which the activity plateaued. The increased activation of caspase 3 (Figure 4a) and 9 (Figure 4b), collectively indicated the potentiality of methanolic extract to induce apoptosis through the intrinsic pathway of caspase activation.

**Figure 1: Effect of leaf ethyl acetate and methanolic extract of *Garcinia cowa* on two Different Cancer Cell Lines**

Effect of leaf ethyl acetate and methanolic extract of *Garcinia cowa* (5-100μg/ml) by MTT assay against HCT 116 (a) and Jurkat (b) cell lines at 24th time point. Each line graph represents the Mean value ± Standard Deviation of three individual experiments in duplicate.

**Table 1: Anti-proliferative effect of leaf methanolic extract of *Garcinia cowa* on different cancer cell lines**

| Cell Line       | Concentration (μg/mL) | IC\(_{50}\) Value (μg/mL) |
|-----------------|-----------------------|---------------------------|
| HCT 116         | 5                     | 16.0 ± 2.4                |
|                 | 10                    | 12.0 ± 1.2                |
|                 | 50                    | 6.0 ± 0.5                 |
|                 | 100                   | 3.0 ± 0.2                 |
| Jurkat          | 5                     | 32.0 ± 4.6                |
|                 | 10                    | 28.0 ± 3.9                |
|                 | 50                    | 14.0 ± 1.7                |
|                 | 100                   | 7.0 ± 0.4                 |
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Figure 2: Effect of leaf methanolic extract of Garcinia cowa on five Different Cancer Cell Lines. Effect of methanolic extract of Garcinia cowa (5-100µg/ml) by MTT assay against A549, MCF-7, MOLT-4, HepG2, MDA-MB-468, and normal PBMC isolated from healthy donors at 24h (a) and 48h (b) time point. Each line graph represents the Mean value ± Standard Deviation of three individual experiments in duplicate. *p < 0.01 as compared to normal PBMC.

Figure 3: Fluorescence Microscopic Images of A549 Cells Treated with or without methanolic extract at Different Concentrations. Control A549 cells (A), Gc ME treated A549 cells at concentrations 5 μM (B), 10 μM (C) and 50μM (D). The control cells were with intact nucleus and gave bright blue fluorescence; whereas treated cells showed intense fragments of nucleus indicated by yellow arrows as signs of apoptosis.

Table 1: IC₅₀ values (µg/ml) of the leaf Methanolic Extracts of Garcinia cowa obtained from the MTT assay for the following cell lines. All the data are the mean value of three independent experiments with standard deviation.

| Cell lines | A549 | MCF-7 | HepG2 | MOLT-4 | MDA-MB-43S |
|------------|------|-------|-------|--------|-------------|
| IC₅₀ value (µg/ml) | 0.58 ± 0.47 | 5.775 ± 1.66 | 5.86 ± 0.82 | 8.51 ± 0.05 | 72.64 ± 3.29 |

To confirm the role of caspases in the methanolic extract induced anti-proliferative activity, A549 cells were co-incubated for 24 h with methanolic extract, in the absence/presence of a nontoxic concentration of Z-VAD-FMK, a pan-caspase inhibitor and cell viability was measured by MTT assay. The IC₅₀ value of anti-proliferative activity was increased from 3.334 µg/mL to 36.93 µg/mL upon treatment with Z-VAD-FMK validating that induction of apoptosis, in this case, is a caspase-dependent phenomenon in A549 cells (Figure 4d).

Metabolomic profiling of the methanol and ethyl acetate extract through GC/MS

Further, it was evaluated for the characterization of the phytoconstituents of ethyl acetate and methanolic extract of the leaf specimen of Garcinia cowa. Complete metabolomic profiling was carried out through GC/MS analysis. GC/MS chromatograms of methanol and ethyl acetate extract of G. cowa according to the aforementioned experimental procedure showed various peaks indicating the presence of a different group of phytochemicals in the extracts. The methanol and ethyl acetate extract of G. cowa leaves revealed the presence of 97 (Figure 5) and 106 (Figure 6) different compounds, respectively which were characterized and identified by analysing their mass fragmentation patterns in the NIST MS Search 2.2 database. List of compounds present in the methanol and ethyl acetate extracts has been given in Table 2 and Table 3 respectively. Among the individual components in both the extracts, it has been noted that 52 compounds are common to both the extracts. 45 compounds are found to be exclusive to methanolic extract and 54 compounds for ethyl acetate extract (Figure 7). In this investigation, we have taken those major compounds for analysis, which possessed 1% and above area percentage of the total area of the peaks. By which, 23 compounds in case of methanolic extract and 21 compounds in the case of ethyl acetate extract have been recorded.

Further, the identified major compounds were investigated for their biological activities through a literature survey. There we found that most of them possess a diverse range of positive pharmacological and therapeutic properties. Details of the compounds and their reported biological activities have been enlisted in Table 4 for methanolic extract and Table 5 for ethyl acetate extract.
Table 2: List of compounds identified from the leaf methanolic extract of *Garcinia cowa* by GC/MS analysis.

| Sl No. | Retention time | Peak Area | Peak Area (%) | Compound Detected |
|-------|----------------|-----------|---------------|-------------------|
| 1     | 4.589          | 1373180   | 0.251457      | Hexanoic acid     |
| 2     | 4.698          | 976203    | 0.178763      | Phenol            |
| 3     | 5.463          | 7487579   | 1.371129      | α-Cymene          |
| 4     | 5.535          | 2079358   | 0.380773      | Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)- |
| 5     | 5.575          | 2971432   | 0.54413       | Benzyl alcohol    |
| 6     | 5.655          | 12760944  | 2.33679       | 2-Pyrrolidinone, 1-methyl- |
| 7     | 5.972          | 1123366   | 0.205711      | Bicyclo[3.1.0]hex-3-en-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2α,5α)- |
| 8     | 6.147          | 4067637   | 0.744867      | Acetophenone      |
| 9     | 6.518          | 1635828   | 0.299554      | Benzene, 1-methyl-4-(1-methylethenyl)- |
| 10    | 7.65           | 4789928   | 0.877006      | Benzoic acid      |
| 11    | 8.178          | 4983414   | 0.912565      | Naphthalene       |
| 12    | 8.247          | 1303677   | 0.23849       | α-Terpineol       |
| 13    | 8.327          | 3930191   | 0.719698      | Dodecane          |
| 14    | 8.735          | 1225119   | 0.24344       | Triethylene glycol|
| 15    | 10.012         | 1470131   | 0.269211      | Tridecane         |
| 16    | 10.325         | 1998047   | 0.365883      | Naphthalene, 2-methyl- |
| 17    | 11.512         | 822967    | 0.150702      | 1-Tetradecane     |
| 18    | 11.639         | 14817656  | 2.713416      | Tetradecane       |
| 19    | 11.77          | 979995    | 0.179439      | Diphenyl ether    |
| 20    | 11.832         | 1236722   | 0.226469      | Naphthalene, 1,7-dimethylen- |
| 21    | 12.069         | 892137    | 0.163369      | Naphthalene, 1,4-dimethylen- |
| 22    | 12.123         | 830732    | 0.152124      | Naphthalene, 2,6-dimethylen- |
| 23    | 12.618         | 938795    | 0.175575      | Tetradecane, 2,6,10-trimethyl- |
| 24    | 12.902         | 766347    | 0.140334      | (4s,4αR,6R)-4,4α-Dimethyl-6(prop-1-en-2-y1)-1,2,3,4,4α,5,6,7-octahydronaphthalene) |
| 25    | 12.997         | 731199    | 0.133897      | 1,2,4-Benzenetriol |
| 26    | 13.193         | 3531479   | 0.646686      | Naphthalene, decahydro-4α-methyl-1-methylen-7-(1-methylethenyl)-, [4αR-(4αα,7α,8αβ)]- |
| 27    | 13.805         | 1064841   | 0.194994      | Undec-10-ynoic acid, octadecyl ester |
| 28    | 14.07          | 719864    | 0.131822      | Dodecanedioic acid |
| 29    | 14.565         | 1434078   | 0.262609      | Cetene            |
| 30    | 14.675         | 16014049  | 2.9325        | Hexadecane        |
| 31    | 14.74          | 1144519   | 0.209585      | Benzene, (1-methylnonadecyl)- |
| 32    | 15.177         | 937799    | 0.17173       | Benzene, (1-pentylheptyl)- |
| 33    | 15.228         | 2289552   | 0.419264      | Benzene, (1-butylheptyl)- |
| 34    | 15.37          | 2138430   | 0.39159       | Benzene, (1-propiloctyl)- |
| 35    | 15.665         | 1474681   | 0.270044      | Benzene, (1-ethylnonyl)- |
| 36    | 15.988         | 4552691   | 0.833691      | 13-Octadecanal,(Z)- |
| 37    | 16.079         | 1153200   | 0.211175      | Oxirane, tetradecyl- |
| 38    | 16.178         | 3732557   | 0.683508      | Benzene, (1-methylnonadecyl)- |
| 39    | 16.294         | 1245116   | 0.228006      | Pentadecanal- |
| 40    | 16.327         | 2890228   | 0.52926       | Benzene, (1-pentylheptyl)- |
| 41    | 16.593         | 2627996   | 0.48124       | Benzene, (1-butyloctyl)- |
| 42    | 16.76          | 2296938   | 0.420166      | Benzene, (1-propynolyl)- |
| 43    | 16.884         | 4982718   | 0.912438      | Tetradecanoic acid |
| 44    | 17.051         | 1618062   | 0.2963        | Benzene, (1-ethyldecyl)- |
| 45    | 17.328         | 1054656   | 0.193129      | Hexadecen-1-ol, trans-9- |
| 46    | 17.419         | 10098676  | 1.849274      | Nonadecane        |
| 47    | 17.561         | 3515482   | 0.643757      | Benzene, (1-methylundecyl)- |
| 48    | 17.827         | 3066537   | 0.561546      | Benzene, (1-propyloctyl)- |
| 49    | 17.947         | 20733653  | 3.800419      | Neophytadiene     |
| 50    | 18.023         | 3046644   | 0.557903      | 2-Pentadecaneone, 6,10,14-trimethyl- |
| 51    | 18.085         | 1493665   | 0.275321      | Benzene, (1-propyloctyl)- |
| 52    | 18.387         | 1164381   | 0.213222      | Benzene, (1-ethylundecyl)- |
| 53    | 18.493         | 11280523  | 2.065695      | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol |
| 54    | 18.875         | 2428502   | 0.444708      | Benzene, (1-methyldecyl)- |
| 55    | 19.02          | 15568671  | 2.850942      | n-Hexadecanoic acid methyl ester |
| 56    | 19.515         | 6279278   | 11.48335      | n-Hexadecanoic acid |
| 57    | 19.85          | 7048102   | 1.290652      | Hexadecanoic acid, ethyl ester |
Table 3: List of compounds identified from the leaf ethyl acetate extract of Garcinia cowa by GC/MS analysis.

| Sl No. | Retention time | Peak Area | Peak Area (%) | Compound Detected                      |
|-------|----------------|-----------|---------------|----------------------------------------|
| 1     | 4.625          | 3977105   | 0.448488      | Pentanoic acid                         |
| 2     | 4.709          | 2334761   | 0.263285      | Phenol                                 |
| 3     | 5.47           | 8124782   | 0.91621       | α-Go-Myrene                            |
| 4     | 5.539          | 2839489   | 0.320202      | D-Limonene                             |
| 5     | 5.67           | 5441295   | 0.613601      | 2-Pyrrolidinone, 1-methyl-             |
| 6     | 5.979          | 1260103   | 0.142099      | Myrtenyl 3-methylvalerate              |
| 7     | 6.158          | 59637194  | 6.72513       | Acetophenol                            |
| 8     | 6.46           | 2087385   | 0.235389      | Butanamide, N-formyl-2-hydroxy-3-methyl-2-(1-methylethyl)- |
| 9     | 6.62           | 1977022   | 0.222944      | Benzoic acid, methyl ester             |
| 10    | 6.875          | 2748995   | 0.309997      | Hexanoic acid, 2-ethyl                 |
| 11    | 7.038          | 1307666   | 0.147462      | 2-Butenioic acid, 3-methoxy-4-nitro-, (E)- |
| 12    | 7.97           | 8030274   | 0.95553       | Heptanediamide,N,N'-di-benzoxyloxy-    |
| 13    | 8.181          | 5961198   | 0.672229      | Naphthalene                            |
| 14    | 8.247          | 3552182   | 0.40057       | L-a-Terpineol                          |
| No. | Retention Time | FID Signal | FID Response | Compound Description |
|-----|----------------|------------|--------------|----------------------|
| 15  | 8.331          | 5490738    | 0.619176     | Dodecane             |
| 16  | 8.564          | 7210205    | 0.813076     | 2-Hexanol, 2,5-dimethyl- (S)- |
| 17  | 9.033          | 1930500    | 0.217697     | 1,2,3-Propanetriol, 1-acetate |
| 18  | 9.084          | 2068892    | 0.233304     | 1-Butanone, 3-methyl-2-nitro-1-phenyl |
| 19  | 9.146          | 10457906   | 1.179311     | 1-Phenoxypropan-2-ol  |
| 20  | 9.419          | 404820     | 0.04565      | Desulphosinigrin      |
| 21  | 9.452          | 906133     | 0.102182     | Nonanoic acid         |
| 22  | 9.564          | 1276487    | 0.143946     | Nonanal dimethyl acetal |
| 23  | 9.033          | 3366048    | 0.37958      | Naphthalene, 2-methyl- |
| 24  | 9.643          | 24929784   | 2.811266     | Tetradecane           |
| 25  | 11.77          | 1671564    | 0.188498     | Diphenyl ether        |
| 26  | 11.832         | 2814687    | 0.317405     | Naphthalene, 1,7-dimethyl- |
| 27  | 12.069         | 2005849    | 0.226154     | Naphthalene, 1,7-dimethyl- |
| 28  | 12.127         | 1960048    | 0.221029     | Naphthalene, 1,5-dimethyl- |
| 29  | 12.349         | 2785541    | 0.311148     | Ethanone, 2-(acetoxy)-1-phenyl |
| 30  |                | 7867643    | 0.887213     | Acetophenone, 2-chloro- |
| 31  | 12.622         | 1442921    | 0.162714     | Tetradecane, 2,6,10-trimethyl- |
| 32  | 12.906         | 2962573    | 0.334082     | (4a,4aR,6R)-4,4a-Dimethyl-6-prop-1-ene-2-yl, 1,2,3,4,4a,5,6,7-octahydropyridine |
| 33  | 13.193         | 1274308    | 0.1437       | Naphthalene, decahydro-4a-methyl-7-(1-methylethenyl) [4aR-(4aα,7α,8aβ)]- |
| 34  | 13.397         | 2808964    | 0.31676      | 2,4-Di tert-butylphenol |
| 35  | 13.805         | 4332912    | 0.486611     | Benzene, (1-butylhexyl)- |
| 36  | 13.936         | 2506222    | 0.28262      | Benzene, (1-propylethyl)- |
| 37  | 14.099         | 2000330    | 0.225572     | Dodecanoic acid        |
| 38  | 14.212         | 1810807    | 0.2042       | Benzene, (1-ethylcyloxy)- |
| 39  | 14.314         | 1826084    | 0.205923     | 2-Octa-3-azabicyclo[4.4.0]dec-3-ene, 5-methyl-1-trimethylsiloxy-N-oxide |
| 40  | 14.565         | 1980941    | 0.223386     | Cetene                |
| 41  | 14.678         | 24101900   | 2.717908     | Hexadecane            |
| 42  | 14.74          | 4002869    | 0.451393     | Benzene, (1-methylnonadecyl)- |
| 43  | 15.177         | 3121857    | 0.352044     | Benzene, (1-pentylhexyl)- |
| 44  | 15.228         | 7804376    | 0.880079     | Benzene, (1-butylethyl)- |
| 45  | 15.373         | 7296672    | 0.822827     | Benzene, (1-propylethyl)- |
| 46  | 15.665         | 5702595    | 0.645067     | α-acorenole            |
| 47  | 15.988         | 4012511    | 0.45248      | Dodecyl acrylate       |
| 48  | 16.178         | 10332643   | 1.165185     | Benzene, (1-methyldecyl)- |
| 49  | 16.527         | 7820428    | 0.881899     | Benzene, (1-pentylheptyl)- |
| 50  | 16.593         | 7445409    | 0.839599     | Benzene, (1-butylethyl)- |
| 51  | 16.76          | 5551836    | 0.626066     | Benzene, (1-propynyl)- |
| 52  | 16.913         | 11378991   | 1.283179     | Tetradecanoic acid     |
| 53  | 17.051         | 4080996    | 0.460203     | Benzene, (1-ethyldecyl)- |
| 54  | 17.328         | 1388505    | 0.156578     | 1-Nonadecene           |
| 55  | 17.419         | 14194866   | 1.600718     | Octadecane             |
| 56  | 17.561         | 8465396    | 0.954621     | Benzene, (1-methylndecyl)- |
| 57  | 17.83          | 7345117    | 0.82829      | Benzene, (1-ethylheptyl)- |
| 58  | 17.947         | 23533260   | 2.653784     | Neophytadiene          |
| 59  | 18.023         | 3211467    | 0.362149     | 2-Pentadecanone, 6,10,14-trimethyl- |
| 60  | 18.085         | 3415481    | 0.385153     | Benzene, (1-propyldecyl)- |
| 61  | 18.383         | 1540439    | 0.173711     | Benzene, (1-ethylundecyl)- |
| 62  | 18.496         | 13072273   | 1.471262     | Neophytadiene          |
| 63  | 18.584         | 1308986    | 0.147611     | (3S,3aS,6R,7R,9aS)-1,1,7-Trimethyldecahydro-3a,7-methanocyclopenta[8]annulene-3,6-diol |
| 64  | 18.875         | 5339542    | 0.602126     | Benzene, (1-methylnecyl)- |
| 65  | 19.02          | 3699311    | 0.417162     | Methyl 9-methyltetradecanoate |
| 66  | 19.544         | 123247324  | 13.89828     | α-Hexadecanoic acid    |
| 67  | 19.85          | 1342233    | 0.15136      | Hexadecanoic acid, ethyl ester |
| 68  | 19.916         | 2763588    | 0.311643     | Heptadecane            |
| 69  | 19.948         | 6201235    | 0.699297     | Chlorpyrifos           |
| 70  | 20.331         | 5856208    | 0.660389     | 4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-1-methylethyl ester |
| 71  | 20.625         | 1690074    | 0.190585     | Nonanedioic acid, dibutyl ester |
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| No. | Retention Time (min) | Peak Area (counts) | Relative Intensity (RI) | Compound Name |
|-----|----------------------|--------------------|------------------------|---------------|
| 1   | 21.262               | 21.262             | 4.536937               | Phytol        |
| 2   | 21.488               | 21.488             | 0.304314               | 9,12-Octadecadienoic acid (Z,Z)- |
| 3   | 21.572               | 21.572             | 1.339764               | Oleic Acid    |
| 4   | 21.823               | 21.823             | 2.239891               | Octadecanoic acid |
| 5   | 22.198               | 22.198             | 0.310944               | Pentadecane   |
| 6   | 22.341               | 22.341             | 0.277194               | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol |
| 7   | 22.111               | 22.111             | 0.031496               | Columbin      |
| 8   | 22.264               | 22.264             | 0.037428               | Heptadecane, 9-hexyl- |
| 9   | 23.468               | 23.468             | 0.040465               | Oleyl chloride |
| 10  | 23.57                | 23.57              | 0.073774               | Pentadecanal- |
| 11  | 23.708               | 23.708             | 0.233601               | Eicosane, 2-cyclohexyl- |
| 12  | 24.198               | 24.198             | 0.531036               | 4,8,12,16-Tetramethylheptadecan-4-olide |
| 13  | 24.298               | 24.298             | 0.163352               | Heptadecane, 9-hexyl- |
| 14  | 24.607               | 24.607             | 0.083451               | Heptadecanal- |
| 15  | 25.284               | 25.284             | 0.065058               | Octadecane, 3-ethyl-5-(2-ethylbutyl)- |
| 16  | 25.608               | 25.608             | 0.246146               | Eicosanol-    |
| 17  | 25.812               | 25.812             | 2.408768               | Phthalic acid, di(2-propylpentyl)ester |
| 18  | 25.978               | 25.978             | 0.198259               | y-Tocopherol  |
| 19  | 26.303               | 26.303             | 0.47058                | Cholesta-4,6-dien-3-ol, (3β)- |
| 20  | 26.944               | 26.944             | 1.341724               | dl-a-Tocopherol |
| 21  | 32.036               | 32.036             | 0.288983               | Stigmastadiene-3-one |
| 22  | 32.502               | 32.502             | 1.642749               | 2-Methyl-4,7-Dien-3-One |
| 23  | 32.837               | 32.837             | 0.431679               | 4-Campestene-3-one |
| 24  | 32.971               | 32.971             | 0.243051               | 9,19-Cyclohexan-24-en-3-ol, acetate, (3β)- |
| 25  | 33.091               | 33.091             | 0.226909               | 4,22-Stigmastadiene-3-one |
| 26  | 33.262               | 33.262             | 2.109739               | Stigmasta-3,5-Dien-7-One |
| 27  | 33.302               | 33.302             | 1.946938               | dl-a-Tocopherol |
| 28  | 33.488               | 33.488             | 1.090609               | 9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl-,acetate,(3β,4α,5α)- |
| 29  | 33.623               | 33.623             | 5.512032               | 9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl-,acetate,(3β,4α,5α)- |
| 30  | 34.191               | 34.191             | 1.239562               | Friedelan-3-one |
| 31  | 34.427               | 34.427             | 0.306566               | Friedelan-3-one |
| 32  | 34.853               | 34.853             | 0.321171               | Phenol, 2,6-bis (1,1-dimethylethyl)- |

**Figure 4:** Effect of methanolic extract on Caspase Activity. Lysates of A549 cells following treatment with methanolic extract were used to study the activity of caspase 3 (a), caspase 9 (b), and caspase 8 (c) as described in Materials and methods. Effect of Z-VAD-FMK on cell viability (d). A549 cells were incubated with methanol extract (5-100µg/ml) and Z-VAD-FMK (20mM) for 24 h and cell viability measured by the MTT assay as described in Materials and methods. Each point corresponds to the mean ± SD of at least three experiments in duplicate.
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**Figure 5**: GCMS Chromatogram of the Methanolic extract of *Garcinia cowa*.

**Figure 6**: GCMS Chromatogram of the Ethyl Acetate extract of *Garcinia cowa*.

**Figure 7**: Venn diagrammatic representation of total number of compounds present in the methanol and ethyl acetate extract of leaf specimen of *Garcinia cowa*.
Table 4: Major compounds identified in the methanolic extract of *Garcinia cowa* by GC/MS analysis and their reported biological activities.

| Sl No | Peak RT (min) | Peak Area (%) | Compound Detected | Mol. Formula | Mol. Wt. | CAS No. | Type of compound | Reported Biological Activity | Reference |
|-------|---------------|---------------|-------------------|--------------|----------|---------|------------------|-------------------------------|-----------|
| 1     | 5.463         | 7487579       | o-Cymene          | C_{10}H_{14}  | 134.222  | 527-84-4 | Essential oil    | Antitumor activity             | 15        |
| 2     | 5.655         | 12760944      | 2-Pyrrolidinone, 1-methyl-Tetradecane | C_{7}H_{10}NO | 99.13  | 872-50-4 | Cyclic amide    | Anticancer activity            | 16        |
| 3     | 5.738         | 14817656      | 4,4-Dimethyl-2-pentene | C_{10}H_{20} | 142.28 | -       | Alkane            | Antimicrobial activity          | 17        |
| 4     | 6.106         | 16014049      | Hexadecane        | C_{16}H_{34}  | 226.442 | 544-76-3 | Alkane hydrocarbon | Antibacterial, antioxidant activities | 18        |
| 5     | 6.167         | 10098676      | Hexadecane        | C_{16}H_{34}  | 226.442 | 544-76-3 | Alkane hydrocarbon | Antibacterial, antioxidant activities | 19        |
| 6     | 17.947        | 20735655      | Neophytadiene     | C_{20}H_{38}  | 278.5157 | 504-96-1 | Essential oil    | Antimicrobial, anticancer, anti-inflammatory, antimicrobial, and antioxidant activity | 20        |
| 7     | 18.493        | 11280525      | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C_{20}H_{40}O | 296.5310 | 102608-53-7 | Aliphatic diterpenoid alkene alcohol | Antimicrobial, anticancer, anti-inflammatory, anti-diuretic, Antioxidant, Hypocholesterolemic activity | 21        |
| 8     | 19.075        | 15568671      | n-Hexadecanoic acid methyl ester | C_{17}H_{34}O_{2} | 270.4507 | 112-39-0 | Fatty acid methyl esters | Antioxidant activity | 22        |
| 9     | 19.515        | 62709278      | n-Hexadecanoic acid | C_{17}H_{34}O_{2} | 270.4507 | 112-39-0 | Fatty acid methyl esters | Antioxidant activity | 22        |
| 10    | 19.85         | 7048102       | Hexadecanoic acid, ethyl ester | C_{18}H_{36}O_{2} | 284.4772 | 628-97-7 | Fatty acid ethyl esters | Antioxidant, Hypocholesterolemic, Flavor, Nematicide, Anti-androgenic activity | 24        |
| 11    | 21.259        | 48302849      | Phytol            | C_{20}H_{40}O | 296.5310 | 150-86-7 | Acyclic diterpene alcohol | Cytotoxic activity | 25        |
| 12    | 21.55         | 6508952       | 9-Octadecanoic acid, (E)- | C_{18}H_{36}O | 254.4241 | 57-10-3 | Linear chain saturated fatty acids | Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Anti-androgenic activity | 23        |
| 13    | 21.808        | 11564505      | Octadecanoic acid | C_{18}H_{36}O_{2} | 284.4772 | 57-11-4 | Straight-chain saturated fatty acid | Antimicrobial activity, Antimicrobial, anti-inflammatory, anticancer and anti-diuretic properties | 22        |
| 14    | 22.431        | 7634300       | Phytol, acetate   | C_{20}H_{40}O | 338.5677 | -       | Acyclic diterpene alcohol | Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Anti-androgenic activity | 24        |
| 15    | 25.608        | 6531831       | Eicosanol         | C_{20}H_{40}O | 296.5310 | 2400-66-0 | - | NR | - | 22 |
| 16    | 30.944        | 22359687      | dl-α-Tocopherol   | C_{20}H_{40}O | 430.7 | 10191-41-0 | Natural vitamin E | Natural antioxidant activity, strong antioxidant activity and antibacterial activity against multidrug resistant mycobacteria | 21        |
| 17    | 32.039        | 11112777      | Stigmasterol      | C_{29}H_{48}O | 412.7 | 83-48-7 | Phytosterol | Anti-diabetic activity | 27        |
| 18    | 32.527        | 29906534      | γ-Sitosterol      | C_{29}H_{48}O | 414.7067 | 83-47-6 | Triterpenoid | Anti-diabetic activity | 27        |
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Table 5: Major compounds identified in the Ethyl Acetate extract of *Garcinia cowa* by GC/MS analysis and their reported biological activities.

| Sl No. | Peak RT (min) | Peak Area | Peak Area (%) | Compound Detected | Mol. Formula | Mol. Wt. | CAS No. | Type of compound | Biological Activity | Reference |
|-------|--------------|-----------|--------------|-------------------|--------------|---------|---------|------------------|---------------------|-----------|
| 1     | 6.158        | 59637194  | 6.72         | Acetophenone      | C₈H₈O       | 120.1485| 98-86-2 | aromatic ketone   | NR                  | -         |
| 2     | 18.010749    | 3.03      |              | Stigmasta-3,5-Dien-7-One | C₂₉H₄₆O₄     | 410.7   | 2034-72-2 | -                | Free radical scavenging, Antidiabetic, Anticancer | 23        |
| 3     | 977425       | 1.67      |              | dl-α-Tocopherol   | C₂₀H₃₂O₃     | 430.7   | 10191-41-0 | natural vitamin E | Natural antioxidant activity | 26        |
| 4     | 51442110     | 8.65      |              | 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenyloctadec-4-enyl) cyclohexane | -           | -       | -       | Steroid          | NR                  | -         |
| 5     | 16473067     | 2.77      |              | γ-Sitosterone     | C₂₉H₄₆O₄     | 412.6908| 84924-96-9 | Steroid           | NR                  | -         |

(NR: Not Reported)
In this study, our preliminary vision was to check the anti-proliferative potential of the methanolic and ethyl acetate extract of *Garcinia cowa* leaves. For this, we checked the anti-proliferative activity of both the extracts through MTT assay in two different cancer cell line viz. HCT 116 and Jurkat. HCT 116 (human colon cancer cell line) is a solid tumour cancer cell and is an adherent culture. On the contrary, we have taken human acute T cell leukemic cell Jurkat which is a suspension culture. In both cases, it has been noted that methanolic extract has shown to be more potent in the anti-proliferative activity. Hence, we have opted leaf methanolic extract as our experimental material for further analysis of the anti-proliferative activity in different human cancer cell lines. Here appreciable activity of methanol extract against cancer cells has been recorded. Anti-proliferative activity was cancer cell-specific as negligible anti-proliferative activity was observed in case of normal PBMC cells isolated from healthy donors. A549 cell was the most sensitive cell line and the lowest IC_{50} was recorded from this cell line. Being a triple-negative breast cancer cells MDA-MB-468 cells generally shows poor prognosis. Here the methanol extracts also showed good anti-proliferative activity against MDA-MB-468. Hence, it is evident that leaf methanolic extract of *Garcinia cowa* is a good repository of anti-proliferative property-rich molecules.

DAPI (4', 6-diamidino-2-phenylindole) is a fluorescent stain. It binds preferentially to the AT-rich regions of dsDNA. Cells undergoing apoptosis will have nuclear fragmentation. Nuclear fragmentation was also observed by DAPI staining in the most sensitive cell line A549. Clear fragmentation of the nucleus was visualized and concentration dependant increase of nuclear fragmentation has been observed.

One of the distinctive features of the intrinsic pathway of programmed cell death is the release of cytochrome C from the mitochondria into the cytosol which in turn forms complexes with Apaf-1. This complex cleaves and activates Procaspase-9 into Caspase-9. Activated Caspase-9 ultimately cleaves and activates Caspase-3 into the executioner Caspase-3 leading to DNA fragmentation and cellular death. In this investigation, we have also observed the increase of caspase-9 and caspase-3 activity post methanolic extract treatment in A549 cells as compared to untreated control. No upregulation or downregulation of caspase 8 has been observed. It has also substantiated that the reduction of cell viability was caspase-mediated as the methanolic extract induced anti-proliferative activity was attenuated when it was co-incubated with a pan-caspase inhibitor, validating the intrinsic pathway of caspase dependant apoptosis.

Now, to check the individual components of ethyl acetate and methanol extracts, total metabolomic profiling of the leaves of *G. cowa* has been made through GC/MS analysis. Mainly Fatty acids, steroids, alkane groups, essential oil, triterpenoid, diterpenoid, phytosterol group of compounds have been identified. The relative abundance of different groups of components in methanol and ethyl acetate extract has been represented graphically in figure 8 (Figure 8). According to the reported biological activities, compounds are mainly reported with antitumor, antimicrobial and antioxidant properties. Presence of o-Cymene; 2-Pyrrolidinone 1-methyl-; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Phyitol; Phytol acetate; 9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl-acetate,(3β,4α,5α)- are the probable reason of appreciable anti-proliferative activity shown by the methanolic extract in MTT assay as all these compounds are previously reported with anti-cancer activities. Anticancer property-rich compounds like o-Cymene; 2-Pyrrolidinone, 1-methyl-; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol are present exclusively in the methanol extract which corroborates the potent anti-proliferative activity of methanolic extract than ethyl acetate extract in the result section. dl-α-Tocopherol; Stigmasterol; Hexadecane; Nonadecane; Neophytadiene; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; n-Hexadecanoic acid methyl ester; n-Hexadecanoic acid; ethyl ester are reported with antioxidant properties. A compound like dl-α-Tocopherol is a natural form of vitamin E which is a fat-soluble potent antioxidant molecule. Methanolic extract exclusively contains Stigmasterol, Nonadecane, n-Hexa decanoic acid methyl ester and Hexadecanoic acid, ethyl ester. All these compounds are reported with good antioxidant properties. Especially stigmastanol is reported with strong antioxidant properties. There are several compounds like γ-Sitostenone; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; 9-Octadecanoic acid, (E)- are compounds which have no bioactivity record till date and yet to be explored.

**DISCUSSION**

In this study, our preliminary vision was to check the anti-proliferative potential of the methanolic and ethyl acetate extract of *Garcinia cowa* leaves. For this, we checked the anti-proliferative activity of both the extracts through MTT assay in two different cancer cell line viz. HCT 116 and Jurkat. HCT 116 (human colon cancer cell line) is a solid tumour cancer cell and is an adherent culture. On the contrary, we have taken human acute T cell leukemic cell Jurkat which is a suspension culture. In both cases, it has been noted that methanolic extract has shown to be more potent in the anti-proliferative activity. Hence, we have opted leaf methanolic extract as our experimental material for further analysis of the anti-proliferative activity in different human cancer cell lines. Here appreciable activity of methanol extract against cancer cells has been recorded. Anti-proliferative activity was cancer cell-specific as negligible anti-proliferative activity was observed in case of normal PBMC cells isolated from healthy donors. A549 cell was the most sensitive cell line and the lowest IC_{50} was recorded from this cell line. Being a triple-negative breast cancer cells MDA-MB-468 cells generally shows poor prognosis. Here the methanol extracts also showed good anti-proliferative activity against MDA-MB-468. Hence, it is evident that leaf methanolic extract of *Garcinia cowa* is a good repository of anti-proliferative property-rich molecules.

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One of the distinctive features of the intrinsic pathway of programmed cell death is the release of cytochrome C from the mitochondria into the cytosol which in turn forms complexes with Apaf-1. This complex cleaves and activates Procaspase-9 into Caspase-9. Activated Caspase-9 ultimately cleaves and activates Caspase-3 into the executioner Caspase-3 leading to DNA fragmentation and cellular death. In this investigation, we have also observed the increase of caspase-9 and caspase-3 activity post methanolic extract treatment in A549 cells as compared to untreated control. No upregulation or downregulation of caspase 8 has been observed. It has also substantiated that the reduction of cell viability was caspase-mediated as the methanolic extract induced anti-proliferative activity was attenuated when it was co-incubated with a pan-caspase inhibitor, validating the intrinsic pathway of caspase dependant apoptosis.

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**CONCLUSION**

This study implies that *Garcinia cowa* is a potent medicinal plant as it shows a noticeable anti-proliferative property particularly against human lung cancer cell line, A549. Modest anti-proliferative potential...
was also noticed in MCF – 7, HepG2, MOLT – 4 and some exhibits minimal effect as MDA-MB-468. Anti-proliferative activity, nuclear morphology, caspase activity of the leaf methanolic extract infers that the extract possesses potentially rich bioactive molecules. GC-MS analysis also substantiates the fact. Further, the GC/MS analysis revealed the presence of many compounds like γ -Sisostenone; 1,1,6,13-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenyl-10,14-dimethylene-pentadec-4-enyl)cyclohexane; Eicosanal-; 9-Octadecanoic acid, (E)- with unknown bioactivity. This adds up to the pharmaceutical importance of the study for further isolation and purification of phytochemicals. Specific anti-proliferative activity towards cancer cells and not with normal cells conclude that the methanolic extract may be tapped as a repository of natural anti-cancer molecules in the development of drugs in the future of lung cancer treatment in particular.

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AUTHORS’ CONTRIBUTION
Anirban Chouni has collected the specimen, performed the assays processed the data and written the manuscript. Maintenance of the cell culture and the analysis of caspase was done by Ms. Amrita Pal and Dr. Priya K Gopal. Prof. Santanu Paul has given the idea, gone through the data and edited the manuscript. He has guided the entire work and assessed the manuscript.

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CONFLICTS OF INTEREST
None.

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**GRAPHICAL ABSTRACT**

*Garcinia cowa* Leaves

**Anti-proliferative and Apoptotic potential**

**GC/MS Analysis**
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