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Original Article

GC-MS Analysis and Screening of Anti-Proliferative Potential of Methanolic Extract of *Garcinia cowa* on Different Cancer Cell Lines

Anirban Chouni, Amrita Pal, Priya K Gopal, Santanu Paul*

ABSTRACT

Introduction: *Garcinia cowa* (Clusiaceae) is popular among integrative medicine in several communities. This study undertook to evaluate the anti-proliferative activity on cancer cells and its cytotoxic effect on normal cells. Here we are reporting for the first time the metabolomic profiling of *G. cowa* leaf. **Methods:** Anti-proliferative potential of ethyl acetate and methanol extract of *Garcinia 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 methanolic 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 *Garcinia 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: *Garcinia cowa*, Cancer, GC-MS, Metabolomic profiling, Anti-proliferative.

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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 of a natural origin [1]. Currently, more emphasis has given to natural drug discovery. Natural compounds may have the potential to inhibit cancer [2]. According to food and drug administration, around 40% of the approved molecules are natural compounds or inspired by them. Among the approved natural compounds, 74% are used in cancer treatment. Natural products are more biologically friendly and less toxic to normal healthy cells [3]. In search of potentially bioactive natural compounds, the study of different secondary metabolites like alkaloid, diterpene, triterpene, and polyphenolic compounds is essential. One of the best method for the investigation of the wide range of secondary metabolites is complete metabolomic profiling. We are interested in identifying plants; as a repository of anti-cancer molecules, which will be clinically useful and safe, which could modulate therapeutic response and may have a future in the clinic.

Garcinia cowa Roxb. Ex DC. is a tree species. Locally it 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 occurs in China in the southern and western parts of Yunnan Province. Several bioactivities have been reported from the extracted chemicals from the plant. In several countries, the plant is in use as traditional medicine for a long time [4]. Until the present, a total of 397 accepted species are in the record [5]. About 30 species are indigenous to India. Several bioactivities are in the report from the numerous chemicals, extracted from the plant. In India, *G. cowa* 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 [6]. Previously *G. cowa* reported with good anti-bacterial activities, antiplatelet aggregation capacity, anticancer activity against human colorectal adenocarcinoma cells [7–9]. However, the least information is present about the complete metabolomic profiling and phytochemical potential of *G. cowa*. This study has been done to estimate the anti-proliferative potential

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and metabolomic profiling of different solvent extracts of *G. cowa* leaf. Here, for the first time, we are reporting the metabolomic profiling of *Garcinia 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 *Garcinia 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% CO_2 at 37°C in a CO_2 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. [10]. After the isolation, the cells were washed. After then, it was resuspended in phosphate-buffered saline (PBS). Finally, the cells were suspended in the RPMI 1640 medium. The medium was supplemented with 2mM glutamine, gentamicin, and 10% heat-inactivated FBS.

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay

HCT 116, A549, MCF – 7, HepG2, MDA-MB-468 cells were seeded at $1x10^4$ cells/well, on the other hand, MOLT-4 and Jurkat cells were seeded at $1x10^5$ cells/well in 96-well plate for 24 hours. After then, the cells were treated with different concentrations of leaf methanolic and ethyl acetate extract of *G. cowa* and observed at 24 and 48hrs time point by MTT assay [11]. Three hours before the completion of the time points, 20µl of MTT solution (10% v/v of 5-mg/mL) was added 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:

Cell viability (%) =

$$\frac{(\text{Absorbance of Sample} - \text{Absorbance of 100 \% Lysis})}{(\text{Absorbance of 0\% Lysis} - \text{Absorbance of 100\% Lysis})} \times 100$$

Nuclear morphology analysis with DAPI staining

A549 cells ($1x10^5$ cells/well) were seeded in 6 well plate and treated with different concentrations of leaf methanolic extract of *Garcinia 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 (1×10^5 cell/mL) were treated with methanolic extract (1µg/ml, 18h at 37^0C). 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 paranitroanilide (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

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 2914V; 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 100µg/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 100µg/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 24h 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 24h 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 24h 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 [12]. 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.

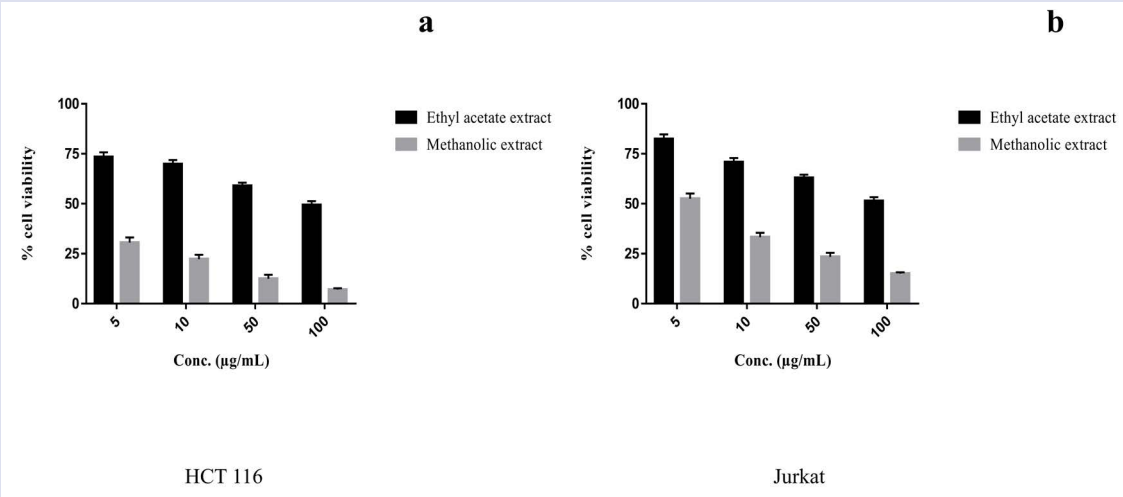

Figure 1: Effect of leaf ethyl acetate and methanolic extract of *Garcinia cowa* on two Different Cancer Cell Lines. Effect of leaf ethyl acetate and methanolic extract of *Garcinia cowa* (5-100µg/ml) by MTT assay against HCT 116 (a) and Jurkat (b) cell lines at 24h time point. Each line graph represents the Mean value ± Standard Deviation of three individual experiments in duplicate.

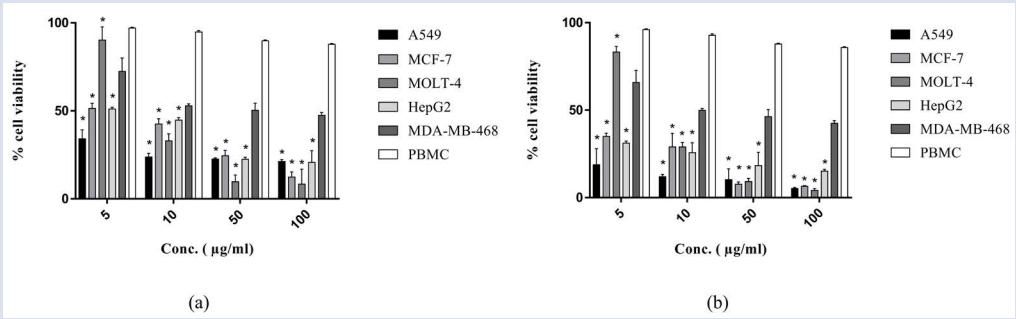

Figure 2: Effect of leaf methanolic extract of *Garcinia cowa* on five Different Cancer Cell Lines. Effect of methanolic extract of *Garcinia cowa* (5-100µg/ml) by MTT assay against A549, MCF-7, MOLT-4, HepG2, MDA-MB-468, and normal PBMC isolated from healthy donors at 24h (a) and 48h (b) time point. Each line graph represents the Mean value ± Standard Deviation of three individual experiments in duplicate. *p < 0.01 as compared to normal PBMC.

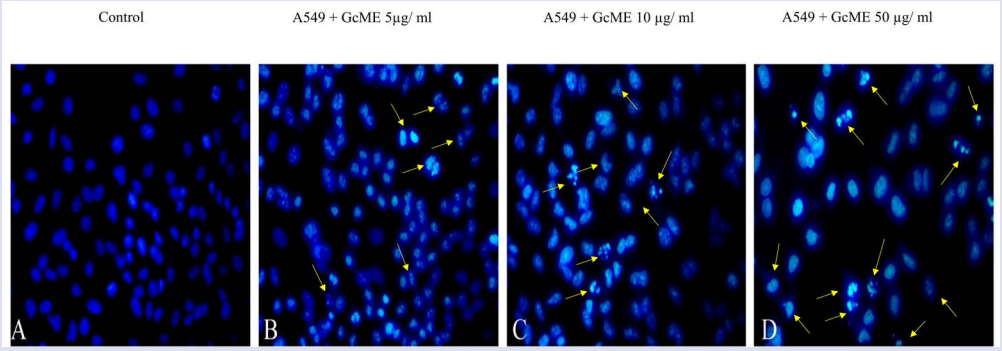

Figure 3: Fluorescence Microscopic Images of A549 Cells Treated with or without methanolic extract at Different Concentrations. Control A549 cells (A), *Gc* ME treated A549 cells at concentrations 5 µM (B), 10 µM (C) and 50µM (D). The control cells were with intact nucleus and gave bright blue fluorescence;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

Table 1: IC_{50} 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-435
IC_{50} 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 IC_{50} 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	o-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	4067636	0.744867	Acetophenone
9	6.518	1635828	0.299554	Benzene, 1-methyl-4-(1-methylethenyl)-
10	7.65	4789228	0.877006	Benzoic acid
11	8.178	4983414	0.912565	Napthalene
12	8.247	1302367	0.23849	α-Terpineol
13	8.327	3930191	0.719698	Dodecane
14	8.735	1225119	0.224344	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-Tetradecene
18	11.639	14817656	2.713416	Tetradecane
19	11.77	979895	0.179439	Diphenyl ether
20	11.832	1236722	0.226469	Naphthalene, 1,7-dimethyl-
21	12.069	892137	0.163369	Naphthalene, 1,4-dimethyl-
22	12.123	830732	0.152124	Naphthalene, 2,6-dimethyl-
23	12.618	958795	0.175575	Tetradecane, 2,6,10-trimethyl-
24	12.902	766347	0.140334	(4s,4aR,6R)-4,4a-Dimethyl-6(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene)
25	12.997	731199	0.133897	1,2,4-Benzenetriol
26	13.193	3531479	0.646686	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ)]-
27	13.805	1064841	0.194994	Undec-10-ynoic acid, octadecyl ester
28	14.07	719864	0.131822	Dodecanoic 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-propyloctyl)-
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	16.527	2890228	0.52926	Benzene, (1-pentylheptyl)-
41	16.593	2627996	0.48124	Benzene, (1-butyloctyl)-
42	16.76	2296938	0.420616	Benzene, (1-propylnonyl)-
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-pentyloctyl)-
49	17.947	20753655	3.800419	Neophytadiene
50	18.023	3046644	0.557903	2-Pentadecanone, 6,10,14-trimethyl-
51	18.085	1493665	0.273521	Benzene, (1-propyldecyl)-
52	18.387	1164381	0.213222	Benzene, (1-ethylundecyl)-
53	18.493	11280525	2.065695	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
54	18.875	2428502	0.444708	Benzene, (1-methyldodecyl)-
55	19.02	15568671	2.850942	n-Hexadecanoic acid methyl ester
56	19.515	62709278	11.48335	n-Hexadecanoic acid
57	19.85	7048102	1.290652	Hexadecanoic acid, ethyl ester

58	19.916	4296196	0.786721	Hexadecane
59	20.618	951450	0.17423	Octadecanoic acid
60	21.113	1073906	0.196654	9-Octadecenoic acid (Z)-, methyl ester
61	21.397	4087010	0.748415	Methyl stearate
62	21.47	1474052	0.269929	9,12-Octadecadienoic acid (Z,Z)-
63	21.55	6508952	1.191922	9-Octadecanoic acid, (E)-
64	21.808	11564505	2.117697	Octadecanoic acid
65	22.143	1857430	0.340133	Octadecanoic acid, ethyl ester
66	22.198	1524380	0.279145	Heptadecane
67	22.431	7634300	1.397996	Phytol, acetate
68	23.377	819725	0.150108	Aspidospermidin-17-ol,1-acetyl-19-21-epoxy-15,16-dimethoxy-
69	23.468	1313043	0.240445	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate
70	23.57	4291327	0.78583	Eicosanal-
71	23.705	3407212	0.62393	Ethanol,2-(9-octadecenyloxy)-,(Z)-
72	23.887	2822651	0.516885	4,8,12,16-Tetramethylheptadecan-4-olide
73	24.607	3334931	0.610694	Octadecanal
74	25.401	3869936	0.708664	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
75	25.608	6531831	1.196112	Eicosanal-
76	25.812	2947781	0.539799	Diisooctyl phthalate
77	26.569	1687310	0.308981	Eicosonal-
78	27.494	1272245	0.232974	Eicosonal-
79	28.909	823805	0.150856	1-Eicosanol
80	29.139	1351038	0.247403	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-
81	30.278	3121862	0.571677	γ-Tocopherol
82	30.573	2968436	0.543581	Cholesta-4,6-dien-3-ol, (3β)-
83	30.744	1086993	0.199051	Stigmasta-3,5-diene
84	30.809	1664670	0.304835	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-
85	30.944	22359687	4.094516	dl-α-Tocopherol
86	31.788	2754046	0.504322	Campesterol
87	32.039	11112777	2.034977	Stigmasterol
88	32.527	29806534	5.458186	γ- sitosterol
89	32.84	4201897	0.769453	4-Campestene-3-one
90	32.975	1390059	0.254548	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3β,4α,5α)-
91	33.091	2269462	0.415585	4,22-Stigmastadiene-3-one
92	33.262	18010749	3.298136	Stigmasta-3,5-Dien-7-One
93	33.302	14044031	2.571749	dl-α-Tocopherol
94	33.488	9974925	1.826613	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3β,4α,5α)-
95	33.626	51442110	9.420102	γ- Sitostenone
96	34.191	16473067	3.016555	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane
97	34.427	2674294	0.489718	Friedelan-3-one
	Total	546088660	100	

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	o-Cymene
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	Acetophenone
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-Butenoic acid, 3-methoxy-4-nitro-, (E)-
12	7.97	80302742	9.05553	Heptanediamide,N,N'-di-benzoyloxy-
13	8.181	5961198	0.672229	Naphthalene
14	8.247	3552182	0.40057	L-α-Terpineol

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	9.626	1276487	0.143946	Nonanal dimethyl acetal
23	10.012	1805675	0.203621	Tridecane
24	10.038	3813122	0.429996	1H-Indene, 1-ethylidene-
25	10.325	3366048	0.37958	Naphthalene, 2-methyl-
26	11.643	24929784	2.811266	Tetradecane
27	11.77	1671564	0.188498	Diphenyl ether
28	11.832	2814687	0.317405	Naphthalene, 1,7-dimethyl-
29	12.069	2005489	0.226154	Naphthalene, 1,7-dimethyl-
30	12.127	1960048	0.221029	Naphthalene, 1,5-dimethylm
31	12.349	2785541	0.314118	Ethanone, 2-(acetyloxy)-1-phenyl-
32		7867643	0.887213	Acetophenone, 2-chloro-
33	12.622	1442921	0.162714	Tetradecane, 2,6,10-trimethyl-
34	12.906	2962573	0.334082	(4s,4aR,6R)-4,4a-Dimethyl-6(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene)
35	13.179	1274308	0.1437	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ)]-
36	13.193	606609	0.068406	1,4-Dimethyl-7-(prop-1-en-2-yl)decahydroazulen-4-ol
37	13.397	2808964	0.31676	2,4-Di-tert-butylphenol
38	13.805	4332912	0.488611	Benzene, (1-butylhexyl)-
39	13.936	2506222	0.28262	Benzene, (1-propylheptyl)-
40	14.099	2000330	0.225572	Dodecanoic acid
41	14.212	1810807	0.2042	Benzene, (1-ethyloctyl)-
42	14.314	1826084	0.205923	2-Oxa-3-azabicyclo(4.4.0)dec-3-ene,5-methyl-1-trimethylsilyloxy-,N-oxide
43	14.565	1980941	0.223386	Cetene
44	14.678	24101900	2.717908	Hexadecane
45	14.74	4002869	0.451393	Benzene, (1-methylnonadecyl)-
46	15.177	3121857	0.352044	Benzene, (1-pentylhexyl)-
47	15.228	7804376	0.880079	Benzene, (1-butylheptyl)-
48	15.373	7296672	0.822827	Benzene, (1-propyloctyl)-
49	15.665	5702595	0.643067	α-acorenol
50	15.988	4012511	0.45248	Dodecyl acrylate
51	16.178	10332643	1.165185	Benzene, (1-methyldecyl)-
52	16.527	7820428	0.881889	Benzene, (1-pentylheptyl)-
53	16.593	7445409	0.839599	Benzene,(1-butyloctyl)-
54	16.76	5551836	0.626066	Benzene, (1-propylnonyl)-
55	16.913	11378991	1.283179	Tetradecanoic acid
56	17.051	4080996	0.460203	Benzene, (1-ethyldecyl)-
57	17.328	1388505	0.156578	1-Nonadecene
58	17.419	14194866	1.600718	Octadecane
59	17.561	8465396	0.954621	Benzene, (1-methylundecyl)-
60	17.83	7345117	0.82829	Benzene, (1-hexylheptyl)-
61	17.947	23533260	2.653784	Neophytadiene
62	18.023	3211467	0.362149	2-Pentadecanone, 6,10,14-trimethyl-
63	18.085	3415481	0.385155	Benzene, (1-propyldecyl)-
64	18.383	1540439	0.173711	Benzene, (1-ethylundecyl)-
65	18.496	13072273	1.474126	Neophytadiene
66	18.584	1308986	0.147611	(3S,3aS,6R,7R,9aS)-1,1,7-Trimethyldecahydro-3a,7-methanocyclopenta[8]annulene-3,6-diol
67	18.875	5339542	0.602126	Benzene, (1-methylundecyl)-
68	19.02	3699311	0.417162	Methyl 9-methyltetradecanoate
69	19.544	123247324	13.89828	n-Hexadecanoic acid
70	19.85	1342233	0.15136	Hexadecanoic acid, ethyl ester
71	19.916	2763588	0.311643	Heptadecane
72	19.948	6201235	0.699297	Chlorpyrifos
73	20.531	5856208	0.660389	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-,1-methylethyl ester
74	20.625	1690074	0.190585	Nonanedioic acid, dibutyl ester

75	21.262	40232711	4.536937	Phytol
76	21.488	2698599	0.304314	9,12-Octadecadienoic acid (Z,Z)-
77	21.572	11880775	1.339764	Oleic Acid
78	21.823	19862932	2.239891	Octadecanoic acid
79	22.198	2757390	0.310944	Pentadecane
80	22.431	2458101	0.277194	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
81	23.111	279303	0.031496	Columbin
82	23.264	331907	0.037428	Heptadecane, 9-hexyl-
83	23.468	358839	0.040465	Oleoyl chloride
84	23.57	654211	0.073774	Pentadecanal-
85	23.708	2071529	0.233601	Eicosane, 2-cyclohexyl-
86	23.89	4709125	0.531036	4,8,12,16-Tetramethylheptadecan-4-olide
87	24.298	1448574	0.163352	Heptadecane, 9-hexyl-
88	24.607	740026	0.083451	Eicosanal-
89	25.284	576919	0.065058	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
90	25.608	2182775	0.246146	Eicosanal-
91	25.812	21360504	2.408768	Phthalic acid, di(2-propylpentyl)ester
92	30.278	1758124	0.198259	γ-Tocopherol
93	30.573	4173015	0.47058	Cholesta-4,6-dien-3-ol, (3β)-
94	30.944	11898160	1.341724	dl-α-Tocopherol
95	32.036	2562644	0.288983	Stigmasterol
96	32.502	14567590	1.642749	γ- sitosterol
97	32.837	3828051	0.431679	4-Campestene-3-one
98	32.971	2155331	0.243051	9,19-Cyclolanost-24-en-3-ol, acetate, (3β)-
99	33.091	2012188	0.226909	4,22-Stigmastadiene-3-one
100	33.262	18708773	2.109739	Stigmasta-3,5-Dien-7-One
101	33.302	17265078	1.946938	dl-α-Tocopherol
102	33.488	9671320	1.090609	9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl-,acetate,(3β,4α,5α)-
103	33.623	48879667	5.512032	γ- Sitostenone
104	34.191	10992207	1.239562	Friedelan-3-one
105	34.427	2718574	0.306566	Friedelan-3-one
106	34.853	2848083	0.321171	Phenol, 2,6-bis (1,1-dimethylethyl)-
Total		886781285	100	

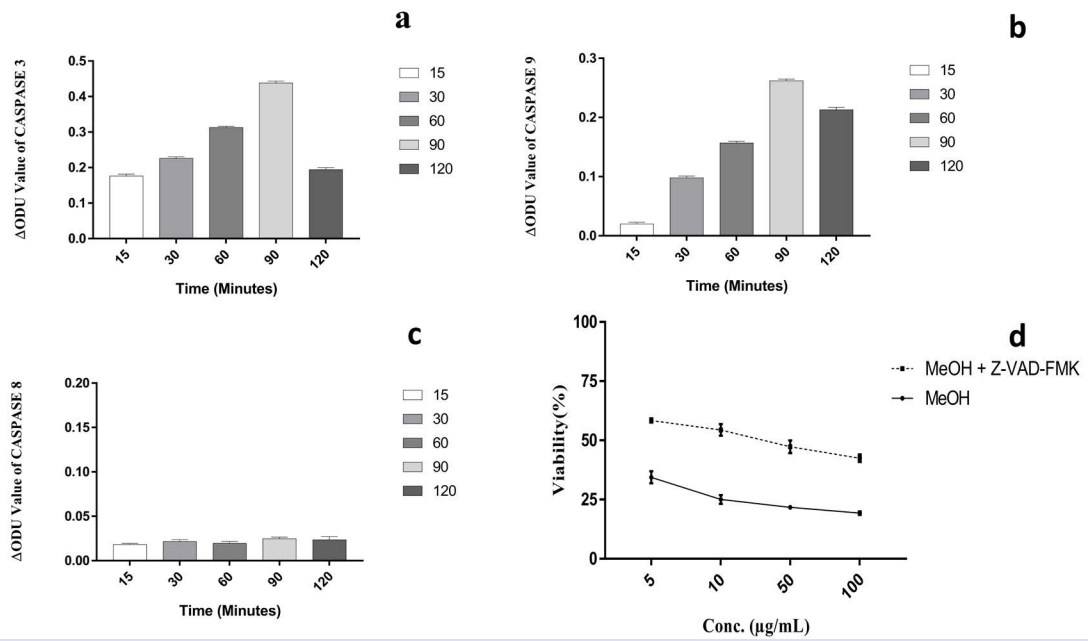

Figure 4: Effect of methanolic extract on Caspase Activity. Lysates of A549 cells following treatment with methanolic extract were used to study the activity of caspase 3 (a), caspase 9 (b), and caspase 8 (c) as described in Materials and methods. Effect of Z-VAD-FMK on cell viability (d). A549 cells 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.

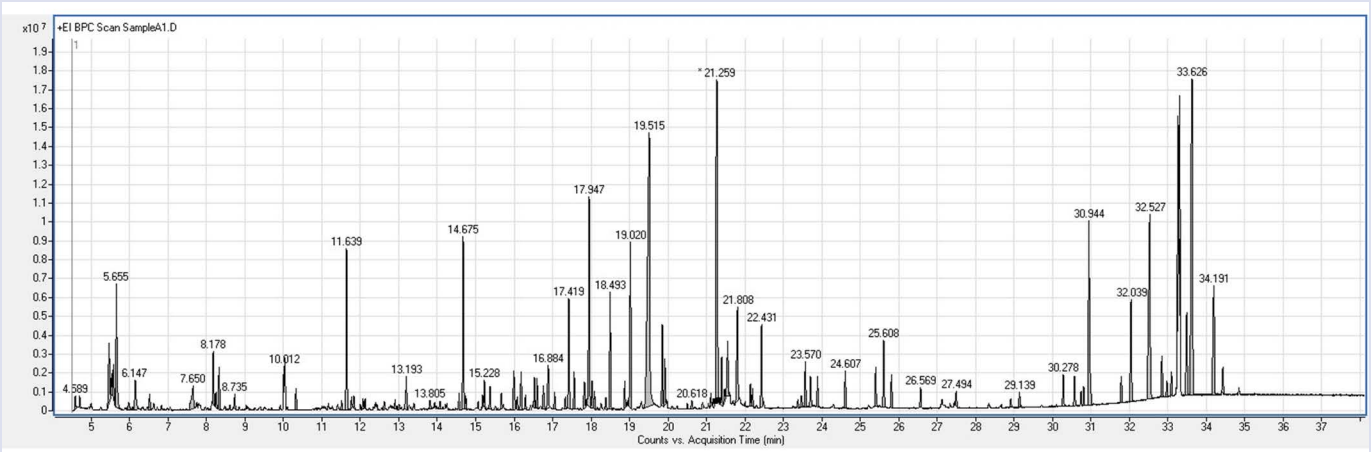

Figure 5: GCMS Chromatogram of the Methanolic extract of *Garcinia cowa*.

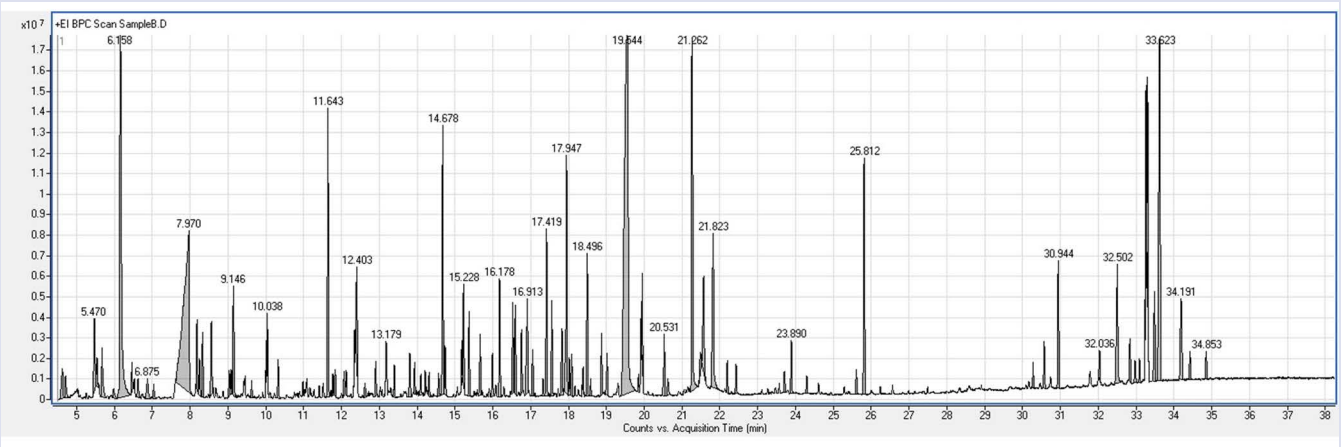

Figure 6: GCMS Chromatogram of the Ethyl Acetate extract of *Garcinia cowa*.

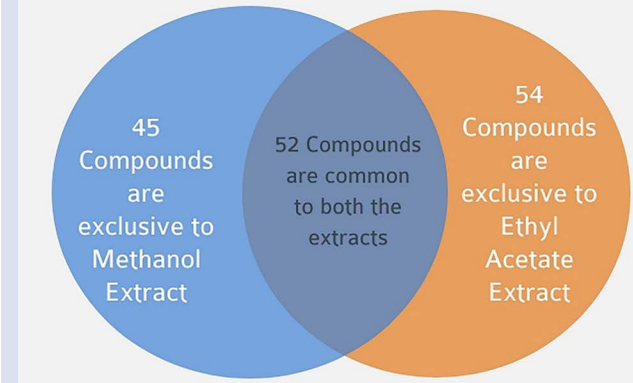

Figure 7: Venn diagrammatic representation of total number of compounds present in the methanol and ethyl acetate extract of leaf specimen of *Garcinia cowa*.

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	Peak Area (%)	Compound Detected	Mol. Formula	Mol. Wt.	CAS No.	Type of compound	Reported Biological Activity	Reference
1	5.463	7487579	1.26	o-Cymene	$C_{10}H_{14}$	134.222	527-84-4	Essential oil	Antitumor activity	15
2	5.655	12760944	2.14	2-Pyrrolidinone, 1-methyl-	C_5H_9NO	99.13	872-50-4	Cyclic amide	Anticancer activity	16
3	11.639	14817656	2.49	Tetradecane	$C_{14}H_{30}$	198.3880	629-59-4	straight chain alkane	Antimicrobial activity	17
4	14.675	16014049	2.69	Hexadecane	$C_{16}H_{34}$	226.4412	544-76-3	alkane hydrocarbon	Antibacterial, antioxidant activities	18
5	17.419	10098676	1.69	Nonadecane	$C_{19}H_{40}$	268.5209	629-92-5	alkane hydrocarbon	Antioxidant, Antibacterial activity	19
6	17.947	20753655	3.49	Neophytadiene	$C_{20}H_{38}$	278.5157	504-96-1	essential oil	analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant activity	20
7	18.493	11280525	1.89	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	19.02	15568671	2.62	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	10.55	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	57-10-3	linear chain saturated fatty acids	Anti-inflammatory, Antioxidant, hypocholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor, , potent mosquito larvicide	23
10	19.85	7048102	1.18	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4772	628-97-7	fatty acid ethyl esters	Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Anti-androgenic activity	24
11	21.259	48302849	8.12	Phytol	$C_{20}H_{40}O$	296.5310	150-86-7	acyclic diterpene alcohol	Cytotoxic activity	25
12	21.55	6508952	1.09	9-Octadecanoic acid, (E)-	-	-	-	Long-chain fatty acids	NR	-
13	21.808	11564505	1.94	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	57-11-4	straight-chain saturated fattyacid	Antimicrobial activity	23
14	22.431	7634300	1.28	Phytol, acetate	$C_{22}H_{42}O_2$	338.5677	-	acyclic diterpene alcohol	Antimicrobial, anti-inflammatory, anticancer and antidiuretic properties	22
15	25.608	6531831	1.09	Eicosanal-	$C_{20}H_{40}O$	296.53	2400-66-0	-	NR	-
16	30.944	22359687	3.76	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.7	10191-41-0	natural vitamin E	Natural antioxidant activity	26
17	32.039	11112777	1.87	Stigmasterol	$C_{29}H_{48}O$	412.7	83-48-7	phytosterol	strong antioxidant activity and antibacterial activity against multidrug resistant mycobacteria	21
18	32.527	29806534	5.01	γ- sitosterol	$C_{29}H_{50}O$	414.7067	83-47-6	triterpenoid	Anti- diabetic activity	27

19	33.262	18010749	3.03	Stigmasta-3,5-Dien-7-One	$C_{29}H_{46}O$	410.7	2034-72-2	-	Free radical scavenging, Anti-diabetic, anticancer	[23]
20	33.302	14044031	2.36	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.7	10191-41-0	natural vitamin E	Natural antioxidant activity	[26]
21	33.488	9974925	1.67	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3β,4α,5α)-	$C_{32}H_{52}O_2$	468.7541	10376-42-8	Steroid	Antimicrobial, Anti – inflammatory, Anticancer, Antiasthma, Hepatoprotective, Diuretic activity	[28]
22	33.626	51442110	8.65	γ- Sitostenone	$C_{29}H_{48}O$	412.6908	84924-96-9	Steroid	NR	-
23	34.191	16473067	2.77	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl) cyclohexane	-	-	-	-	NR	-

(NR- Not Reported)

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	C_8H_8O	120.1485	98-86-2	aromatic ketone	NR	-
2	7.97	80302742	9.05	Heptanediamide,N,N'-di-benzoyloxy-	$C_{21}H_{22}N_2O_6$	398.415	-	-	NR	-
3	9.146	10457906	1.18	1-Phenoxypropan-2-ol	$C_9H_{12}O_2$	152.1904	770-35-4	-	NR	-
4	11.643	24929784	2.81	Tetradecane	$C_{14}H_{30}$	198.3880	629-59-4	straight chain alkane	Antimicrobial activity	[17]
5	14.678	24101900	2.72	Hexadecane	$C_{16}H_{34}$	226.4412	544-76-3	straight chain alkane	Antibacterial, antioxidant activities	[18]
6	16.178	10332643	1.16	Benzene, (1-methyldecyl)-	$C_{17}H_{28}$	232.4042	4536-88-3	Alkyl benzene	NR	-
7	16.913	11378991	1.28	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.3709	544-63-8	saturated fatty acid	Larvicidal and repellent activity	[23]
8	17.419	14194866	1.60	Octadecane	$C_{18}H_{38}$	254.4943	593-45-3	straight-chain alkane	Hypoglycaemic activity, Antimicrobial activity and Antioxidant activity	[29]
9	17.947	23533260	2.65	Neophytadiene	$C_{20}H_{38}$	278.5157	504-96-1	essential oil	analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant activity	[20]
10	19.544	123247324	13.89	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	57-10-3	linear chain saturated fatty acids	Anti-inflammatory, Antioxidant, hypocholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor, , potent mosquito larvicide	[23]
11	21.262	40232711	4.53	Phytol	$C_{20}H_{40}O$	296.5310	150-86-7	acyclic diterpene alcohol	Cytotoxic activity	[25]
12	21.572	11880775	1.33	Oleic Acid	$C_{18}H_{34}O_2$	282.4614	112-80-1	Natural fatty acid	NR	
13	21.823	19862932	2.24	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	57-11-4	straight-chain saturated fattyacid	Antimicrobial activity	[23]
14	25.812	21360504	2.41	Phthalic acid, di(2-propylpentyl)ester	$C_{24}H_{38}O_4$	390.5561	-	alkyl aryl esters	NR	-
15	30.944	11898160	1.34	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.7061	10191-41-0	Natural Vitamin E	Natural antioxidant activity	[26]
16	32.502	14567590	1.64	γ- sitosterol	$C_{29}H_{50}O$	414.7067	83-47-6	triterpenoid	Anti- diabetic activity	[27]

17	33.262	18708773	2.11	Stigmasta-3,5-Dien-7-One	$C_{29}H_{46}O$	410.7	2034-72-2	phytosterol	Free radical scavenging, Anti-diabetic, anticancer	[23]
18	33.302	17265078	1.94	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.7061	10191-41-0	Natural Vitamin E	Natural antioxidant activity	[26]
19	33.488	9671320	1.09	9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl-,acetate,(3β,4α,5α)-	$C_{32}H_{52}O_2$	468.7541	10376-42-8	Steroid	Antimicrobial, Anti – inflammatory, Anticancer, Antiasthma, Hepatoprotective, Diuretic activity	[28]
20	33.623	48879667	5.51	γ- Sitostenone	$C_{29}H_{48}O$	412.6908	84924-96-9	-	NR	-
21	34.191	10992207	1.24	Friedelan-3-one	$C_{30}H_{50}O$	426.7174	559-74-0	triterpenoid	antifeedant, anti-inflammatory, anticancer, hepatoprotective, antimicrobial and anticandidal activities	[30]

(NR- Not Reported)

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.

DAPI (4', 6-diamidino-2-phenylindole) is a fluorescent stain. It binds preferentially to the AT-rich regions of dsDNA [13]. 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 Procaspase-3 into the executioner Caspase-3 leading to DNA fragmentation and cellular death [14]. 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; Phytol; 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; 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 stigmasterol is reported with strong antioxidant properties. There are several compounds like γ- Sitostenone; 1,1,6-trimethyl-3-methylene-2-(3,6,9,13 tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane; Eicosanal-; 9-Octadecanoic acid, (E)- are compounds which have no bioactivity record till date and yet to be explored.

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

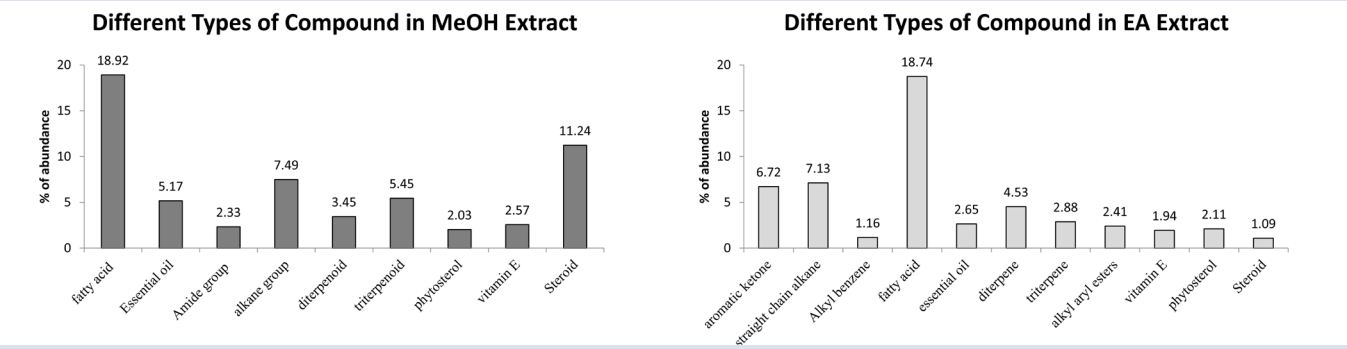

Figure 8: Graphical representation of the percentage of different types of compounds present in the methanolic (MeOH) and ethyl acetate (EA) extract.

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- Sitostenone; 1,1,6-trimethyl-3-methylene- 2-(3,6,9,13 tetramethyl-6-ethenye-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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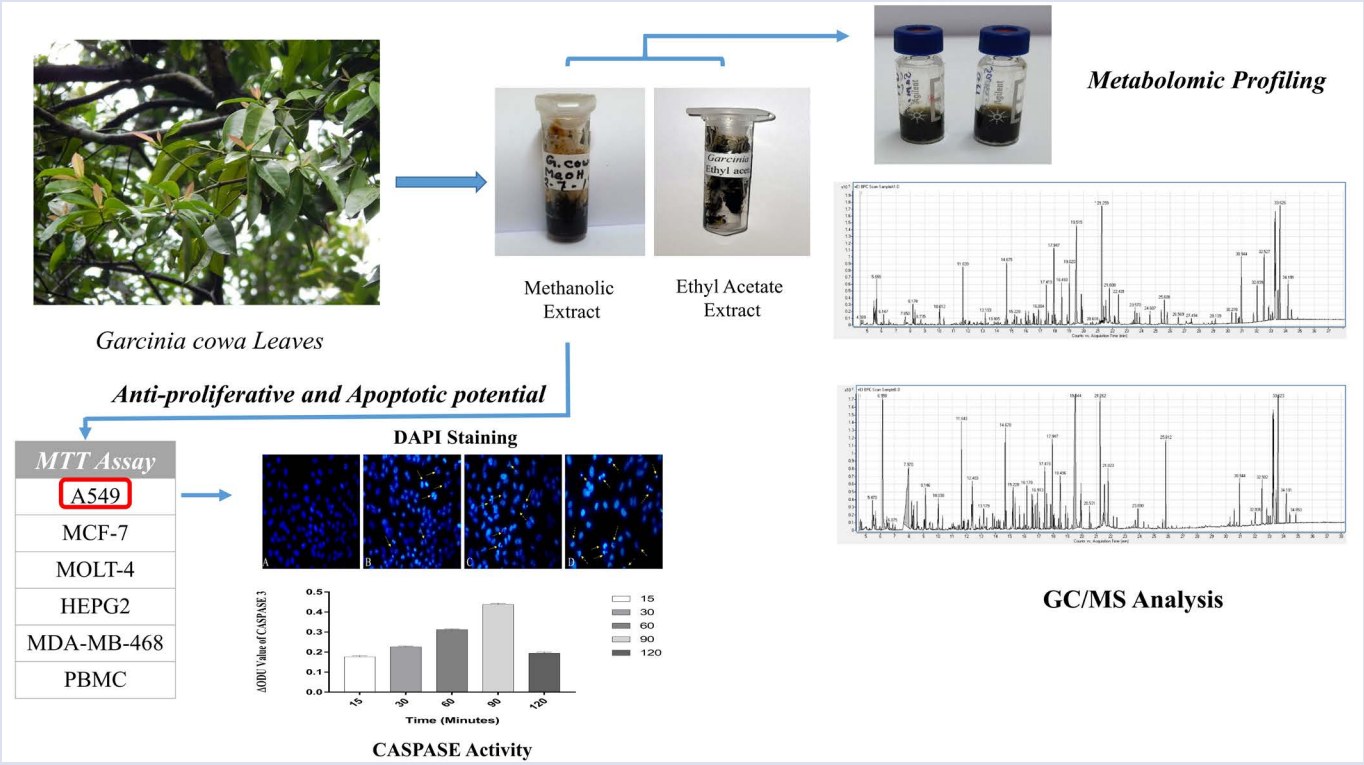
GRAPHICAL ABSTRACT



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