Screening Data Reveals that *Spirogyra triplicata*, a Fresh Water Algae Induces Robust Anti-Proliferative Activity Against A549 Cells

Ankita Mridha, Priya K Gopal, Santanu Paul*

ABSTRACT

**Introduction:** Algae comprise a promising source of novel components with potent therapeutic agents. In particular, algae have been considered as a potential source of new bioactive compounds. The antioxidant data of our previous study with six different algal methanolic extract reveals the presence of high antioxidant, total phenol content and total flavonoid content in *Spirogyra triplicata*. Thus, we further focused on screening the anti-proliferative activity of six different green algae on five different cancer cell lines like MCF7, A549, HEPG2, REH, MOLT4.

**Methods:** To fulfill our aim we performed MTT assay for testing anti-proliferative activity and DAPI staining for observing nuclear morphology. We also looked into the metabolomic profiling of *Spirogyra triplicata* by GC-MS chemometric study. **Results:** The result indicates that after 24 hours of treatment with methanolic extract of *Spirogyra triplicata* A549 was the most sensitive cell line with IC$_{50}$ value of 24.07 ± 1.09 μg/ml. Followed by *Rhizoclonium fontinale* and *Hydrodictyon reticulatum* with IC$_{50}$ value of 25.97 ± 1.94 μg/ml and 32.50 ± 1.97 μg/ml respectively. The HEPG2 cell line was the second most sensitive cell line against *S. triplicata* with IC$_{50}$ Value of 30.20 ± 1.45 μg/ml. The MOLT4 cell line was detected as most resistant cell line against the green algal extract in this study. Though the methanolic extracts of six green algae showed maximum to moderate anti-proliferative activity on different cancer cell line but no significantly affect on normal PBMC was observed. Nuclear fragmentation was observed in a dose dependent fashion by DAPI staining on A549 cells treated with methanolic extract of *Spirogyra triplicata*. We further looked into the chemo profiling of *Spirogyra triplicata* by GC-MS analysis. The result of GC-MS clearly indicates presence of nineteen major components and twenty-three minor components which have more or less bioactivity and would help in theapeutics in future. **Conclusions:** In brief this study indicates for the first time that green algae *Spirogyra triplicata* induces anti-proliferative activity specifically against A549 cell but not in normal PBMC. It can be concluded that *Spirogyra triplicata* holds a great promise as a good repository of anti cancer compounds which may be used in future drug discovery.

**Key words:** *Spirogyra triplicata*, Anti-proliferative, A549, GCMS.

INTRODUCTION

Cancer is a major health problem characterized by uncontrolled cell proliferation and its propagation.1 Statistical report of GLOBOCAN reveals that 18.1 million new cancer cases evolved within which 9.6 million cancer deaths eventuated in 2018 worldwide.2 Cancer treatment follows chemotherapy using cytotoxic drugs, radiation therapy and surgery.3 Though all these procedure have been reported to combat this dreaded disease, people still suffers with undesirable side effects.4 The use of plant-based bio-products for cancer treatment is rapidly growing in medical practices.5 Thus our vision was to hunt more natural sources for the treatment of cancer with minimum side effects. Scientists worldwide are engaged in designing the targeted therapies that can exterminate cancer cells without harming normal one. Natural products like algae,6 angiosperms,7 lichens,8 Mushroon9 have been a continuous source of medicines to treat diseases and injury. Epidemiological studies proclaimed that the people of Asian countries who consumes high amount of fish and seafood have low incidence of particular type of cancers such as lung, breast, colorectal and prostate cancers.10 Reports consider algae as a repository of promising novel phytochemical as a source of biomedicine.11 Such therapeutics primarily try to understand how and by what mean cancer cells are different from normal non-transformed cells. It is explicit that cancer cells and normal cells are morphologically and behaviorally different. Current information suggests that bioactive components extracted from algae, as well as methyl jasmonate (a natural compound derived from the plant of jasmonates family), seem to have anti-cancer activities through multiple mechanisms of action, including downregulation of cancer-cell proliferation and metastasis, and through the promotion of apoptosis of cancerous cells.12 Many algae are still untouched to unmask. After getting positive results in antioxidiant activity of some green algae, our focus was to check the anti-proliferative activities of six green algae in human cancer and metabolomic studies of the most potent...
green methanolic algal extract. The result of which could be a great source of future medical discovery.

**MATERIALS AND METHODS**

**Sample collection, identification and extraction procedure**

Green algae were collected from several lentic and slowly lotic sources of India. The collected samples viz. *Rhizoclonium crassipellitum (RC)*, *Spirogyra triplicata (ST)*, *Pithophora cleaviana (PC)*, *Rhizoclonium fontinale (RF)*, *Cladophora glomerata (CG)* Hydrodictyon reticulatum (HR) were identified by physcology specialist and voucher specimens deposited at Phycology lab, Botany Dept, University of Calcutta. The collected materials were processed and extracted according to our previous study.13

**Cell and cell culture reagents and chemicals**

MCF7, A549, HEPG2, REH, MOLT4 cell lines were kindly gifted from Dr. Sanjit Dey department of Physiology University of Calcutta and Mitali Chatterjee IPGMER, Kolkata. Cell lines were cultured at 1×10^6 cells/ml in RPMI 1640 (Sigma Aldrich) or Dulbecco’s Modified Eagle’s medium (DMEM) (Sigma Aldrich) depending upon cell line, supplemented with 10% fetal bovine serum (FBS) (Invitrogen), 2mM L-glutamine, 10 U/ml penicillin and streptomycin (Sigma Aldrich) and maintained in humidified 5% CO₂ incubator (HF-90). Percoll (Sigma Aldrich), glutamine (Sigma aldrich), gentamicin (Sigma aldrich), Trypan blue (Sigma aldrich), gentamicin (Sigma aldrich), Trypan blue (Sigma aldrich), MTI 3-(4, 5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma Aldrich), DAPI and PI for microscopic analysis of nuclear fragmentation were purchased from Sigma Aldrich.

**Isolation of peripheral blood mononuclear cells (PBMC)**

Peripheral blood mononuclear cells constitute the cellular part of the blood containing all blood cells with a round nucleus. PBMCs are mainly invading cells that comprised of monocytes, T cells, B cells, natural killer (NK) cells, and dendritic cells. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood of healthy normal volunteers by percoll density (Sigma aldrich) gradient centrifugation (d=1.082 g/L) creating prominent layers containing specific cell populations. The cells can then be isolated by extracting the respective layer. The total cell number and the number of viable cells were determined with Trypan blue (Sigma aldrich) exclusion. PBMC were then recovered at the interface and the retrieved cells were washed and re-suspended in phosphate-buffered saline (PBS) and finally fixed in RPMI 1640 (Sigma aldrich) supplemented with 2mM glutamine(Sigma aldrich), gentamicin (Sigma aldrich) and 10% heat inactivated FBS (invitrogen).13

**Cell viability assay**

To check the viability of cells after treating with methanol extracts, we performed the cell viability assay. Cells were harvested 96-well plat with increasing concentrations of STME and observed at 24 Hours of time point by MTT assay. In brief cells were incubated in *triplicata* in a 96-well plate at different concentrations of methanolic algal extract in a final volume of 200µl for 24 hrs at 37°C. Three hours before the completion of time points, 20µl of MTT solution (5mg/mL) was added to each well. Formazone crystals formed, representing percentage of cell viability was assessed at 560 nm. Cell apoptosis (100%) was obtained by lysis of cells in 5% SDS lysis buffer. All treatments were performed in *triplicata*. The percentage of cell viability was calculated as mentioned below:

\[
\% \text{ cell viability} = \frac{\text{O.D. sample-O.D. 100% lysis}}{\text{O.D. 0% lysis-O.D. 100% lysis}}
\]

**Nuclear staining with DAPI**

Nuclear fragmentation is an important phenomenon of apoptosis and can be visualized by DAPI staining.17 Cells were seeded in 12 well plate and treated with STME at different concentrations for 24h. After treatment, cells were collected, washed with 1 X phosphate buffered saline (PBS). DAPI (1µg/mL in PBS) was added and incubated for 15 min at room temperature. The stained cells were observed under a fluorescence microscope.

**Microscopic staining of apoptotic cells by PI**

Based on our MTT data, we found that A549 cell lines are mostly affected by *Spirogyra triplicata*. Thus we took control and three increasing concentration of STME treated A549 cell well plate. The treated 1×10^5 A549 cells were stained with freshly prepared PI (1µg/mL) and incubated for 10 min. after washing, the cells were visualized under fluorescence microscope.16 (Dewinter).

**Metabonomic study by GC/MS study**

From our previous study, after getting positive antioxidant result and high amount of total phenol and flavonoid content, our next aim was to analyze the chemometric activity by GC-MS study.

**Sample preparation for GC/MS analysis**

The 10mg algal methanol extract was dissolved in 1 ml of the HPLC grade methanol, vortexed properly for well mixing and finally filtered through 0.22 µm syringe filter (Millipore Corp., Bedford, MA, USA).

**Instrumentation and chromatographic conditions**

Agilent Technologies 7890A GC System liquid autosampler was used for GC/MS analysis. The gas chromatograph was interfaced to a mass spectrometer instrument utilizing the following conditions viz. HP-5 ms column (30 m× 0.25 mm× 0.25 µm), fitted with silica and coupled to an Agilent triple quadrupole Mass selective Detector MSD7000, ionization voltage 70 eV, electron multiplier energy 2000 V; transfer line temperature,295°C. Helium as carrier gas (1 ml/min). The initial temperature was 60°C for 0 min, then gradually increased to 320°C automaticaly and in the split ratio mode (10:1), transfer line temperature,295°C. Statistical analysis

The results were expressed as mean ± standard deviation. Descriptive statistics was used to analyze the mean, standard deviation, variation, and level of statistical significance between groups. When p < 0.05 and p < 0.01, it was considered statistically significant for analysis of percent inhibition of cell growth.

**RESULTS**

**Differential anti-proliferative activity of algal extract by MTT assay**

To fish out the most potent anti-cancerous extract, we investigated the anti-proliferative effects of six different green algal methanolic fraction and tested on five different cancer cell lines e.g. MCF-7, A549, HEpg2, REH, MOLT4. Each cell line was treated with six different algal extract for 24-hour time points followed by MTT assay. Results of the MTT assay proved that A549 was the most sensetive cell line
Hydrodictyon reticulatum, Rhizoclonium crassipellitum most by 2.67 μg/ml respectively. The anti-proliferative activity of A549 cell line methanolic Pithophora cleveana did not show significant anti-proliferative activity having IC₅₀ values of against A549 cell line with IC₅₀ value of 24.07 ± 1.09 μg/ml. Followed methanol extract (STME) having maximum anti-proliferative activity against HEPG2 cell line and Rhizoclonium frontinale against A549 cell line with IC₅₀ value of 25.97 ± 1.94 μg/ml, 30.2 ± 1.45 μg/ml and 30.45 ± 2.67 μg/ml respectively. The anti-proliferative activity of A549 cell line mostly by Hydrodictyon reticulatum, Rhizoclonium crassipellitum and Pithophora cleveana methanolic extract with IC₅₀ values of 32.50 ± 1.35 μg/ml, 34.71 ± 1.79 μg/ml and 37.54 ± 1.47 μg/ml respectively. STME shows moderate anti-proliferative activity against MCF-7 cell line with IC₅₀ value 47.28 ± 1.98 μg/ml but against REH and Molt 4 cell lines did not show significant anti-proliferative activity having IC₅₀ values of 82.68 ± 2.38 and 89.71 ± 1.82 respectively (Figure 1b). Rhizoclonium frontinale (RFME) also exhibited lower anti-proliferative activity against REH, and MOLT4 cells (Figure 1d). From the Figure 1 we can conclude that cancer cell viability decreased with the dose dependent fashion. However, anti-proliferative activity of Cladophora glomerata, Pithophora cleveana, Rhizoclonium crassipellitum, Hydrodictyon reticulatum were found to be of least anti-proliferative efficacies against MOLT4 cell line with IC₅₀ values of 72.82 ± 1.3 μg/ml, 202.8 ± 1.89 μg/ml, 213.7 ± 2.83 μg/ml, 165.9 ± 1.34 μg/ml respectively (Table 1). Thus it is proved from the data that MOLT4 cells were most resistant (Table 1) to all the extracts tested. In a nutshell from the MTT assay results we observe that S. triplicata possesses most promising Anti-cancer potentiality followed by R. frontinale (Figure 1d) in A549 cells. Whereas no significant anti proliferative effect was observed in normal PBMC isolated from healthy donor.

**Table 1.** IC₅₀ values of six different green algal extract on different cell lines.

| Algae                          | MCF-7 | A549 | HEPG2 | REH | MOLT4 |
|--------------------------------|-------|------|-------|-----|-------|
| Rhizoclonium crassipellitum    | 42.35±1.3 | 34.71±1.79 | 37.63±1.82 | 136.2±1.26 | 381.3±1.9 |
| Spirogyra triplicata           | 47.28±1.98 | 24.07±1.09 | 30.2±1.45 | 82.68±1.39 | 89.71±1.82 |
| Pithophora cleveana            | 80.48±2.7  | 37.54±1.47 | 57.19±1.68 | 82.29±1.37 | 99.04±1.48 |
| Rhizoclonium frontinale        | 58.19±1.6  | 25.97±1.94 | 30.45±2.67 | 99.04±1.48 | 93.81±1.38 |
| Cladophora glomerata           | 70.24±1.96 | 48.03±1.42 | 39.07±1.38 | 78.46±1.62 | 72.82±1.3 |
| Hydrodictyon reticulatum       | 77±1.54  | 32.50±1.97 | 111±2.58 | 62.04±1.39 | 165.9±1.34 |

**Figure 1:** Effect of different algal extract on MCF7, A549, HEPG2, REH, MOLT4 Cell Lines and normal PBMC. Effect of six different algal methanolic extract (A-F), (25 μg/ml-100 μg/ml) as determined by MTT assay at 24 h time point. PBMC cells were unaffected but A549 cells are mostly affected. a- Rhizoclonium crassipellitum (RC), b- Spirogyra triplicata (ST), c- Pithophora cleveana (PC), d- Rhizoclonium frontinale (RF), e- Cladophora glomerata (CG), f- Hydrodictyon reticulatum (HR).
Effects of *S. triplicata* methanolic extract on cellular morphological changes of A549 cells

As the methanolic extract of *S. triplicata* showed the most potential anti-proliferative activity we further looked at the nuclear fragmentation of A549 cells upon treatment with *S. triplicata*. The characteristic nuclear fragmentation induced during apoptosis was measured using DAPI staining. DAPI is a nuclear stain that can be visualized under UV fluorescence when excited under fluorescence microscope. In our study, DAPI staining revealed the changes related with apoptosis in A549 cells treated with the methanolic fraction of *S. triplicata*. The morphological changes associated with apoptosis such as chromatin condensation, nuclear fragmentation, and margination of nucleus are evident in A549 cells upon treatment (Figure 2).

Observation of A549 cells by PI staining

PI is a membrane impermeant stain; it can’t penetrate through the living cell membrane. When the cell undergoes apoptosis, the cell membrane becomes leaky and the stain can enter into the cell. PI staining can be used to exhibit the morphologic condition of DNA after treatment with methanolic extract. Thus to observe the amount of dead cells we performed the PI staining. In our study four groups of cells were considered in which first one untreated group i.e. no STME concentration was given to A549 cells and three ascending concentration of STME were given to rest three cell groups. In control cells presence of little amount of PI stained cells were visualized which suggests that there was no such apoptotic cells. As most of the cells were alive, PI did not get into the cells. Whereas the number of PI stained cells increased according to the increase in the concentration of STME treatment (Figure 2a). The figure clearly represents the scenario where the control cell with little PI stained cell, means most of the cells having membrane integrity, thus PI stain was unable to enter into maximum cells. Whereas the increasing concentration of STME changes the cellular membrane integrity thus allowed to enter PI within the cell and looks red in colour under fluorescence microscope and % cell death gradually increased (Figure 3b, 3c, 3d).

Chemo profiling analysis by GC-MS

Previous analysis with STME indicates higher percentages of antioxidant capacities as well as the total phenolic content and total flavonoid content. Henceforth, it was further investigated for metabolomic profiling by GC/MS analysis. GC/MS chemometric profiling of STME as per aforesaid experimental procedure showed numerous different peaks indicating the presence of diversified phyto chemotypes (Figure 4). The methanol extract of STME revealed the presence of different chemotypes which were characterized and identified by comparison of their mass fragmentation patterns with the similar compounds present in the NIST database. Of these nineteen different chemotypes, 1,4-Phosphasilacyclohexane, 4,4-dimethyl-1-phenyl- (27.33%), Linoleic acid ethyl ester (8.57%), (Z,Z,Z)-9,12,15-Octadecatrienoic acid (7.61%), 9,12,15-Octadecatrienoic acid, methyl ester, (9Z,12Z,15Z)- (6.33%), Pentadecanoic acid, 13-methyl-, methyl ester (6.04%), (Z,Z,Z)-9,12,15-Octadecatrienoic acid (4.98%), 7,10,13-Eicosatrienoic acid, methyl ester (4.23%), Neophytadiene(3.73%), 1,2,3-Benzatrol (3.49%), Hexylene glycol(2.72%), Doconexten(2.53%), Azulene (2.53%), 10,13-Eicosadienoic acid, methyl ester (2.32%), Cyclopentanetridecanoic acid, methyl ester (1.99%), Methyl octyl phthalate (1.48%), 2-Pyrrolidinone, 1-methyl- (1.34%), 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone (1.32%), 6, 19-Cycloandrostan-3,7-diol, 3β-methoxy- (1.26%), Methyl tetradecanoate (1.15%) were found to be the major constituents (Table 2a). Along with the nineteen major components, twenty-three minor...
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DISCUSSION

The use of naturally occurring plant-based products has shown promising results in the treatment of cancer. Due to the complexity of cancer, novel bioactive compounds with multi targeting efficacy are the need of the hour. In this paper we looked into the anti-proliferative effect of freshwater green algae Spirogyra triplicata by MTT assay. Previously we performed a pilot study with both Ethyl acetate and methanol extract of Spirogyra triplicata and our findings demonstrate that the methanolic fraction of S. triplicata possesses the most potent anticancer activity against human lung cancer cells (A549) and comparable activity in few other cancer cell lines. In contrast no significant cytotoxicity was observed in the normal PBMC cells. Next, we used DAPI, a fluorescent DNA-binding agent to visualize morphological changes of the nucleus. Nucleus gets fragmented with increase in concentration of the methanolic extracts. The GC/MS chemometric profiling is a useful technique to detect the volatile compounds present in natural products. The GC/MS fingerprinting of methanolic fraction of S. triplicata revealed the presence of nineteen major bioactive components in large amount along with twenty-three minor components in trace amount. The major phytochemical groups namely fatty acid esters, sterols,
Table 2a: GC-MS chemo profiling of major component of *Spirogyra triplicata* methanolic extract.

| Sl No | Peak RT (min) | Peak Area | Peak Area% | Compound Name | Mol. Formula | Mol. Wt. (g/mol) | CAS No. | Chemotype | Activity |
|-------|---------------|-----------|------------|---------------|--------------|-----------------|---------|------------|----------|
| 1     | 18.36         | 116361042 | 27.33      | 1,4-Phosphasilacyclohexane, 4,4-dimethyl-1-phenyl- | C<sub>20</sub>H<sub>22</sub>PSi | 222             | 111514-26-2 | -          | -        |
| 2     | 18.83         | 36485993  | 8.57       | Linoleic acid ethyl ester | C<sub>20</sub>H<sub>36</sub>O<sub>2</sub> | 308.4986 | 544-35-4 | fatty acid ethyl ester | human metabolite and a plant metabolite<sup>20</sup> |
| 3     | 20.88         | 32403118  | 7.61       | (Z,Z,Z)-9,12,15-Octadecatrienoic acid | C<sub>28</sub>H<sub>30</sub>O<sub>2</sub> | 278.4 | 463-40-1 | Fatty acid | -        |
| 4     | 20.53         | 26983285  | 6.33       | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | C<sub>29</sub>H<sub>32</sub>O<sub>2</sub> | 292.5 | 301-00-8 | Fatty acid methyl ester | -        |
| 5     | 18.79         | 25733184  | 6.04       | Pentadecanoic acid, 13-methyl-, methyl ester | C<sub>17</sub>H<sub>34</sub>O<sub>2</sub> | 270.4507 | 5487-50-3 | Fatty acid ester | -        |
| 6     | 18.98         | 21237407  | 4.98       | (Z,Z,Z)-9,12,15-Octadecatrienoic acid | C<sub>28</sub>H<sub>30</sub>O<sub>2</sub> | 278.4 | 463-40-1 | Fatty acid | -        |
| 7     | 20.63         | 18029271  | 4.23       | 7,10,13-Eicosatrienoic acid, methyl ester | C<sub>21</sub>H<sub>36</sub>O<sub>2</sub> | 320 | 30223-51-9 | Fatty acid ester | -        |
| 8     | 17.97         | 15915194  | 3.73       | Neophytadiene | C<sub>20</sub>H<sub>30</sub> | 278 | 504-96-1 | sesquiterpenoids | -        |
| 9     | 12.52         | 14860304  | 3.49       | 1,2,3-Benzentriol | C<sub>7</sub>H<sub>12</sub>O<sub>3</sub> | 126.11 | 87-66-1 | - | -        |
| 10    | 5.57          | 11582577  | 2.72       | Hexylene glycol | C<sub>6</sub>H<sub>10</sub>O<sub>3</sub> | 118.1742 | 107-41-5 | Glycol | -        |
| 11    | 20.06         | 10801913  | 2.53       | Doconexent | C<sub>22</sub>H<sub>32</sub>O<sub>2</sub> | 328.4883 | 6217-54-5 | very long-chain fatty acid | antioxidant, antipyretic, anti-inflammatory, antimicrobial, antidiabetic, anti-inflammatory, antiarthritic, and anticancer activities<sup>21</sup> |
| 12    | 9.95          | 10797783  | 2.53       | Azulene | C<sub>10</sub>H<sub>8</sub> | 128.1705 | 257-51-4 | hydrocarbon | -        |
| 13    | 20.46         | 9902887   | 2.32       | 10,13-Eicosadienoic acid, methyl ester | C<sub>21</sub>H<sub>32</sub>O<sub>2</sub> | 322 | 30223-50-8 | Fatty acid methyl ester | -        |
| 14    | 20.73         | 8494916   | 1.99       | Cyclopentanetridecanoic acid, methyl ester | C<sub>19</sub>H<sub>36</sub>O<sub>2</sub> | 296 | 24828-61-3 | Fatty acid methyl ester | -        |
| 15    | 16.21         | 6325633   | 1.48       | Methyl octyl phthalate | C<sub>17</sub>H<sub>30</sub>O<sub>4</sub> | 292 | 91485-83-5 | Diisononyl ester | Plasticizer<sup>25</sup> |
| 16    | 7.58          | 5716847   | 1.34       | 2-Pyrrolidinone, 1-methyl- | C<sub>8</sub>H<sub>10</sub>NO | 99 | 872-50-4 | - | -        |
| 17    | 24.28         | 5621412   | 1.32       | 3',8,8'-Trimethoxy-3'-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetone | C<sub>26</sub>H<sub>22</sub>NO<sub>5</sub> | 487 | 127611-84-1 | - | -        |
| 18    | 20.81         | 5368432   | 1.26       | 6,19-Cycloandrostan-3,7-diol, 3β-methoxy- | C<sub>25</sub>H<sub>32</sub>O<sub>4</sub> | 320 | - | fatty acid methyl ester | -        |
| 19    | 16.74         | 4916926   | 1.15       | Methyl tetradecanoate | C<sub>15</sub>H<sub>30</sub>O<sub>2</sub> | 242 | 124-10-7 | fatty acid methyl ester | -        |
Table 2b: GC-MS chemoprofiling of minor component of *Spirogyra triplicata* methanolic extract.

| Sl No | PeakRT (min) | Peak Area | Peak Area% | Compound Name | Mol. Formula | Mol. Wt. (g/mol) | CAS No. | Chemotype |
|-------|--------------|-----------|------------|---------------|--------------|-----------------|---------|-----------|
| 1     | 16.39        | 4216235   | 0.99       | 3-Hydroxydodecanoic acid | C₁₂H₂₄O₃ | 216.32 | 1883-13-2 | Fatty acid |
| 2     | 25.38        | 3741161   | 0.87       | Lycopersene | C₆H₁₀O₂ | 547      | 502-62-5 | acyclic carotene |
| 3     | 6.532        | 3687989   | 0.86       | Valeric acid | C₅H₁₀O₂ | 102.13 | 109-52-4 | saturated fatty acid |
| 4     | 17.099       | 2928528   | 0.688017846| Isopropyl palmitate | C₃₃H₆₆O₂ | 298.5 | 142-91-6 | |
| 5     | 20.00        | 2876594   | 0.67       | Ethyl arachidonate | C₃₂H₅₂O₄ | 332.5 | 1808-26-0 | fatty acid ethyl ester |
| 6     | 11.56        | 2485142   | 0.58       | 1-ETHYLIDENE-1H-INDENE | C₁₉H₃₀O₂ | 298.5 | 142-91-6 | |
| 7     | 18.63        | 1777880   | 0.41       | 13-HEPTADECYNYL-1-OL | C₂₂H₄₄O₂ | 352.5 | 56554-77-9 | |
| 8     | 15.647       | 1580754   | 0.37       | Dodecanoic acid,1-methylethyl ester | C₁₁H₂₀O₂ | 242 | 10233-13-3 | fatty acid ester and an isopropyl ester |
| 9     | 18.9         | 1420852   | 0.33       | Ethyl linoleate | C₃₂H₆₆O₂ | 308.5 | 544-35-4 | fatty acid ethyl ester |
| 10    | 18.598       | 1369414   | 0.32       | 1-MONOLINOLENOYL-RAC-GLYCEROL | C₃₂H₅₄O₄ | 352.5 | 18465-99-1 | |
| 11    | 18.23        | 1071873   | 0.25       | 13-HEPTADECYNYL-1-OL | C₂₃H₄₆O₂ | 298.5 | 56554-77-9 | |
| 12    | 10.47        | 1000281   | 0.23       | 5-HYDROXYMETHYLFURFURAL | C₅H₁₀O₂ | 126.11 | 67-47-0 | furan |
| 13    | 16.47        | 907653    | 0.21       | Geranyl isovalerate | C₁₇H₂₆O₂ | 238.3 | 109-20-6 | |
| 14    | 7.41         | 870297    | 0.20446445 | 2-Ethylhexyl trichloroacetate | C₁₄H₂₆Cl₂O₂ | 275.6 | 16397-79-8 | |
| 15    | 8.06         | 824996    | 0.19       | 4-Chlorobutyrophenone | C₁₄H₂₀ClO | 182.64 | 939-52-6 | |
| 16    | 7.39         | 787620    | 0.18       | P-Cymene | C₁₀H₁₄ | 134.22 | 99-87-6 | |
| 17    | 10.79        | 684364    | 0.16       | 1-Phenoxy-2-propanol | C₉H₁₆O₂ | 152.19 | 770-35-4 | |
| 18    | 14.20        | 550520    | 0.12       | Piperidine-4-carboxylic acid, 1-{2-methyl-2,3-dihydroindol-1-yl}-2-oxoethyl-, amide | C₁₇H₂₃NO₂ | 301 | - | |
| 19    | 8.79         | 477712    | 0.11       | 2-Ethylhexanoic acid | C₁₂H₂₄O₂ | 144.21 | 149-57-5 | |
| 20    | 11.80        | 455883    | 0.10       | Bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene | C₁₅H₂₄O₂ | 142.2 | 2443-46-1 | |
| 21    | 14.67        | 452629    | 0.10       | Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8-a-trimethyl- | C₁₄H₂₀O₂ | 240 | 54344-83-1 | |
| 22    | 6.60         | 400001    | 0.09       | Phenyl beta-D-glucopyranoside | C₁₅H₂₀O₄ | 256.25 | 1464-44-4 | |
| 23    | 26.323       | 338854    | 0.07       | Lycopersene | C₂₁H₃₄O₂ | 347 | 502-62-5 | |

unsaturated alcohols, alkynes etc. were found to be the dominant components and these are known for their positive biological functions. Neophytadiene, 2-Pyrrolidinone, 1-methyl-, Methyl tetradecanoate, Doconexent and Azulene were the major phytocomponents exhibiting bioactivity. The Neophytadiene shows antioxidant, analgesic antipyretic, anti-inflammatory, antimicrobial, anti-diabetic, anti-inflammatory, anti-arthritis and anti-cancer activity. 2-Pyrrolidinone, 1-methyl- exhibits immunomodulatory activity and known as antimonyloma compound. Methyl tetradecanoate is being used as a flavouring and fragrancing agent. Doconexent with mixture of other oil act as a very good supplement of DHA (which have anti inflammatory effect in brain). Azulene- acts as a volatile oil component. Data taken together reflects that methanol extract of *S. triplicata* proves to be a good repository of phytochemicals that holds a great promise in the treatment of cancer in future. However, further in depth studies need to be performed to fully discover its anticancer properties by looking at the different cell signaling pathways linked with cancer development.

**AUTHORS CONTRIBUTION**

Ankita Mridha performed all assays and data collection. Priya K Gopal maintained the cell culture. Santanu Paul made over all data analysis and assessment of manuscript.

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**CONFLICTS OF INTEREST**

There are no conflicts of interest.

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Algae - MTT assay result

Best Result

Nuclear Fragmentation by DAPI staining
Apoptosis by PI staining

Major Components

GCMS chemometric profiling of STME

1. L-Phe-nalbutylacetate/1,4-dimethoxybenzene

2. L-Proline/glutamine

3. Glucose/lactic acid

4. Glucose/palmitic acid

5. Malic acid

1. L-Leucine

2. L-Isoleucine

3. L-Valine

4. L-Threonine

5. L-Asparagine

6. L-Alanine

7. L-Aspartic acid

8. L-Glutamic acid

9. L-Histidine

10. L-lysine

11. L-proline

12. L-serine

13. L-tryptophan

14. L-tyrosine

15. L-ornithine

16. L-arginine

17. L-cystine

18. L-methionine

19. L-valine

20. L-isoleucine

21. L-phenylalanine

22. L-tyrosine

23. L-tryptophan

24. L-asparagine

25. L-glutamic acid

26. L-serine

27. L-cystine

28. L-histidine

29. L-lysine

30. L-aspartic acid

31. L-glutamic acid

32. L-serine

33. L-cystine

34. L-histidine

35. L-lysine

36. L-aspartic acid

37. L-glutamic acid

38. L-serine

39. L-cystine

40. L-histidine

41. L-lysine

42. L-aspartic acid

43. L-glutamic acid

44. L-serine

45. L-cystine

46. L-histidine

47. L-lysine

48. L-aspartic acid

49. L-glutamic acid

50. L-serine

51. L-cystine

52. L-histidine

53. L-lysine

54. L-aspartic acid

55. L-glutamic acid

56. L-serine

57. L-cystine

58. L-histidine

59. L-lysine

60. L-aspartic acid

61. L-glutamic acid

62. L-serine

63. L-cystine

64. L-histidine

65. L-lysine

66. L-aspartic acid

67. L-glutamic acid

68. L-serine

69. L-cystine

70. L-histidine

71. L-lysine

72. L-aspartic acid

73. L-glutamic acid

74. L-serine

75. L-cystine

76. L-histidine

77. L-lysine

78. L-aspartic acid

79. L-glutamic acid

80. L-serine

81. L-cystine

82. L-histidine

83. L-lysine

84. L-aspartic acid

85. L-glutamic acid

86. L-serine

87. L-cystine

88. L-histidine

89. L-lysine

90. L-aspartic acid

91. L-glutamic acid

92. L-serine

93. L-cystine

94. L-histidine

95. L-lysine

96. L-aspartic acid

97. L-glutamic acid

98. L-serine

99. L-cystine

100. L-histidine

101. L-lysine

102. L-aspartic acid

103. L-glutamic acid

104. L-serine

105. L-cystine

106. L-histidine

107. L-lysine

108. L-aspartic acid

109. L-glutamic acid

110. L-serine

111. L-cystine

112. L-histidine

113. L-lysine

114. L-aspartic acid

115. L-glutamic acid

116. L-serine

117. L-cystine

118. L-histidine

119. L-lysine

120. L-aspartic acid

121. L-glutamic acid

122. L-serine

123. L-cystine

124. L-histidine

125. L-lysine

126. L-aspartic acid

127. L-glutamic acid

128. L-serine

129. L-cystine

130. L-histidine

131. L-lysine

132. L-aspartic acid

133. L-glutamic acid

134. L-serine

135. L-cystine

136. L-histidine

137. L-lysine

138. L-aspartic acid

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140. L-serine

141. L-cystine

142. L-histidine

143. L-lysine

144. L-aspartic acid

145. L-glutamic acid

146. L-serine

147. L-cystine

148. L-histidine

149. L-lysine

150. L-aspartic acid

151. L-glutamic acid

152. L-serine

153. L-cystine

154. L-histidine

155. L-lysine

156. L-aspartic acid

157. L-glutamic acid

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159. L-cystine

160. L-histidine

161. L-lysine

162. L-aspartic acid

163. L-glutamic acid

164. L-serine

165. L-cystine

166. L-histidine

167. L-lysine

168. L-aspartic acid

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171. L-cystine

172. L-histidine

173. L-lysine

174. L-aspartic acid

175. L-glutamic acid

176. L-serine

177. L-cystine

178. L-histidine

179. L-lysine

180. L-aspartic acid

181. L-glutamic acid

182. L-serine

183. L-cystine

184. L-histidine

185. L-lysine

186. L-aspartic acid

187. L-glutamic acid

188. L-serine

189. L-cystine

190. L-histidine

191. L-lysine

192. L-aspartic acid

193. L-glutamic acid

194. L-serine

195. L-cystine

196. L-histidine

197. L-lysine

198. L-aspartic acid

199. L-glutamic acid

200. L-serine

201. L-cystine

202. L-histidine

203. L-lysine

204. L-aspartic acid

205. L-glutamic acid

206. L-serine

207. L-cystine

208. L-histidine

209. L-lysine

210. L-aspartic acid

211. L-glutamic acid

212. L-serine

213. L-cystine

214. L-histidine

215. L-lysine

216. L-aspartic acid

217. L-glutamic acid

218. L-serine

219. L-cystine

220. L-histidine

221. L-lysine

222. L-aspartic acid

223. L-glutamic acid

224. L-serine

225. L-cystine

226. L-histidine

227. L-lysine
ABOUT AUTHORS

Ankita Mridha is a Senior Research Fellow funded by Indian Council of Medical Research at the Department of Botany, University of Calcutta, India.

Dr. Priya K Gopal is currently an Assistant Professor of Microbiology at the Muralidhar Girls College, Kolkata, India and has done her PhD under the supervision of Dr. Santanu Paul.

Dr. Santanu Paul is a Professor of Botany, University of Calcutta, India and also the chief of the "Laboratory of Cell and Molecular Biology." Has immense knowledge and experience in the molecular mechanism of apoptosis induction in cancer cells induced by compounds from plants.

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