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Research Article

Phytochemical Profiling and *In-vitro* Assessment of Antiproliferative Effects of Extracts of Freshwater Microalgae, *Poterioochromonas malhamensis*

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ABSTRACT

The development of anticancer drugs remains a formidable challenge. *Poterioochromonas malhamensis*, known for its predatory nature on microbes and plankton, was investigated for anticancer properties. This study screened and quantified phytochemicals using LC-MS and evaluated the anticancer effects of aqueous ethanol extracts of *P. malhamensis* from Tavanampalli's freshwater bodies. Phytochemical analysis by HR-LCMS detected proteins, phenols, flavonoids, and alkaloids. The extract contained 87.75 mg of quercetin equivalents, 134.55 mg of gallic acid equivalents, and 72.64 mg of bovine serum albumin equivalents per gram of dry weight. Liquid chromatography-mass spectrometry (LC-MS) also identified amino acids, fatty acids, amides, and esters in the extract. Antiproliferative activity was assessed using MTT assay, AO/ETBR, and Annexin V/Propidium iodide (PI) staining assays. The extract exhibited IC_{50} values of 95.09, 103.63, 162.14, 339.30, and 355.18 µg with A375, HepG2, HeLa, HT29, and A549 cell lines, respectively, outperforming cisplatin, which had IC_{50} values of 3.56, 4.87, 3.88, 7.23, and 4.65 µg. This study is the first to report the antiproliferative activity of *P. malhamensis*, broadening the scope of research in anticancer compound discovery.

Introduction

Photosynthetic organisms found in aquatic habitats include algae. Microalgae are minuscule and differ from macroalgae because they are unicellular and visible to the human eye. They can be eukaryotic, like chlorophyta, or prokaryotic, like chloroxybacteria. Being autotrophs by nature, they are frequently referred to as phytoplanktons.[1] The number of studies on the exploitation of these organisms surged when it was discovered that microalgae could persist in a variety of typical conditions, including variable pH, temperature, light intensities, salinity, fresh to marine water niches, and marsh areas to arid locations. Algae are categorized into six different groups based on their pigmentation: chlorophyta, chrysophyta, rhodophyta, cyanophyta, phaeophyta, and pyrrophyta.[2] Since Chrysophyta serves as photosynthesizers and the pinnacle of the food chain, they are the most often studied of them. Chrysophyta are unique in containing the green pigment chlorophyll a and the glucose polymer, chrysolaminarin. They are classified as diatoms and chrysophyceae, often termed golden algae.[3]

Generally, microalgae are used as food, fodder, and supplements for humans and animals. They are the main source of biofuel products like biochar, biosyngas, biodiesel, bioethanol, and biohydrogen. Being autotrophic, they utilize sunlight to synthesize many bioactive compounds that are widely used as antioxidants, polyunsaturated fatty acids, vitamin sources, coloring agents, antimicrobial drugs, and several other treatments.[4] The algal pigments, chlorophyll, β carotene, fucoxanthin, phycocyanin, astaxanthin, and phycoerythrin impose several health benefits such as protection against neurological, dermal

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and photosensitive disorders, antioxidant (peroxidase, catalase, and superoxide dismutase), anticancer, anti-inflammatory (lowered amounts of tumor necrosis factor and interleukins), antiobesity (inhibition of 3T3-L1 differentiation), and immune-boosting activities.[2]

The development of anticancer medications is one of the medical field's constraints. In India, cancer has become a deadly disease, accounting for 9% of deaths caused by 71% of non-communicable diseases, with 13,92,179 patients reported in India in 2020. Cancer in the breast, mouth, lungs, and tongue are some of the most common cancer types reported in India. Despite being a threat to human life, cancer has become an economic burden, too in terms of the cost of treatment.[5] Research on the potential applications of naturally occurring bioactive compounds has become a vital area in cancer research.

The mixotrophic nature of *Poterioochromonas malhamensis*, has been extensively researched. They are spherical, yellowish-green cells with a diameter of 5 to 25 µm and two heterodynamic flagella. A periplast, two chromatophores, a food vacuole, a single golgi body, and mitochondria are present in these cells. There are no cell walls, pyrenoids, scales, or spines.[6] *Chlorella sorokiniana, Chlamydomonas reinhardtii, Dictyosphaerium* sp., *Nannochloropsis oceanica, Phormidium mucicola, Scenedesmus acuminatus, Synechocystis* sp., and *Synechococcus* are known to be predators of *P. malhamensis.*[7] *P. malhamensis* is widely exploited only for its predatory nature on microbes and plankton. In the present study, novel attempts were made to exploit the predatory activities of *P. malhamensis* on cancer cell lines.

Prior research conducted on the presence of microalgae in Tavanampalli has confirmed the existence of *Chlorochromonas danica*, a type of microalgae that possesses strong anticancer characteristics. Their extract was analyzed using LC-MS, which revealed the presence of a variety of bioactive compounds that proved anticancer properties.[8] This study served as a precedent for the current investigation into the pharmacological characteristics of other microalgae. *In-vitro* and *in-vivo* studies show that microalgae produce carotenoids, polysaccharides, and PUFAs that fight cancer.[9] They kill cancer cells without harming healthy cells with fewer harmful effects on cancer patients than traditional chemotherapy. This is important because cancer cells can defy conventional treatments. Several phytochemicals, such as phycobiliproteins, chlorophylls, and carotenoids from microalgae, fight cancer. Fucoxanthin and astaxanthin from brown algae and *Haematococcus pluvialis*, alginate, carrageenan, and fucoidan,[10] omega-3 fatty acids alter cancer-promoting cellular signaling pathways and have anti-inflammatory properties as well.[11] Microalgae may treat cancer, but more research is needed to develop drugs. Optimizing growth conditions for high bioactive chemical yields, improving extraction methods, and increasing preclinical and clinical studies are hurdles.[12]

Hence, the current study was designed with objectives such as (i) isolation, identification, and culture of the microalgae from freshwater bodies in Tavanampalli, Andhra Pradesh, India, (ii) phytochemical analysis in the aqueous ethanol extracts of *P. malhamensis,* (iii) quantitative estimation of the phytochemicals present, and (iv) evaluation of *P. malhamensis* aqueous ethanol extracts' antiproliferative effects on cancer cell lines.

Materials And Methods

Isolation of Microalgae

Water samples for the study were taken at Tavanampalli, Andhra Pradesh, India. Every month, samples were collected from the same source using a phytoplankton gauze filter and put in a sterile container. Samples were taken to the lab after being fixed in 4% formalin. Algal samples were washed well before being processed. The collected samples were grown in Bold's basal medium (BBM) to isolate and purify the algae.[13]

Identification of Microalgae by Morphological and Phylogenetic Techniques

Under a light microscope, the cultivated algae were morphologically identified pertaining to their size, shape, and colony formation. MEGA11 was used to construct the phylogenetic tree.[14] The scanning electron microscope (SEM) (JEOL JMS-6390, Greensmed Labs, Chennai) was used to study the surface morphology of the microalgae that were identified based on their morphology.

Preparation of *P. malhamensis* Extract

The collected algal samples were thoroughly cleaned with water and allowed to air dry. The dried algae were crushed and kept in a sterile container at room temperature. In the water bath, 10 g of the dried algae were refluxed with aqueous ethanol (9:1) for about 8 hours. The mixture was subjected to filtration by a Whatman filter paper. Rotary evaporators were used to dry the filtrate. The resulting extract was thoroughly desiccated in a vacuum chamber.[15]

Phytochemical Screening of the Aqueous-ethanol Extract of *P. malhamensis*

The phytochemicals such as flavonoids, alkaloids, quinones, phenols, steroids, tannins, saponins, terpenoids, proteins, and cardiac glycosides present in the aqueous ethanol extract of *P. malhamensis* were qualitatively analyzed by following the standard procedures.[16]

Estimation of Total Flavonoids

After adding aluminum chloride (2%) to 200 µg of powdered algae, it was left undisturbed for an hour. The measurement of absorbance was conducted at a wavelength of 420 nm. Using quercetin at concentrations spanning between 10 to 320 µg/mL, the standard curve was developed.[17]

Estimation of Total Phenol

The estimation of total phenol was performed using the Folin-Ciocalteau reagent. Water and Folin-phenol Ciocalteu's reagent (in the ratio of 1:1) were added to 200 g powdered algae. After 5 minutes, sodium carbonate and distilled water (1:5) were added. The color that developed after being kept in darkness for 30 minutes was analyzed at a wavelength of 765 nm. A standard curve with gallic acid (2–64 g/mL) was generated.[17]

Estimation of Total Protein

The amount of protein present was determined by Lowry's technique. After adding 5 mL of alkaline-copper sulfate solution to powdered microalgal extract, it was kept for 15 min at standard temperature. Folin-Ciocalteau reagent was added, and 30 minutes were spent incubating. At 660 nm, absorbance was finally measured. To create a standard curve, bovine serum albumin (BSA) was employed at the concentration range of 10 to 320 g/mL.[18]

LC-MS Analysis

At SAIF, IIT Powai, Mumbai, the sample was analyzed using a Hypersil Gold 3micron (100x2.1MM) column, LC-ESI-Q-TOF-MS (Agilent Technologies 6550 i-Funnel) system. Data collection and processing were done with Thermo Scientific Xcalibur (4.2.28.14) and Compound Discoverer (3.2).[19]

Anticancer Assay

Cancer cell line culture

The VERO cell lines (non-cancerous) and the cancer cell lines of humans such as A549 (lung adenocarcinoma), A375 (melanoma), HeLa (Cervical adenocarcinoma), HepG2 (liver cancer), and HT29 (colorectal adenocarcinoma) from the National Center for Cell Science, India were cultured until confluent in Dulbecco's Modified Eagle medium containing 10% inactive fetal bovine serum, penicillin (100 IU/mL), and streptomycin (100 µg/mL) at 37°C in humid conditions with 5% CO_2.[20]

Cytotoxic activity assessment

The cytotoxic efficacy of the microalgal extract was investigated through MTT test.[20] Trypsinizing the monolayer of cell culture in 10% FBS media gave a cell suspension of 1.0X 10^5 cells/mL. Within a day, a partial monolayer had developed. Then 100 µL of test samples (3.125, 6.25, 12.5, 25, 50, and 100 µg) were added individually to the monolayer after removing the supernatant. The control was cisplatin. For one day, the plate was kept at 37°C utilizing 5% CO_2. Following incubation, MTT (20 µL in each well) in place of the testing solutions. The plate was maintained in 5% CO_2 at 37°C for 4 hours. Take off the supernatant, mix in 100 µL of DMSO, and gently shake the plate to dissolve the formazan. The absorbance was then recorded at 570 nm. Using this formula, the percent viability was determined.:

Percent viability = (treatment cell absorbance/control cell absorbance) x 100

AO/ETBR staining

On a 6-well plate (3×10^4/well), A375 cell lines, including the positive control, were exposed to varying concentrations of the drug in a CO_2 incubator for 48 hours. The cells were fixed at room temperature for 30 minutes in a 3:1 mix of methanol and glacial acetic acid. They were then washed with PBS and stained with AO/EtBr. Cells that had been stained were rinsed with PBS and examined under the fluorescence microscope. Number of apoptotic cells in the field is represented as a percentage.[20]

PI-Annexin V staining

In this procedure, A375 cells were seeded in 1.5 mL microtubes with RPMI-1640 and 10% FBS. Samples at 47.5 and 95 µg/mL concentrations were then added. Samples are maintained at 24°C for 24 hours. Cells underwent three rounds of washing with PBS and were resuspended in binding buffer. Then 5 µL of annexin V was introduced and maintained at 25°C in dark circumstances for 20 minutes. Cell Quest and flow cytometry were used to examine the results. In parallel, untreated cells were employed as negative control and doxorubicin as positive control.[10]

Statistical Analysis

These trials were done in triplicates and shown as mean ± SEM. ANOVA was used to evaluate values and find statistically significant variations between means ($p < 0.05$).

Results

Morphological Characterization and Phylogenetic Analysis of Isolated Microalgae

The exploitation of natural freshwater habitats for the isolation of microalgae of high medical importance is an effective way to discover eco-friendly, compatible, and potent therapeutics. In this context, microalgae were isolated from the freshwater Lake Tavanampalli, Andhra Pradesh, India. The morphological features as analyzed through 40X magnification of the light microscope, revealed the spherical, yellowish-green cells of the microalgae (Fig. 1a), which was confirmed through the scanning electron microscopic analysis (Fig. 1b). The forward and reverse 18s rRNA sequences were contig constructed using CAP3 sequence assembly software, the BLAST analyzed, and the sequences were submitted to GenBank in FASTA format. The phylogenetic analysis by neighbor-joining method revealed the species as *P. malhamensis* (Fig. 2).

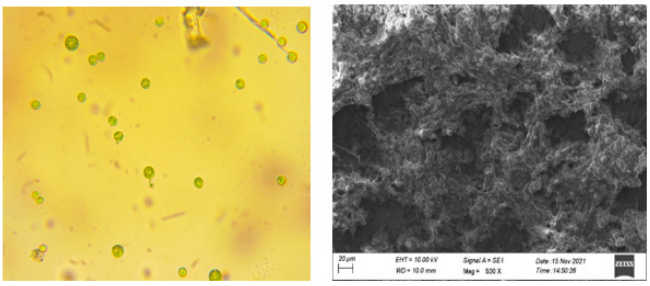


Fig. 1: Microscopic view of *P. malhamensis;* (a) Yellowish green cells viewed at 40X magnification in the light microscope; (b) spherical cells viewed at 500X magnification in SEM

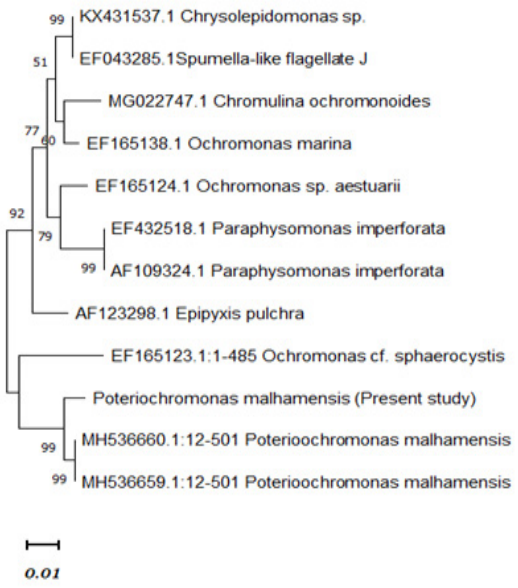


Fig. 2: Evolutionary analysis of *P. malhamensis* by constructing a phylogenetic tree by the neighbor-joining method with a bootstrap value of 1000

Phytochemical Profile of the Aqueous Ethanol extract of *P. malhamensis*

For phytochemical assessment, the aqueous-ethanol extract of *P. malhamensis* was prepared, whose dried powder weighed around 0.360 g. The phytochemical analysis revealed the presence of several phytochemicals (Table 1).

Quantitative Estimation of Phytochemicals

The amount of flavonoids, phenol, and protein in an aqueous ethanol extract of *P. malhamensis* was estimated by standard protocols (Table 2). The total amount of flavonoid was determined using the calibration curve model, y = 0.0191x + 0.0438, where x denotes the concentration of quercetin and y denotes the absorbance (420 nm). The R^2 value was 0.9919. The extract has 87.75 mg quercetin equivalent/g dry weight.

The total amount of phenol was determined using the calibration curve model, y = 0.0139x+ 0.0244, where x denotes the concentration of gallic acid and y denotes the absorbance (765 nm). The R^2 value was 0.9918. The extract has 134.55 mg gallic acid equivalent/g dry weight.

The total amount of protein was determined using the calibration curve model, y = 0.0051x+0.0515, where x denotes the concentration of BSA and y denotes the absorbance (700 nm). The R^2 value was 0.9834. The extract has 72.64 mg of BSA equivalent/g dry weight.

Table 1: Phytochemical composition of the aqueous-ethanolic extract of *P. malhamensis*

S.No	*Phytochemical*	*Extract*
1	Alkaloids	-
2	Flavonoids	+
3	Saponin	-
4	Tannin	-
5	Phenol	+
6	Cardiac glycosides	+
7	Steroids	+
8	Terpenoids	+
9	Quinones	-
10	Proteins	+

LC-MS Assessment

The aqueous-ethanol extract of *P. malhamensis* underwent LC-MS analysis, which identified 17 potent compounds (Table 3 and Fig. 3).

Cytotoxic Activities of *P. malhamensis* Extract on Various Cancer Cell Lines

Cytotoxicity of *P. malhamensis* aqueous ethanolic extract was assessed by MTT assay on A375, HepG2, HeLa, HT29, and A549 cell lines. The positive standard was cisplatin and the non-cancerous standard was VERO cell lines. IC_{50} values of the extract on each cancer cell line were calculated based on inhibition percentage. The IC_{50} value exhibited by the aqueous ethanolic extract of *P. malhamensis* and cisplatin against the A375, HepG2, HeLa, HT29, and A549 cell lines were 95.09, 103.63, 162.14, 339.30, 355.18 and 3.56, 4.87, 3.88, 7.23, and 4.65 µg, respectively. The *in-vitro* cultured cancer cell lines exhibited shrinkage followed by detachment from the adhered surface when treated with the extract of *P. malhamensis* (Fig. 4). This signifies the cytotoxic effect of the *P. malhamensis* extract.

Table 2: Quantitative analysis of phytochemicals in the aqueous-ethanolic extract of *P. malhamensis*

S. No.	*Phytochemical*	*Amount (in mg of standard equivalent/g dry weight of the extract)*
1	Flavonoids	87.75 ± 0.20
2	Phenol	134.55 ± 0.22
3	Proteins	72.64 ± 0.15

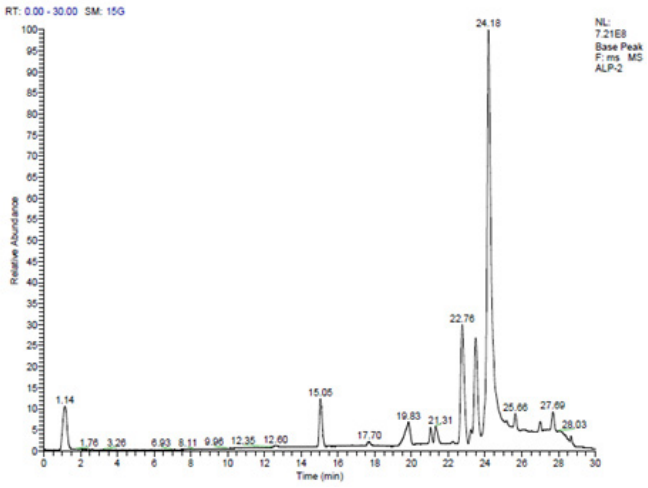


Fig. 3: LC-MS spectra of *P. malhamensis* aqueous-ethanolic extract

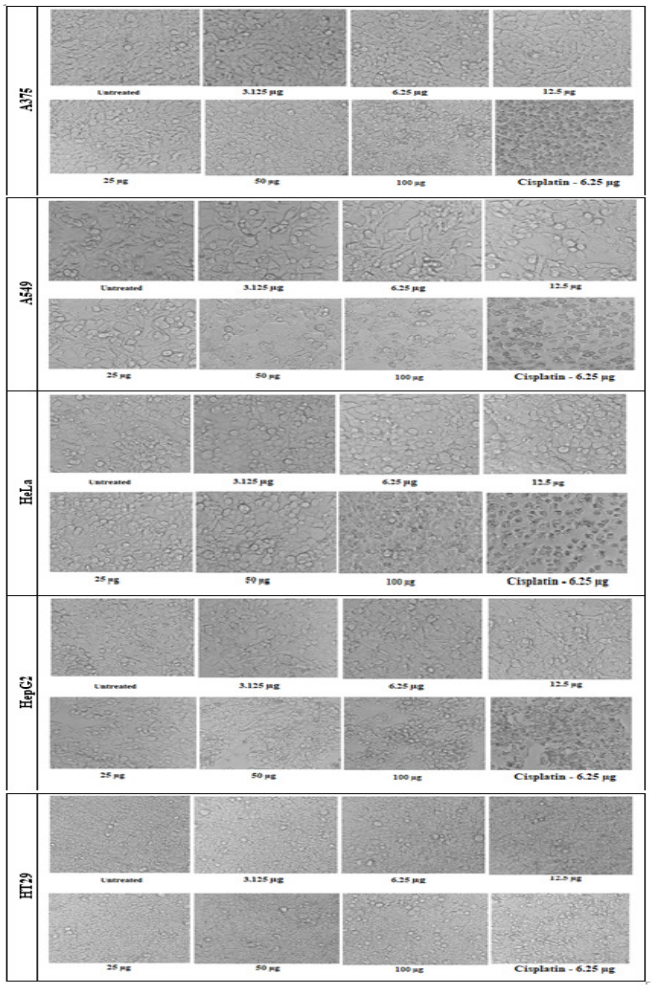


Fig. 4: Cytotoxic efficacy of different concentrations of the aqueous-ethanol extract of *P. malhamensis* on tested cancer cell lines

Concentration-dependent (3.125, 6.25, 12.5, 25, 50, 100 µg) cytotoxicity was observed with the extract against studied cancer cell lines, *viz.,* A375 (98.82 ± 0.002, 98.28 ± 0.005, 95.35 ± 0.002, 93.63 ± 0.005, 85.58 ± 0.004, 41.88 ± 0.002), A549 (99.79 ± 0.003, 98.84 ± 0.003, 97.10 ± 0.002, 93.22 ± 0.004, 89.56 ± 0.004, 86.38 ± 0.003), HeLa (99.50 ± 0.002, 98.94 ± 0.003, 97.88 ± 0.003, 93.41 ± 0.002, 87.69 ± 0.005, 68.67 ± 0.004), HepG2 (98.83 ± 0.004, 96.31 ± 0.002, 94.42 ± 0.003, 90.36 ± 0.004, 69.56 ± 0.005, 54.01 ± 0.005), and HT29 (99.47 ± 0.005, 98.53 ± 0.005, 96.75 ± 0.004, 93.88 ± 0.005, 88.52 ± 0.002, 85.77 ± 0.003) (Fig. 5). With increasing *P. malhamensis* aqueous-ethanol extract concentrations, VERO cell viability increased *viz.,* 97.92 ± 0.002, 94.09 ± 0.003, 92.09 ± 0.002, 86.35 ± 0.005, 74.12 ± 0.002, 60.38 ± 0.003.

AO/ETBR Staining

EtBr penetrates dead cells and stains the nucleus, which fluoresces green and reddish orange. Dead and living cells are both stained by acridine orange. The results of AO/EtBr staining of A375 cell lines depicted the completely green stained cells with bright green patches in control, which shows that all are live cells. The doxorubicin-treated cells and algal extract-treated cells exhibited green-colored live cells along with reddish-orange cells depicting apoptosis (Fig. 6).

PI-Annexin V Staining

Annexin V/PI labeling was used to evaluate apoptosis. Flow cytometry was used to examine *P. malhamensis* extract-treated A375 cell monolayers labeled with Annexin V and PI. Untreated cells did not apoptose, while *P. malhamensis*

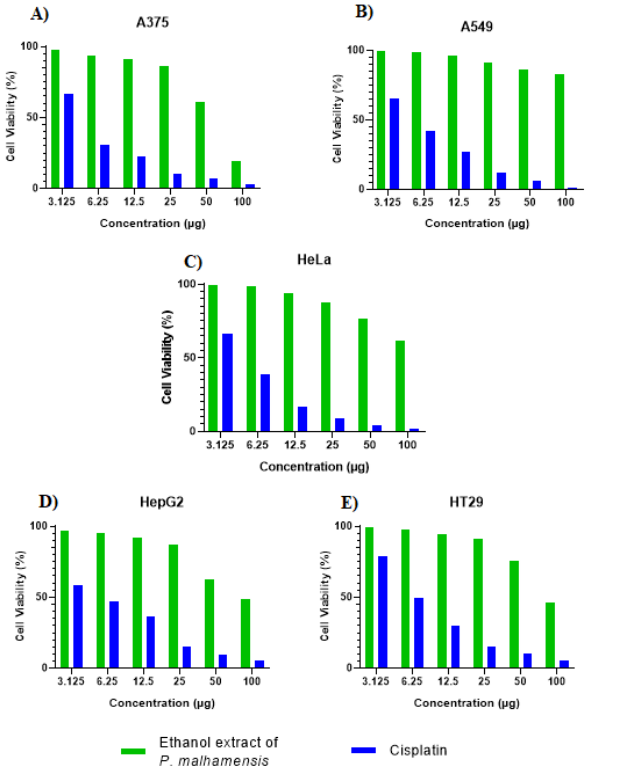
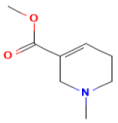
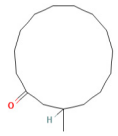
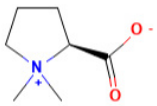
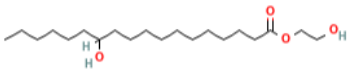
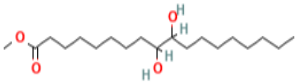
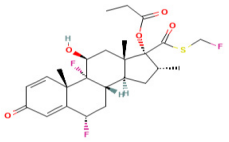
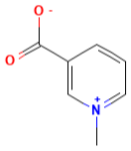
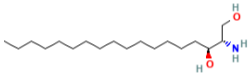
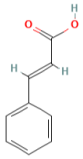
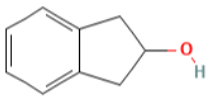
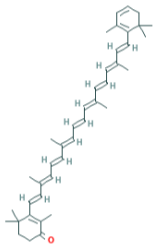
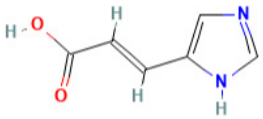
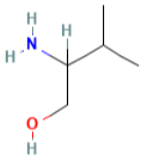
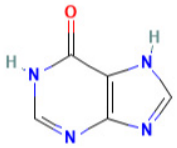
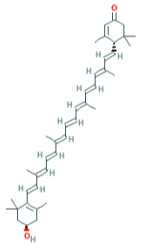
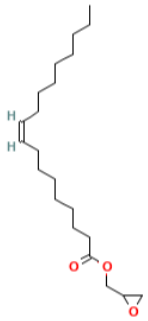
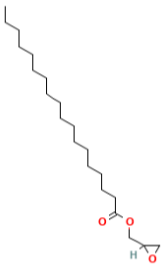


Fig. 5: Comparison of cytotoxicity of aqueous-ethanol extract of *P. malhamensis* and cisplatin on the viability of tested cancer cell lines by MTT assay

Table 3: Major compounds identified through LC-MS analysis of aqueous-ethanol extracts of *P. malhamensis*

S. No	*Name of the compound*	*Structure of compound*	*Formula*	*Molecular weight*	*RT [min]*
1	Arecoline		C8 H13 N O2	155.09444	1.192
2	Muscone		C16 H30 O	238.2292	22.755
3	Stachydrine		C7 H13 N O2	143.09447	1.011
4	2-Hydroxyethyl 12-hydroxyoctadecanoate		C20 H40 O4	344.29211	23.145
5	Methyl 9,10-dihydroxystearate		C19 H38 O4	330.27639	28.684
6	Fluticasone propionate		C25 H31 F3 O5 S	500.18379	18.427
7	Trigonelline		C7 H7 N O2	137.04752	1.173
8	Safingol		C18 H39 N O2	301.29753	19.209
9	Cinnamic acid		C9 H8 O2	148.05231	21.282
10	2-Indanol		C9 H10 O	134.07302	16.764
11	Beta-caroten-4-one		C40 H54 O2	566.41149	23.977

12	Urocanic acid		C6 H6 N2 O2	138.04276	1.241
13	Valinol		C5 H13 N O	103.09954	1.132
14	Hypoxanthine		C5 H4 N4 O	136.03839	1.122
15	3-Hydroxy-3'-oxo-beta, epsilon-carotene		C40 H54 O2	566.41167	23.407
16	Glycidyl oleate		C21 H38 O3	338.28154	25.737
17	Glycidyl stearate		C21 H40 O3	340.29711	24.083

extract-treated cells did. The percentage of apoptosis exhibited by *P. malhamensis* extract was found to be 27.3% at 47.5 µg and 13.6% at 95 µg (Fig. 7).

Discussion

P. malhamensis has been isolated from various freshwater resources previously and structural descriptions were reported.[21,22] The prevalence of *Chlorochromonas danica, and* other microalgae in Lake Tavanampalli has been previously reported.[8] This is in accordance with another study reporting the pH-dependent coexistence of *Ochromonas danica* and *P. malhamensis* in lake water.[23] The total flavonoid content in *C. danica*, another member of the Chrysophyceae family, isolated from the same lake was estimated to be 158.65 mg/g in the earlier study.[8] Similarly, the flavonoid contents of various extracts of

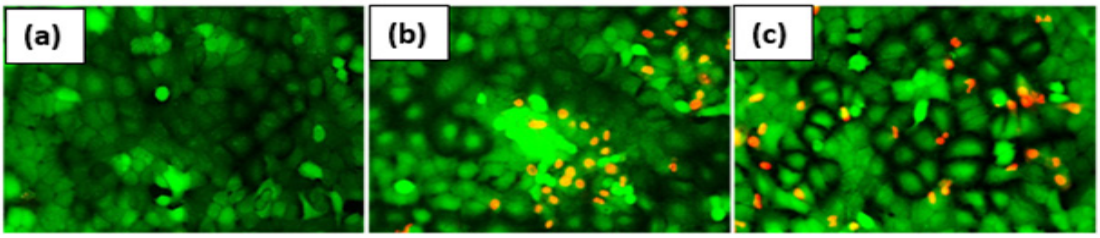


Fig. 6: Acridine Orange/EtBr staining-based apoptotic study of A375 cell lines; (a) Untreated control A375 cells fluorescing in green color showing the absence of apoptosis, (b) A375 cell lines treated with doxorubicin, (c) A375 cell lines treated with aqueous-ethanol extract of *P. malhamensis*

other microalgae, such as *Gracilaria* sp., *Ulva rigida, Fucus vesiculosus, Nannochloropsis gaditana,* and *Chlorella* sp., were determined to be 0.28 to 7.38 mg/g extract dry weight.[24] Flavonoids and their subclass compounds are reported to be potent anticancer agents by regulating the free radical scavenging enzymes, inducing apoptosis, and suppressing the proliferation of cancerous cells.[25,26]

Flavonoids and phenols are considered as the most abundant phytochemicals in the plants. The phenol content in *Chlorochromomas danica* isolated from the same water source, Lake Tavanampalli was reported to be 15.75 mg/g extract's dry weight.[8] The amount of phenolics in 23 different microalgae extracts (in 1 g) was found to be between 0.95 mg and 17.24 mg gallic acid equivalent.[27] With varying amounts of sodium nitrate, *Spirulina maxima* was shown to augment the synthesis of phenolic compounds. The resulting phenol levels were found to be between 4.51 and 16.96 mg per gram of dry extract.[28]

C. danica from the same lake Tavanampalli contained 134.65 mg/g of protein by dry weight.[8] The total amount of phenol, flavonoids, and protein in *P. malhamensis* was found to be significantly higher than other algae of other classes. Particularly, the total phenolics and total protein content were higher than the *C. danica* isolated from the same water source. This suggests that *P. malhamensis* may have a greater potential to synthesize protein and phenol using available nutrients.

Most of the compounds identified through GCMS were reported to be therapeutically important. In particular, many of these compounds, such as arecoline,[29,30] muscone,[31,32] trigonelline,[33,34] safingol,[35] beta-carotene,[11,36] and hypoxanthine derivatives[37,38] inhibit the multiplication of various types of cancer cells. A recent study found that arecoline can prevent human basal cell carcinogenesis by lowering IL-6, increasing p53, and inducing cell cycle arrest and apoptosis.[39] The new muscone derivative ZM-32 targets breast cancer and may be a promising alternative to anticancer drugs.[31] Muscone has low cytotoxicity, anti-dementia, antitumor, anti-cerebral ischemia, diabetic neuropathy, and anti-early pregnancy effects. This promotes cell growth, angiogenesis, apoptosis, and inflammation reduction.[32]

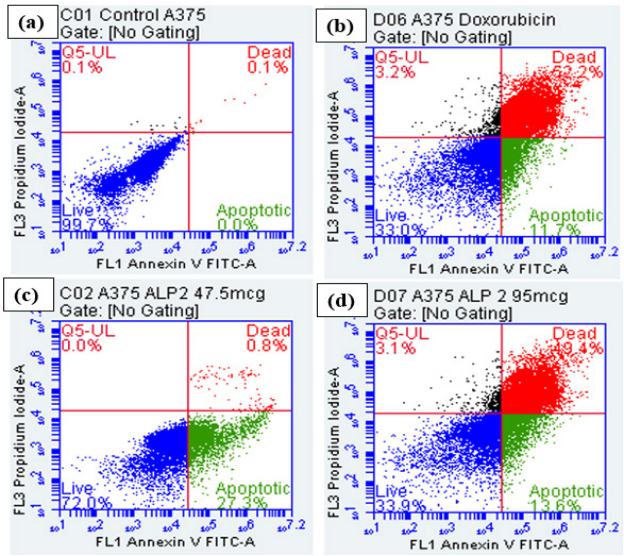


Fig. 7: Annexin V propidium iodide double staining assay-based apoptotic study of A375 cell lines depicting the flow cytometry scattergram; (a) untreated A375 cell lines, (b) A375 cell lines treated with doxorubicin, (c) A375 cell lines treated with 47.5 µg of extract of *P. malhamensis*, (d) A375 cell lines treated with 95 µg of extract of *P. malhamensis*

The anticancer properties of *P. malhamensis* are uncertain. The aqueous ethanol extract of *P. malhamensis* has substantial anticancer activity against all cancer cells examined *in-vitro*, with the highest activity against A375 cells (IC_{50} = 95.09 µg). Earlier findings on A375 cell lines with *Euastrum* sp. methanol extract indicated activity against cancer [IC_{50} = 1.04 mg/mL].[10] The LC-MS study of *P. malhamensis* aqueous ethanol extract revealed different carotenoids. Carotenoids were reported to exhibit high anticancer activity against melanoma cells (A375 cell lines) [IC_{50} : 5–125 mg/mL].[21]

This is the first report of the existence of *P. malhamensis* in India. Additionally, the identification of pharmacologically active substances linked with *in-vitro* anticancer activity against numerous cancer cell lines. The reports on the anticancer activity of melanoma cells are highly limited and have been addressed in this study. This study may advance anticancer medication research by focusing on microalgal phytocompounds. Despite frequently coexisting

with *O. danica*, *P. malhamensis* is always regarded as a contaminant.[40] Even though they produce a significant amount of biomass with other types of algae, they also use up nutrients and cause the production of various toxins in the surrounding water.[41] This contaminant can be effectively used up from wastewater sources as an efficient anticancer agent against various cancers. Among them, *P. malhamensis* extracts were proven to be effective against melanoma cell lines (A375), which is one of the serious types of cancer. The exploitation of this abundant resource may end up not only in an effective anticancer drug but also in the cleaning up of environmental pollution.

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