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In Vitro Antioxidant and Anti-cancer Activity Against MG-63 Cell Line Using Ethanol Extract of *Desmodesmus sp*. PGDR1 Isolated from Retteri Lake

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ABSTRACT
Microalgae are emerging as valuable sources of bioactive compounds with potential therapeutic applications. This study investigated the ethanol extract of *Desmodesmus* sp. PGDR1 for its phytochemical composition, antioxidant potential, and anticancer activity against MG-63 osteosarcoma cells, along with molecular characterisation. Phytochemical screening revealed the presence of tannins, steroids, terpenoids, and proteins among ten tested compounds. Antioxidant activity was evaluated using the DPPH and ABTS assays, with IC_{50} values of >320 µg/ml for both assays, indicating moderate scavenging activity compared to the reference standard, ascorbic acid (IC_{50} values of 19.97 µg/ml for DPPH and 20.46 µg/ml for ABTS). The anticancer potential was assessed using the MTT assay, and the extract demonstrated dose-dependent cytotoxicity against MG-63 cells, with IC_{50} values >100 µg/ml and a maximum inhibition of 99.08% at 6.25 µg/ml. Molecular identification was performed through 18S rDNA sequencing, and the resulting sequence was submitted to NCBI GenBank. Phylogenetic analysis using MEGA 7 confirmed the isolate's affiliation with the *Desmodesmus* genus, clustering closely with reference sequences. These results indicate that *Desmodesmus* sp. PGDR1 possesses bioactive phytochemicals with moderate antioxidant activity and significant anticancer effects against MG-63 osteosarcoma cells, highlighting its potential as a natural source for therapeutic applications.

Key words: *Desmodesmus* sp., Phytochemicals, 18S rRNA gene, Antioxidant, Anticancer, MG-63 cell line

INTRODUCTION

Microalgae are cosmopolitan organisms that occur in a wide range of environments, including freshwater, marine ecosystems, and soil, and possess numerous valuable biotechnological applications (Ghasemi et al. 2007). Their broad utility is largely attributed to their remarkable adaptability to diverse environmental conditions, including variations in temperature, nutrient availability, and culture regimes. Among these, *Desmodesmus* is a widely distributed genus of green microalgae (Chlorophyta, family Scenedesmaceae), commonly found in stagnant or slow-moving freshwater bodies, brackish habitats, and eutrophic soils (Demura et al. 2024). A distinctive characteristic of *Desmodesmus* species is their pronounced phenotypic plasticity; in response to grazing pressure from zooplankton such as *Daphnia*, these microalgae undergo morphological modifications, including spine formation and colony enlargement, as an effective defensive strategy (Lürling 2003).

Microalgae are increasingly recognised as a rich source of bioactive compounds with antioxidant and antimicrobial properties, including carotenoids, tocopherols (vitamin E), ascorbic acid (vitamin C), and a wide range of phenolic compounds (Goiris et al. 2015). Oxidative stress plays a crucial role in cancer initiation and progression by promoting genomic instability, uncontrolled cell proliferation, and resistance to programmed cell death (apoptosis) (Offer and Diasio 2018). In this context, naturally derived compounds that are readily metabolised and exhibit lower toxicity than synthetic drugs are gaining considerable attention as potential therapeutic agents.

Despite growing interest in microalgal bioactives, limited information is available on the antioxidant and anticancer potential of native *Desmodesmus* strains from urban freshwater ecosystems. Therefore, the present study aimed to isolate *Desmodesmus* sp. PGDR1 from Retteri Lake and evaluate the antioxidant and anticancer activities of its ethanol extract, using the MG-63 osteosarcoma cell line

under in vitro conditions. This work provides new insights into the biomedical potential of indigenous

MATERIAL AND METHODS

Collection and isolation

Rettai Eri, locally known as Retteri, is a lake located in the Kolathur area of Chennai, India, which is visible from the 100-ft road. Water samples were collected from the lake using a 0.2 mm mesh net for phytoplankton and stored in sterile vials. The samples were transported to the laboratory and centrifuged at 2000 rpm for 10 min to concentrate the biomass. The sample was then inoculated into BBM. The resulting sediments were cultured on solid BBM medium and incubated at 25°C for 5-7 days. Pure cultures were obtained through serial dilution and the streak plate method.

Morphological identification

The cultured algal strains were identified under 40X magnification of a light microscope based on the size and shape of the cells and colony formation. A microphotograph was taken using a Microvision industrial digital camera.

Molecular Identification using 18S rRNA

Extraction and determination of the quality of DNA

The Genomic DNA was extracted from a 13-15 day-old log-phase culture. It was extracted using a meticulously prepared extraction buffer for a 500 ml volume, which comprised 20 mM Na2EDTA (3.7 g) and 100 mM Tris-HCl (6 g). The pH was adjusted to 8, followed by the addition of 1.4 M NaCl (40 g) and 2% (w/v) CTAB (10 g). The extraction buffer was preheated to 60°C, and the sample was thoroughly mixed and incubated for 1 hour at 60°C with intermittent shaking every 10 min. The mixture was cooled down to 37°C. To 2 ml of this mixture, 1 ml of chloroform: isoamyl alcohol (24:1) was added and gently mixed by inverting the tubes to form an emulsion. It was then centrifuged at 5000xg for 15 min. The clear aqueous phase was transferred to a fresh tube, and 150 µl of 6 M NaCl was added and mixed. To this, 1 ml of ice-cold ethanol was added, and the mixture was refrigerated for 1 hr at -20°C. The pellet was washed several times with ethanol and then resuspended in 100 µl of elution buffer. The DNA samples were stored at 4°C for further analysis (Jagielski et al. 2017). The quality of extracted DNA was checked on 0.8% agarose gels. Agarose powder was dissolved in 1X TAE buffer and boiled until it turned into a clear solution. Once it was cooled to 50°C, ethidium bromide was added and mixed well. The gel was cast in the gel tray and soaked in 1X TAE buffer in the electrophoresis tank. 3 µl of DNA with 3 µl of gel loading dye was loaded in the wells and run at 70 V for 15 to 20 min. The DNA bands were observed as orange-coloured bands under a UV-transilluminator (Genei) (Lee et al. 2012).

Amplification and analysis of DNA by PCR

The Polymerase Chain Reaction (PCR) was conducted in a 25 µl volume using the GeneAmp PCR System 9700. The reaction mix included 1X PCR buffer, 0.2 mM of each dNTP, 1 µl of DNA template, 0.2 µl of Phire Hotstart II DNA polymerase, and 10 pM each of the 18S F and 18S R primers. The thermal cycling protocol involved an initial denaturation at 95°C for 30 sec, followed by 35 cycles of 30 sec each at 95°C (denaturation), 56°C (annealing), and 72°C (extension), with a final 10 min extension at 72°C.

The resulting PCR products were then verified by electrophoresis on a 1.2% agarose gel prepared with 0.5X TBE buffer and 0.5 µg/ml ethidium bromide, using a 2-log DNA ladder as a molecular standard and visualised the bands with a UV transilluminator (McInerney et al. 2014). For subsequent steps, the PCR product was purified by mixing 5 µl of the product with 2 µl of ExoSAP-IT (containing Exonuclease I and Shrimp Alkaline Phosphatase). This mixture was incubated at 37°C for 30 min to digest any remaining primers and dNTPs, followed by enzyme inactivation at 80°C for 15 min.

Sequencing of purified DNA using BigDye Terminator v3.1

The sequencing of the ExoSAP-treated PCR product was conducted using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The 10 µl sequencing reaction included 10 ng of DNA template, 3.2 pM of primer, sequencing mix, DMSO, and 5X reaction buffer. The thermal profile consisted of 30 cycles with a 50°C annealing step and a 4 min final extension at 60°C (McInerney et al. 2014). To purify the sequencing product for analysis, an ethanol

precipitation protocol was followed. This involved the addition of a master mix containing EDTA, sodium acetate (pH 4.6), and ethanol to the 10 µl reaction volume. After a 30 min room temperature incubation, the product was pelleted by centrifugation, washed with 70% ethanol, air-dried, and loaded onto an ABI 3500 DNA analyser. The final sequence data quality was assessed using the dedicated Sequence Scanner Software v1 (Biswas et al. 2015).

Phylogenetic analysis using BLAST and phylogenetic tree construction

The amplified DNA sequences, identified as part of the 18S rRNA gene, were initially confirmed using the NCBI's BLAST program to establish their similarity to existing reference species. The sequences were then aligned and edited using the MEGA 7 software. Finally, MEGA 7 was also employed to construct the phylogenetic tree based on the processed sequence data.

Phytochemical extraction and quantitative analysis from *Desmodesmus* sp. PGDR1

To extract compounds from the microalgae, an 8.2 g sample was combined with 50 ml of aqueous ethanol solvent. This mixture was agitated on a rotary shaker at 150 rpm for 72 hrs at 25°C. Following agitation, the mixture was filtered, and the resulting filtrate was concentrated to dryness using a vacuum evaporator, a procedure slightly modified from Pandey et al. (2020). The resulting crude extract was sealed in airtight glass vials and stored at 4°C until it was needed for further analysis. The extracted crude was analysed for the presence or absence of phytochemical constituents by following the method of Harborne (1973).

In vitro antioxidant activity

DPPH assay

The antioxidant capacity of the crude sample was estimated *in vitro* using the DPPH radical scavenging assay, a procedure modified slightly from that described by Perumal and Saravanabhavan (2018). In this test, a 0.135 mM methanolic DPPH solution was used. The sample was tested across a concentration range (5 to 320 µg/ml) and compared to a standard, ascorbic acid. Each test concentration was mixed with 2.0 ml of the DPPH solution, and after a 30 min incubation at room temperature, the absorbance was measured at 517 nm. The effectiveness of the sample was quantified as the percentage of DPPH inhibition:

% DPPH inhibition = [(OD of control - OD of test) / (OD of control)] ×100

ABTS assay

Antioxidant activity was also measured using a modified ABTS radical scavenging assay (Perumal and Saravanabhavan 2018). The ABTS working solution was generated from a 7 mM ABTS stock activated with 140 mM potassium persulfate. The crude sample, across concentrations from 5 to 320 µg/ml, and the Ascorbic acid standard were each mixed with 2.0 ml of the ABTS solution. After 20 min of room-temperature incubation, the absorbance was measured at 734 nm using a UV-visible spectrophotometer. The results were expressed as the ABTS radical scavenging effect using following equation:

ABTS radical scavenging effect (%) = [(A0 - A1) / A0] ×100.

where, A0 is the control; A1 is the test

Anticancer activity against the MG63 cell line

Cell lines and culture medium

The Human Osteosarcoma cell line (MG63) was obtained from the National Centre for Cell Science in Pune, India. These cells were maintained as a stock culture in MEM medium enriched with 10% heat-inactivated Fetal Bovine Serum (FBS) and antibiotics (penicillin at 100 IU/ml and streptomycin at 100 µg/ml). Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 until they reached confluency (Sodde et al. 2015).

MTT assay

For the MTT assay, a stock of test samples was prepared through a serial two-fold dilution, spanning a concentration range of 6.25 to 100 µg. First, the cells were prepared: the monolayer culture was trypsinised, and the cell density was adjusted to 1.0×10^5 cells/ml in the appropriate 10% FBS medium. 100 µl of this suspension, resulting in 1×10^4 cells per well, was then dispensed into each well of a 96-well microtiter plate. After a 24 hr incubation period, allowing a partial monolayer to form, the supernatant was removed. The cells were washed once with fresh medium before 100 µl of the test sample (at various concentrations) was added. The

plate was incubated for another 24 hrs at 37°C in 5% CO_2. Following the second incubation, the test solutions were discarded, and 20 µl of the MTT solution (2 mg/ml in PBS) was added to each well. The plate was incubated for 4 hrs under the same conditions (37°C, 5% CO_2). Finally, the supernatant was removed, and 100 µl of DMSO was added to dissolve the formed formazan crystals. Absorbance was measured at 570 nm using a microplate reader, with Doxorubicin serving as the reference standard. Cell viability was calculated using the formula:

% viability = Sample abs/Control abs x 100

RESULTS

Morphological identification

The isolated microalga was cultivated on a BBM liquid medium, resulting in green biomass, and streaked onto a BBM agar plate. The genus *Desmodesmus* (Chodat) S.S.An, T.Friedl & E.Hegewald, 1999, which has Colonies of 4 (or 2, 8, 16) cells attached side by side, arranged linearly or zigzag; the cell body is elliptical in shape; and the terminal cells lack any spiny projections. The cell dimension is 22 x 51 µm (Fig. 1).

Molecular identification

The DNA sequence was submitted to the NCBI GenBank database under the accession number PX376984. Phylogenetic analysis was performed using MEGA 12 software (Fig. 2). A total of 14 nucleotide sequences were retrieved from GenBank, and a phylogenetic tree was constructed. The tree with the highest log likelihood (0.00050) is presented here. Initial trees for the heuristic search were generated automatically using the Neighbour-joining and BioNJ algorithms, based on pairwise distances estimated with the Maximum Composite Likelihood (MCL) method. The topology with the superior log likelihood value was then selected for the final phylogenetic tree. The phylogenetic tree revealed that *Desmodesmus* sp. PGDR1 clustered closely with other *Desmodesmus* strains available in GenBank, confirming its taxonomic placement within the genus. This strong clustering with reference sequences supports the accurate identification of the isolate as a member of *Desmodesmus*.

Figure 1. Microscopic image of *Desmodesmus* sp. PGDR1 under 45X magnification

Qualitative analysis

The ethanol extract of *Desmodesmus* sp. PGDR1 was found to contain various phytochemicals. A total of ten phytochemicals were screened in this study, of which only four tannins, steroids, terpenoids, and protein were detected, while the remaining compounds were absent (Table 1).

In vitro antioxidant activity

DPPH Assay

The antioxidant potential of *Desmodesmus* sp. PGDR1 ethanol extracts were evaluated using the DPPH assay. The IC_{50} values of *Desmodesmus* sp. PGDR1 and the reference standard ascorbic acid were >320 and 19.97 µg/ml, respectively. The

Table 1. Qualitative phytochemical analysis of *Desmodesmus* sp. PGDR1

Phytochemicals	Status
Alkaloids	-
Flavonoids	-
Saponins	-
Tannins	+
Phenols	-
Cardiac glycosides	-
Steroids	+
Terpenoids	+
Quinones	-
Proteins	+

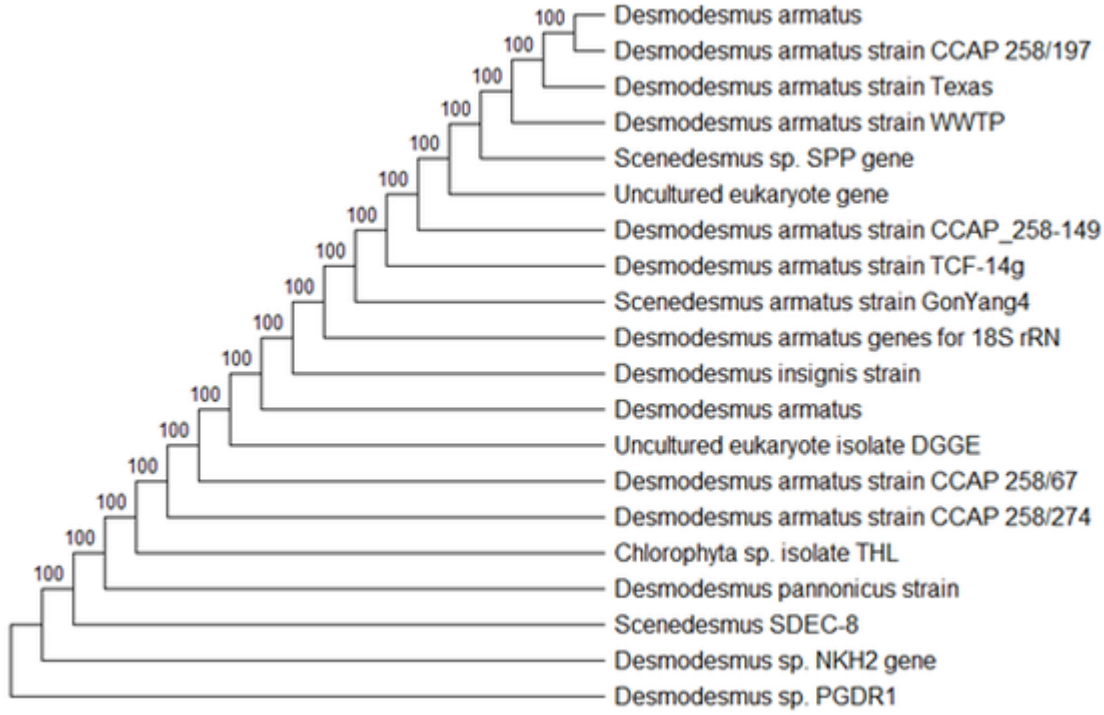

Figure 2. Phylogenetic tree of *Desmodesmus* sp. PGDR1

Table 2. Inhibition percentage of *In vitro* Anti-oxidant using DPPH assay

Sample	Concentration (µg/ml)							
	5	10	20	40	80	160	320	IC_{50}
Ascorbic acid	7.11	30.6	63.8	78.85	85.46	91.24	93.94	19.97
***Desmodesmus* sp.**	0.5	0.55	1.24	1.65	1.65	2.98	4.5	>320

Table 3. Inhibition percentage of *In vitro* Anti-oxidant using ABTS assay

Sample	Concentration (µg/ml)							
	5	10	20	40	80	160	320	IC_{50}
Ascorbic acid	3.41	39.06	61.62	75.34	82	88.72	95.67	20.46
***Desmodesmus* sp.**	1.99	3.13	5.3	6.78	18.68	29.16	37.64	>320

antioxidant capacity of the crude extracts increased with increasing concentration. The maximum antioxidant activity (4.5%) was observed at 320 µg/ml, whereas ascorbic acid exhibited 93% activity at the same concentration. These results confirm that *Desmodesmus* sp. PGDR1 possesses relatively low antioxidant activity compared to the natural antioxidant, ascorbic acid (Table 2).

ABTS Assay

The antioxidant activity of the ethanol extracts was evaluated using the ABTS assay. The IC_{50} values of *Desmodesmus* sp. PGDR1 and the standard drug (ascorbic acid) were found to be >320 and 20.46 µg/ml, respectively. The highest antioxidant activity of the extract (37.6%) was observed at 320 µg/ml, whereas the natural antioxidant ascorbic acid exhibited 95.6% activity at the same concentration. Extracts obtained using other solvents showed comparatively lower antioxidant capacities (Table 3).

Anticancer activity against the MG-63 cell line

The percentage of cell growth inhibition by *Desmodesmus* sp. PGDR1 extracts at a concentration

Table 4. Viability percentage of Anti-cancer activity using MTT assay

Sample	Concentration (µg/ml)					
	6.25	12.5	25	50	100	IC_{50}
Doxorubicin	54.54	48.78	34.25	28.8	15.86	9.52
***Desmodesmus* sp.**	99.08	92.8	86.77	82.12	78.9	>100

of 100 µg/ml was found to be 78.93% after 48 hrs. The ethanolic extracts of *Desmodesmus* sp. PGDR1 showed significant cytotoxic activity against the MG-63 cell line in the MTT assay. Both the crude extract and Doxorubicin (reference standard) demonstrated dose-dependent inhibition, with IC_{50} values of >100 and 9.52 µg/ml, respectively. The maximum cell growth inhibition (99.08%) was observed at a concentration of 6.25 ìg/ml (Table 4).

DISCUSSION

This study represents the first biotechnological investigation of the microalgal strain *Desmodesmus* sp. PGDR1 isolated from Retteri Lake. Previous studies on Retteri Lake primarily addressed environmental aspects, such as physico-chemical characteristics (Thangamalathi and Anuradha 2018) and the quality of surrounding groundwater, which was reported to range from excellent (19%) and good (62%) to poor or very poor (19%) (Rathinam et al. 2024). In contrast, the present work extends existing knowledge by demonstrating the biomedical potential of a native microalgal isolate from this ecosystem.

The antioxidant scavenging activity of *Desmodesmus* sp. PGDR1 extracts exhibited an apparent concentration-dependent increase, with the lowest activity observed at 5 µg/ml and the highest at 320 µg/ml. Although the activity was lower than that of the ascorbic acid standard, this trend is consistent with earlier reports on related taxa. Basha et al. (2024) reported modest DPPH radical-scavenging activity (up to 22.32%) in *Desmodesmus* (SP04) and *Acutodesmus obliquus*. Similarly, Almendinger et al. (2021) attributed variations in antioxidant capacity among microalgal strains to differences in the concentration of soluble bioactive compounds, with reported values ranging from 12 to 14 µmol/g ascorbic acid equivalents. Furthermore, the efficiency of antioxidant extraction is strongly influenced by methodological factors such as solvent type, extraction time, and temperature (Maadane et al. 2015), which may account for inter-study variability. In this context, previously reported IC_{50} values of 1.53 mg/l for *Arthrospira platensis* and 0.95 mg/l for *Chlorella vulgaris* further highlight the species-specific nature of their antioxidant potential. The observed anticancer activity of *Desmodesmus* sp. PGDR1 appears to be closely associated with its antioxidant properties, as oxidative stress modulation is a well-established mechanism underlying the inhibition of cancer cells. The algal extract induced a dose-dependent reduction in cell viability, a characteristic commonly observed for natural bioactive compounds. This finding is in agreement with earlier studies on limnic microalgae (KACC 2 and KACC 23), which demonstrated increased cytotoxicity against MG-63 osteosarcoma cells with rising extract concentrations (James and Thomas 2019).

Desmodesmus species have been reported to exhibit stronger antiproliferative effects against MCF-7 breast cancer cells than closely related species such as *Acutodesmus obliquus* (Basha et al. 2024). The present results further support the growing body of evidence highlighting microalgae as promising sources of anticancer agents. For instance, methanolic extracts of *Dunaliella tertiolecta* have shown efficacy against A2058 human melanoma cells, while crude ethanol extracts of *Chaetoceros calcitrans* significantly inhibited MCF-7 cell proliferation, potentially through apoptosis induction (Nigjeh et al. 2013). Comparable studies have reported low IC_{50} values, such as 97.33 ± 3.75 µg/mL and 59.33 ± 3.3 µg/ml against MCF-7 cells using the MTT assay (Sodde et al. 2015), underscoring the therapeutic relevance of microalgal bioactive compounds. Overall, the findings of this study suggest that *Desmodesmus* sp. PGDR1 isolated

from Retteri Lake is a promising candidate for further exploration as a natural source of antioxidant and anticancer compounds, warranting future investigations into compound isolation, mechanistic pathways, and in vivo efficacy.

CONCLUSION

Desmodesmus sp. PGDR1 exhibits a versatile morphology and considerable biotechnological potential, particularly for its antioxidant and anticancer properties. The extract exhibited moderate antioxidant potential, as demonstrated by DPPH and ABTS assays, and significant anticancer activity against MG-63 osteosarcoma cells in a dose-dependent manner. Its adaptability, combined with ongoing advances in molecular taxonomy and cultivation techniques, makes it a promising subject for both fundamental research and applied purposes.

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