Biol. Mar. Mediterr. (2026), 30 (1): 208-211 www.biologiamarinamediterranea.it

54° Congresso SIBM: Plancton

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PROMISING ANTIPROLIFERATIVE ACTIVITY OF THE MARINE MICROALGA *DIACRONEMA LUTHERI*

PROMETTENTI ATTIVITÀ ANTIPROLIFERATIVE DELLA MICROALGA MARINA DIACRONEMA LUTHERI

Abstract - *Marine microalgae are photosynthetic eukaryotic organisms that have been shown to produce primary and secondary metabolites with possible bioactivities useful for human health. Recently, there has been a growing interest from the scientific community in the search for natural compounds with potential applications in the field of cancer therapy, due to the high incidence of cancer in the population. Marine microalgae are attractive sources of compounds with biological activity thanks to their great biodiversity in terms of species, compounds produced and thanks to the possibility of growing them in large quantities. The aim of the present study was to evaluate the biological activity of the marine Haptophyta* Diacronema lutheri *(formerly known as* Pavlova lutheri*). We prepared and tested extracts of this microalga on human cells and found interesting activities against human lung carcinoma cells. These promising extracts will be further considered for chemical identification of bioactive components.*

Keywords: *marine microalgae, antiproliferative activity, MTT assay*

Introduction – Cancer is one of the major causes of death worldwide. Considering the high incidence in the population and side effects of current treatments, the interest of the scientific community is focused on identifying new solutions. Currently, there are on the market 17 drugs originally isolated from marine organisms (https://www.marinepharmacology.org/approved; accessed on 6 August 2025), of which twelve for various cancer treatments (Cytosar-U®, Halaven®, Adcentris®, Yondelis®, Aplidin®, Polivy™, PADCEV™, Zepzelca™, Aidixi™, TIVDAK™, EMRELIS™, Blenrep™), three for Hypertriglyceridemia (Lovaza®, Vasepa®, Epanova®), one for sever pain (Prialt®) and one as antiviral (Arasena A®). They have been originally isolated from sponges, fishes, tunicates, cyanobacteria associated to mollusks, but to our knowledge, there are still no molecules from marine microalgae as drug commercially available (D'Incalci *et al.,* 2004; Nigam *et al*., 2019).

Several studies have shown that microalgae may produce compounds as defence against predators or as antimicrobial which can also have biotechnological applications (Lauritano *et al*., 2015; Miralto *et al*., 1999). These molecules include lipids, carbohydrates, nucleosides, pigments and also complex polyketides with bioactivities useful for human health. Studies are ongoing to understand specificity, concentrations, mechanism of action, possible cytotoxicity and genetic engineering to implement production of molecules of interest (Bazzani *et al*., 2021).

In the current study, we focused on the Haptophyta *Diacronema lutheri* (Droop) Bendif & Véron, a microalgal species known to be used in aquaculture and studied as a possible model for lipid biosynthesis (Hulatt *et al*., 2021). Previous studies have shown that *D. lutheri* had antiviral activity against the herpes simplex virus type 1 (HSV-1 strain SC16) (Montuori *et al*., 2025) and beneficial effects for the possible prevention of obesity and metabolic syndrome in high-fat-fed wistar rats (Mayer *et al*., 2022). Our aim was to evaluate a possible antiproliferative activity of *D. lutheri* against human lung epidermoid carcinoma cell line Calu-1. Lung carcinoma is one of the most common cancer worldwide, and according to World Cancer Research Fund

(https://www.wcrf.org/preventing-cancer/cancer-statistics/lung-cancer-statistics/), in 2022, there were 2,480,675 new cases of lung cancer.

Materials and Methods - Microalga cultivation - The microalga *Diacronema lutheri* (CCMP1325) was cultivated in triplicate 2 L - polycarbonate bottles in Guillard's medium without silicates constantly bubbled with air filtered through 0.2 µm membrane filters. Bottles were kept in a climate chamber at 19 °C on a 12:12 h light/dark cycle at 100 µmol photons m^{-2} s^{-1}. Initial cell concentrations were about 5000 cells/mL. To reduce culturing differences, each replicate was centrifuged at the end of stationary phase for 15 min at 4 °C at 3900× g and the pellets kept at −80 °C until chemical extraction. Chemical extraction – The microalgal pellet was macerated for 1h in methanol 100% and subsequently the organic mixture was sonicated with three bursts of 30 s in an icy water bath, centrifuged at 3800 rpm at room temperature to precipitate the solid material in suspension that was discerned. The organic phase was transferred in a glass vials, decanted and dried under vacuum at reduced pressure. The extracts were stored at -20°C, until use.
Chemical Fractionation– Fractionation of raw extract was performed by solid phase extraction (SPE) using CHROMABONDR HR-X cartridges (6 mL/500 mg) as in Cutignano *et al.* (2015). The cartridge was conditioned with 6 mL of MeOH and equilibrated with 12mL of distilled water. The extract was prepared by the addition of 1 mL of distilled water and sonicated for a few seconds in a bath of icy water before loading it into the cartridge. After the adsorption of extract by the resin, the following elution consist in five step to obtain five different fractions: 100% H_2O (12 mL, fraction A); CH_3OH/H_2O (50:50, 18 mL, fraction B); CH_3CN/H_2O (70:30, 12 mL, fraction C); 100% CH_3CN (12 mL, fraction D); CH_2Cl_2/CH_3OH (90:10, 12 mL, fraction E). The fraction, collected in vials, was decanted and dried under vacuum at reduced pressure, and preserved at -20°C.
MTT Assay - Human cells were bought at ATCC (https://www.atcc.org/). Calu-1 (Human lung epidermoid carcinoma; ATCCR HTB-54™) and HaCaT cell (Spontaneously immortalized keratinocytes from adult skin) were cultured in DMEM High Glucose supplemented with 10% Fetal Bovine Serum, 1% L-Glutamine and 1% Pen-Strep solution in a humidified incubator at 37 °C and 5% CO_2. To estimate the *in vitro* antiproliferative effects HaCaT and Calu-1 cells were seeded in 96-well microtiter plate, at concentration of $1x10^4$ cells/well and incubated at 37°C to allow for cell adhesion in the plates. After 24 h, the medium was replaced with fresh medium containing increasing concentrations of the total extract and fractions (6,25 µg/mL, 12,5 µg/mL, 25 µg/mL, 50 µg/mL and 100 µg/mL) dissolved in dimethyl sulfoxide (DMSO) and further incubated for 24 h. The maximum concentration of DMSO used was 0,5% (v/v). Each concentration was tested at least in triplicate. After 24 h, cell viability was assessed using the MTT test (3-(4,5-dimethyl-2-thizolyl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT, A2231,0001, Applichem Panreac Tischkalender, Darmstadt, GmbH). The medium was replaced with medium containing MTT solution 1:10 (stock solution 5 mg/mL) and the plates were incubated for 3 h at 37°C. After incubation, cells were treated with isopropyl alcohol (used as MTT solvent) for 1h at room temperature. Absorbance was measured at OD = 570 nm and 630 nm using a microplate reader (MultiskanTM FC Microplate Photometer, Thermo Fisher Scientific, Waltham, MA, United States). Cell survival was expressed as a percentage of viable cells in the presence of the tested samples, with respect to untreated control cultures with only DMSO. The percentage of

cell viability was calculated as follows: mean (A570–A630) and compared to cells supplemented with DMSO alone. Values shown in the plot are mean ± SD of six determinations. Mean and the standard deviation was calculated on biological triplicates, each in technical triplicate, using Microsoft Excel. Statical analysis was performed by GraphPad Prism8 software (Two-way ANOVA, Dunnett's test)

Results – Cell viability of human cells after 24 h treatment with *D. lutheri* extract and fractions is shown in Figure 1 and 2. Results showed that the most active extract was Fraction C which induced a reduction in lung carcinoma cell viability below 30% (Fig. 1) after only 24 h of treatment at 100 µg/mL. Between the other fractions, another one that induced a reduction to about 40% in cell viability was Fraction D at 50 µg/mL. However, for both we did not observe a dose-dependent response.

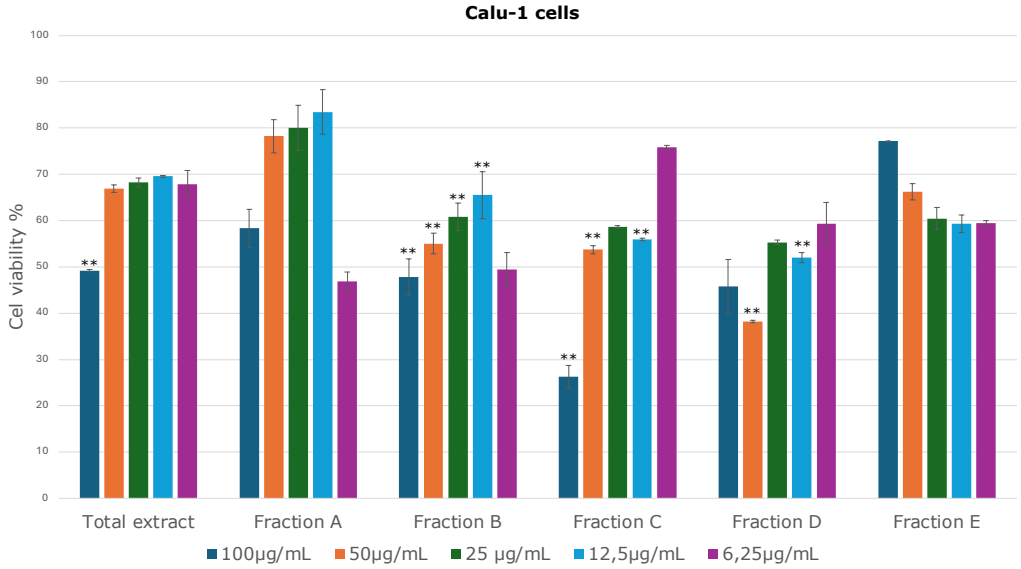

Fig. 1 - Cell viability assay on lung cells (Calu-1). The figure shows the effects of raw extracts and fractions of *Diacronema lutheri* at increasing concentrations (6,15 µg/mL, 12,5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL) on cell-viability of Calu-1 cells after 24h of treatment. Cell viability was normalized to control cells (DMSO addition). Results are expressed as percent survival (n = 3; * for p<.033; ** for p < 0.002 and *** for p < 0.001, Ordinary two-way ANOVA, Dunnett's test).
Test di vitalità cellulare su cellule polmonari (Calu-1). La figura mostra gli effetti di estratti grezzi e frazioni di Diacronema lutheri *a concentrazioni crescenti (6,15 µg/mL, 12,5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL) sulla vitalità cellulare delle cellule Calu-1 dopo 24 ore di trattamento. La vitalità cellulare è stata normalizzata rispetto alle cellule di controllo (aggiunta di DMSO). I risultati sono espressi come percentuale di sopravvivenza (n = 3; * per p < 0,033; ** per p < 0,002 e *** per p < 0,001, ANOVA a due vie, test di Dunnett).*

The same raw extract and fractions were also tested on a normal cell line (HaCaT) to evaluate general cytotoxicity. Results showed less toxicity on normal cells. Cell viability of HaCaT cells for the same active fractions was about 70% (Fig. 2).

Conclusion - The aim of the current work was to identify bioactive microalgal extracts with anticancer activity. These preliminary results helped to identify key fractions (fraction C and fraction D) with which to proceed with further chemical investigation to identify new possible anticancer compounds and for which investigate the mechanism of action of cell death.

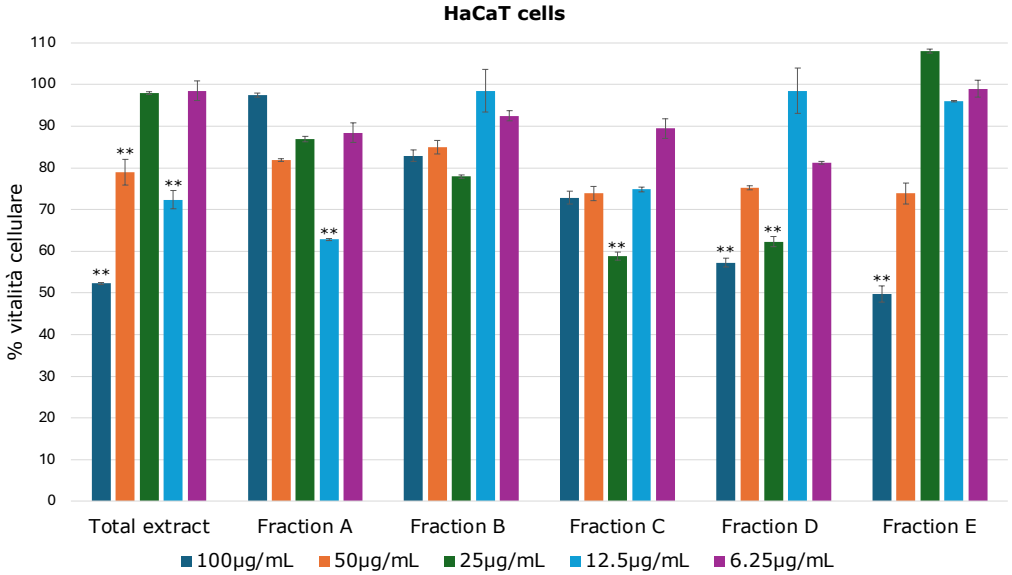

Fig. 2 - Cell viability assay on normal cells (HaCaT). The figure shows the effects of raw extracts and fractions of *Diacronema lutheri* at increasing concentrations (6,15 µg/mL, 12,5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL) on cell-viability of HaCaT cells after 24h of treatment. Cell viability was normalized to control cells (DMSO addition). Results are expressed as percent survival (n = 3; * for p<.033; ** for p < 0.002 and *** for p < 0.001, Ordinary two-way ANOVA, Dunnett's test).
Test di vitalità cellulare su cellule normali (HaCaT). La figura mostra gli effetti di estratti grezzi e frazioni di Diacronema lutheri *a concentrazioni crescenti (6,15 µg/mL, 12,5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL) sulla vitalità cellulare delle cellule HaCaT dopo 24 ore di trattamento. La vitalità cellulare è stata normalizzata rispetto alle cellule di controllo (aggiunta di DMSO). I risultati sono espressi come percentuale di sopravvivenza (n = 3; * per p < 0,033; ** per p < 0,002 e *** per p < 0,001, ANOVA a due vie, test di Dunnett).*

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