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First insights on chemical nature and bioactivity of surface mucus from the Antarctic sponges *Mycale* (Oxymycale) *acerata* and *Dendrilla antarctica*

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Abstract

The mucus produced by the Antarctic sponge species *Mycale (Oxymycale) acerata* and *Dendrilla antarctica* was analyzed to investigate its chemical composition and assess potential cytotoxic activity against human tumor cell lines. The mucus samples exhibited a high-water content (98–99%), while inorganic salts represented most of the total dry weight (67.1 ± 2.3%). Sodium was the most abundant element, accounting for 75% in *D. antarctica* and up to 82% in *M. acerata*. Other major elements—including magnesium, potassium, and calcium—comprised 3–10% of the inorganic fraction. Protein content was estimated at 0.44% for *M. acerata* and 5.63% for *D. antarctica*, with a carbon-to-nitrogen (C/N) ratio between 3 and 5. Elemental analysis was supported by μFT-IR spectroscopy, which confirmed the presence of inorganic salts, water, and proteinaceous materials. Further characterization by ^{1}H NMR spectroscopy and LC–MS revealed the presence of amino acids, peptides, sugars, carboxylic acids, nitrogenous bases, and their derivatives. Notably, oxylipin concentrations ranged between 1 and 3 ng/L. In addition, *M. acerata* mucus demonstrated antiproliferative activity, selectively inhibiting the growth of Malme-3 M melanoma cell lines, thus suggesting a potential for cytotoxic effects. Overall, these findings provide the first chemical characterization of Antarctic sponge mucus, providing first insights for its potential exploitation as a novel source of bioactive compounds.

Keywords Antarctic · *Mycale acerata* · *Dendrilla antarctica* · Mucus · Chemical analysis · Bioactivity · Melanoma

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Introduction

Marine sponges (phylum Porifera), among the oldest extant multicellular animal groups, are key constituents of benthic ecosystems, where they contribute significantly to habitat complexity and nutrient cycling. They are classified into three classes, namely the *Hexactinellida* (glass sponges), *Calcarea* (calcareous sponges), and *Demospongiae* (demosponges), which include the highest number of species (Borchiellini et al. 2001; Hooper and Soest 2002). Sponges are sessile organisms obtaining nutrients from the surrounding environment by filtering large volumes of water (Rizzo and Lo Giudice 2018) through their aquiferous system. Marine sponges, as sessile invertebrates, employ multiple strategies for adaptation and defense, among which is the secretion of a surface mucus layer composed predominantly of water, polysaccharides, and proteins. A broad spectrum of functions have been suggested in marine invertebrates (mainly cnidarians, polychaetes, and echinoderms), namely adhesion enhancement, water preservation, movement helping, and heterotrophic feeding (Stabili et al. 2014). Mucus layers could act like a physical barrier by providing mechanical protection against infectious agents and debris accumulation on the body surface (Stabili et al. 2014, 2009; Iori et al. 2014; Martin and Walther 2003). This is possible thanks to the framework structure and the slimy consistency that catches and holds extraneous particles, preventing their entry into the deeper cellular layers. Several studies have reported the presence of genetic elements involved in the regulation of mucus production and chemical composition in Porifera, including precursors of mucin-like proteins. In addition, mucus secretion has been documented in various sponge species (Biggerstaff et al. 2017; McGrath et al. 2017), even if without further insights into the role or chemical composition. In summary, the real role of mucus production, which is widespread in many marine organisms, has not been clearly deciphered, with most studies that have been conducted in temperate and tropical areas, while polar regions remain largely unexplored in this context.

The real interest in these biological fluids is moving towards their chemical composition and biotechnological potential. It has been established that the mucus of marine organisms contains chemical compounds, including metabolites and enzymes, which provide a disgusting, poisonous, or urticant nature to mucus producers (Derby 2007). Moreover, it has been demonstrated that mucus produced by many marine organisms contains lytic compounds, antimicrobial molecules, and antiadhesive compounds (Aneiros and Garateix 2004; Mayer et al. 2013; Smith et al. 2010; Lopes et al. 2024). These properties could be justified by ecological requirements, being sessile marine invertebrates fixed to the substrate, therefore particularly vulnerable to external stressors such as pathogens, predators, parasites, and environmental disturbances. As an example, sponges, in particular, filter large volumes of seawater containing dissolved organic matter, viruses, bacteria, phytoplankton, and suspended sediments, which can accumulate on or within their bodies (Strehlow et al. 2017; Welsh et al. 2020; Wooster et al. 2019).

Recent papers have used a multidisciplinary approach by combining natural product characterization with in -silico analyses such as molecular docking, density functional theory calculations and biological activities, such as for antituberculosis, antibacterial and antifungal activities (Geng et al. 2024; El-Attar et al. 2023).

Nowadays researchers are strongly encouraged to search for new molecules of natural origin with high specificity and biodegradability, with potential application in the biotechnological field. The exploration of under-investigated environments, such as the polar regions, is an even more appealing approach, since the comprehension of ecological significance and chemically mediated interactions at the base of mucus production and properties in extreme habitats could reveal new processes and new compounds. In the field of biodiscovery, the exploration of new and unexplored sources represents a crucial step to enhance the isolation of new compounds. Moreover, the understanding of the mechanism of action of an active molecule enables it to be replicated in laboratory conditions using more sophisticated synthetic biology or recombinant DNA techniques.

Diverse Arctic and Antarctic marine benthic organisms have been investigated as sources of bioactive molecules, including Porifera, Cnidaria, Mollusca, and Echinodermata. However, studies specifically addressing the mucus produced by polar organisms remain scarce, despite their potential ecological and pharmacological significance. To the best of our knowledge, we only have information on a self-cleaning and solid waste-removal mechanisms described in the non-polar sponge *Aplysina archeri*, involving a continuous expulsion of particulate matter embedded in a mucus stream (Kornder et al. 2022). This finding suggests that the mucus layer may function not only in contaminant retention but also as a medium for the release of sponge-derived metabolites. However, the study is based on experimental data obtained from aquarium experiments, as well as visual observations of time-lapse videos and analyses of organic matter trapped in the mucus secreted by the sponge. Other available insights come from studies on polar invertebrates not included in the Porifera group. Slattery et al. (Slattery et al. 1997) reported the waterborne antibacterial metabolite secreted into the mucus of the Arctic soft coral *Gersemia fruticosa*. In the Antarctic nemertean species *Parborlasia corrugatus*, the mucus has been shown to contain cytotoxic peptides with hemolytic activity toward

erythrocytes (Göransson et al. 2019). The lack of knowledge related to Antarctic sponges, highlights the importance of investigating sponge-derived mucus in polar regions, particularly in Antarctica, where extreme environmental conditions may drive the evolution of unique chemical adaptations. The present study addresses the significant gap in our understanding of the ecological role and chemical composition of Antarctic sponge-derived mucus. To our knowledge, this is the first investigation focusing on the surface mucus produced by Antarctic sponges *Mycale (Oxymycale) acerata* and *Dendrilla antarctica*, intending to elucidate both its chemical composition and potential bioactivity. By combining ecological observations with chemical and biological analyses, this work provides novel insights into the multifunctional nature of sponge mucus and its potential as a source of pharmacologically relevant compounds. In doing so, it contributes to advancing our knowledge of polar marine biochemistry and highlights the untapped biotechnological potential of benthic Antarctic invertebrates.

Materials and methods

Materials

Water, acetonitrile and methanol (LC–MS grade) were from Carlo Erba (Milan, Italy). Formic acid and ammonium formate were from Sigma-Aldrich (Darmstadt, Germany). Solid phase extractions cartridges Oasis PRiME HLB (150 mg), Oasis MCX Plus (225 mg) and Oasis MAX Plus (225 mg) were from Waters. Oxylipin standards, EPA CYP450 Oxylipin LC–MS Mixture, containing (±)8(9)-EpETE, (±)11(12)-EpETE, (±)14(15)-EpETE, (±)17(18)-EpETE, (±)5(6)-DiHETE, (±)11(12)-DiHETE, (±)8(9)-DiHETE, (±)14(15)-DiHETE, (±)17(18)-DiHETE, were from Cayman Chemical (Herlev, Denmark).

Sample collection

The mucus samples were collected as additional activity within the scientific activities envisaged in the context of the XXXIV Antarctic Campaign at Thetys Bay (Ross Sea, Antarctica), near the Italian Research Station "Mario Zucchelli". Sponge specimens of *Mycale* (Oxymycale) *acerata* (Kirkpatrick, 1907) (three specimens, MNA_CIBAN XXXIV-1388; MNA_CIBAN XXXIV-1389; MNA_CIBAN XXXIV-1390) and *Dendrilla antarctica* (Topsent, 1905) (three specimens, MNA_CIBAN XXXIV-1386; MNA_CIBAN XXXIV-1387; MNA_CIBAN XXXIV-1391) were collected by scuba divers (74° 42.067′; 164° 02.518′), and transported directly to the laboratory at 4 °C for processing (Lo Giudice et al. 2024; Costa et al. 2023). Upon arrival in the laboratory, an unexpectedly abundant mucus release by the sponges was observed in two *M. acerata* and one *D. antarctica* specimens (Figure S1). Thus, mucus samples were collected in sterile tubes (approximately 100 mL) under aseptic conditions and stored at − 20 °C. Sponge specimens belong to the National Antarctic Museum (MNA).

Electrical conductivity, pH, inorganic and organic contents

Mucus viscosity was measured at 200 rpm in 1 mL mucus aliquots with a cone-plate viscometer (cone angle of 1.565°, model LVT-C/P 42, Brookfield Engineering Laboratories, Middleboro, MA, USA) connected to a circulating water bath (Thermoline, Wetherill Park, Sydney, Australia) set at 17 ± 0.1 °C. Osmolarity was measured using a VAPRO vapour pressure osmometer (model 5520, WESCOR, Logan, UT, USA). Electrical conductivity was measured using a GLP 31 conductometer (Crison, Barcelona, Spain). The water content was determined by measuring the wet weight of mucus on an analytical balance. After dehydration in a SpeedVac, their dry weight (DW) was detected. All measurements were carried out in triplicate.

Microwave Plasma-Atomic Emission Spectrometry (MP-AES) using an Agilent 4200 MP-AES equipment (Agilent, Victoria, Australia) was employed to determine the inorganic composition of mucus samples. Briefly, the samples were transferred into the microwave digestion flask and nitric acid were added. The samples were digested in a microwave at a ramp temperature to 200 °C with a hold for 4 min. Digested samples were then transferred to volumetric flasks with a 5% (v/v) nitric acid solution and the elements Na, Mg, K, Ca, Se, Zn, Cd, V, Ag, Ba, Cu, Ni, Co, U, Th, Pb, Tl, Mn, Cr and Al were quantified using working standards prepared from certified standard solutions. Results were corrected using a blank solution. Analyses were performed in triplicate.

CHN determination

The organic fraction of dried mucus samples was determined by elemental analysis using a Elementar Vario EL III equipment (950 °C), Hanau–Germany. Nicotinamide was used as a reference compound.

Fourier-transform infrared spectroscopy (micro-FTIR)

The main chemical features of mucus samples were studied by infrared microscopy (micro-FTIR) using a Thermo iN10 IR microscope (Madison, WI, USA). One drop of mucus was placed on an aluminum surface slide and allowed to

dry. Spectra of the dried residue were then obtained under reflection and/or ATR sampling. Data were collected using MCT-A detector and processed using Omnic Picta software (Madison, WI, USA).

Nuclear magnetic resonance analysis (NMR)

NMR spectra were obtained at room temperature using a 500 MHz JEOL system (Tokyo, Japan) equipped with a Royal HFX probe. ^{1}H spectra were obtained for raw samples, lyophilates recovered in D_2O, and chloroform extracts. Data were processed using Delta 5.3.1 software (Tokyo, Japan).

Liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis

Preparation of mucus samples

The samples were submitted to SPE (solid-phase extraction) extraction. The solid phases used were Oasis PRiME HLB (150 mg), Oasis MCX Plus (225 mg), and Oasis MAX Plus (225 mg). For comparison purposes, the raw samples were also analyzed after 10 times dilution.

Analysis

The chromatographic separation of mucus samples was performed on a Thermo Scientific ultimate 3000 UHPLC (Thermo Scientific, Bremen, Germany). The LC analysis of the raw samples, extracts obtained with Oasis Prime HLB cartridges, and extracts of neutral compounds obtained with Oasis MCX Plus and Oasis MAX Plus cartridges was performed with a Thermo Scientific Accucore RP-18 column (2.1 × 100 mm, 2.6 µm). The mobile phase composition was prepared with water (A) and acetonitrile (B), both containing 0.1% formic acid. The gradient (in v/v%) started with 100% of A for 2 min. Then B increased linearly to 30% in 13 min, then to 100% in 16 min, and maintained at 100% for an additional 4 min. The mobile phase then returned to 100% of A in 1 min and was maintained at 100% of A for 4 min. The flow rate was 0.3 mL/min. The injection volume was 5 mL.

The LC analysis of polar extracts from Oasis MCX Plus and Oasis MAX Plus cartridges was performed with a TSK-gel Amide-80 HILIC column with 15.0 cm length, 2 mm internal diameter, and 3 µm particle diameter. A mobile phase comprising water (A) and acetonitrile with 5% water (B), both with 2 mM ammonium formate and 0.05% formic acid was used. The gradient started with 20% of A for 1 min and then changed to 50% of A in 8 min. This mobile phase composition was kept for 1 min and then changed to 60% of A in 0.5 min. This latter (60% of A) was maintained for 2.5 min and then changed to the initial composition in 0.5 min. Finally, the system was allowed to stabilize for an additional 4 min before the next run.

Mass analysis (HRMS) was performed on an Orbitrap Elite mass spectrometer (Thermo Scientific, Bremen, Germany) with a Heated EletroSpray Ionization source (HESI-II). Spectra were acquired using the following ionization parameters: spray voltages, 3.7 kV (positive polarity) and 4.0 kV (negative polarity); sheath gas, 40 arbitrary units; auxiliary gas, 10 arbitrary units; heater temperature, 300 °C; capillary temperature, 350 °C; S-Lenses RF level, 64.9%. Scan range was 100–1000 m/z. The mass spectrometer was run in data-dependent acquisition by selecting the three most intense ions under dynamic exclusion and collision-induced dissociation (CID) activation. The m/z range was between 100 and 1500.

Selected oxylipins were quantified by LC-HRMS, preparing calibration curves using standards. The concentration range was between 0.01 and 0.25 ng/mL. The column was a Thermo Scientific Accucore RP-18, and the separation was achieved using a mixture of water and acetonitrile, both with 0.1% formic acid. The gradient was the following: the mobile phase started with 20% of acetonitrile for 1 min, then rose to 80% of acetonitrile in 4 min, and then to 100% of acetonitrile in an additional 2 min. The mobile phase was kept at 100% acetonitrile for 2 min, then recovered to the initial conditions in 1 min and finally kept the mobile phase composition for an additional 4 min before next run. The LC-HRMS quantitation was performed by running the system in Multi Reaction Monitoring (Costa et al. 2023) under negative polarity. The transitions were the following: (±)8(9)-EpETE, 317→155; (±)11(12)-EpETE, 317→195; (±)14(15)-EpETE, 317→248; (±)17(18)-EpETE, 317→215; (±)5(6)-DiHETE, 355→145; (±)11(12)-DiHETE, 355→167; (±)8(9)-DiHETE, 355→185; (±)14(15)-DiHETE, 355→211; (±)17(18)-DiHETE, 355→203.

Data were analyzed using Xcalibur™ 4.1 Software (Thermo Scientific, Bremen, Germany). The annotation of metabolites was performed using Compound Discoverer 3.3 (Thermo Scientific, Bremen, Germany) by processing the data dependent profiles using maximum ID, metabolomics and lipidomics workflows. The processing of the profiles was performed using a workflow for identification (MaxID) which performs unknown compound detection and predicts elemental compositions for all compounds, annotates using the mzCloud (ddMS2), ChemSpider and Mass Lists (formula or exact mass), performs similarity search for all compounds with ddMS2 data using mzCloud, and applies mzLogic algorithm to rank order ChemSpider and Mass List results (Viant et al. 2017).

In vitro cytotoxic assay

The in vitro cytotoxicity assay has been performed only on *M. acerata* mucus, based on the available amount material. The total extract of freeze-dried samples of Antarctic marine sponges' mucus was obtained in duplicate by chemical extraction in methanol (100%). After a period of maceration in the solvent, the aqueous phase was separated from the organic phase of the extract by shaking. The organic phase was recovered and dried at low pressure. Once obtained, the total extracts were dissolved in sterile ultrapure water (MilliQ) and tested for their possible cytotoxicity on different cell lines, such as the normal fibroblasts MRC-5 cell lines and two human melanoma A2058 and Malme-3 M cell lines. Considering the protective activity of the mucus versus external agents, we focused on the possible application against two melanoma cell lines. The three cell lines were incubated in the presence of three concentrations for each sample (1 µg/mL, 10 µg/mL, and 100 µg/mL) and in the absence of them. After 48 h of incubation at 37 °C with the sample, cell survival was measured with MTT test. The screening was performed using three biological replicates.

Chemical extraction

Sponge mucus lyophilized pellets were extracted by soaking in methanol, with 1 h of maceration, followed by agitation, sonication with three bursts of 30 s. Samples were then centrifuged at 3400 rpm at 4 °C. The organic phase was evaporated under reduced pressure as in Martinez et al. (Martínez Andrade et al. 2018).

Cell culture

Human melanoma cells (A2058; ATCC® CRL-11147™), human primary fibroblast MRC-5 (ATCC® CCL-171™) cells (used as a control) were cultured in DMEM high glucose supplemented with 10% Fetal Bovine Serum, 1% L-glutamine, 1% Pen-Strep solution. Human melanoma Malme-3 M cells (ATCC® HTB-64™) were cultured in ISCOVE's DMEM medium supplemented with 10% Fetal Bovine Serum and 1% Pen-Strep solution in a humidified incubator at 37 °C and 5% CO_2.

Determination of viable cell density by trypan blue dye assay

In a microtube, 1 part of 0.4% trypan blue and 1 part of cell suspension were mixed. The mixture was loaded into a Bürker counting chamber and, within 5 min of mixing, the dead cells and the total number of cells were counted to evaluate the percentage of viable cells (Martínez et al. 2022).

MTT cell viability assay

To estimate the in vitro cytotoxic effects, the human melanoma cells A2058 and Malme-3 M, and the human primary fibroblast MRC5 cells were seeded in 96-well microtiter plate at density of 1×10^4 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 extracts (1 µg/mL, 10 µg/mL, and 100 µg/mL) dissolved in sterilized water (milliQ), and further incubated for 48 h. Each concentration was tested at least in triplicate. After 48 h of treatment with the extracts, cell viability was assessed using the MTT test 3-(4,5-dimethyl-2-thizolyl)-2,5-diphenyl-2H-tetrazolium bromide; A2231,0001, Applichem Panreac Tischkalender, Darmstadt, GmbH). MTT/PBS solution (0.5 mg/mL) was then added to the wells and incubated for 3 h at 37 °C in a humidified atmosphere. The reaction was stopped by removal of the supernatant and the formazan products were dissolved with 100 µL of isopropyl alcohol. Absorbance was measured at OD = 570 nm using a microplate reader (MultiskanTM FC Microplate Photometer, Thermo Fisher Scientific, Waltham, MA, United States). The assay was performed according to the manufacturer's instructions. Cell survival was expressed as a percentage of viable cells in the presence of the tested samples, with respect to untreated control cultures. Doxorubicin was used as standard (reference drug) which consisted of a dose–response curve using doxorubicin at 5 mM with an 8-point serial dilution (1:2 dilutions) (Lauritano et al. 2020). Extracts with cell viabilities below 50% were considered active. Experiments were performed in triplicates and the statistical analysis was performed by GraphPad Prism (version 8.1.2, GraphPad Software Inc., San Diego, CA, USA) by Two-way ANOVA following Dunnett's test.

Results

Mucus physico-chemical properties and organic/inorganic content

The mucus samples revealed a high-water content, ranging from 98 to 99%. For *Mycale* (*Oxymycale*) *acerata*, the electrical conductivity, pH, and total dissolved solids (TDS) were measured at 27.51 ± 12 µS/cm, 7.3 ± 0.3, and 18.11 ± 6.2 mg/L, respectively. In *Dendrilla antarctica*, these values were slightly lower for conductivity and TDS,

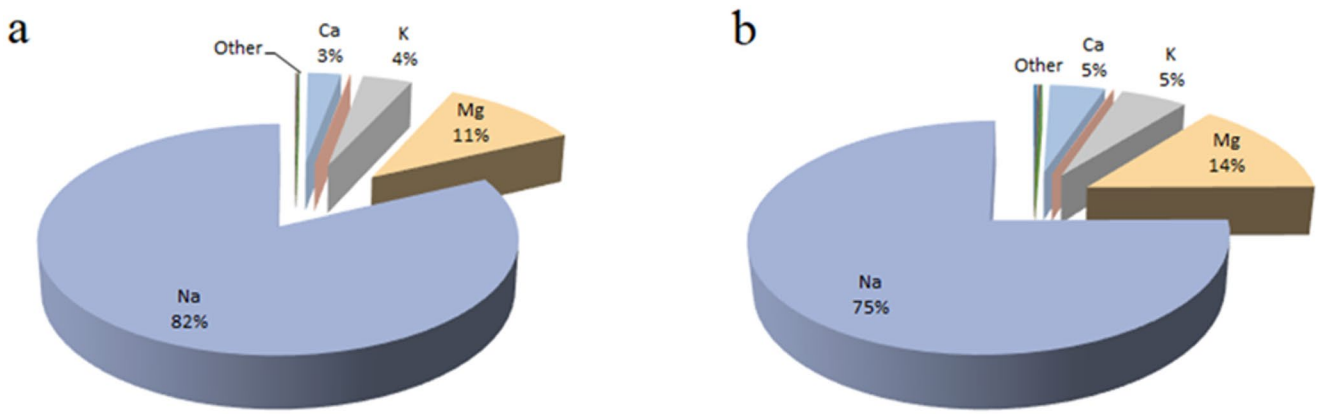

Fig. 1 Mineral composition (%) of mucus released by the sponges *M. acerata* (**a**) and *D. antarctica* (**b**)

Table 1 Elements (%) detected in sponge mucus samples in comparison with the composition of mucus from other marine organisms (data for *M. acerata* mucus are presented as average values.)

This study			Kang et al. (2019)	Liao et al. (2024)
Element	*D. antarctica*	*M. acerata*	*A. equina*	*M. infundibulum*
C	2.85	0.31	2.13	2.34
H	2.17	1.61	1.53	1.79
N	0.90	0.07	0.45	0.66
Fe	0.11	0.56	0.00	0.00
Ca	4.55	2.64	0.71	1.08
Mg	14.17	10.98	2.41	2.82
Zn	0.02	0.00	0.06	0.04
Cu	0.00	0.00	0.00	1.35
K	5.46	4.14	2.11	35
Na	75.11	82.07	13.38	14.87
Cl	nd	nd	44.48	34.13
P	0.31	0.05	0.00	0.00
Sn	nd	nd	0.00	0.00
Se	0.10	0.08	0.00	0.00
Mn	0.00	0.00	nd°	nd
Ba	0.00	0.00	nd	nd
Al	0.07	0.05	nd	nd
V	0.07	0.05	nd	nd
Ni	0.00	0.00	nd	nd
Pb	0.01	0.01	nd	nd
Cr	0.00	0.00	nd	nd
Cd	0.00	0.00	nd	nd

nd not determined

with measurements of 21.51 ± 9.0 µS/cm, 7.5 ± 0.1, and 16.02 ± 4.6 mg/L, respectively.

The C, H and N composition, and the C/N ratio, indicate that the organic content of mucus obtained from high in *D. antarctica* mucus. Based on the N content (5) and using a factor of 6.25 the protein contents were estimated to be 0.44 and 5.63% for *M. acerata* and *D. antarctica*, respectively. The C/N ratios were between 3 and 5, values in the range of amino acid matrices. Inorganic salts represented the main part (67.1 ± 2.3%) of the total mucus dry weight (DW) (Fig. 1). Mean values of the detected elements are listed in Table 1. Na was the most abundant element in all samples, ranging from 75 (*D. antarctica*) to 82% (*M. acerata*). Other major elements (e.g., Mg, K and Ca) represented 3–10% of the inorganic content (Fig. 1). Minor elements (e.g. Fe, Se, and V) were also detected (Table 1).

Fourier-transform infrared spectroscopy (m-FTIR)

Analysis by m-FTIR (Fig. 2) showed the presence of a broad absorbance between 3700 and 2700 cm^{-1}. On the other hand, the absorbance between 1700 and 1600 cm^{-1} showed the features typical of the peptide linkage.

Nuclear magnetic resonance (NMR)

Figure S2 shows the ^{1}H NMR spectra of the mucus of the two sponges. Besides the expected signal of water, the samples presented signals between 0 and 5 ppm, the region where compounds such as amino acids, peptides, sugars, carboxylic acids, nitrogen bases, and their derivatives show $^{1}HNMR$ signals. No signals were detected in the aromatic region.

LC–MS

Compound annotation

All samples, the diluted of mucus as well as the extracts prepared using the different SPE matrices, were analysed by LC-HRMS under data-dependent acquisition to annotate the main compounds in both sponge mucus. The compounds annotated using the mzCloud with match 85% or higher are listed in Table 2 (see chemical structures in Supplementary Table S1). Different compound classes were annotated, ranging from acids to amino acids and amino

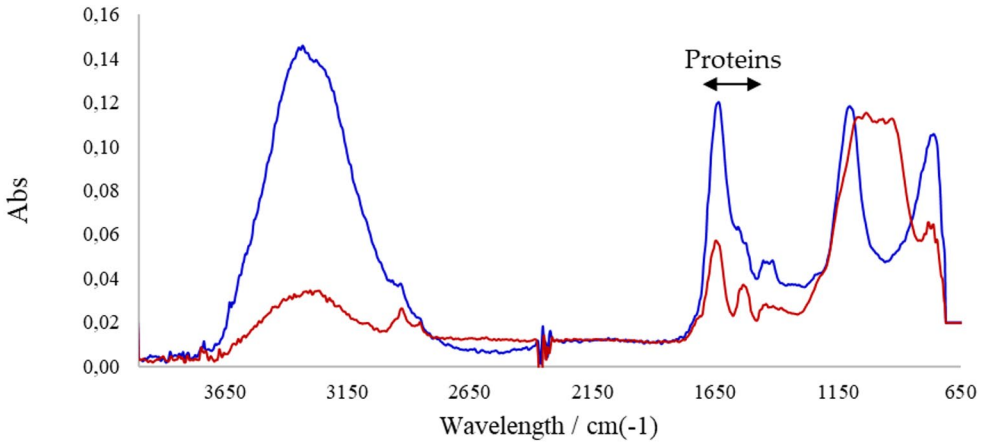

Fig. 2 FTIR spectra of the solid residue of mucus samples. Spectra were obtained using a MCT-A detector and ATR sampling. Blue—*D. antarctica*; red—*M. acerata*

acid derivatives, and nitrogen bases and derivatives, which also generally agree with NMR data. A list of potential biological roles/functions for class compounds is provided in Supplementary Table S2 (Yu et al. 2025; Kang et al. 2019; Liao et al. 2024; Zeng et al. 2025; Mills and O'Neill 2014; Ghisletti and Russo 2025).

Quantification of oxylipins

Both mucus samples showed the presence of polyunsaturated fatty acids and some related oxidation products known as oxylipins (Table 3 and supplementary Fig. S3). These products are formed enzymatically and might have bioactivities; therefore, it was decided to proceed with further analysis of quantification. A selection of both epoxides and di-hydroxy oxylipin compounds were studied. None of the epoxides—(±)8(9)-EpETE, (±)11(12)-EpETE, (±)14(15)-EpETE, and (±)17(18)-EpETE—were detected. The di-hydroxy compounds (±)5(6)-DiHETE, (±)11(12)-DiHETE, (±)8(9)-DiHETE, (±)14(15)-DiHETE, and (±)17(18)-DiHETE were detected and quantified. Table 3 presents the concentration of some of the quantified compounds.

In vitro cytotoxic assay

The histograms of Fig. 3a and b show cytotoxic effects of sponges' mucus extract on melanoma Malme-3 M and A2058 cell lines. Bioactivity screening on Malme 3 M cells showed that the extracts of mucus samples significantly reduced cell proliferation only at 1 µg/mL. In particular, mucus sample A was more active by inducing a reduction of cell viability to about 40% (Fig. 3a), while *M. acerata* mucus B induced 50% cell viability at 1 µg/mL (control was constituted by the cells with only the medium). Both mucus A and B induced cell viability higher than 70% at 10 and 100 µg/mL. Regarding A2058 cells (Fig. 3b), the two *M. acerata* mucus samples A and B did not reduce cell viability after 48 h treatment. On the contrary, human cancer cell proliferation increased. In particular, both mucus A and B increased cell viability from 100 to 150% when tested at 1, 10, 100 µg/mL for 48 h. Bioactivity screening was also performed on MRC-5 normal fibroblasts (Fig. 3c), used as a control, and results showed that the extracts were less toxic to normal cells compared to Malme-3 M. Reduction of cell viability was never below the cutoff of 50%. In particular, mucus A induced cell viability to about 80, 100 and 100 when tested at 1, 10, 100 µg/mL, respectively, for 48 h, respectively, while mucus B to 60, 100 and 110% when tested at 1, 10, 100 µg/mL, respectively, for 48 h.

Discussion

Marine invertebrates produce a large variety of mucus secretions, generally rich in glycoproteins, which are probably involved in defensive strategies adopted for survival (Bhagwat et al. 2024; Mentino et al. 2025; Alesci et al. 2024). Beyond the suggested primary roles involved in adhesion, water preservation, and movement, mucus also appears to participate in a range of ecological processes, contributing to chemical and immunological defense, mediating interactions with the associated microbiota, and influencing particle trapping and nutrient acquisition within complex benthic environments. Furthermore, due to the presence of bioactive compounds with antimicrobial, antioxidant, or cytotoxic properties, invertebrate-derived mucus represents a promising source of pharmacologically relevant molecules, highlighting its potential for future biotechnological and biomedical applications. In the present study, we focused on two species of Antarctic sponges, i.e. *M. acerata* and *D. antarctica*, which secreted a quite large amount of mucus during and after the collection. This work involved the chemical characterization of mucus samples and the evaluation of their bioactive potential. To date, sponge-derived mucus remains largely unexplored, with no studies specifically

Table 2 Compounds identified (ID) or annotated (mzCloud match ≥ 85%) in the analyzed sponge mucus samples

Retention time (min)	Compound	Formula	m/z (±)	Annotation/Identification
Polyamines, nitrogen bases and derivatives				
1.3	Uridine	$C_9H_{12}N_2O_6$	279.039 (−)	mzCloud 99.5%
1.3	1-Methylguanine	$C_6H_7N_5O$	166.072 (+)	mzCloud 94.5%
1.3	Xanthine	$C_5H_4N_4O_2$	153.040 (+)	mzCloud 91.8%
			151.026 (−)	mzCloud 95.8%
1.4	Thymidine 5'-monophosphate	$C_{10}H_{15}N_2O_8P$	321.049 (−)	mzCloud 94.5%
2.3	Guanosine	$C_{10}H_{13}N_5O_5$	282.084 (−)	mzCloud 99.9%
3.5	2'-Deoxyinosine	$C_{10}H_{12}N_4O_4$	251.078 (−)	mzCloud 99.3%
4.5	N(2)-Methylguanosine	$C_{11}H_{15}N_5O_5$	298.114 (+)	mzCloud 100.0%
			296.100 (−)	mzCloud 99.7%
12.0*	Spermidine	$C_7H_{19}N_3$	146.165 (+)	mzCloud 97.9%
9.1*	Spermine	$C_{10}H_{26}N_4$	203.223 (+)	mzCloud 99.8%
Acids				
1.1	Citric acid	$C_6H_8O_7$	191.020 (−)	mzCloud 99.2%
1.1*	gamma-Aminobutyric acid (GABA)	$C_4H_9NO_2$	104.070 (+)	mzCloud 99.7%
1.3	Succinic acid	$C_4H_6O_4$	117.019 (−)	mzCloud 99.7%
10.0	Phenylpyruvic acid	$C_9H_8O_3$	163.040(−)	mzCloud 99.5%
10.0	Kynurenic acid	$C_{10}H_7NO_3$	190.050 (+)	mzCloud 98.3%
			188.035 (−)	mzCloud 99.5%
12.8	Suberic acid	$C_8H_{14}O_4$	173.086 (−)	mzCloud 99.3%
15.0	Azelaic acid	$C_9H_{16}O_4$	187.097 (−)	mzCloud 99.6%
20.8	Dodecanedioic acid	$C_{12}H_{22}O_4$	229.146 (−)	mzCloud 98.8%
22.9	(±)17(18)-DiHETE,	$C_{20}H_{32}O_4$	335.223 (−)	ID
23.0	(±)12-HpETE	$C_{20}H_{32}O_4$	335.222 (−)	mzCloud 89.0%
23.13	Tetradecanedioic acid	$C_{14}H_{26}O_4$	257.175 (−)	mzCloud 97.7%
23.26	(±)11(12)-DiHETE,	$C_{20}H_{32}O_4$	335.223 (−)	ID
23.36	(±)8(9)-DiHETE	$C_{20}H_{32}O_4$	335.223 (−)	ID
23.59	15(S)-HpEPE	$C_{20}H_{32}O_4$	333.206 (−)	mzCloud 85.5%
23.61	(±)5(6)-DiHETE	$C_{20}H_{32}O_4$	335.223 (−)	ID
25.04	(±)18-HEPE	$C_{20}H_{30}O_3$	317.212 (−)	mzCloud 98.6%
25.56	(±)8-HEPE	$C_{20}H_{30}O_3$	317.211 (−)	mzCloud 85.0%
26.52	11,12-Epoxy-(5Z,8Z,11Z)-icosatrienoic acid	$C_{20}H_{32}O_3$	319.227 (−)	mzCloud 96.5%
30.19	Arachidonic acid	$C_{20}H_{32}O_2$	303.232(−)	mzCloud 99.6%
Amino acids and derivatives				
1.1	Histidine	$C_6H_9N_3O_2$	156.076 (+)	mzCloud 89.0%
1.2	Pyroglutamic acid	$C_5H_7NO_3$	130.050 (+)	mzCloud 99.4%
1.2*	Valine	$C_5H_{11}NO_2$	118.086 (+)	mzCloud 99.9%
1.2	Proline	$C_5H_9NO_2$	116.070 (+)	mzCloud 99.9%
1.3*	1-Methylhistidine	$C_7H_{11}N_3O_2$	170.092 (+)	mzCloud 99.3%
1.4	Norleucine	$C_6H_{13}NO_2$	132.102 (+)	mzCloud 99.7%
1.4	Tyrosine	$C_9H_{11}NO_3$	182.080 (+)	mzCloud 97.5%
1.8*	Lysine	$C_6H_{14}N_2O_2$	147.113 (+)	mzCloud 99.8%
1.9*	2-Aminoisobutyric acid	$C_4H_9NO_2$	104.070 (+)	mzCloud 100.0%
2.2*	N,N-Dimethylglycine	$C_4H_9NO_2$	104.070 (+)	mzCloud 85.0%
2.8	Phenylalanine	$C_9H_{11}NO_2$	166.086 (+)	mzCloud 99.7%
3.4*	Tyrosylalanine	$C_{12}H_{16}N_2O_4$	251.104 (−)	mzCloud 94.2%
3.7	Valylproline	$C_{10}H_{18}N_2O_3$	215.139 (+)	mzCloud 99.9%
3.9	Glycyl-leucine	$C_8H_{16}N_2O_3$	189.123 (+)	mzCloud 99.4%
			187.109 (−)	mzCloud 99.0%
4.9*	Alanylalanine	$C_6H_{12}N_2O_3$	159.080 (−)	mzCloud 96.5%
5.0	Phenethylamine	$C_8H_{11}N$	122.094 (+)	mzCloud 99.9%
5.1*	Glutamic acid	$C_5H_9NO_4$	146.046 (−)	mzCloud 87.0%
8.0	Tryptophan	$C_{11}H_{12}N_2O_2$	203.083 (−)	mzCloud 99.5%
8.6	Leucylproline	$C_{11}H_{20}N_2O_3$	229.154 (+)	mzCloud 100.0%
			227.140 (−)	mzCloud 97.3%

Table 2 (continued)

Retention time (min)	Compound	Formula	m/z (±)	Annotation/Identification
11.4	N-Acetylalloisoleucine	$C_8H_{15}NO_3$	172.098 (−)	mzCloud 99.0%
13.8	N-Acetyl-tryptophan	$C_{13}H_{14}N_2O_3$	245.094 (−)	mzCloud 97.6%
14.8	Indole-3-acetic acid	$C_{10}H_9NO_2$	176.070 (+)	mzCloud 99.7%
Other				
1.1	4-Indolecarbaldehyde	C_9H_7NO	144.046 (−)	mzCloud 90.9%
1.2	4-Guanidinobutyric acid	$C_5H_{11}N_3O_2$	146.092 (+)	mzCloud 98.6%
1.6*	Carnitine	$C_7H_{15}NO_3$	162.112 (+)	mzCloud 97.2%
2.1*	Leucineamide	$C_6H_{14}N_2O$	131.118 (+)	mzCloud 96.1%
1.2*	Creatine	$C_4H_9N_3O_2$	132.077 (+)	mzCloud 94.3%

*Retention time under HILIC chromatography

Table 3 Concentration of di-hydroxy oxylipins in mucus samples (ng/mL)

Compound	*M. acerata*	*D. antarctica*
(±)5(6)-DiHETE	<QL	3
(±)11(12)-DiHETE	<QL	2
(±)8(9)-DiHETE	<QL	1
(±)14(15)-DiHETE	<QL	<QL
(±)17(18)-DiHETE	<QL	2
Total	–	8

addressing specimens from Antarctic environments. Current knowledge indicates that mucus secretions from various marine invertebrates—including starfish species (e.g., *Marthasterias glacialis*, *Porania pulvillus*), brittlestars (e.g., *Ophiocomina nigra*) (Bavington et al. 2004), anthozoans (e.g., *Actinia equina*) (Stabili et al. 2015), annelids (e.g., *Sabella spallanzanii*, *Myxicola infundibulum*) (Stabili et al. 2019), corals (e.g., *Acropora pharaonis*, *Galaxea fascicularis*, *Pocillopora verrucosa*, *Porites lobata*, and *Stylophora pistillata*) (Hadaidi et al. 2019), and the sea urchin *Paracentrotus lividus* (Santos et al. 2009)—have been investigated for their chemical composition and diverse biological activities. The water content measured in mucus samples was in line with the composition of other mucus of marine origin, accounting for the 97–98% of total mucus volume (Stabili et al. 2015, 2019, 2011), as well as the high percentage of inorganic material and elemental composition agreed with previous results reported for mucus from the marine species *A. equina* (Stabili et al. 2015) and *M. infundibulum* (Stabili et al. 2019), as evidenced in Table 1. The results indicated that the organic content of mucus obtained from *D. antarctica* was much higher than that of *M. acerata*, and the detected C/N ratios suggested that a significant fraction of the organic composition was of protein nature.

The amounts of some elements (such as Mg, Ca, and K) were higher than those reported for *A. equina*. The notably high percentage of Na (about 75, 79, and 82% of the mineral fraction) can be attributed to the marine environment and may also result from residual dried salts remaining within the mucus matrix.

Analysis by m-FTIR showed the presence of the broad absorbance between 3700 and 2700 cm^{-1} suggesting a possible assignment to inorganic salts and water (Farvid et al. 2013), while the absorbance between 1700 and 1600 cm^{-1} is consistent with protein-type materials and in agreement with the results obtained by elemental analysis (Singh 1999).

The results obtained from FTIR analysis confirmed that the mucus matrix contained proteins, as observed in the mucus from cnidarians (Stabili et al. 2015) and annelids (Stabili et al. 2019). Stabili et al. (Stabili et al. 2019) and Santos et al. (Santos et al. 2009) detected the presence of valine, leucine, and alanine as the most abundant amino acids in the annelid *M. infundibulum* and sea urchin *P. lividus* footprint mucus, respectively. The presence of proteins and related compounds was further confirmed by LC-HRMS, which allowed the annotation of several amino acids and derivatives.

Marine sponges are renowned as a source of bioactive compounds with wide applications, but most of the compounds currently known were isolated from sponge tissues or were proven to be produced by symbiotic bacteria (Rizzo and Lo Giudice 2018; Piel et al. 2004; Unson and Faulkner 1993; Unson et al. 1994). According to Varijakzhan et al. (Varijakzhan et al. 2021), sponges produce mucus containing toxins composed of several cytotoxic chemicals, as a defensive barrier against other species to preserve their water-pumping activity that could be affected by biofilm formation. Moreover, sponges are also able to regulate the toxin production by avoiding damage to themselves (Perdicaris et al. 2013). The comparison between bacterial communities associated with the sponge mesohyl and those present in the mucous layer revealed significant differences in both community composition and bioactivity (*data not shown*). Based on the current evidence, it is likely that the observed bioactivity primarily arises from the mucus itself and its specific chemical composition, rather than from the microbial communities associated with either the mucus or the sponge mesohyl. However, further investigations are needed to confirm this hypothesis and to better clarify

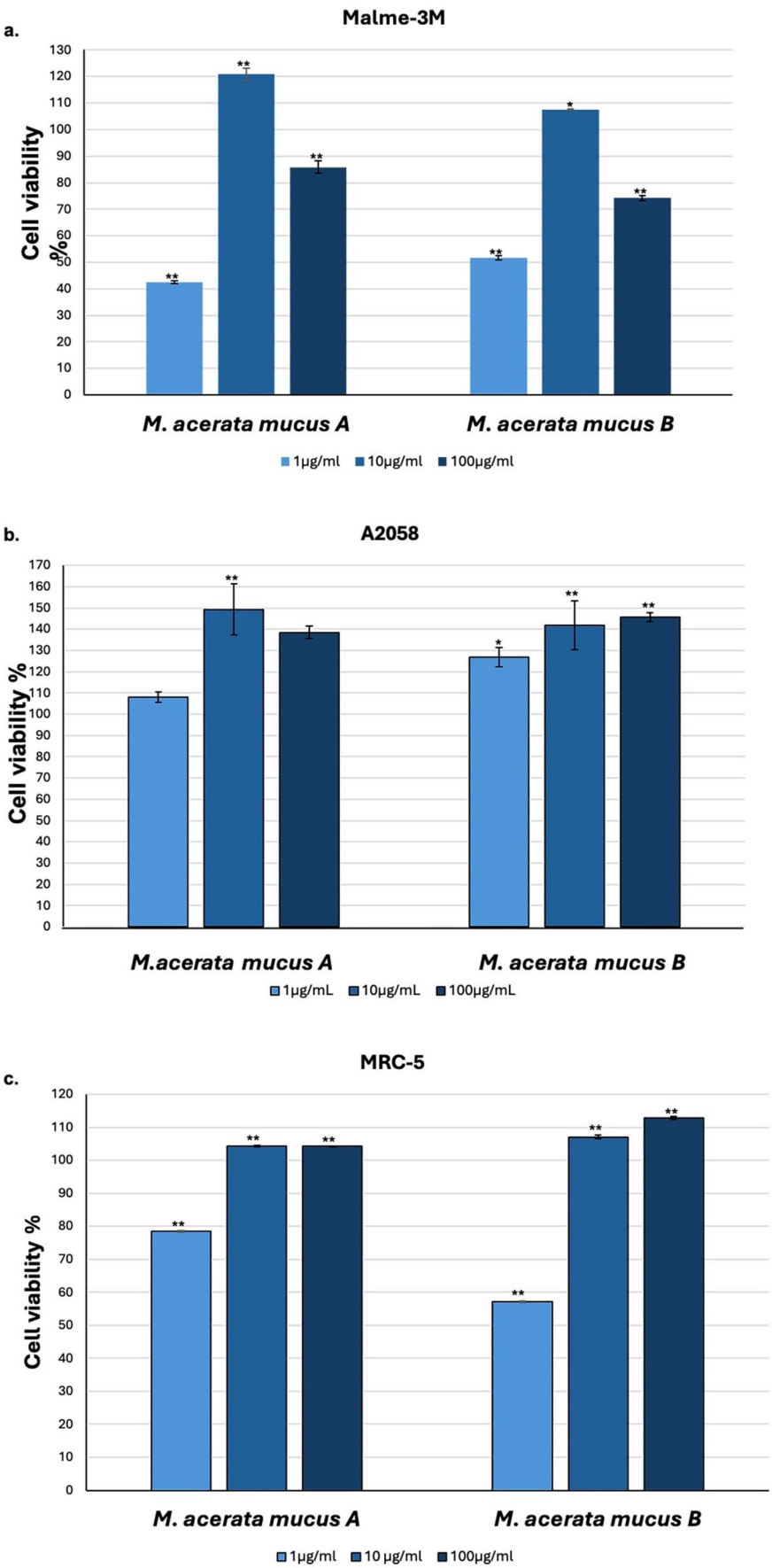

Fig. 3 In vitro cytotoxicity assay. The histograms show cytotoxic effects of sponges' mucus extract on (**a**) A2058 and (**b**) Malme-3 M human melanoma cell lines and (**c**) MRC-5 primary fibroblast cell line. Results are expressed as percentage of cell survival ± SD after 48 h exposure (n = 3). Cell growth was expressed as the percentage of cell viability in comparison with the control for each concentration (*$p < 0.05$, **$p < 0.01$; Two-way ANOVA, Dunnett's test)

the respective contributions of the host, the mucus, and the associated microbiota.

The composition of Antarctic sponge mucus also provided information on the adaptive strategies of these organisms in cold environments. The presence of modified nucleosides could be correlated with modification occurring in cold environments, as in the case of N2-methylguanosine (m2G) (see Table 2), one kind of post-transcriptional modification which plays crucial roles in the control and stabilization of tRNA. It has been described that modifications in the structure of tRNA, specifically methylations of nucleosides at the junctions of the internal structure, are crucial for the correct secondary and tertiary folding tRNAs achievement. Specifically, the modified nucleosides, N2-methylguanosine (m2G), N2,N2-dimethylguanosine (m22G), and N2,N2,2′-O-trimethylguanosine (m22Gm), have conserved positions in the tRNA structure and control the L-fold in the tertiary tRNA structure in all three domains of life (Noon et al. 2003). Different tRNA methyltransferases appear to play key roles in various tRNA modifications in thermophilic microorganisms, which thrive in extremely high and inhospitable temperatures (Tomikawa et al. 2010; Väre et al. 2017). Another abundant group of compounds identified in our samples was oxylipins, metabolites derived from lipid peroxidation. Numerous studies have reported the release of oxylipins by marine organisms, and their potential applications for human health have also been explored (Orefice et al. 2022). Some studies have reported that some oxylipins may induce programmed cell death in human tumour cells. Many oxylipins can be produced by marine organisms, and new ones are yet to be discovered and characterized. Isolation, purification and bioactivity screening of each of them could clarify anticancer-specific activity, selectivity, and efficacy in future studies (Lauritano et al. 2016; Linares-Maurizi et al. 2023; Ruocco et al. 2020; Saide et al. 2021).

Among acids, kynurenic acid showed a high mzCloud match. Interestingly, it is a bioactive compound that possesses neuroactive activity. It acts as an anti-excitotoxic and anticonvulsant, most likely through acting as an antagonist at excitatory amino acid receptors. It has been isolated for the first time from horseshoe crab *Tachypleus tridentatus* by Li et al. (2021), and its beneficial effects on obesity, hyperlipidemia, and gut microbiota in high-fat diet-fed mice have been proven. Mucus samples showed the presence of polyunsaturated fatty acids and some oxidized related products. These products are formed enzymatically and were detected in MeOH extracts of the marine sponge *Topsentia* sp. (Luo et al. 2006), which showed moderate cytotoxicity towards five human solid tumour cell lines. In line with this information, the data obtained from the cytotoxicity assay showed promising biological activities that deserve future investigations. It was interesting to note that the total extract of *M. acerata* mucus showed cytotoxic activity only on one of two human melanoma cell lines, Malme-3 M, denoting a target selectivity. According to the cell sheets, the A2058 cells exhibit epithelial morphology on samples isolated from the skin, with nerve growth factor (NGF) and laminin as reported expression markers (https://www.atcc.org/products/crl-3601#detailed-product-information; accessed on 9 August 2024), while Malme-3 M are fibroblasts isolated from the skin, metastatic, with antigen expression HLA A2, Aw30, B13, B40(±), DRw7 (https://www.atcc.org/products/htb-64; accessed on 9 August 2024). Our data suggest a possible specific mechanism of action of the extracts, which needs to be further explored at the genetic and protein levels. The reduction of cell viability could be related to the interaction of the compounds constituting the extract with mediators of the apoptotic mechanism, determining the cell death, or mediators of cell proliferation mechanism, repressing it. The cytotoxic effect observed with the MTT standard assay could be due to the activation/repression of specific molecular mechanisms (such as apoptosis). Cell treatment with the extracts could induce the interaction of apoptosis mediators but also the interference of other pathways such as the PI3K/Akt/mTOR pathway, already know to be activated by other marine compounds (Damiano et al. 2025). It is important to note that our results refer to the treatment of human cells with total extracts, therefore the activity could derive from the synergistic action of multiple compounds present in it. Future investigations, when collected Antarctic biomass will be enough, can be focused on fractions obtained by raw extracts, in order to narrow the search of the bioactive molecule responsible for the observed activity. In addition, our results showed that the activity was higher at lower concentrations. This can be explained considering the concept of "hormesis" that consists in the presence of different therapeutic modes of action for different doses of the same compound. In cancer therapy, this phenomenon is very common. A practical example is cyclophosphamide which at high doses is toxic to the bone narrow with possible fatal consequences, while at low doses contributes to the antitumor immunity (Gaya et al. 2015). We can also hypothesize that at higher concentrations there can be the formation of oligomers/micelles with limited access to membranes, as commonly reported for some proteins (Mahler et al. 2009; Hofmann et al. 2016). Future studies will be addressed on the bioactivity investigation of specific compounds, such as specific nucleoside or oxylipin. In addition, thanks to the LC–MS analysis, we detected in our samples the presence of spermidine and spermine too. Spermidine is the precursor of spermine, both are polyamines and have been reported to induce a dose-dependent cytotoxic effect in vitro human intestinal cell model (Rio et al. 2018). In addition, spermidine showed a role in immune cell regulation, which makes

it a very good and promising candidate for cancer immune therapeutic applications (Chamoto et al. 2024).

As reported in a very recent study by Hofer et al. (2024), spermidine has been associated to autophagy enhancement, which is well-known to be associated to cancer being a degradation process crucial to maintain metabolic homeostasis (Pooya et al. 2025), geroprotection and reduced incidence of cardiovascular and neurodegenerative diseases.

Conclusions

This study represents a first attempt to explore the mucus of Antarctic sponges as a potential source of novel bioactive compounds. The possibility of cultivating these organisms either in their natural environment or under controlled conditions in aquaria offers a sustainable strategy for the continued investigation of these rare biological resources, minimizing the impact on wild populations. Our chemical analyses revealed compounds that may be involved in the adaptive and defensive strategies of Antarctic sponges, as suggested by the presence of molecules previously isolated from non-polar sponge species. Notably, preliminary evidence demonstrated selective cytotoxic activity of the total mucus extract against human melanoma cell lines, indicating potential biomedical applications. Although further studies are needed to better characterize the chemical nature and biological roles of these compounds, the data presented here provide a solid foundation for future research. Results reported in the current work seem promising and future attempts should implement these preliminary antiproliferative results on human cells by fractionation or isolation of pure molecules, studying the mechanism of action and the molecular targets. Overall, these findings are promising and support the potential exploitation of polar sponge mucus as a reservoir of new bioactive substances with pharmacological relevance.

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Data availability The original data presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Declarations

Conflict of interest The authors declare no conflict of interest.

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