**ORIGINAL PAPER**

# Experimental evidence of antimicrobial activity in Antarctic seaweeds: ecological role and antibiotic potential

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## Abstract

Seaweeds contain a wide range of secondary metabolites which serve multiple functions, including chemical and ecological mediation with microorganisms. Moreover, owing to their diverse bioactivity, including their antibiotic properties, they show potential for human use. Nonetheless, the chemical ecology of seaweeds is not equally understood across different regions; for example, Antarctic seaweeds are among the lesser studied groups. With the aim of improving our current understanding of the chemical ecology and potential bioactivity of Antarctic seaweeds, we performed a screening of antibiotic activity using crude extracts from 22 Antarctic macroalgae species. Extractions were performed separating lipophilic and hydrophilic fractions at natural concentrations. Antimicrobial activity assays were performed using the disk diffusion method against seven Antarctic bacteria and seven human pathogenic surrogates. Our results showed that red seaweeds (especially *Delisea pulchra*) inhibited a larger number of microorganisms compared with brown seaweeds, and that lipophilic fractions were more active than hydrophilic ones. Both types of bacteria tested (Gram negative and Gram positive) were inhibited, especially by butanolic fractions, suggesting a trend of non-specific chemical defence. However, Gram-negative bacteria and one pathogenic fungus showed greater resistance. Our study contributes to the evidence of antimicrobial chemical interactions between Antarctic seaweeds and sympatric microorganisms, as well as the potential of seaweed extracts for pharmacological applications.

**Keywords** Antibiotic activity · Rhodophyta · Phaeophyceae · Chemical ecology · Pathogenic microorganisms

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## Introduction

Seaweeds possess a wide range of secondary metabolites used in chemically mediated interactions with other organisms which allow them to thrive in their environment and adapt to potential changes (Amsler 2008). For example, several putative interactions between bacteria and seaweeds have been proposed for the genus *Ulva* (Kessler et al. 2018; Ghaderiardakani et al. 2020). However, compared with other groups of organisms, there is a scarcity of studies on chemical activity and interactions of seaweeds, especially for species of high latitudes like the polar regions (Avila et al. 2008; Vankayala et al. 2017; Angulo-Preckler et al. 2018a; Solanki et al. 2018). In the case of the Antarctic marine flora, there is a high degree of endemism favoured by the prolonged isolation and polar conditions (Wiencke and Clayton 2002; Oliveira et al. 2020; Pellizzari et al. 2020). In their native environment, Antarctic seaweeds are exposed to a wide range of microorganisms present in the surrounding water, including bacteria, fungi and microalgae (Singh et al. 2016; Gaitan-Espitia and Schmid 2020). These Antarctic microorganisms are present in high concentrations in the marine water column (Zdanowski 1995; Jenkins et al. 1998; Bej et al. 2010), and they are known to interact with macroalgae in different ways (Amsler 2008; Alvarado et al. 2018; Gaitan-Espitia and Schmid 2020). They even have the potential to modulate functions such as seaweed's growth, adaptation to altered environmental parameters (e.g. salinity) or even reproduction (Gaitan-Espitia and Schmid 2020). Even though seaweed chemodiversity itself is indeed very high (Young et al. 2015; Bernardi et al. 2016; von Salm et al. 2018; Benites Guardia 2019; Carroll et al. 2020), owing to the close relationship between seaweeds and their microbiome, it can be difficult to discern which part of the holobiont (the combination of seaweed and associated microorganisms) is responsible for producing a specific compound in an ecologically relevant chemical interaction between organisms and environment. Nevertheless, it is reasonable to think that many interactions will be dominated by compounds produced and mainly modulated by the seaweed itself. Elucidating the mechanism of chemical activity in seaweed extracts is crucial to understanding the dynamics of polar coastal ecosystems. Aside from the ecological implications, the diversity of these seaweed compounds also presents a high potential for the discovery of useful bioactive molecules, which could be vital for fields like medicine and the fight against antibiotic-resistant microorganisms (D'Costa et al. 2011). New sources of potentially useful compounds are needed as new resistant pathogenic strains are identified each year (Pang et al. 2019; Ragheb 2019; Andersson et al. 2020). In this context, the two main objectives of our study were: first, to elucidate the chemical activity of Antarctic seaweed extracts against sympatric microorganisms to add evidence for potential ecological relationships, and second, to evaluate the antibiotic potential of these macroalgae extracts against surrogates of common human pathogens, which would serve as a reference for future assays to discover new antibiotic compounds.

## Materials and methods

Species for the antimicrobial assay were selected by considering the lack of previous information on their bioactivity and, when there was any previous report on specific taxa, their potentially interesting chemistry. The species selected for the bioassay were 22 Antarctic macroalgae (Table 1), 14 of which were Rhodophyta and 8 Phaeophyceae (Ochrophyta). Seaweed sampling was performed during several Antarctic cruises (along the west Antarctic Peninsula and South Shetland Islands archipelago) in the framework of the ACTIQUIM and DISTANTCOM projects, during the austral summers of 2012, 2013, 2016 and 2017 (Fig. 1, Table 1). The samples were collected by scuba diving for subtidal specimens and by hand for intertidal species. The samples (whole individuals) were immediately frozen (−20 °C) until the chemical study was performed. Once in the laboratory, and before chemical extractions, fragments of various sizes depending on the taxa of the frozen samples were used to perform taxonomic identification at the species level following Wiencke and Clayton (2002) along with specific taxonomic monographies for each species to avoid misidentification. For this, morphology and microscopic anatomy of the sampled species was studied with histologic preparations and optical microscopy of the fragments taken, always making sure enough material was left frozen for the chemical extractions, following Angulo-Preckler et al. (2015). The fragments used for identification were conserved as herbarium vouchers for consultation in the herbarium of the University of Barcelona (BCN-Phyc).

Chemical extractions were made following a modification of Angulo-Preckler et al. (2015), which was based on previous studies (Avila et al. 2000; Bhosale et al. 2002; Iken et al. 2002; Murugan and Ramasamy 2003). The algal material was first cleaned of epiphyte organisms, and then weighed to obtain the wet weight. Then, the samples were fragmented for homogenization before grinding with acetone. The resulting mixture was then filtered with filter paper and treated with ultrasonic waves (for 5 min) to increase the breakup of cells initiated by the acetone. This step was repeated three times per sample, obtaining a liquid solution with algal solid residue, which was left to dry and weighed. The acetone was then evaporated *in vacuo* in

**Table 1** Seaweed sampling and extraction data

| Seaweed species | Location | Station | Latitude | Longitude | Dry weight (g) | Natural concentration (mg per g of dry weight) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Ethereal | Butanolic |
| Rhodophyta | | | | | | | |
| *Neuroglossum delesseriae* | Deception Island | Neptune's Bellows | 63° 00′ 00″ S | 60° 34′ 00″ W | 15.73 | 12.51 | 5.32 |
| *Iridaea cordata* | Livingston Island | Half Moon Island | 62° 35′ 44″ S | 59° 54′ 12″ W | 3.05 | 0.45 | 19.94 |
| *Palmaria decipiens* | Antarctic Peninsula | Cierva Cove | 64° 09′ 03″ S | 60° 57′ 04″ W | 1.06 | 22.57 | 46.79 |
| *Curdiea racovitzae* | Antarctic Peninsula | Cierva Cove | 64° 09′ 03″ S | 60° 57′ 04″ W | 2.49 | 28.57 | 15.88 |
| *Phyllophora ahnfeltioides* | Deception Island | Fildes Point | 62° 59′ 28.55″ S | 60° 33′ 39.6″ W | 0.47 | 28.17 | 12.16 |
| *Pantoneura plocamioides* | Deception Island | Whaler's Bay | 62° 58′ 53.91″ S | 60° 33′ 44.46″ W | 1.98 | 0.05 | 0.03 |
| *Hymenocladiopsis prolifera* | Deception Island | Whaler's Bay | 62° 58′53,91″ S | 60° 33′44,46″ W | 5.07 | 45.14 | 3.54 |
| *Leniea lubrica* | Deception Island | Whaler's Bay | 62° 58′ 53.91″ S | 60° 33′ 44.46″ W | 9.25 | 70.77 | 2.85 |
| *Delisea pulchra* | Deception Island | Whaler's Bay | 62° 58′ 53.91″ S | 60° 33′ 44.46″ W | 0.35 | 47.89 | 3.10 |
| *Sarcothalia papillosa* | Deception Island | Whaler's Bay | 62° 58′ 53.91″ S | 60° 33′ 44.46″ W | 9.92 | 10.75 | 3.51 |
| *Phycodrys austrogeorgica* | Deception Island | Whaler's Bay | 62° 58′ 53.91″ S | 60° 33′ 44.46″ W | 0.27 | 16.96 | 30.84 |
| *Rhodymenia subantarctica* | Deception Island | Whaler's Bay | 62° 58′ 53.91″ S | 60° 33′ 44.46″ W | 0.52 | 283.61 | 14.18 |
| *Sarcopeltis antarctica* | Deception Island | Whaler's Bay | 62° 58′ 53.91″ S | 60° 33′ 44.46″ W | 14.14 | 11.04 | 0.24 |
| *Plocamium* sp. | Antarctic Peninsula | Paradise Bay | 64° 51′ 57″ S | 62° 51′ 55″ W | 0.46 | 11.11 | 1.75 |
| Phaeophyceae | | | | | | | |
| *Desmarestia menziesii* | Antarctic Peninsula | Cierva Cove | 64° 09′ 03″ S | 60° 57′ 04″ W | 0.29 | 47.99 | 55.06 |
| *Desmarestia antarctica* | Deception Island | Fildes Point | 62° 59′ 28.55″ S | 60° 33′ 39.6″ W | 0.49 | 205.15 | 80.48 |
| *Desmarestia anceps* | Deception Island | Fildes Point | 62° 59′ 28.55″ S | 60° 33′ 39.6″ W | 0.77 | 66.15 | 44.10 |
| *Cystosphaera jacquinotii* | Deception Island | Whaler's Bay | 62° 58′ 53.91″ S | 60° 33′ 44.46″ W | 10.59 | 28.95 | 1.12 |
| *Himantothallus grandifolius* | Deception Island | Fildes Point | 62° 59′ 27″ S | 60° 33′ 19″ W | 13.54 | 92.68 | 2.59 |
| *Ascoseira mirabilis* | Deception Island | Descubierta Point | 62° 59′ 45″ S | 60° 43′ 31″ W | 3.70 | 59.55 | 14.03 |
| *Phaeurus antarcticus* | Deception Island | Whaler's Bay | 62° 58′ 53.91″ S | 60° 33′ 44.46″ W | 9.15 | 40.73 | 3.05 |
| *Adenocystis utricularis* | Deception Island | Telephone Bay | 62° 55′ 12″ S | 60° 39′ 37″ W | 0.14 | 262.94 | 53.59 |

a rotary evaporator. Then, the hydrophilic and lipophilic fractionations were separated using specific solvents for each type of extract. For lipophilic compounds, diethyl ether 100% (Et$_2$O) was used, and the process was repeated three times. The resulting ethereal solution was evaporated again *in vacuo* and transferred to pre-weighed vials. The content of the vials was again evaporated and weighed to obtain the ethereal extract weight. Then, they were stored frozen (−20 °C) until used for the antimicrobial assays. The hydrophilic compounds were separated using butanol 100% (BuOH), twice. After this, similarly to the lipophilic compounds, the butanolic solution containing hydrophilic fractions was transferred to the pre-weighed vials, adding trichloromethane for easier evaporation. Then, the fractions were lyophilized and weighed again before being stored frozen.

The resulting algal solid residue was weighed to obtain the total dry weight (DW$_T$, sum of the weights of algal solid residue and dry Et$_2$O crude extracts, dry weight of BuOH and dry weight of aqueous residue). This is necessary to calculate the extract natural concentration (that is, concentration of the phase extract in the original sample, Table 1), which will be further used in the microbial experiments to simulate the real concentration in nature.

Antimicrobial activity inhibition was performed by using the crude extracts with the agar disk diffusion method on isolated cultures of a variety of microorganisms (Fig. 2), as described in the literature (Acar 1980; Álvarez 1990; Salvador, et al. 2007; Figuerola et al. 2014, 2017; Angulo-Preckler et al. 2018b). The microorganisms were selected by their availability in the culture collections at the University of Barcelona from the pathogen surrogate's storage collections
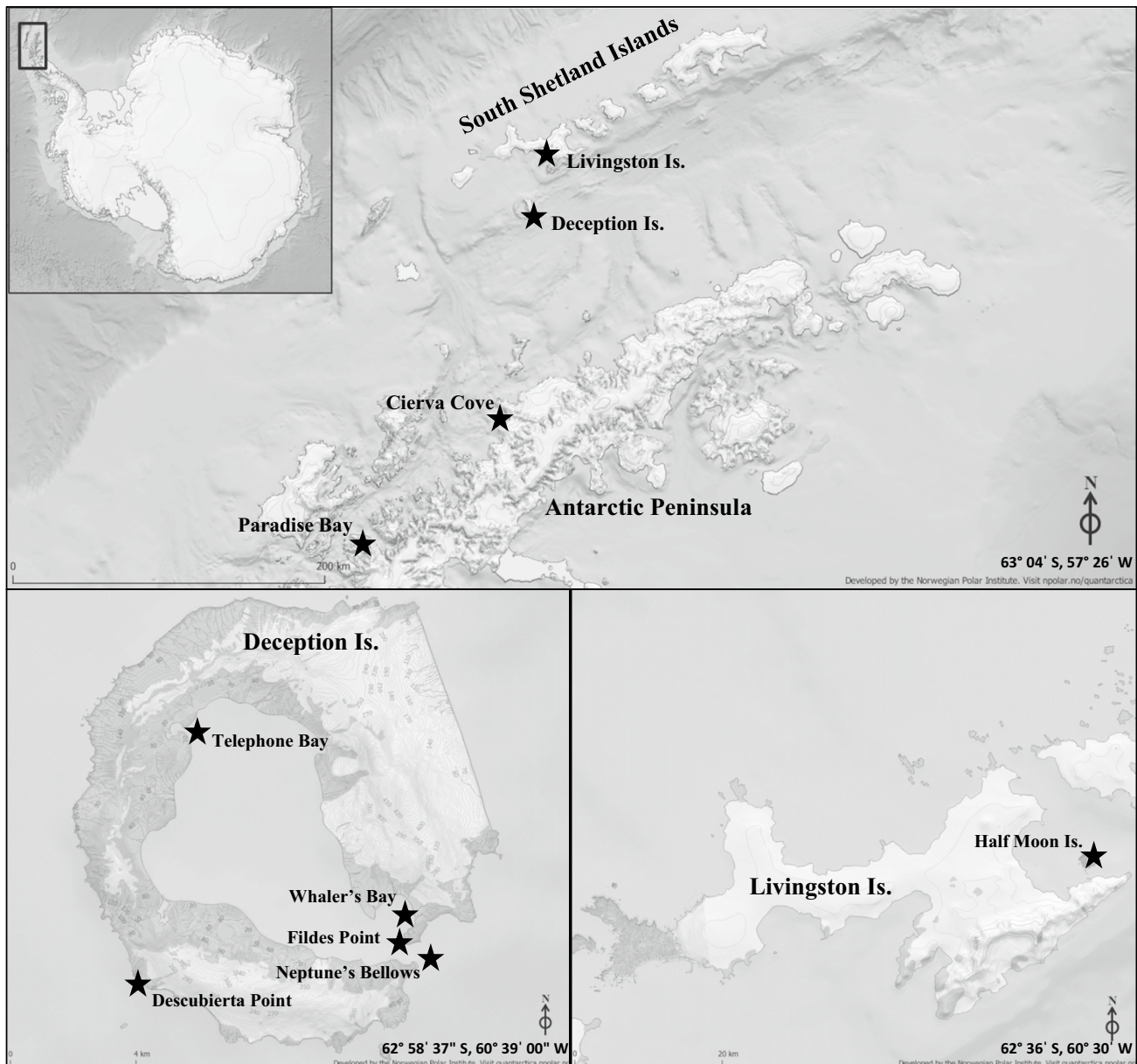
**Fig. 1** Maps of the sampling locations and stations listed in Table 1 (marked with stars). **a** Antarctic continent. **b** Antarctic Peninsula region and South Shetland archipelago. **c** Deception Island. **d** Livingston Island. Map constructed with QGIS software (v. 3.16) with Quantarctica package

and successive Antarctic campaigns isolated strains. The taxa used in the tests consisted of 13 different bacteria (seven Antarctic isolates and six strains of pathogen surrogates) and one pathogenic fungus (Table 2). Antarctic isolates included *Psychrobacter* sp., *Paracoccus* sp., *Oceanobacillus* sp., *Bacillus aquamaris*, *Micrococcus* sp. and two strains of *Arthrobacter* sp., previously isolated from different Antarctic organisms and substrata (Table 2). The other six bacteria strains used here were *Vibrio cholerae* CECT 657, *Escherichia coli* O157:H7, ATCC 43,888, *Pseudomonas aeruginosa* NCTC 10332 T, *Escherichia coli* CECT 515, *Bacillus cereus* CECT 4014, *Staphylococcus aureus* CECT 59 and

the fungus *Candida albicans* CECT 1001. As described by Angulo-Preckler et al. (2015), the Antarctic bacteria were incubated for 48 h on marine agar medium at 20 °C as this temperature is well within their growth range (Straka and Strokes 1960; Morita 1975) and allows for faster testing, while the pathogenic representatives were incubated at 37 °C for 24 h in Mueller–Hinton medium. To achieve a turbidity of 0.5 McFarland, the microorganisms were transferred to a dilution medium with different NaCl concentrations (1.5% for the Antarctic strains and 0.85% for the pathogenic ones). Then, microorganisms were distributed homogeneously in separated testing plates with the corresponding agar medium
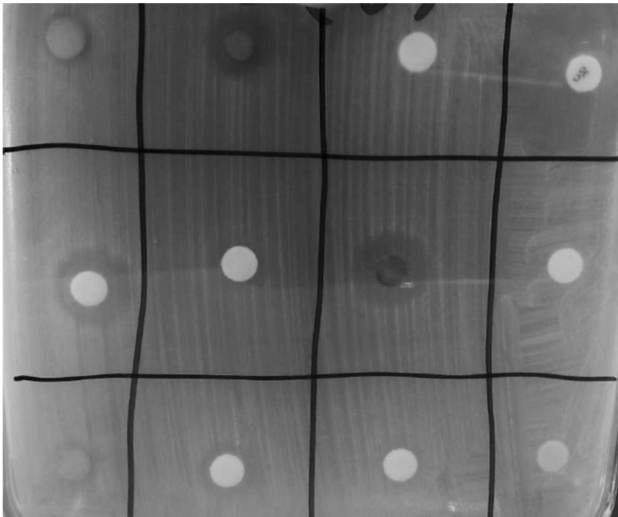
**Fig. 2** Examples of microbial inhibition test plates with ethereal extracts (with inhibition halii) and control disks in *Psychrobacter* sp. strain (A strain)

to perform the agar disk diffusion tests. For each microbial strain and fraction, three replicates of the bioassay were performed.

For each microbial strain and fraction, the bioassay consisted of three replicates. The extracts at natural concentration equivalents were inoculated on diffusion paper disks (6 mm, PRAT DUMMAS *France*) using Hamilton syringes, avoiding saturation of the disks. Methanol was used as solvent to infuse the two different types of extracts in the disks owing to its rapid evaporation. Furthermore, for each test, three controls were used for each replicate: one positive (impregnated with chloramphenicol) and two negatives, one consisting of a blank disk and another soaked only with

the corresponding solvent used for the extractions (diethyl ether for the ethereal extracts and methanol for the butanolic ones). The prepared disks were then placed on the testing plates with the different microorganisms for the inhibition test. The inoculated plates with the disks were incubated for 48 h at 20 °C for Antarctic bacteria, and for 24 h at 37 °C for the pathogenic microorganisms. After incubation, the size in millimetres of the inhibition halii surrounding the paper disk was measured to assign antimicrobial activity for each extract. Antibacterial activity was established following Mahon et al. (2003) criteria to define inhibition categories: weak inhibition 0.1–1 mm (+), moderate inhibition 1.1–1.9 mm halii (++) and strong inhibition > 2.0 mm (+++). Additionally, as some of our extracts exceeded measures of 4 mm, we classified them as very strong inhibition (++++).

Once the intensity of inhibition was assessed, the percentages of inhibition of the two types of seaweed (rhodophytes and Phaeophyceae), the two types of extract (hydrophilic and lipophilic) and the types of microorganism (Antarctic strains, pathogenic surrogates and Gram staining) were compared for statistical significance or $p$ value ($p$) using the "$N - 1$" chi-squared test, as described in the literature (Campbell 2007; Richardson 2011; Altman et al. 2013).

## Results

Of the 22 macroalgae species studied here (14 Rhodophyta and 8 Phaeophyceae), a total of 44 extracts (22 hydrophilic and 22 lipophilic) were tested against 14 microbial strains (Tables 3 and 4). A total of 14 of the 22 macroalgae studied showed antimicrobial activity against at least one microbial strain (Fig. 3). The species with the largest number of

**Table 2** List of tested microorganisms: strain code, species name, source, classification and cell wall type

|  | Strain | Microorganism | Isolated from | Type | Gram staining |
|---|---|---|---|---|---|
| Antarctic | A | *Psychrobacter* sp. | *Dendrilla antarctica* (sponge) | Bacteria | − |
|  | B | *Paracoccus* sp. | Stone | Bacteria | − |
|  | C | *Arthrobacter* sp. | *Axinella crinita* (sponge) | Bacteria | + |
|  | D | *Arthrobacter* sp. | *Axinella crinita* (sponge) | Bacteria | + |
|  | E | *Oceanobacillus* sp. | *Haliclona* sp. (sponge) | Bacteria | + |
|  | F | *Bacillus aquamaris* sp. | Sediment | Bacteria | + |
|  | G | *Micrococcus* sp. | Sediment | Bacteria | + |
| Pathogenic | H | *Vibrio cholerae* CECT 657 | Culture collection | Bacteria | − |
|  | I | *Escherichia coli* O157:H7, ATCC 43,888 | Culture collection | Bacteria | − |
|  | J | *Pseudomonas aeruginosa* NCTC 10332 T | Culture collection | Bacteria | − |
|  | K | *Escherichia coli* CECT 515 | Culture collection | Bacteria | − |
|  | L | *Bacillus cereus* CECT 4014 | Culture collection | Bacteria | + |
|  | M | *Staphylococcus aureus* CECT 59 | Culture collection | Bacteria | + |
|  | N | *Candida albicans* | Culture collection | Fungus | N/A |

**Table 3** Antibiotic activity of butanolic extracts of seaweed species against microorganism strains

| Species | Antarctic strains* | | | | | | | Pathogenic strains* | | | | | | | Σ Inhibited strains |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | |
| **Rhodophyta** | | | | | | | | | | | | | | | |
| *Neuroglossum delesseriae* | – | – | ++ | ++ | ++ | + | +++ | – | – | – | – | – | – | – | 5 |
| *Iridaea cordata* | – | – | – | – | – | – | – | ++ | – | – | – | – | – | – | 1 |
| *Palmaria decipiens* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Curdiea recovitzae* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Phyllophora ahnfeltioides* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Pantoneura plocamioides* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Hymenocladiopsis prolifera* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Leniea lubrica* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Delisea pulchra* | ++ | +++ | +++++ | +++++ | +++++ | ++++ | ++++ | ++++ | – | – | – | +++ | +++++ | +++ | 11 |
| *Sarcothalia papillosa* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Phycodrys austrogeorgica* | – | – | + | – | – | – | – | – | – | – | – | – | – | – | 1 |
| *Rhodymenia subantarctica* | – | – | – | ++ | – | – | – | – | – | – | – | – | – | – | 1 |
| *Sarcopeltis antarctica* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Plocamium* sp. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| **Phaeophyceae** | | | | | | | | | | | | | | | |
| *Desmarestia menziesii* | – | – | ++ | + | – | + | – | – | – | – | – | + | – | – | 4 |
| *Desmarestia antarctica* | – | – | ++ | ++ | – | + | ++ | +++ | – | – | – | + | +++ | – | 7 |
| *Desmarestia anceps* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Cystosphaera jacquinotii* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Himantothallus grandifolius* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Ascoseira mirabilis* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Phaeurus antarcticus* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| *Adenocystis utricularis* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| Total active extracts | 1 | 1 | 5 | 5 | 2 | 4 | 3 | 3 | 0 | 0 | 0 | 3 | 2 | 1 | |

Data expressed as in Mahon et al. (2003)

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**Table 4** Antibiotic activity of ethereal extracts of seaweed species against microorganism strains

| Species | Antarctic strains* | | | | | | | Pathogenic strains* | | | | | | | Σ Inhibited strains |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | |
| **Rhodophyta** | | | | | | | | | | | | | | | |
| Neuroglossum delesseriae | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| Iridaea cordata | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| Palmaria decipiens | ++ | – | – | + | – | – | – | – | – | – | – | – | – | – | 2 |
| Curdiea recovitzae | + | – | – | – | – | – | – | – | – | – | – | – | – | – | 1 |
| Phyllophora ahnfeltioides | +++ | ++ | – | – | – | – | – | – | – | – | – | – | – | – | 2 |
| Pantoneura plocamioides | + | + | ++ | +++ | – | + | +++ | – | – | – | – | – | – | – | 5 |
| Hymenocladiopsis prolifera | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| Leniea lubrica | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| Delisea pulchra | ++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | – | – | – | +++ | ++++ | ++++ | 11 |
| Sarcothalia papillosa | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| Phycodrys austrogeorgica | – | – | ++ | – | – | – | + | – | – | – | – | – | – | – | 2 |
| Rhodymenia subantarctica | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| Sarcopeltis antarctica | – | – | – | – | + | – | + | + | – | – | – | – | – | – | 3 |
| Plocamium sp. | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| **Phaeophyceae** | | | | | | | | | | | | | | | |
| Desmarestia menziesii | +++ | +++ | ++ | ++ | ++ | – | + | – | – | – | – | – | – | – | 6 |
| Desmarestia antarctica | +++ | – | ++ | +++ | + | + | +++ | – | ++ | – | +++ | ++ | +++ | – | 10 |
| Desmarestia anceps | ++ | – | + | – | – | + | – | – | – | – | – | – | – | – | 2 |
| Cystosphaera jacquinotii | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| Himantothallus grandifolius | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| Ascoseira mirabilis | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| Phaeurus antarcticus | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0 |
| Adenocystis utricularis | – | – | – | – | – | – | – | +++ | – | – | – | – | – | – | 1 |
| Total active extracts | 8 | 4 | 7 | 6 | 5 | 5 | 7 | 4 | 1 | 0 | 1 | 3 | 3 | 1 | |

Data are expressed following Mahon et al. (2003)
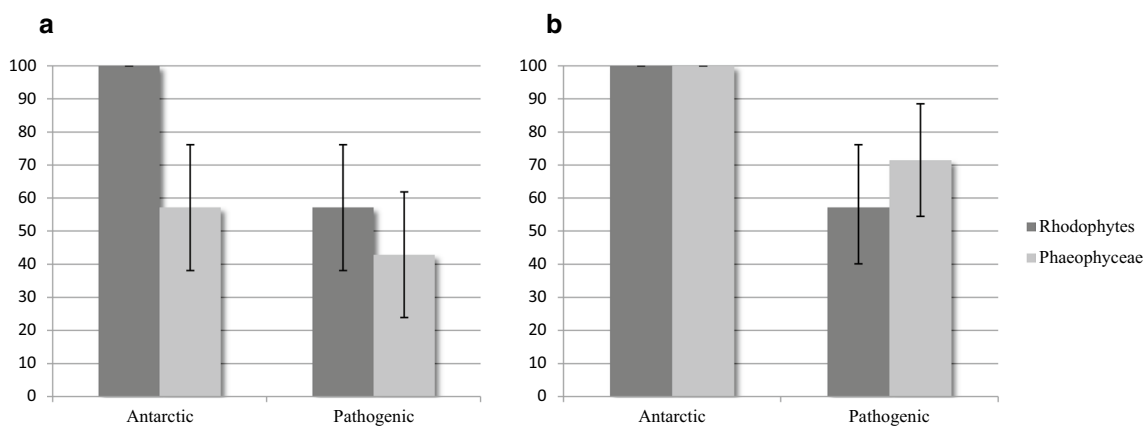
**Fig. 3** Percentages of inhibition with standard error bars of seaweed extracts on Antarctic and pathogenic microorganisms. **a** Percentages for butanolic (hydrophilic) extracts; **b** percentages for ethereal (lipophilic) extracts

microorganism strains inhibited was the red algae *Delisea pulchra*, which inhibited 11 of the 14 microorganism strains tested with both lipophilic and hydrophilic extracts. *D. pulchra* was also the only tested algae that showed inhibition against the fungus *C. albicans* and presented the greatest halii inhibition size against *V. cholerae*. For the brown seaweeds, the most active taxon was *Desmarestia antarctica*, which presented the strongest intensity in its lipophilic extracts against *Psychrobacter* sp. (Fig. 2). In contrast, several species showed no activity, including four brown algae (*Cystosphaera jacquinotii*, *Himantothallus grandifolius*, *Ascoseira mirabilis* and *Phaeurus antarcticus*) and four red algae (*Hymenocladiopsis prolifera*, *Sarcothalia papillosa*, *Leniea lubrica* and *Plocamium* sp.). As a rule, variability between the three replicates on each seaweed extract tested was very low (with overall mean differences between samples < 0.5 mm in the halii). Nonetheless, some individual replicates showed higher variation in the tests (*Desmarestia menziesii* and *Phyllophora ahnfeltioides* against *Psychrobacter* sp. and *Delisea pulchra* against *Vibrio cholerae*) greater than 1 mm, compared with the other two replicates of the same tests. However, as the rest of the replicates for the other tests and species showed no major variability, these individual replicates were not included in the study.

When compared with the "$N - 1$" chi-squared test, the percentages of inhibition of the extracts according to the seaweed type (Rhodophyta or Phaeophyceae), showed no significant differences ($p = 0.32$). Nonetheless, as stated before, a large number of Rhodophyta were active compared with the Phaeophyceae tested. In percentages, 71% of rhodophytes (10 out of 14) showed activity of any type, whereas 50% (4 out of 8) of the Phaeophyceae displayed inhibition in their extracts. Similarly, we assessed whether red or brown algae inhibited Antarctic or pathogenic microorganisms to a significantly greater extent, finding that red seaweed extracts tended to inhibit both types of microorganism to

a greater extent than did brown seaweed species. The percentages obtained were 64.28% of inhibition for Antarctic microorganisms and 21.42% for pathogenic surrogates in the case of rhodophyte extracts, and 37.50% for both types of microorganism for the Phaeophyceae extracts. Nonetheless, neither Antarctic nor pathogenic surrogate microbes were significantly more inhibited by red or brown algae ($p = 0.23$ for Antarctic microorganisms and $p = 0.42$ for the comparison of pathogenic surrogates). We cannot discard that the small sample size played a role in the lack of significance.

On the other hand, our results showed significant differences when comparing the percentages of inhibition on the type of extract: 50% (11 out of 22) ethereal (lipophilic) extracts were active, compared with 31.81% (7 out of 22) of active butanolic (hydrophilic) extracts ($p = 0.02$). It also worth mentioning that nearly 30% of the active lipophilic extracts exhibited strong inhibition (+ + +), compared with 13% of hydrophilic extracts. It is worth noting that the taxa with stronger activity in the lipophilic extracts also displayed higher levels of inhibition in the hydrophilic fractions (e.g. *Delisea pulchra* and *Desmarestia antarctica*). Our results also show that Antarctic microorganisms were more inhibited than pathogenic surrogates for both types of extract as Antarctic bacteria were always inhibited by at least one extract of each type, where pathogenic surrogates showed 57.14% (four out of seven) for butanolic extracts and 85.71% (six out of seven) for ethereal extracts. When comparing these percentages with the chi-squared test, only the comparison between ethereal extracts of both types of microorganism was significantly different ($p = 0.03$). Nonetheless, although for the butanolic extracts the comparison between the two types of microbe was not statistically significant, the very low $p$ of 0.06 may indicate a tendency towards significance which is not fully achieved owing to the small sample size. Our results also show that the type of extract affected the tested bacteria differently depending on their

Gram staining type. For both butanolic and ethereal extracts, Gram-positive bacteria were inhibited in larger numbers (in all cases, they were inhibited at least by one extract of each type). However, when comparing the percentages of inhibition, they were only significant in the case of the butanolic extracts ($p = 0.04$), whereas the ethereal extracts showed no significance ($p = 0.28$). We must note that the only strain that showed any inhibition by any of the macroalgal extracts tested was *P. aeruginosa*, which belongs to the Gram-negative bacteria. Similarly, in the tests with hydrophilic extracts, there were three strains that showed no inhibition, which were again *P. aeruginosa* and the two strains of *E. coli*. Apart from that, the fungus *C. albicans* was only inhibited by the two fractions of *D. pulchra*.

It is also worth noting that the natural concentration (mg/g of dry weight) of the different chemical extracts of our samples showed that lipophilic fractions (ethereal extracts) were found in higher concentrations than hydrophilic ones for both Rhodophyta and Phaeophyceae (see natural concentrations in Table 1). This was especially the case for Phaeophyceae, as ethereal extracts were more than three times more concentrated than butanolic ones.

## Discussion

More than half of the Antarctic macroalgae tested here (14 out of 22 species) showed antimicrobial activity against at least one microbial strain, with stronger inhibition found in lipophilic extracts than in hydrophilic ones. These results are in agreement with previous studies using different algal species and also some of the taxa we used here, where the lipophilic compounds also presented stronger activities (Ankisetty et al. 2004; Salvador et al. 2007; Amsler et al. 2008, 2009; Young et al. 2015; Shannon and Abu-Ghannam 2016; Sacristán-Soriano et al. 2017). This could be partially attributed to the larger lipophilic concentrations found in the extracts (Table 1) compared with the hydrophilic ones, or to the fact that lipophilic compounds are present in seaweed cell walls in larger amounts compared with hydrophilic ones (Wiencke and Clayton 2002, 2014; Gómez and Huovinen 2020), hence explaining the higher natural concentration in the extracts. However, we cannot speculate which individual compounds may be responsible for the activity observed in each case. In the same manner, as we worked with the whole holobiont, we cannot discard that some of the compounds originate or are affected by the microbiome present on the algal surfaces (Sacristán-Soriano et al. 2017; Califano et al. 2020). Even so, previous studies have described molecules linked to inhibition activity (Young et al. 2007; Amsler et al. 2008, 2009; von Salm et al. 2018; Carroll et al. 2020), especially lipophilic molecules, that could be responsible for our results here. These molecules may

include isoprenoids (e.g. terpenoids, diterpenes, meroterpenoids, carotenoids and steroids), aromatic compounds (such as tannins), polyphenols (such as phlorotannins) and/or acetogenins. Moreover, previous studies reported inhibitory activity from secondary metabolites of lipophilic nature in other types of Antarctic organisms (Figuerola et al. 2014, 2017; Angulo-Preckler et al. 2015, 2018b; Sacristán-Soriano et al. 2017), thus reinforcing the main role of these lipophilic compounds in chemical interactions. On the other hand, the lower inhibitory activity observed in hydrophilic compounds could be explained by (1) hydrophilic molecules, being polar compounds, diluting easily in seawater, leading to a lower concentration in the extracts compared with lipophilic compounds (Sotka et al. 2009), and/or (2) hydrophilic compounds being sensitive to temperature changes during the extraction process (Cox et al. 2012), which could reduce their activity. In addition, another plausible factor is related to the above-mentioned larger proportion of lipophilic compounds in cell surfaces and walls compared with hydrophilic compounds, suggesting that the latter are less available to participate in the activity. We must note here that the statistical significance of our tests is to be treated with caution, as the small sample size (number of seaweed species available) may be a limiting factor masking the tendencies that the overall numbers (absolute number of inhibition of extracts per seaweed type) seem to indicate. Also, the differences in natural concentration of the extracts could be affecting the significance observed between the extract types. We also cannot discard the potential effects of seasonality derived from environmental variation, which could affect the natural concentrations of different compounds in the studied species. Hence, as our sampling cannot exclude these potential effects, we must be cautious when interpreting our results.

Concerning the microorganisms used, our results show that Antarctic bacteria were inhibited in more tests than the pathogenic strains (all the Antarctic strains were inhibited by at least one extract, compared with 86% of pathogenic microorganisms), and in general, Gram-negative bacteria seem to be more resistant to macroalgal extracts than Gram-positive ones. This could be due to Gram-negative microorganisms having a more complex cell membrane and wall structure, which grants them an increased resistance when exposed to potentially antibiotic compounds or combinations of compounds. In any case, our results agree with former works on bacteria and Atlantic macroalgae (Freile-Pelegrín and Morales 2004) as well as other Antarctic macro- and microorganisms (Amsler 2008; Amsler et al. 2009; Figuerola et al. 2014, 2017; Young et al. 2015; Solanki et al. 2018; von Salm et al. 2018). Since Antarctic seaweeds evolved alongside sympatric bacteria, it is not unreasonable to think that the sensitivity of Antarctic bacteria observed here could be related to specific compounds that seaweeds evolved to inhibit potentially detrimental microbes (Aguila-Ramirez

et al. 2012; Pérez et al. 2016; Shannon and Abu-Ghannam 2016). Nevertheless, to understand these phenomena, future research must focus on the chemical isolation of specific compounds and further assays must be performed with the bacteria studied. However, we must acknowledge that, for further research, the current availability of Antarctic microorganism cultures or sequences may be a limiting factor, which increases the importance of these kind of studies.

Regarding the specific pathogenic surrogates strains tested, *Pseudomonas* sp. was not inhibited by any seaweed, in contrast to previous tests with *Pseudomonas* where activity was reported in non-Antarctic seaweeds, including Rhodophyta and Phaeophyceae (Pérez et al. 2016; Shannon and Abu-Ghannam 2016). Specificity in bacteria or seaweed species used in the studies may be behind these differences. In contrast, our *Psychrobacter* sp. was inhibited by a similar number of lipophilic extracts from three brown seaweeds (*Desmarestia*) and five Rhodophyta (Table 4). These findings are quite interesting when we compare them to those of previous studies, such as Salvador et al. (2007), where Gram-negative bacteria tested against macroalgae from the Iberian Peninsula were mainly inhibited by Rhodophyta instead of brown algae. In the same work, a larger number of Iberian ochrophytes having inhibitory activity against Gram-positive microorganisms was also observed. However, in our case, red and brown seaweeds seemed to affect mainly Gram-positive bacteria, which raises the need of further experimentation with Antarctic species to fully understand this. The pathogenic fungus *C. albicans* was inhibited by only one macroalgal species with the two different extracts (*D. pulchra*), similarly to findings reported previously (Pérez et al. 2016; Shannon and Abu-Ghannam 2016) for the fungus *Saccharomyces*, which was more difficult to inhibit than other fungi (e.g. *Cryptococcus neoformans*). These results seem to reinforce the idea that unicellular fungi (at least non-Antarctic ones) are more resistant than bacteria to seaweed chemical activity. Further tests with Antarctic marine unicellular fungi may shed light on the specific relationships that may occur between these two groups of organisms.

*Desmarestia* species showed a wide range of activity (similar to red seaweeds), in both types of extract. Our results agree with previous records of antimicrobial activity on *Desmarestia* species (Hornsey and Hide 1974), with some of the taxa we used, including *D. menziesii* (Ankisetty et al. 2004), and other species of the genus such as *Desmarestia aculeata* (Linnaeus) J.V. Lamouroux and *Desmarestia ligulata* (Stackhouse) J.V. Lamouroux, (Benites Guardia 2019) and *Desmarestia confervoides (Bory)* M.E. Ramírez & A.F. Peters (Hornsey and Hide 1974; Benites Guardia 2019). Nonetheless, in the latter study, antimicrobial activity was found on the desmarestial *H. grandifolius*, a species that showed no antimicrobial activity in our tests. This difference may be due to a combination of

several factors, such as organism variability (e.g. the specific bacteria strains used). Our results also differ from the study of Iken et al. (2011), which found activity in both types of extract in *Desmarestia anceps*. Contrastingly, our results did not show any hydrophilic antimicrobial activity in this species. The main reason for this could be the higher dilution of the hydrophilic molecules compared with lipophilic ones (Sotka et al. 2009), although other factors may be involved. Also, unlike Iken et al. (2011), we found that *D. antarctica* was one the most active brown algae. *Adenocystis utricularis* also showed antimicrobial activity, even if it acted only against *Vibrio cholerae* with strong inhibitory action. Antiviral activity in *A. utricularis* has been linked to fucoidans (Ponce et al. 2003), opening an interesting field of study on the chemistry of this particular species. Apart from the differences mentioned above, our findings on Phaeophyceae are in general well in line with what is known about brown seaweed chemistry (Golan et al. 2011). Again, even though our study focuses on the general activity of natural extracts and we cannot discard the effect of the microbiome of the seaweeds, as mentioned earlier, several specific compounds from brown macroalgae could be behind the activity observed. Examples may include phenolic molecules and phlorotannins (Boland et al. 1982; Rivera et al. 1990; Fairhead et al. 2005a; Amsler and Fairhead 2006; Pérez et al. 2016; Benites Guardia 2019). Additionally, Phaeophyceae are rich in polysaccharides such as alginates, laminarin and fucoidin that have been also linked to antimicrobial activity in the past (Baba et al. 1988; Kadam et al. 2015; Vieira et al. 2017; Hamrun et al. 2020).

Regarding Rhodophyta, their higher level of activity compared with brown seaweeds here is similar to that found in previous studies (Caccamese et al. 1980, 1981; Bouhlal et al. 2013; Sacristán-Soriano et al. 2017). For example, species of the order Bonnemaisoniales, such as *Bonnemaisonia asparagoides* (Woodward) C. Agardh, *Bonnemaisonia hamifera* Hariot and *Asparagopsis armata* Harvey, which live in warmer latitudes, have shown antimicrobial activity in the past (Paul et al. 2006; Salvador et al. 2007). Previous studies are available for only two of the red seaweeds tested here, *D. pulchra* and *Plocamium* sp. For the former (which also belongs to Bonnemaisoniales), antifouling and antibiotic properties have been reported (Maximilien et al. 1998; Ren et al. 2002, 2004; Hentzer and Givskov 2003; Ankisetty et al. 2004). These results are consistent with the great inhibitory activity found in our samples, reinforcing the idea that *D. pulchra* has a rich diversity of chemical interactions with Antarctic microorganisms. However, it is worth mentioning that, contrary to previous data reported on this species (Hentzer and Givskov 2003), our samples produced inhibition on the pathogenic surrogate of *Pseudomonas aeruginosa*. This dissimilarity could be attributed to factors such as

chemical variability of certain secondary metabolites on the species, as reported in previous works (Fairhead et al. 2005b; Longford et al. 2007).

For *Plocamium* sp., it is worth noting that, though in the past *Plocamium* from Antarctica had been classified as *Plocamium cartilagineum*, in recent times, evidence has grown that the populations of this species in Antarctica are a separate taxon, and thus the current general trend is to use the term "*Plocamium* sp." (Wiencke and Clayton 2002; Hommersand et al. 2009; Dubrasquet et al. 2018; Guillemin et al. 2018). From this genus, several compounds with antimicrobial activity were found previously (Rovirosa et al. 1990; Cueto et al. 1991; Ankisetty et al. 2004; Harden et al. 2009). However, in our study, *Plocamium* sp. extracts showed no antimicrobial activity. One reason for this dissimilarity could be attributed to differences in the protocols used. Nonetheless, some of these studies used species of *Plocamium* from warmer environments. Therefore, chemical differences between species of the genus from different habitats could also explain the contrast in the results. As mentioned earlier, although we cannot discard an effect of the algal microbiome, previous works reported several active secondary metabolites that could be good candidates for the antimicrobial activity observed in our red algae samples. These compounds would include halogenated isoprenoids such as monoterpenes and carotenoids from several Rhodophyta families with Antarctic representatives (Amsler 2008; Amsler et al. 2009; Young et al. 2013, 2015; Carroll et al. 2020). Also, red seaweed polysaccharide compounds (such as carrageenans, sulphated galactans and galactofucans) with established antimicrobial activity could be good candidates for the type of results we observed here (Harden et al. 2009).

## Conclusions

Our observations indicate that extracts from Antarctic macroalgae are as bioactive as seaweeds from other parts of the world, even though studies on their chemical activity are scarcer than in other geographical areas. Several factors could modulate the concentration or activity of the potential compounds present in the extracts. For this reason, further analyses of very active species, such as *D. pulchra* and the genus *Desmarestia*, will prove vital to comprehending their chemical ecology and the role of the microbiome in Antarctic algae to a full extent. Our results also provide new insights into the role of macroalgae as a source of bioactive compounds potentially useful in research on antibiotic-resistant bacteria. Further studies may elucidate new applications derived of the isolation of compounds present in Antarctic seaweed extracts.

**Availability of data and material** All herbarium vouchers from the samples studied are available for consulting in the seaweed collection of the Centre de Documentació de Biodiversitat Vegetal (CedocBiv; https://crai.ub.edu/en/about-crai/CeDocBiV) from the Centre de Recursos per a l'Aprenentatge i la Investigació (CRAI) of the University of Barcelona. Chemical data and measurements are available from the corresponding author.Code availability Not applicable.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Ethical approval** Not applicable.

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