



Article

Bioprospecting of an Antarctic *Pseudogymnoascus* Isolate for Antibacterial and Antioxidant Activities

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Key Words	Abstract
Antarctica, psychrophilic fungi, antibacterial activity, antioxidant activity, bioprospecting, secondary metabolites.	Antarctic environments harbour cold-adapted microorganisms with considerable potential for the production of bioactive secondary metabolites. In the present study, a culturable <i>Pseudogymnoascus</i> sp. was isolated from soil collected near the Bharati Research Base, Antarctica, and evaluated for its antibacterial and antioxidant potential. The fungal isolate was morphologically characterized and cultivated at 20 ± 2 °C, followed by sequential extraction using methanol, ethyl acetate, and hexane. The methanolic, ethyl acetate, and hexane extracts showed crude extract recoveries of approximately 0.67, 0.61, and 0.57 mg/mL, respectively. Among the extracts, only the methanolic fraction exhibited detectable antibacterial activity against the tested bacteria. The highest zone of inhibition was observed against <i>Escherichia coli</i> MTCC 739 (17.32 ± 0.6 mm), followed by <i>Staphylococcus aureus</i> MTCC 96 (16.4 ± 1.1 mm) and <i>Bacillus cereus</i> MTCC 430 (12.4 ± 0.9 mm). No inhibition was detected against <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae</i> , <i>Enterococcus faecalis</i> , <i>Micrococcus luteus</i> , or MDR <i>E. coli</i> . The methanolic extract showed a minimum inhibitory concentration of 2560 µg/mL against <i>E. coli</i> . In the DPPH assay, the methanolic extract exhibited concentration-dependent radical-scavenging activity, increasing from $4.54 \pm 0.8\%$ at 10 µg/mL to $60.32 \pm 1.4\%$ at 2560 µg/mL. The ethyl acetate and hexane extracts showed substantially lower antioxidant activity. These findings indicate that Antarctic <i>Pseudogymnoascus</i> sp. represents a promising source for further investigation of fungal metabolites with antibacterial and antioxidant properties.

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1. Introduction

Antarctica represents one of the most pristine ecosystems globally, where diverse extreme environmental conditions prevail (Rosa et al. 2019). Antarctica remains one of the least explored terrestrial ecosystems and the microorganisms present in these environments must cope with extremely low temperatures, wide temperature fluctuations, frequent freeze and thaw

events during the austral summer, strong winds, intense solar and UV irradiations, different pH conditions, desiccation, and locally high salinity (Zucconi et al., 2020 and Varrella et al., 2021). In these extreme environmental conditions, extremophilic microorganisms can successfully colonize organic materials. Two groups of microorganisms can be found in these environments. Psychrophiles, which are defined by an optimum growth temperature of 15 °C, a maximum growth temperature below 20 °C, and a minimum growth temperature equal to or below 0 °C, and psychrotrophic or psychrotolerant microorganisms, which are cold-adapted mesophiles, and compete well for resources (Abrashev et al., 2025; Alborés et al., 2018).

The microbial biodiversity of Antarctica is mainly concentrated in ice-free areas, which represent less than 1% of the continent. Despite such harsh environmental conditions, Antarctic soils harbor diverse microbial communities, particularly fungi, which possess unique physiological and metabolic adaptations for survival. These harsh conditions generate oxidative stress and have driven Antarctic fungi to evolve unique physiological and biochemical

adaptations, including highly efficient antioxidant defense systems and the production of diverse secondary metabolites with potential biotechnological applications (Abrashev et al., 2025). Antarctic fungi have attracted considerable scientific interest due to their ability to produce cold-active enzymes and structurally diverse secondary metabolites with a wide range of bioactivities, including antibacterial, antifungal, antiviral, antioxidant, cytotoxic, antitumor, and anti-inflammatory effects (Ferrarezi et al., 2019; Camacho et al., 2024; Evidente, 2024 and Cortez et al., 2026). These fungi have even successfully survived in man-made environmental impacts such as heavy metal pollution, antibiotics, and other pharmaceutically active substances. Such exposures qualify for fungi as potential sources of metabolites with differing biological activities (Svahn et al., 2015). Godinho et al. (2015), for example, investigated fungi from oligotrophic soils of continental Antarctica and demonstrated the bioprospecting potential of Antarctic fungal isolates, suggesting that pristine Antarctic environments may represent valuable reservoirs for the discovery of novel bioactive molecules.

The Antarctic fungal communities comprise representatives of genera and species from the major fungal phyla Ascomycota and Basidiomycota, with additional fungal lineages reported from diverse terrestrial and marine habitats traditionally studied by mycologists. Antarctic fungi exist in perpetual extreme conditions; consequently, they may have developed unique or unusual metabolic pathways to produce novel bioactive compounds (Santiago et al. 2012), which may have potential

applications in the development of new drugs and pesticides for pharmaceutical or agrochemical purposes (Goncalves et al., 2015).

Fungi belonging to genera such as *Penicillium*, *Aspergillus*, *Cladosporium*, *Geomyces*, *Trichoderma*, and *Pseudogymnoascus* have been frequently reported from various Antarctic habitats, including soils, marine sediments, macroalgae, lichens, and glacial ice (Cavalcante et al., 2024; Varrella et al., 2021; Satriawan et al., 2024). Many of these species produce structurally unique metabolites such as polyketides, alkaloids, terpenoids, peptides, and phenolic compounds. These metabolites not only contribute to the fungi's ecological survival under extreme conditions but also exhibit therapeutic activities of pharmaceutical interest. For example, bis-anthraquinones from *Penicillium chrysogenum* and diketopiperazines from *Penicillium citreonigrum* display strong cytotoxic and antitumor effects, while carotenoid pigments from *Sporobolomyces salmonicolor* act as powerful antioxidants (Dimitrova et al., 2013; Rosa, 2019; De Menezes et al., 2020).

The species *Pseudogymnoascus* is known as a psychrophilic pathogenic fungus with a ubiquitous distribution in Antarctica (Santos et al., 2020; Rosa et al., 2021; Martorell et al., 2021). *Pseudogymnoascus* was first reported by Raillo in 1929 (Villanueva et al., 2021), which was previously known as *Geomyces*. The extracts of some of *Pseudogymnoascus* strains exhibit potent bioactivities, such as antimicrobial, herbicidal, and antitumoral activities (Henríquez et al., 2014; Gonçalves et al., 2015; Gomes et al., 2018). Members of the

genus *Pseudogymnoascus* are of particular interest among Antarctic fungi because of their association with cold environments and their capacity to produce specialized metabolites. Antarctic *Pseudogymnoascus* isolates have yielded structurally diverse secondary metabolites, including polyketides, sesquiterpenoids, and steroidal compounds. For example, investigations of *Pseudogymnoascus* sp. HSX2#-11 resulted in the isolation of previously undescribed polyketides, while subsequent chemical investigation of the same fungal strain identified new tremulane sesquiterpenoids and other metabolites with biological activities. These findings highlight the chemical potential of cold-adapted fungal taxa and support the importance of combining fungal isolation, molecular identification, metabolite extraction, and biological screening in Antarctic bioprospecting studies (Shi et al., 2021).

The antibacterial potential of Antarctic fungi has been demonstrated against a range of bacterial pathogens. Purić et al., (2018) evaluated crude intracellular and extracellular extracts from filamentous fungi isolated from Antarctic soil and marine sediments and reported antibacterial activity against phytopathogenic *Xanthomonas* species. More recently, Ordóñez-Enireb et al., (2022) genetically characterized four Antarctic fungal isolates and demonstrated variable antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Staphylococcus aureus*. Such findings support the continued investigation of Antarctic fungi for antimicrobial metabolite discovery, particularly at a time when the search for

alternative sources of antibacterial compounds remains important.

In recent years, the increasing emergence of multidrug-resistant pathogens and the demand for novel natural bioactive compounds have intensified the exploration of extremophilic microorganisms from polar environments. Molecular biology techniques are widely employed in the taxonomic identification of Antarctic fungi. Genomic DNA is commonly extracted from fungal mycelia using standardized extraction protocols, followed by amplification and sequencing of the Internal Transcribed Spacer (ITS) region. The ITS region is considered the most reliable and universally accepted DNA barcode marker for fungal identification due to its high interspecific variability and taxonomic resolution and has therefore been extensively utilized for the molecular characterization of fungal isolates (Rosa et al., 2019). Although molecular approaches have enhanced our understanding of Antarctic microbial diversity, culture-dependent isolation remains essential for obtaining fungal strains for bioprospecting studies (Varrella et al., 2021).

Therefore, the present study aimed to isolate and identify culturable fungi from Antarctic soil samples and to evaluate their biotechnological potential through assessment of Antibacterial and Antioxidant activities. The study involved morphological characterization of the isolate, followed by cultivation and sequential extraction of fungal metabolites using methanol, ethyl acetate, and hexane. The resulting fractions were evaluated for antibacterial activity against

selected Gram-positive and Gram-negative bacterial strains, followed by MIC determination. Their antioxidant potential was assessed using the DPPH radical-scavenging assay.

2. Materials and methods

2.1 Chemicals, reagents, and cultured media

Ethanol, Methanol, Ethyl acetate, Hexane, Dimethyl sulphoxide(DMSO) and other chemicals, reagents used in the study were of analytical grades and procured from Merck and Sigma- Aldrich, India. Media were used such as Nutrient Agar (NA), Muller Hinton Agar (MHA), Chloramphenicol Yeast Glucose Agar (CYGA), Muller Hinton Broth (MHB), Chloramphenicol Yeast Glucose Broth (CYGB). According to the manufacturer's instructions, all media were prepared.

2.2 Sample collection and Isolation

The fungal strain utilized in this study was originally isolated from the soil samples that were collected from the vicinity of the Bharati Research Base, Antarctica during the Indian Scientific Expedition to Antarctica and were transported in an unbroken freeze chain to our laboratory. For the present study, the strain was revived from the stocks preserved in paraffin oil at -70°C .

The streak plate method was used to perform pure culture isolation. Fungal isolates were removed from the freezer and allowed to thaw briefly at room temperature. Using a sterile inoculating loop, a small aliquot of the stock was aseptically streaked onto Chloramphenicol Yeast Glucose Agar (CYGA) plate. The inoculated plates were incubated at $20 \pm 2^{\circ}\text{C}$ for 5–7 days

until visible fungal growth was observed. Mixed colonies appearing on the plates were sub-cultured to obtain pure fungal cultures. The remaining stock was immediately returned to -70°C to avoid repeated freeze–thaw cycles.

2.3 Morphological characterization

Pure isolate was identified based on the macroscopic as well as its microscopic properties. The macroscopic properties of the fungal colony like colony colour, texture, pigmentation, margin, elevation, surface appearance, and growth pattern, were recorded after incubation. Colony diameter and growth rate were also observed periodically. For microscopic characterization, a small portion of fungal mycelium was taken from the actively growing culture and stained using lactophenol cotton blue stain. The stained fungal structures were observed under a light microscope to examine hyphal characteristics, septation, conidiophores, spores, and reproductive structures.

2.4 Mass cultivation of fungi

The isolate was inoculated into CYGB (Chloramphenicol Yeast Glucose Broth) and incubated at 20°C on a rotatory shaker for 15 days facilitate fungal biomass production and metabolite secretion. Following incubation, the fungal cultures were harvested and subjected to solvent extraction for the recovery of bioactive secondary metabolites. The extraction was performed

using solvents of different polarities, Methanol(polar), Ethyl acetate(bipolar), and Hexane(non-polar), to obtain fractions containing metabolites with varying physicochemical properties.

2.5 Extraction of Fungal Metabolites and Bioactivity Screening

The cultivated fungal biomass was subjected to sequential solvent extraction using solvents of different polarities, namely methanol, ethyl acetate, and hexane, resulting in three distinct crude extract fractions designated as PMeOH, PEtOAc, and PHex, respectively. Methanol was initially added to the culture, followed by sonication for 10 min at 25°C to facilitate the release of fungal metabolites. The mixture was subsequently centrifuged for 15 min, resulting in separation of the methanolic supernatant from the fungal biomass. The fungal biomass remaining after methanolic extraction was further subjected to extraction with ethyl acetate. The biomass was sonicated in ethyl acetate and subsequently centrifuged to separate the solvent fraction from the residual biomass. The recovered supernatant was collected as the ethyl acetate fraction (PEtOAc) and concentrated to obtain the corresponding crude extract. The residual biomass was subsequently extracted with hexane following the same sonication and centrifugation procedure. The resulting supernatant was collected as the hexane fraction (PHex) and concentrated to obtain the crude hexane extract.

Thus, sequential extraction with solvents of differing polarity generated three fractions, PMeOH, PEtOAc, and PHex, which were subsequently evaluated for their antibacterial, antioxidant, and chemical profiles. The dried extracts were weighed and stored appropriately until further analysis. The following formula was used to determine the extraction yield:

$$\text{Percentage Yield (\%)} = \text{E/R} \times 100$$

E = weight of the extracted residue, and R = weight of the dried biomass.

The crude extracts were dissolved in Dimethyl sulfoxide (DMSO) at a concentration of 20 mg/mL (100 mg crude extract in 5 mL DMSO) to prepare stock solutions for bioactivity screening. The extracts were subsequently evaluated for antimicrobial and antioxidant activities using standard in-vitro assays.

2.6 Antibacterial

Activity

2.6.1 Bacterial strain and inoculum preparation

Eight test microorganisms used in this study including Gram-positive and Gram-negative bacteria as well as a MDR (Multi drug resistant) strain. *Staphylococcus aureus* MTCC 96, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453, *Klebsiella pneumoniae* MTCC 3384, *Enterococcus faecalis* MTCC 439, *Bacillus cereus* MTCC 430, *Micrococcus luteus* MTCC 2470 and MDR *Escherichia coli* DDWI-01 were cultured overnight on a Sterile Nutrient Agar slant and diluted with 5 ml saline solution.

2.6.2 Antibacterial assay

The Antibacterial activity of the crude fungal extracts obtained using methanol, ethyl acetate, and hexane was evaluated against seven bacterial strains using the Agar Well Diffusion Method described in (Henríquez et al., 2014). Inoculation of bacteria was done by mixing 100 µL of diluted bacterial culture and 100 ml of Muller Hinton Agar (MHA) and poured in the petri plates (90 mm in diameter). Wells of approximately 6 mm diameter were punched into the agar using a sterile cork borer. 100 µL of

each crude extract (Methanol, Ethyl Acetate and Hexane)

was pipetted into the wells. DMSO served as a negative control, while a standard antibiotic (Ciprofloxacin) was used as a positive control. The plates were then incubated at 37 °C for 24 h and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition (mm) around each well. A digital Vernier's calliper was used to measure the zone of inhibition that was produced (NCCLS, 1997). The size of the zone indicates the antibacterial activity of the fungal extract.

2.6.3 Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of the isolated strain was determined using the broth microdilution method. Serial two-fold dilutions (e.g., 10- 2560 µg/mL) of the crude extracts were prepared in sterile broth medium to obtain a range of concentrations. A standardized microbial inoculate of 5ml was added to each and incubated at 37 °C for 24 hours. A growth control containing bacterial inoculum without fungal extract and a solvent control containing the corresponding concentration of DMSO were included. Following incubation, bacterial growth was assessed based on the presence or absence of visible turbidity. The MIC was defined as the lowest concentration of extract that inhibited visible microbial growth.

2.7 Antioxidant

Activity

The antioxidant activity of the isolated strain was assessed by evaluating the percentage inhibition of free radical scavenging activity. The DPPH (2,2- diphenyl-1-picrylhydrazyl) free

radical scavenging assay was conducted following the method first outlined by (Brand-Williams et al., 1995). A 0.1 mM solution of DPPH was prepared in methanol and kept in the dark to prevent photodegradation. The fungal extract was dissolved in methanol to obtain different concentrations (e.g., 10- 2560µg/mL). Ascorbic acid was used as a standard antioxidant under similar conditions. For the assay, 1 mL of DPPH solution was mixed with 1 mL of the extract at various concentrations. A control was prepared by mixing 1 mL of DPPH solution with 1 mL of methanol. The reaction mixtures were incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a UV-Visible spectrophotometer. All experiments were carried out in triplicate, and the average values were recorded.

2.7.1 Calculation of Percentage Inhibition

The free radical scavenging activity was calculated using the following formula:

$$\% \text{Inhibition} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

Where, A_{control} = Absorbance of control,

A_{sample} = Absorbance of sample

3. Results

3.1 Isolation of Antarctic Fungal Strains

A Fungus belonging to the genus *Pseudogymnoascus* (formerly *Geomyces*) was

isolated from the soil sample. A distinct fungal isolate was successfully obtained and purified through

repeated subculturing. Further, morphological characterization was done by using Lactophenol Cotton Blue. The isolate exhibited visible colony morphology characterized by white cottony often with lighter or white hyphal margins. The fungal colony demonstrated growth under low-temperature conditions, suggesting adaptation to cold environmental conditions.

The purified isolate was maintained on CYGA slants for further characterization and downstream bioprospecting studies, including antimicrobial, antioxidant, molecular identification, and metabolite profiling analyses.

3.2 Morphological characterization

The fungal isolate exhibited rapid growth on Chloramphenicol Yeast Glucose Agar (CYGA) at $20 \pm 2^\circ\text{C}$ forming dense, cottony (floccose) colonies with abundant white aerial mycelium. The colony surface was uniformly white to creamy white, lacking visible diffusible pigmentation into the surrounding medium. The colony margin was irregular, and adjacent colonies coalesced with continued incubation. The texture and rapid mycelial coverage are consistent with fast-growing filamentous fungi commonly isolated from cold-soil environments.

Table 1: Morphological characteristics of the Antarctic fungal isolate *Pseudogymnoascus* sp.

Characteristic	Observation
Colony colour	White to off-white/creamy
Colony texture	Cottony to floccose (fluffy, wool-like aerial mycelium)
Colony margin	Irregular, slightly undulating; colonies appear to be coalescing across the plate
Colony elevation	Raised, with dense aerial mycelium
Growth pattern	Rapid, dense radial growth covering most of the plate surface

Characteristic	Observation
Sporulation	powdery texture suggesting sporulation
Pigment diffusion	No obvious diffusible pigment visible in the medium
Exudate droplets	Not observed



Figure 1: Macroscopic colony morphology (obverse view) of *Pseudogymnoascus* sp. cultured on Chloramphenicol Yeast Glucose Agar (CYGA) after 7 days of incubation at $20 \pm 2^\circ\text{C}$, showing White to off-white/creamy, powdery colonies with sporulation.

3.3 Extraction

Concentration of this fraction to dryness using a rotary evaporator yielded approximately 100 mg of crude methanolic extract (PMeOH), corresponding to a crude extract recovery of approximately 0.67 mg/mL of methanolic supernatant.

Sequential solvent extraction of the cultivated fungal culture using solvents of different polarities resulted in three crude metabolite fractions: methanol (PMeOH), ethyl acetate (PEtOAc), and hexane (PHex). Approximately 150 mL of each solvent fraction was recovered and concentrated to dryness using a rotary evaporator.

The methanolic fraction (PMeOH) yielded approximately 100 mg of crude extract, followed

by 90 mg for the ethyl acetate fraction (PEtOAc) and 80 mg for the hexane fraction (PHex). Based on the volume of solvent fraction recovered, the corresponding crude extract recoveries were 0.67, 0.60, and 0.53 mg/mL for PMeOH, PEtOAc, and PHex, respectively. Among the extracts obtained, the methanolic fraction (PMeOH) showed comparatively greater recovery of crude metabolites, whereas the ethyl acetate (PEtOAc) and hexane (PHex) fractions yielded comparatively different amounts of extract. The extract appeared as a dark brown residue and was subsequently dissolved in Dimethyl sulfoxide (DMSO) for antibacterial and antioxidant.

Table 2: Recovery of crude fungal metabolite extracts obtained by sequential solvent extraction.

Fraction	Solvent volume	Crude extract obtained	Recovery
PMeOH	~150	~100mg	~0.67mg/ml
PEtOAc	~150	~92mg	~0.61mg/ml
PHex	~150	~86mg	~0.57mg/ml

PMeOH, methanolic fraction; PEtOAc, ethyl acetate fraction; PHex, hexane fraction. Crude extract recovery was calculated as the mass of dried crude extract obtained per volume of solvent

used (mg/mL). Values represent approximate recovery based on the measured solvent volume and crude extract mass.

3.4 Antibacterial Activity

Among the three fungal extracts tested, the methanolic extract (PMeOH) exhibited antibacterial activity, whereas the ethyl acetate (PEtOAc) and hexane (PHex) extracts did not

produce detectable zones of inhibition against any of the tested bacterial strains. The highest antibacterial activity of PMeOH was observed against *E. coli* MTCC 739, with a ZOI of 17.32 ± 0.6 mm, followed by *S. aureus* MTCC 96 (16.4 ± 1.1 mm) and *B. cereus* MTCC 430 (12.4 ± 0.9 mm). No inhibition was observed against *P. aeruginosa* MTCC 2453, *K. pneumoniae* MTCC 3384, *E. faecalis* MTCC 439, *M. luteus* MTCC 2470, or MDR *E. coli* DDWI-01. None of the solvent controls showed inhibition, confirming the observed activity was originated from the fungal metabolites.

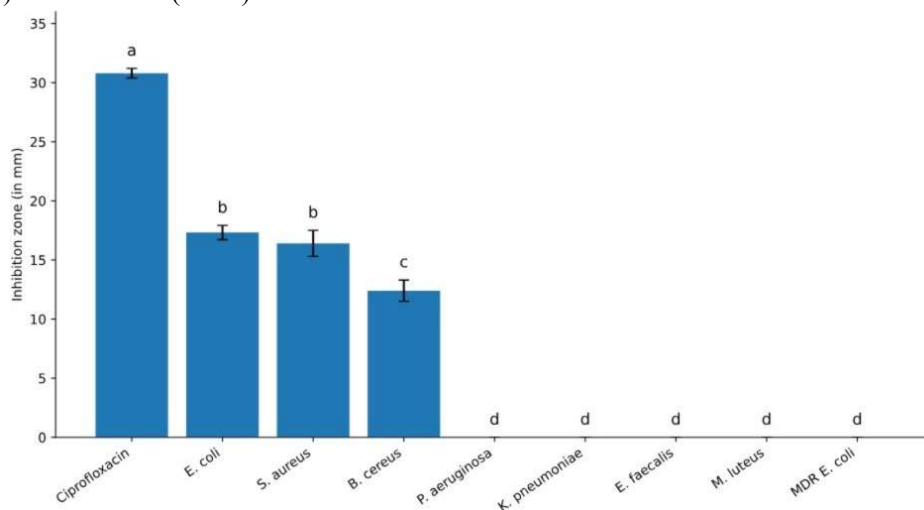


Figure 2: Antibacterial activity of methanolic (PMeOH), ethyl acetate (PEtOAc), and hexane (PHex) extracts of Antarctic *Pseudogymnoascus* sp. against selected bacterial strains.

3.4.1 Minimum Inhibitory Concentration (MIC)

Methanolic extract showed the lowest MIC-2560 μ g/mL against *Escherichia coli* MTCC 739

among the tested microorganisms as shown in Table 3.

Table 3: Minimum Inhibitory Concentration of active extract of Antarctic *Pseudogymnoascus* sp. against bacteria

Extract	Test organism	10	20	40	80	160	320	640	1280	2560	MIC μ g/ml
PMeOH	<i>Staphylococcus aureus</i> MTCC 96	+	+	+	+	+	+	+	+	+	>2560
	<i>Escherichia coli</i> MTCC 739	+	+	+	+	+	+	+	+	-	2560
	<i>Bacillus cereus</i> MTCC 430	+	+	+	+	+	+	+	+	+	>2560

+ (Growth observed) – (No growth observed); *Pseudogymnoascus* methanolic extract

3.5 Antioxidant Potential

The antioxidant potential of the fungal extracts was evaluated using the DPPH radical scavenging assay. The extract was diluted from a stock solution (2560 µg/mL to 10 µg/mL) to calculate the antioxidant potential. The extracts demonstrated varying degrees of antioxidant activity depending on the solvent used for extraction. Among the tested solvent fractions, the methanolic extract (PMeOH) exhibited the

highest DPPH radical scavenging activity. The AP in terms of % DPPH free radical scavenging activity was recorded in the range of $4.54 \pm 0.8\%$ at 10 µg/mL to $60.32 \pm 1.4\%$ at 2560 µg/mL. The PEtOAc and PHex extracts exhibited comparatively lower antioxidant activity. No detectable DPPH radical-scavenging activity was observed for either extract at concentrations from 10 to 640 µg/mL. At 1280 µg/mL, PEtOAc and PHex exhibited $3.43 \pm 0.7\%$ and $2.76 \pm 0.4\%$ inhibition, respectively, which increased to $12.32 \pm 1.2\%$ and $8.32 \pm 1.3\%$, respectively, at 2560 µg/mL shown in a Figure 3.

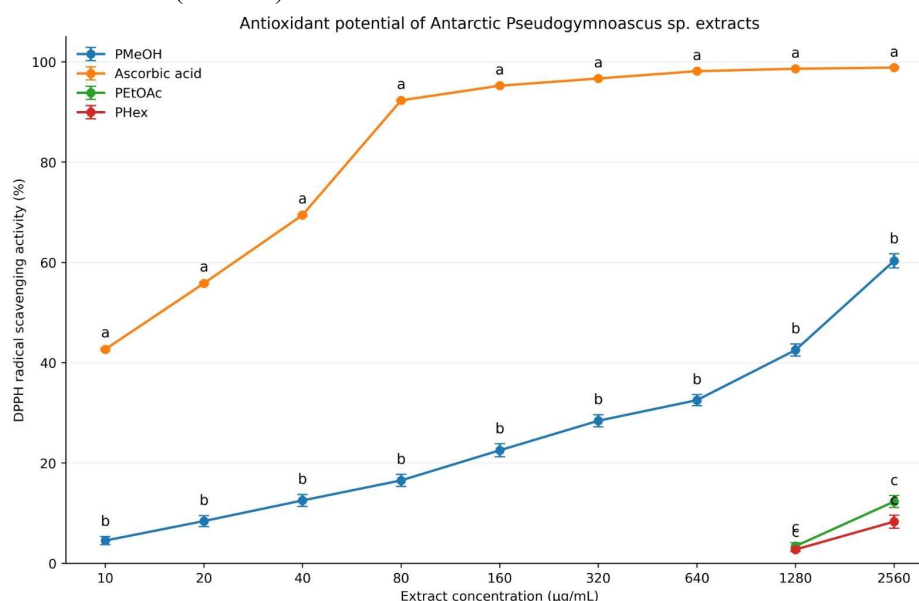


Figure 3: DPPH radical-scavenging activity of methanolic (PMeOH), ethyl acetate (PEtOAc), and hexane (PHex) extracts of Antarctic *Pseudogymnoascus* sp. at different concentrations. Ascorbic acid was used as the positive control. Data is presented as mean $\pm$ SD. Different lowercase letters indicate statistically significant differences among treatments ($p < 0.05$).

4. Discussion

Antarctic terrestrial environments represent important reservoirs of cold-adapted microorganisms because the extreme environmental conditions prevailing in these habitats impose strong selective pressures on microbial communities. *Pseudogymnoascus* sp. is among the most widespread fungi, inhabiting

diverse environments worldwide and exerting significant economic and health impacts. In Antarctica, they are commonly found in soil, snow, ice, seawater, marine and freshwater sediments, lakes, plants, and animals (Santiago et al., 2015; Gomes et al., 2018; Rosa et al., 2019; Ogaki et al., 2020; Rosa et al., 2020). The identification of the fungal isolate as

Pseudogymnoascus is consistent with previous reports describing this genus as an important component of the culturable fungal community in Antarctic terrestrial environments. Durán et al., (2019) recovered 54 culturable fungal strains representing 19 genera from soils collected across several Antarctic locations, including King George Island, Deception Island and Robert Island. Notably, *Pseudogymnoascus* was among the genera recovered, while a substantial proportion of the isolates exhibited psychrophilic or psychrotrophic growth characteristics, highlighting the prevalence of fungi adapted to low-temperature environments. The ecological relevance of *Pseudogymnoascus* in Antarctic soils is further supported by Santos et al., (2020) who reported *Pseudogymnoascus* as one of the most abundant fungal genera recovered from soils associated with the retreating Collins Glacier on King George Island, indicating its capacity to persist under the harsh environmental conditions prevailing in Antarctic soils. More recently, Childress et al. (2025) described three new *Pseudogymnoascus* species from Antarctic soils and demonstrated that Antarctic populations encompass previously undescribed phylogenetic diversity. The widespread occurrence of *Pseudogymnoascus* across different Antarctic soil environments may be associated with its adaptation to low temperatures and other environmental stresses characteristic of polar habitats. In the present study, the recovery of *Pseudogymnoascus* from Antarctic soil further supports its ecological significance in cold terrestrial ecosystems (Villanueva et al., 2021).

The dominance of *Pseudogymnoascus* isolates in this study matches earlier research on Antarctic permafrost and active layer soils, which found *Geomyces* to be one of the most common fungal genera. The common presence of this genus in Antarctic ecosystems shows how well it adapts to harsh environments. This also highlights its potential as a source of biologically active secondary metabolites (Da Silva et al., 2020).

The antibacterial screening demonstrated that the metabolites produced by the investigated *Pseudogymnoascus* isolate possess inhibitory activity against several of the bacterial strains evaluated, with the methanolic extract showing the most pronounced activity. Shi et al. (2021) reported antibacterial activity of metabolites isolated from *Pseudogymnoascus* sp. HSX2#-11 against *S. aureus*, *Aeromonas salmonicida* including activity associated with a newly identified polyketide, pseudophenone A, and a benzoic acid derivative. In addition, pannomycin isolated from *P. pannorum* MF7016 demonstrated antibacterial activity against *S. aureus*, *Enterococcus faecalis* and *Bacillus subtilis*. More recently, four Antarctic *Pseudogymnoascus* isolates obtained from Collins Glacier were reported to inhibit *S. aureus* ATCC 6538, with the active compounds including asterric acid, violaceol, mollicellin, psegynamide A, diorcinol, and thailandolide A, further demonstrating the capacity of this genus to produce antibacterial metabolites. (Gomes et al., 2018) reported *Pseudogymnoascus* sp. F09-T18-1 showed activity against both Gram-positive and Gram-negative bacteria, including *S. aureus*, *Pseudomonas*

aeruginosa, *Acinetobacter baumannii* and *Escherichia coli*. A crude extract from an Antarctic *Pseudogymnoascus* isolate effectively suppressed *Xanthomonas citri*, and the active metabolite, bisdechlorogedin, was shown to inhibit bacterial growth by disrupting the cell membrane. These findings suggest that the antimicrobial activity of Antarctic fungal extracts is frequently associated with structurally diverse secondary metabolites that target essential cellular processes (Ferrarezi et al., 2024). Similar antibacterial activity has been reported for Antarctic *Pseudogymnoascus* isolates, whose extracellular pigment extracts inhibited *Staphylococcus aureus*. These observations support the susceptibility of Gram-positive bacteria to metabolites produced by Antarctic fungi (Cavalcante et al., 2024). The antioxidant enzyme activity observed in this study matches findings from Antarctic fungal isolates reported by (Tosi et al., 2010). They found that six out of thirty strains had high SOD and CAT activity. This may mean that making antioxidant enzymes helps Antarctic fungi adapt to cold environments.

Antarctic fungi continue to yield structurally unique secondary metabolites. For example, novel nitroasteric acid derivatives (pseudogymnoascins A–C and 3-nitroasteric acid) were isolated from an Antarctic *Pseudogymnoascus* species associated with a marine sponge. Although these compounds did not exhibit antibacterial or antifungal activity against the microorganisms tested, their discovery demonstrates the remarkable biosynthetic diversity of Antarctic fungi and highlights the importance of continued

exploration of these organisms for new natural products (Figueroa et al., 2015). The pronounced antibacterial activity observed against *S. aureus* in the present study is consistent with previous investigations demonstrating the antimicrobial potential of Antarctic fungi. Brunati et al. (2009) reported antimicrobial activity in 47 filamentous fungal isolates obtained from Antarctic lake microbial mats, with *S. aureus* representing one of the most frequently inhibited test organisms. The antibacterial activity observed in the present study supports the potential of extremophilic fungi as sources of antimicrobial metabolites. Fungi inhabiting extreme environments are exposed to persistent environmental stresses that may influence their secondary metabolism and promote the production of compounds with antimicrobial properties (Dishliyska et al., 2025).

5. Conclusion

The present study highlights the biotechnological potential of *Pseudogymnoascus* sp. isolated from Antarctic soil. The fungal isolate produced metabolites that showed antibacterial and antioxidant properties, indicating its ability to produce biologically active compounds under laboratory conditions. Among the solvent extracts evaluated, the methanolic extract showed better biological activity compared with the ethyl acetate and hexane extracts. The observed antibacterial activity against selected bacterial strains and the antioxidant response further support the potential of this Antarctic fungus as a source of bioactive metabolites. The findings also demonstrate the value of exploring Antarctic microorganisms for natural products with possible pharmaceutical

and biotechnological relevance. However, the activities observed in the present study are based on crude fungal extracts, and the compounds responsible for these effects have not yet been identified. Further investigations involving detailed chemical profiling, purification, and structural characterization of the active metabolites are required. Evaluation of the purified compounds using broader biological assays will also be important to understand their mechanism of action and application potential. Thus, the investigated *Pseudogymnoascus* isolate represents a promising candidate for further natural-product research and Antarctic microbial bioprospecting.

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