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Screening of antibacterial and antioxidant activities of marine fungi isolated from the North Sea of Vietnam

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ABSTRACT

Marine fungi are recognized as a rich source of pharmacologically active secondary metabolites, different from terrestrial fungi. This study aimed to assess the antimicrobial and antioxidant activities of 61 marine fungal strains isolated from 34 samples collected in the research journey of the Akademik Oparin vessel in the North Sea of Vietnam in 2021. Of these, 23 were from sponges, 21 from seaweeds, 15 from sediments, and only 2 from seawater. The antimicrobial screening results showed that 75.4% (n = 46) of fungal strains exhibited inhibitory activity against at least one test microorganism. The antioxidative properties results indicated the capacity for free DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging of crude extracts with 32.7% (n = 20) and 93.4% (n = 57), respectively. Six isolates with potent antibiotic and antioxidant activities were identified as belonging to the phylum Ascomycota and affiliated with five genera. Our findings indicated that these marine fungi could be impressive sources of multi-functional bioactive compounds and may find applications in the pharmaceutical industry.

Keywords: ABTS, antimicrobial, antioxidant, DPPH, marine fungi.

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INTRODUCTION

The marine environment is the habitat of several organisms that live in complex conditions with extreme variations in pressure, salinity, light, and temperature [1]. The species diversity of marine organisms and their complex living environment enables them to produce novel and unique secondary metabolites with much more potent bioactivities than their terrestrial counterparts [2]. Recently, it has been proven that the marine environment possesses many unique forms of fungi. Marine fungi are widely distributed from shallow water to the deep sea, even to polar ice covers. They are present in various marine substrates, including inorganic matter, microbial communities, plants, invertebrates, and vertebrates [3]. Therefore, marine fungi are a potential source for producing natural compounds with novel structures and various biological activities [4, 5].

The search for new compounds from the marine environment with promising biomedical applications has continually increased. Approximately 100 new compounds produced by marine fungi were described from 2000 to 2005, and 690 metabolites were discovered by 2010. In 2016, 1,277 new marine compounds were reported, of which 36% were from marine fungi, and this figure keeps growing [6]. Many of these metabolites possess novel types and have the potential to be developed as therapeutic agents [7, 8]. The discovery of several new marine fungal taxa with unique metabolic activity and produce novel compounds with various biological activities clearly illustrate that marine fungi are a potential source of novel secondary metabolites.

Vietnam has over 3,600 kilometres of coastline with unique ecosystems such as mangroves, mudflats, coral reefs, bays, lagoons, and estuaries. Thus, the marine environment in Vietnam can be an excellent bio-resource for undiscovered bioactive compounds. Purposing to assess the potential of biodiversity and marine research biochemistry in Vietnam, the Vietnam Academy of Science and Technology has cooperated with the Far Eastern Branch of the Russian Academy of Sciences toward deploying the survey and sample collection onboard Akademic Oparin from 2005 to now. In recent years, numerous studies have shown that Vietnamese marine-derived fungi are one of the significant marine sources of bioactive compounds. Research conducted about the fungal strain Aspergillus flocculosus 01NT.1.1.5 isolated from a sponge Stylissa sp. collected in Nha Trang bay led to the discovery of aspertetranone D, wasabidienone E, and mactanamide showed effective inhibitory activity against osteoclasts [9]. Le et al., (2019) reported that the fungus *Penicillium* sp. M30 was derived from a marine sediment sample collected at a depth of 14 m sea at the Co To island and had significant antimicrobial, α-glucosidase, and α-amylase inhibitory activities [10]. A chemical investigation of the fungal strain *Ascomycota* sp. VK12 was isolated from marine sponge collected from the Quang Nam sea and yielded compounds (3R)-(3,5-dihydroxy phenyl)butan-2-one and AGI-7 showed cytotoxicity toward HepG2, MCF-7, and SKMel2 carcinoma cells [11]. Due to the increasing demand for new antibiotics mechanism of action, antibacterial activity is one of the looks of the properties of marine fungi [12, 13]. Besides, recent studies have confirmed marine fungi are a valuable source of antioxidant compounds with potent biological activity and various structural features, promising sources of novel bioactive compounds [14, 15]. Hence, in this study, we evaluated the potential antibiotic and antioxidant activities of marine fungi isolated during the field survey on the Akademik Oparin vessel in 2021. These results were beneficial information on the bioactivities of marine fungi from the North Sea of Vietnam for further study.

MATERIALS AND METHODS

Sampling collection

Marine samples (sponges, seaweeds, sediments, and seawater) were collected on the various islands at the water depth ranging from 8–15 m in the research journey of the

Akademik Oparin vessel in the North Sea of Vietnam in May 2021 (Table 1). Samples were placed in plastic bags, kept in an ice box, and transported to the laboratory to isolate fungi.

Table 1. A list of sampling sites, marine sources, and fungal strains

Collected sites	Marine samples	Fungal strains
Ba Trai Dao - Hai Phong (20.79199°N-107.09895°E)	Seaweed	2105OF.21.2
Bach Long Vi - Hai Phong (20.14741°N-107.73177°E)	Sponge	2105OF.19.2, 2105OF.19.3, 2105OF.20.1, 2105OF.20.2
Co To Con - Quang Ninh (21.05150°N-107.77650°E)	Seaweed	2105OF.1.3
	Sea water	2105OF.2.1, 2105OF.2.5
	Sediment	2105OF.3.1
	Sponge	2105OF.4.1, 2105OF.4.3, 2105OF.4.5, 2105OF.4.6
Thanh Mai - Quang Ninh (21.03810°N-107.82365°E)	Sediment	2105OF.28.1
	Seaweed	2105OF.29.1, 2105OF.29.2
	Sponge	2105OF.31.2, 2105OF.32.1
Hon Mam Xoi - Quang Ninh (21.03313°N-107.79440°E)	Sediment	2105OF.50.1
Dao Tran - Quang Ninh (21.24189°N-107.95122°E)	Seaweed	2105OF.23.3, 2105OF.24.3, 2105OF.25.6
Nhan Trach - Quang Binh (17.54553°N-106.60096°E)	Sediment	2105OF.10.3, 2105OF.10.4
	Sponge	2105OF.11.3, 2105OF.11.7
Hon Gio - Quang Binh (17.91201°N-106.67337°E)	Sediment	2105OF.12.2, 2105OF.13.6, 2105OF.13.8, 2105OF.13.9
	Sponge	2105OF.15.1, 2105OF.15.2, 2105OF.15.4
Con Co - Quang Tri (17.16059°N-107.33080°E)	Sponge	2105OF.17.1, 2105OF.17.3, 2105OF.17.4, 2105OF.17.5, 2105OF.18.5
Hon Son Cha - Hue (16.21434°N-108.18128°E)	Seaweed	2105OF.6.1, 21105OF.6.2, 2105OF.6.8, 2105OF.6.9
	Sediment	2105OF.7.4, 2105OF.7.5
Bai Ca - Hue (16.21401°N-108.11918°E)	Sediment	2105OF.8.1, 2105OF.8.5, 2105OF.8.6, 2105OF.8.7
	Sponge	2105OF.9.4
Hon Mo - Quang Nam (15.93367°N-108.47440°E)	Seaweed	2105OF.51.2, 2105OF.51.3
	Sediment	2105OF.52.1
Hon Dai - Quang Nam (15.94565°N-108.47782°E)	Sediment	2105OF.49.2, 2105OF.49.4
Ly Son - Quang Ngai (15.39679°N-109.11111°E)	Seaweed	2105OF.35.1, 2105OF.35.5, 2105OF.38.1, 2105OF.38.2, 2105OF.41.2, 2105OF.44.1, 2105OF.45.2

Isolation of marine derived fungi

Sabouraud medium (peptone 10 g, glucose 40 g, agar 18 g dissolved in 1,000 mL natural seawater, pH 6.0–7.0) supplemented with amoxicillin (1 mg/mL) was used to isolate fungi. Sponge and seaweed samples were rinsed with sterile seawater to remove non-attached particles. The samples (10 g) were cut into 0.5 cm × 0.5 cm pieces and ground with 9 mL sterile seawater, then spread on Petri dishes containing Sabouraud medium. For sediment samples, 1 g of sample was diluted in 9 mL sterile seawater in a test tube and spread on an agar plate with a Sabouraud medium. 0.1 mL of seawater was applied directly on a medium plate for the seawater sample. After incubation at 28°C for 5–7 days, all colonies

with different pigmentation and morphology were picked out by hyphal tip isolation and transferred to Sabouraud plates without antibiotics to obtain pure strain [16]. The fungal isolates were stored in 30% glycerol at -80°C at Nha Trang Institute of Technology Research and Application.

Fungal fermentation and extraction

Small-scale fermentation of the isolated fungal strains was carried out on a solid rice medium. Biomass of the isolates from Sabouraud plates was transferred to 500 mL Erlenmeyer flasks, each containing 40 mL of natural seawater (pH 8.0, salinity of 30 g/L) supplemented with 20 g of rice, 20 mg of yeast extract, 10 mg of KH_2PO_4 [17]. The cultures were incubated at room temperature for three weeks. After incubation, the mycelia and medium were homogenized and extracted twice with equal volumes of ethyl acetate. The ethyl acetate extracts were concentrated in vacuo and used for screening biological activities.

Antibacterial screening

All crude extracts were screened for antibacterial activity using a paper disc diffusion assay [18]. The crude extracts were dissolved in ethyl acetate, loaded onto 6 mm diameter sterile Whatman No1 paper discs at a 100 µg/disc concentration and allowed to dry for solvent evaporation. Seven different test organisms were used in the assay, including three Gram-positive bacteria (*Bacillus cereus* ATCC 11778, *Streptococcus faecalis* ATCC 19433, *Staphylococcus aureus* ATCC 25923), and four Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028). The clinical pathogens were purchased from ATCC (Manassas, USA), stored at Nhatrang Institute of Technology Research and Application and used as test microorganisms due to their clinical importance.

The test microorganisms were grown on Mueller Hinton Agar media, and the turbidity of microbe suspensions was adjusted to 10^8 cells/mL using a spectrophotometer at a wavelength of 625 nm. The plates were incubated at 37°C for 24 h. The results were recorded as a diameter in millimeters of the zone of inhibition. Standard streptomycin discs (10 µg/disc) were a positive control, and discs containing only solvent (ethyl acetate) were the negative control.

Antioxidant screening

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The DPPH test is based on the ability of the stable 2,2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors and change from purple to yellow. The analytical procedure was performed using modified methods proposed by Lee et al., [19]. Briefly, 100 µL DPPH solution (0.1 mM) was mixed with 100 µL of the sample (250 µg/mL) in 96-well plates. The reaction was allowed to take place at room temperature in the dark for 30 min. After incubation, the absorbance was measured at 517 nm using an ELISA reader. The positive DPPH free radical scavengers standards were ascorbic acid at 200 µg/mL, and 100% methanol was used as a control. The percentage of DPPH radical scavenging activity was calculated as:

$$Radical\ scavenging\,(\%) = \left[\frac{A_c - A_s}{A_c}\right] \times 100$$

where: A_c and A_s were the absorbance at 517 nm of the control and sample, respectively. The experiment was conducted in triplicates.

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) assay

The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted 1:100 in methanol. 100 µL ABTS reagent was mixed with 100 µL of the sample (250 µg/mL) in 96-well plates and was incubated at room temperature for 6 min to

determine the scavenging activity. After incubation, the absorbance was measured at 734 nm using an ELISA reader [19]. The control was 100% methanol; the standard samples used were ascorbic acid (200 µg/mL). The ABTS scavenging effect was measured using the following formula:

$$Radical\ scavenging(\%) = \left[\frac{A_c - A_s}{A_c}\right] \times 100$$

where: A_c and A_s were the absorbance at 734 nm of the control and sample, respectively. The experiment was conducted in triplicates.

Identification of marine fungi

The selected fungi were identified based on sequence analysis of the ITS region of ribosomal DNA-ITS1-5.8S-ITS2. Genomic DNA was isolated following the protocol proposed by Fredricks et al., [20]. The nuclear ribosomal DNA of the fungal isolate was amplified using the forward primer, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3'), and the reverse primer, ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [21]. Sequences of fungal ITS-rDNA regions were compared with those in the NCBI (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Isolation of marine fungi

Totality 61 fungal strains were isolated from various marine samples encompassing sponges, seaweeds, sediments, and seawater. Among 61 isolates, 23 were from sponges, 21 from seaweeds, 15 from sediments, and only 2 from seawater (Table 1). The isolation frequencies of marine fungi depended on the host, where the highest isolation frequencies were obtained from sponges and seaweeds with 37% and 34%, respectively.

Most studies on marine fungi are associated with marine sediments, with specific substrates like driftwood, algae, corals, and mainly sponges [22]. According to Xu et al., [23], algae and sponges are the most common materials for fungal isolation. Close relations between marine fungi and the host, including symbiotic associations and interactions during sporulation settlement, have been studied that provide insights into the regulation of host symbiotic microbial community interactions. They can colonize inside tissues and organs without causing symptoms or injury to the host. The host protects and gives nutrients to the fungi that protect their host plants from external biotic and abiotic stresses [24]. Because of the specific interactions between the fungi and the algae or sponges, fungi are prospected to produce compounds with novel or notable skeletons [23].

Additionally, sediments are also materials for the isolation of potent fungi. In this study, the isolation rate of marine fungi from sediments was 24%. Sediment-derived fungi inhabit marine sediments in extreme sea environments with sunlight irradiation absent, predominantly low temperature, high hydrostatic pressure, and oligotrophy [25]. A wide variety of inhabitants of marine sediments makes this ecosystem highly competitive, affecting organisms' metabolism [26]. So far, the diversity and composition of marine sedimentary fungi have been reported by many studies, suggesting that these marine sediments harbor high numbers of fungal taxa [27–29].

The isolated fungal strains will be evaluated by screening biological activities to select potential marine-derived fungi for further investigation.

Antibacterial activity of isolated marine fungi

The antimicrobial activity of crude fungal extracts was tested against seven different pathogens. Of the 61 isolates, 46 fungal strains exhibited antibacterial activity to at least one test microorganism. Our study revealed that these marine fungi exhibited a different spectrum of inhibitory activity against pathogens. The results showed that 70.5% (n = 43) of isolates displayed antibacterial activity against *S. aureus*, 65.5%

(n = 40) against *B. cereus*, and 59% (n = 36) against *S. faecalis*. Meanwhile, 62.3% (n = 38) of fungal strains displayed activity against *K. pneumoniae*, only 9.8% (n = 6), 4.9% (n = 3), and 1.6% (n = 1) against *E. coli*, *S. typhimurium*, and *P. aeruginosa*, respectively (Figure 1).

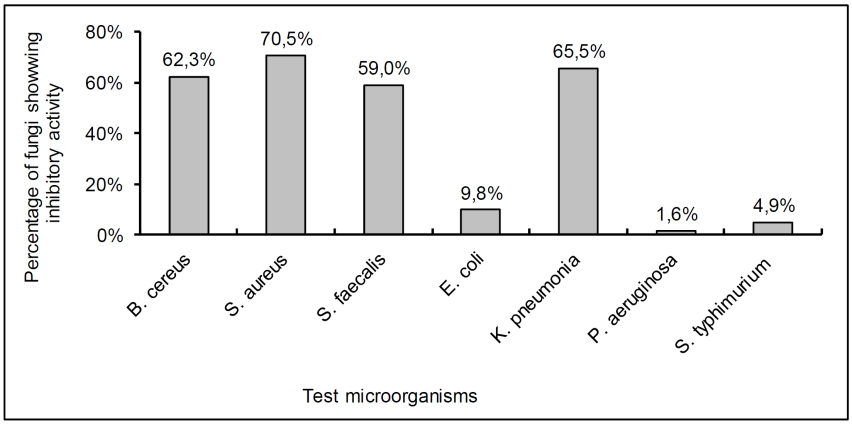


Figure 1. Percentage inhibition of fungal extracts against pathogens

Table 2. Antimicrobial activity of isolated marine fungi against at least 4 pathogens tested

Order	Fungal strains	Zone of inhibiton (mm)						
		BC	SA	SF	EC	KP	PA	ST
1	2105OF.1.3	11	10	10	-	12	-	-
2	2105OF.3.1	10	11	-	11	12	-	-
3	2105OF.4.5	23	21	20	-	20	-	-
4	2105OF.4.6	18	38	24	-	35	-	-
5	2105OF.6.1	11	14	8	-	11	-	-
6	2105OF.8.1	12	16	12	-	12	-	-
7	2105OF.8.6	12	14	11	-	11	-	-
8	2105OF.8.7	14	11	14	-	12	-	-
9	2105OF.10.3	12	14	14	-	11	-	-
10	2105OF.10.4	13	11	10	-	12	-	-
11	2105OF.11.7	15	11	10	9	11	-	-
12	2105OF.13.6	13	11	13	-	11	-	-
13	2105OF.13.9	13	12	12	-	11	-	-
14	2105OF.15.1	14	13	13	-	12	-	-
15	2105OF.15.4	11	13	14	-	12	-	-
16	2105OF.19.3	12	13	9	-	11	-	-
17	2105OF.21.2	11	8	11	-	13	-	-
18	2105OF.23.3	18	34	16	-	20	-	-
19	2105OF.24.3	32	32	42	30	32	11	35
20	2105OF.29.2	21	23	18	18	20	-	11
21	2105OF.35.5	20	16	20	-	20	-	-
22	2105OF.44.1	14	16	12	-	11	-	-
23	2105OF.49.2	8	15	11	-	10	-	-
24	2105OF.50.1	11	12	12	-	12	-	-
25	2105OF.52.1	30	24	28	7	25	-	7

Notes: BC- *Bacillus cereus* ATCC 11778; SA- *Staphylococcus aureus* ATCC 25923; SF- *Streptococcus faecalis* ATCC 19433; EC- *Escherichia coli* ATCC 25922; KP- *Klebsiella pneumoniae* ATCC 700603; PA- *Pseudomonas aeruginosa* ATCC 27853; ST- *Salmonella typhimurium* ATCC 14028; "-": no inhibition.

The number of fungal strains exhibiting a broad antibacterial spectrum (resistant to 4/7 reference strains) accounted for 40.9% (*n* = 25) (Table 2). Among the potent extracts, 2105OF.52.1 and 2105OF.29.2 displayed strong inhibitory ability on the growth of six pathogens. Notably, isolate 2105OF.24.3 exhibited significant activity against all the test microorganisms (Figure 2). In general, fungal extracts were active against Gram-positive bacteria better than Gram-negative ones. A difference in the antimicrobial ability has based on the difference in the cell wall structure. Gram-negative bacteria have lipopolysaccharide in the cell wall as additional protection from antibiotic influences [30–32]. However, many fungal isolates have remarkable activity against *K. pneumonia* (65.5%). The marine fungi isolated from Antarctic sponges also showed antibacterial activity toward *Xanthomonas campestris* (Gram-negative bacterium) higher than *Clavibacter michiganensis* (Gram-positive bacterium) [33]. Differences in the ability of marine fungi to inhibit the growth of microbes may be reasoned by sensitivity to antimicrobial compounds contained in extracts.

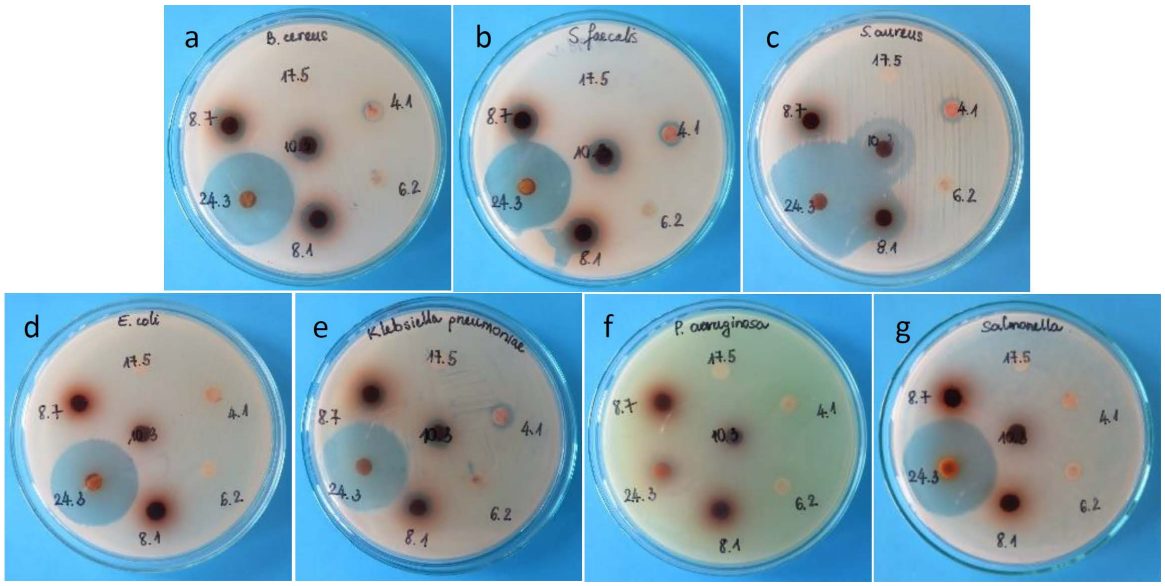


Figure 2. Antibacterial activity of the fungus 2105OF.24.3 against pathogens. (a) *B. cereus*; (b) *S. faecalis;* (c) *S. aureus*; (d) *E. coli*; (e) *K. pneumoniae*; (f) *P. aeruginosa*; (g) *S. Typhimurium*

Several investigations on the antimicrobial efficacy of marine fungi in Vietnam had conducted. According to our previous study, among 73 fungal strains obtained from Son Tra peninsula, and Da Nang, 29 fungal extracts displayed antimicrobial activity against at least two test pathogens. The proportion of the fungal isolates having antimicrobial activity against *B. cereus*, *S. faecalis*, *L. monocytogenes*, *S. aureus*, *E. coli*, *C. albicans*, and *P. aeruginosa* were 42, 33, 31, 22, 7, 5, and 3%, respectively [12]. Moreover, marine fungal strains isolated from sediments in Co To island - Quang Ninh Province also exhibited great antimicrobial activity against reference pathogens [13]. Thus, these results suggest that Vietnam marine fungi have a broad spectrum of antibacterial properties and need further investigation.

Antioxidative activity

The potential free radical scavenging activity of the isolated fungi had evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-amino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS•+) assays. All 61 fungal extracts showed antioxidant activity up to a varying extent.

For the DPPH radical scavenging capacity, twenty fungal extracts showed significant antioxidant activity with SC% values ranging from 50% to 70%. The tested extracts exhibited excellent free radical scavenging properties against ABTS cation radical if compared to DPPH scavenging potential. More than 93.4% (n = 57) of the tested fungal strains recorded high activity in the ABTS assay. Most extracts exhibited ABTS free radical scavenging ability with SC% values > 60% (Table 3). Different results between tested methods caused unequal structure and mechanism of action of both molecules as ABTS cation radical as DPPH radical. The DPPH method has based on the scavenging ability of the free radical DPPH to present a hydrogen-donating antioxidant due to formatting the non-radical form DPPH-H and its color changed from purple to yellow. The antioxidant effect had evaluated by observing the decrease in VIS absorption at 515 nm. This analysis is one of the tests used to prove the ability of the components of the fungal extract to act as donors of hydrogen atoms. In the ABTS test, ABTS is converted into its radical cation (ABTS•+) by adding sodium persulphate. This blue-green radical cation absorbs light at 734 nm. ABTS•+ is reactive toward most antioxidants [34].

Table 3. Antioxidant activity of isolated marine fungi (SC% > 50%)

Order	Fungal strains	Radical scavenging activity (SC%) (250 µg/mL)	
		DPPH	ABTS
1	2105OF.6.8	57.54 ± 0.22	71.94 ± 0.20
2	2105OF.7.4	69.21 ± 0.58	73.22 ± 1.61
3	2105OF.8.6	55.20 ± 0.50	70.00 ± 1.09
4	2105OF.8.7	50.75 ± 2.56	71.29 ± 0.19
5	2105OF.10.3	54.14 ± 0.79	73.87 ± 0.22
6	2105OF.10.4	54.35 ± 0.49	71.60 ± 2.08
7	2105OF.11.3	51.59 ± 0.16	71.29 ± 0.19
8	2105OF.13.8	50.11 ± 0.15	70.65 ± 2.01
9	2105OF.13.9	51.17 ± 0.44	71.94 ± 0.20
10	2105OF.15.1	53.93 ± 0.11	70.00 ± 0.18
11	2105OF.17.1	65.60 ± 0.91	72.58 ± 0.21
12	2105OF.17.3	69.21 ± 0.02	71.62 ± 1.57
13	2105OF.19.3	54.78 ± 0.11	69.03 ± 0.28
14	2105OF.24.3	50.74 ± 0.44	68.07 ± 0.16
15	2105OF.28.1	57.75 ± 0.08	61.94 ± 0.57
16	2105OF.29.2	55.84 ± 0.40	69.36 ± 0.18
17	2105OF.31.2	65.39 ± 0.01	71.29 ± 0.19
18	2105OF.35.5	55.84 ± 0.20	70.00 ± 0.73
19	2105OF.49.2	52.44 ± 0.43	72.25 ± 1.17
20	2105OF.51.2	61.14 ± 0.65	73.55 ± 0.24
21	2105OF.51.3	66.03 ± 0.31	72.58 ± 0.71
22	2105OF.52.1	61.36 ± 0.25	72.90 ± 1.16
23	Ascorbic acid	80.01 ± 0.35	82.55 ± 0.42

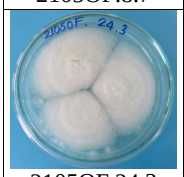
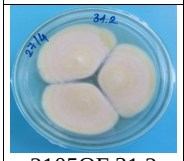
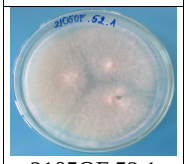
According to our previous investigation, crude extracts from marine fungal strains obtained from Nha Trang bay could reduce DPPH and ABTS free radicals with a

proportion of 61.5% and 80.3%, respectively [14]. Interestingly, five antioxidant compounds such as ochraceopone F, aspertetranone D, cycloechinulin, wasabidienone E, and mactanamide were found in the sponge-derived fungus *Aspergillus flocculosus* from Nha Trang bay [15].

Similar results had recorded by Zhou et al., [35] that the high proportion (84.8%) of the endophytic fungal strains isolated from the South China Sea displayed DPPH and ABTS radical scavenging activities. However, only 17.8% of fungal extracts isolated from the surface of marine invertebrates collected from Kepulauan Seribu Marine National Park in Indonesia exhibited a scavenging ability of DPPH free radicals [36]. These could explain that differences in the biological property of marine-derived fungi are related to the ecological system characteristics of the collected sampling area. Unique and stressful marine habitats affect fungal bioactivity [37].

Identification of the active fungi

Table 4. Identification of selected fungal strains

Fungal strains	Sources of isolation	Scientific name (GenBank accession number)	Closest identified relative	Precent identify
 2105OF.8.7	Sediment	*Penicillium* sp. (OP268180)	*Penicillium javanicum* CMV003F5 (MK450698) *Penicillium javanicum* CBS349 (MH856894) *Penicillium javanicum* CBS291 (MH857207)	99.86% 99.86% 99.19%
 2105OF.24.3	Seaweed	*Aspergillus* sp. (OP268175)	*Aspergillus sclerotiorum* CBS549 (OL772720) *Aspergillus sclerotiorum* NRRL 35202 (EF634389) *Aspergillus sclerotiorum* NRRL 415 (EF661400)	99.56% 99.61% 99.60%
 2105OF.29.2	Seaweed	*Pleosporales* sp. (OP268181)	*Pleosporales* sp. B1a0184PD2CC1074 (KP263116) *Pleosporales* sp. B1B064-2-EM2 (JQ388919) *Pleosporales* sp. P30043SNACC1007 (KP263094)	99.72% 99.58% 95.65%
 2105OF.31.2	Sponge	*Talaromyces* sp. (OP271285)	*Talaromyces macrosporus* CBS580 (MH860585) *Talaromyces macrosporus* CBS353 (MH860495) *Talaromyces macrosporus* CBS226 (MH860463)	99.89% 99.89% 98.97%
 2105OF.35.5	Seaweed	*Aspergillus terreus* (OP268179)	*Aspergillus terreus* DTO403-C9 (MT316343) *Aspergillus terreus* TN01 (JX188057) *Aspergillus terreus* CBS47265 (MW186717)	100% 100% 100%
 2105OF.52.1	Sediment	*Hypomontagnella monticulosa* (OP268183)	*Hypomontagnella monticulosa* Ta-BL161 (LC505293) *Hypomontagnella monticulosa* Ta-BL61 (LC505289) *Hypomontagnella monticulosa* MKC16 (MN427954)	99.47% 99.34% 99.33%

The biological screening showed that several marine fungal strains had promising bioactivities. Based on a combination of antimicrobial activity and free radical scavenging ability, six potential fungal isolates as 2105OF.8.7, 2105OF.24.3, 2105OF.29.2, 2105OF.31.2, 2105OF.35.5, and 2105OF.52.1 were identified as *Penicillium* sp., *Aspergillus* sp., *Pleosporales* sp., *Talaromyces* sp., *Aspergillus terreus*, and *Hypomontagnella monticulosa*, respectively (Table 4). Eurotiales (*Penicillium*, *Aspergillus*, and *Talaromyces*) previously had been described as producers of bioactive natural compounds [38]. To our best knowledge, this is the first report describing the isolation and bioactivities of the fungus *H. monticulosa* from sediment in the Vietnam Sea.

Natural products from fungi are considered a great source of novel antibacterial compounds because of their abundant fungal species diversity, their rich secondary metabolites, and the improvements in their genetic breeding and fermentation processes [23]. Most research on secondary metabolites of marine fungi has focused on a few genera: *Penicillium*, *Aspergillus*, *Fusarium*, and *Cladosporium* [24]. However, studies of natural products from marine fungi continue to increase and have expanded to other genera [22]. In recent years, various studies have focused on intensively investigating compounds with antibacterial activity, of which nearly 76% of new natural products are from marine-derived fungi [39]. Nowadays, as the resistance of bacterial and fungal pathogens has become increasingly significant, there is a growing demand for new antibacterial compounds.

Recent studies have demonstrated that an advance in free radicals and oxidants in the human body causes severe structural changes and functional alterations of cellular organelles and leads to various diseases [40]. Thus, antioxidant activity has become a hot topic and the subject of intensive investigation due to the ever-increasing demand by the food and pharmaceutical industries to develop natural bioactive anti-aging and anti-carcinogenic compounds [41]. Research on molecules with antioxidant effects is a promising strategy for preventing and therapy different diseases. For this reason, there is increasing interest in exploring the possibility of finding novel antioxidant compounds. Based on this work, the identified fungal strains would be good candidates for extensive studies on the production of antibiotic and antioxidant compounds.

CONCLUSION

The present study indicated that marine fungi isolated from the North Sea of Vietnam were valuable sources of biologically active compounds to comprise antibacterial and antioxidant agents. The results showed that there were 75.4% (n = 46) of fungal strains exhibited inhibitory activity against at least one test microorganism, and DPPH and ABTS free radical scavenging capacity was 32.7% (n = 20) and 93.4% (n = 57), respectively. Among the isolates with high biological activities, six fungal strains were identified by ITS gene sequences belonging to five genera *Aspergillus*, *Penicillium*, *Pleosporales*, *Talaromyces*, and *Hypomontagnella*. These findings are preliminary data for further investigating bioactive secondary metabolites from potential fungal strains.

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