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Bioactivity and chemical screening of endophytic fungi associated with seaweeds *Gracilaria* sp. and *Sargassum* sp. of the Bay of Bengal, Bangladesh

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This study explored the great potential of endophytic fungi associated with red seaweed *Gracilaria* sp. and brown seaweed *Sargassum* sp. of the Bay of Bengal, Bangladesh, for the first time. Endophytic fungi were identified taxonomically by morphological features and molecular characterisation (ITS sequence). The identification of six fungal isolates revealed five different fungal species belonging to four genera, namely, *Aspergillus subversicolor*, *A. terreus* and *Cladosporium halotolerans* isolated from *Gracilaria* sp. and *Chaetomium globosum*, *A. terreus* and *Curvularia perotidis* isolated from *Sargassum* sp. The ethyl acetate extracts of fungal endophytes were evaluated for antimicrobial activity, DPPH radical scavenging activity, and brine shrimp lethality bioassay. Amongst all the fungal extracts evaluated in this study, four showed mild to moderate inhibitory activity (10–14 mm) against the tested bacterial strains. Exceptionally, *Chaetomium globosum* exerted significant antibacterial activity with the highest zone of inhibition (21 ± 0.3 mm) against the Gram-negative bacterial strain *Pseudomonas aeruginosa* and also showed moderate antifungal activity (13 ± 0.9 mm) against *A. niger*. *Sargassum* sp.-derived *A. terreus* exhibited the strongest DPPH radical scavenging activity (IC_{50} value of 7.88 ± 0.09 µg/mL). All the fungal extracts have significant lethality against brine shrimp nauplii (LC_{50} value of ≤ 20.39 ± 4.04 µg/mL), while *Curvularia perotidis* and *Cladosporium halotolerans* were the most effective (LC_{50} values of 9.30 ± 2.96 µg/mL and 9.94 ± 3.49 µg/mL, respectively). Gas chromatography-mass spectrometry (GC-MS) analysis of the crude extracts identified the presence of several chemical compounds. These bioactive chemical constituents might contribute to the exhibited bioactivity in this study. The current study's findings support the beneficial impacts of the fungal endophytes on exerting biological activities and consequently as valuable resources of bioactive compounds.

Keywords Endophytic fungi, Seaweeds, *Gracilaria*, *Sargassum*, Antimicrobial activity, DPPH radical scavenging activity, Brine shrimp lethality bioassay, GC-MS

Endophytes, including fungi or bacteria, invade and grow inside the plant tissues, typically inducing harmless interactions[1]. Rather, the endophytic fungi aid the host plant in tolerating environmental stresses and combating pathogenic microbes by synthesizing a plethora of bioactive metabolites[2,3]. However, some endophytes can occasionally become opportunistic under specific conditions like stress or resource scarcity, potentially causing diseases or loss of plant biomass[4,5]. In recent times, endophytic fungi have garnered increasing interest as the fact unveiled that they can produce host plant-derived secondary metabolites[6–8]. They are also suitable for large-scale production using innovative cultivation techniques, improving the chances of discovering new drugs from microbial natural products[9,10]. Unique ecological roles and functional attributes distinguish marine fungi from

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other microbial groups. Their relevance in marine ecosystems, particularly in terms of their interactions with hosts, diversity, and biotechnological potential, often makes them more attractive for metabolite exploitation than marine bacteria or other microbes[11–13]. In this context, researchers worldwide have primarily investigated the endophytic fungal kingdom for active metabolites, intending to discover novel antibacterial[14], antifungal[15], cytotoxic[16], and anticancer compounds[17].

However, the potential of seaweed-associated endophytic fungi, mainly those inhabited in the Bay of Bengal of Bangladesh, has yet to be explored comprehensively. Global changes in geographical conditions, climate, and seawater bring an obvious change in the marine biodiversity of the endophytic fungal community[4,18]. The primary goals of the current study were to isolate the fungal endophytes associated with native seaweed species in the Bay of Bengal, Bangladesh, evaluate their biological potential, and perform a metabolite profiling of the fungal extracts. Seaweeds, also known as marine macroalgae, are considered commercially valuable and environmentally sustainable marine resources. Since ancient times, numerous seaweed species have been utilized as alternative food sources, in cosmetics, pharmaceuticals, fertilizers, biofuel production, and wastewater treatment[19]. Along with various positive impacts, seaweeds provide habitats and food for crucial marine ecosystem components, including endophytic fungi, significantly affecting the climate. The Bay of Bengal, Bangladesh, nourishes over 335 seaweed species[20]. Of these, red and brown seaweeds are most abundantly available in this diverse ecosystem. Previous studies have demonstrated the biological, biochemical, and nutritional analysis of the seaweed species on Bangladesh's coastline[21].

Gracilaria is a genus of red seaweeds (Rhodophyta) in the family Gracilariaceae. It encompasses a diverse array of bioactive compounds, like agarans, phenolic acids, diterpenes, mycosporine-like amino acids, sulfonic acids, lipids, steroids, xanthoproteins, oxylipins, heterosides, and bromophenols[22]. *Sargassum*, a genus of brown seaweeds (Phaeophyta) in the family Sargassaceae, contains a wide variety of novel secondary metabolites such as sulfated polysaccharides, sterols, polyphenols, amino acids, flavonoids, phlobatannin, cardiac glycosides, saponins, terpenoids, fucoxanthin and beta-carotene-like pigments[23–25]. Thus, these two types of edible seaweed have many health and medicinal benefits, such as anti-inflammatory, cytotoxic, antitumor, antioxidant, antimicrobial, antiaging, antidiabetic, analgesic, immunomodulatory, heart disease prevention, improved digestion, obesity control, brain tissue formation, maintaining bone health, anticancer, and anticoagulant effects[19,22–26]. However, the literature needs more evidence regarding the potential of fungal endophytes of these two seaweed species on the Bangladesh coast. Researchers are gradually utilizing naturally grown seaweeds in Bangladesh and their associated endophytic fungi due to their wide variety and abundance of bioactive secondary metabolites.

Thus, it is necessary to assess the effectiveness of endophytic fungi derived from well-known seaweeds. Moreover, the preliminary screening of biological and chemical potentials offers a chance for further investigation into the discovery of novel compounds. The current study represents the isolation of fungal endophytes from the red seaweed *Gracilaria* sp. and the brown seaweed *Sargassum* sp. in the Bay of Bengal, Bangladesh, using a well-established cultivation system, followed by bioactivity screening of fungal crude extracts using standardized protocols to assess antimicrobial, antioxidant, and cytotoxic potentials as well as chemical characterisation using gas chromatography-mass spectrometry (GC-MS) analysis.

Methods

Collection, identification, and extraction of seaweed samples

Fresh seaweed samples were collected from the coastline of Saint Martin's Island, Bangladesh, in November 2021. The sample specimens have been preserved in the Bangladesh National Herbarium. The pigmentation and morphological features primarily lead to the identification of seaweed species. The seaweed samples were properly air-dried first and then oven-dried at 40 ˚C for 24 h before grinding. The powdered samples were extracted with methanol at room temperature. Thus, the seaweed extracts were prepared by filtration and subsequent solvent evaporation.

Isolation and extraction of marine endophytic fungi

Endophytic fungi were isolated from fresh and disease-free seaweed samples. To ensure effective isolation of endophytes, the seaweed samples were surface-sterilized using 70% ethanol followed by 5% sodium hypochlorite solution. After sterilization, the samples were thoroughly rinsed with sterile distilled water to remove any residual chemicals. The seaweed samples were then cut into small pieces, and introduced into the water agar media (16 gm agar in 1000 mL distilled water) supplemented with 0.01% streptomycin[27]. Simultaneously, imprints of the surface-sterilized seaweed samples were prepared on the same culture medium to check for any superficial fungal contaminants. Following the favorable incubation condition (i.e., at 28 ± 2 ˚C in the dark) for 4–6 weeks, visible fungal hyphae growing from the seaweed inoculates were subcultured onto potato dextrose agar (PDA) media to separate each fungal species. The separated fungal endophytes were then streaked or serially diluted to obtain pure cultures.

The isolated endophytic fungi were cultivated on PDA media for 21–28 days at 28 ± 2 °C. The fungal cultures were stored at -20 °C for 48 h and then thawed to room temperature to facilitate the separation of the aqueous phase. After thawing, the culture media containing fungal mycelia were extracted using ethyl acetate[28]. Thus, the fungal crude extracts were prepared by filtration and subsequent solvent evaporation.

Identification of marine endophytic fungi

The isolated endophytic fungi were identified taxonomically based on their morphological and molecular characteristics[27]. The primary recognition of fungal genera was predicted by a thorough investigation of macroscopic and microscopic features of colony morphology, which was further confirmed by molecular analysis. Freshly cultured fungal tissue was frozen in liquid nitrogen and ground into a fine powder with a mortar and

pestle. The mycelial powder was then processed for DNA extraction using the Maxwell™ 16 platform (Maxwell 16 LEV DNA Kit, AS1420, Promega, USA)[29]. The Internal Transcribed Spacer (ITS) region of the isolated fungal DNA was amplified by PCR using the forward primer ITS 5 (5'GGAAGTAAAAGTCGTAACAAGG3') and the reverse primer ITS 4 (5'TCCTCCGCTTATTGATATGC3')[30]. The amplified ITS sequences of fungal DNA were subjected to nucleotide BLAST (Basic Local Alignment Search Tool) analysis against the NCBI (National Center for Biotechnology Information) GenBank databases. Subsequent identification up to the species level was guided by phylogenetic analysis. The phylogenetic tree was constructed in MEGA-X software (version: 10.1.0.1, https://www.megasoftware.net/) using the Neighbor-Joining method[31]. The assembled ITS sequences of each isolate were deposited in the NCBI GenBank, and corresponding accession numbers were received in response.

Antimicrobial activity

The fungal crude extracts were subjected to evaluate antimicrobial activity using the disc diffusion method[32]. Overall, five bacterial strains, i.e., two Gram-positive bacterial strains, *Staphylococcus aureus* (ATCC 9144) and *Bacillus megaterium* (ATCC 13578), and three Gram-negative bacterial strains, *Escherichia coli* (ATCC 11303), *Salmonella typhi* (ATCC 13311), and *Pseudomonas aeruginosa* (ATCC 27833), and two fungal strains, *Aspergillus niger* (ATCC 1004) and *A. flavus* (UCFT 02), were used to assess the antimicrobial activity. The sample discs were prepared with 100 µg extract per disc, using dichloromethane as the solvent. After overnight diffusion from the discs, the bacterial and fungal cultures were incubated for 16–18 h at 37 ± 2 °C and for 44–52 h at 28 ± 2 °C, respectively. The inhibitory activity of the fungal extracts was measured in terms of the zone of inhibition (in mm) and compared to that of the standards, kanamycin and ketoconazole (30 µg/disc), respectively.

Antioxidant activity

The antioxidant activity of the fungal crude extracts was evaluated by testing 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity[33]. This colorimetric method aims to observe how fungal isolates affect the DPPH free radical's ability to oxidize by measuring absorbance with a spectrophotometer at 517 nm. The sample solutions were prepared with varying concentrations from 200 µg/mL to 0.78 µg/mL obtained by serial dilution using methanol as the solvent. These sample solutions were then treated with a DPPH solution (20 µg/mL) in equal volume and allowed to rest for 30 min in the dark. The DPPH radical scavenging activity (RSA) of the sample extracts was estimated by the formula: RSA (%) = $[(A_{control} - A_{test\ sample}) / A_{control}] \times 100$. The IC_{50} values (the concentration that can inhibit oxidation by 50%, in µg/mL) were determined through logarithmic regression analysis of the % inhibition versus concentration curve. These values were then compared with those of two reference antioxidants, ascorbic acid and trolox.

Cytotoxic activity

The cytotoxic activity of the fungal crude extracts was evaluated using the brine shrimp lethality assay[34]. This assay utilizes nauplii of brine shrimp *Artemia salina* to assess the sample's toxicity by measuring the percentage of living nauplii 24 h after the sample treatment. The sample solutions were prepared with concentrations ranging from 400 µg/mL to 1.59 µg/mL by serial dilution using dimethyl sulfoxide (DMSO) as the solvent. These sample solutions were added to brine water (5 mL) containing ten living shrimp nauplii and rested for 24 h. The brine shrimp lethality of the sample extracts was estimated by determining the LC_{50} values (the concentration lethal to 50% brine shrimp nauplii, in µg/mL). These values were then compared with those of the reference cytotoxic agent, vincristine sulfate.

Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectrometry analyses were performed on ethyl acetate extracts of fungal isolates and methanol extracts of host seaweeds using the Clarus® 690 gas chromatograph (PerkinElmer, USA) coupled with a Clarus® SQ 8 C mass spectrophotometer. A sample (1 µL, in splitless mode) was injected at a constant inlet temperature of 280 °C. 99.999% pure helium was used as a carrier gas at a 1 mL/min flow rate for a 40–60 min run time using Elite-35 and HP-5MS columns (30 m X 0.25 mm, 0.25 µm film thickness). The column temperature was raised at a rate of 4–5 °C/min, starting from 60 °C to 240 °C, and maintained for 4–15 min[35]. Electron ionization (EI) mode was applied for sample analysis at an energy of 70 eV. The compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) database, using a minimum match quality of 90% as the criterion.

Data analysis

Each bioactivity test was conducted in triplicate, and the data were recorded accordingly. The logistic regression method was used to calculate the IC_{50} and LC_{50} values. The values are presented as mean ± standard deviation (SD), $n = 3$. Microsoft Excel was used to prepare the calculations and graphs.

Results

The sterilization method proved highly effective in eliminating superficial contaminants, as evidenced by the absence of fungal growth on the control imprints of surface-sterilized seaweed samples. This ensured the successful isolation of six endophytic fungi from *Gracilaria* sp. and *Sargassum* sp. Following purification, the fungal endophytes were preserved at 4 °C for further analysis and maintained by subculturing every two months.

Identification of endophytic fungal isolates

Three endophytic fungi bearing internal strain nos. GE-1, GE-2, and GE-3 were isolated from the red seaweed *Gracilaria* sp. (Herbarium accession no. DACB-90626), which were later identified as *Aspergillus subversicolor*[36], *A. terreus*[37], and *Cladosporium halotolerans*[38], respectively. Another three fungal endophytes bearing internal

strain nos. SE-1, SE-2, and SE-3 were isolated from the brown seaweed *Sargassum* sp. (Herbarium accession no. DACB-90627), which were later identified as *Chaetomium globosum*[39], *A. terreus*[37], and *Curvularia perotidis*[40,41], respectively. The morphological and molecular characteristics of isolated endophytic fungi and their GenBank accession numbers are presented in (Tables 1 and 2). Figures 1 and 2 show pictures of fungal colony surface, colony reverse, microscopic view, and phylogenetic tree reflecting how closely each isolate is related to its best match.

Antimicrobial activity

Most fungal crude extracts revealed mild-to-moderate zone of inhibition (10–15 mm) against tested bacterial strains. Among the samples tested, *Chaetomium globosum* (SE-1) exhibited significant activity against the Gram-negative bacterial strain *Pseudomonas aeruginosa*, with the highest zone of inhibition (21 ± 0.3 mm) (Fig. 3A). The same isolate, SE-1, showed the highest inhibition (13 ± 0.9 mm) against the fungal strain *Aspergillus niger*, while others were primarily inactive against tested fungal strains. The solvent control (dichloromethane) showed no activity.

Antioxidant activity

The antioxidant screening revealed that five of the six fungal crude extracts could scavenge DPPH radicals (IC_{50}, 7.88–178.32 µg/mL) (Fig. 3B). Exceptionally, very strong antioxidant activity was exhibited by *Aspergillus terreus* (SE-2), having the lowest IC_{50} value (7.88 ± 0.09 µg/mL) among all the extracts tested. *Cladosporium halotolerans* (GE-3) had no scavenging activity at the experimental concentration (IC_{50} > 250 µg/mL)[42]. The other fungal extracts had mild to strong antioxidant activity.

Cytotoxic activity

All the fungal crude extracts showed significant cytotoxic activity regarding brine shrimp lethality bioassay (LC_{50}, 9.30–20.39 µg/mL) (Fig. 3C). However, *Curvularia perotidis* (SE-3) exhibited the lowest LC_{50} value (9.30 ± 2.96 µg/mL) among all the extracts tested, indicating potent cytotoxicity. *Cladosporium halotolerans* (GE-3) had similar potency with a lower LC_{50} value of 9.94 ± 3.49 µg/mL. *Aspergillus subversicolor* (GE-1), *A. terreus* (GE-2), *Chaetomium globosum* (SE-1) and *A. terreus* (SE-2) all showed significant cytotoxicity with LC_{50} values of 19.73 ± 4.84 µg/mL, 19.01 ± 1.02 µg/mL, 14.97 ± 4.39 µg/mL, and 20.39 ± 4.04 µg/mL, respectively.

Isolate		**GE-1**	**GE-2**	**GE-3**
Identified fungal taxon		**Aspergillus subversicolor**	**Aspergillus terreus**	**Cladosporium halotolerans**
Macroscopic characteristics*				
Growth rate		Slow	Moderate	Slow
Diameter after 6 days (approx.)		2.1–2.3 cm	4.5–5.5 cm	2.6–2.8 cm
Hyphae		Surficial, submerged	Surficial, submerged	Surficial, submerged
Mycelium depth in agar		Shallow	Shallow	Shallow
Form of colony		Irregular	Filamentous	Irregular, Filamentous
Colour of colony surface		Bluish-green colour with white edge	White initially, then yellowish, finally cinnamon-brown	Smoke-grey
Colour of colony reverse		Yellowish-brown	Yellowish-brown	Black with white margin
Texture of colony surface		Velvety, sulcate	Powdery	Velvety to woolly
Elevation of colony		Raised	Raised	Raised
Margin of colony		Undulate	Filiform	Undulate, filiform
Microscopic characteristics*				
Spore view at		4 days	6 days	5 days
Hyphae		Septate	Septate	Septate, dark
Conidiophores		Unbranched, long, smooth, hyaline, slightly brownish; conidial heads biseriate, loosely radiate, covering most of vesicle	Unbranched, relatively short, smooth, hyaline; conidial heads biseriate, covering the upper half of vesicle, compactly columnar	Branched, erect, dark, producing branching treelike conidial chains
Conidia		Round shaped, hyaline, single celled	Round shaped, hyaline, single celled	Oval shaped, brown, arranged like a string of pearls
Molecular characteristics				
BLAST (ITS sequence)	Total score	952	1109	863
	Query cover/ identity (%)	91/ 98.18	95/ 100	86/ 99.17
Bootstrap support value (%)		82	78	67
GenBank accession no.		OR335219	OR335235	OR338335

Table 1. Morphological and molecular characteristics of marine endophytic fungi associated with *Gracilaria* Sp. *Colonies grown on potato dextrose agar (PDA) media at 28 ± 2 °C.

Isolate		SE-1	SE-2	SE-3
Identified fungal taxon		**Chaetomium globosum**	**Aspergillus terreus**	**Curvularia perotidis**
Macroscopic characteristics*				
Growth rate		Rapid	Rapid	Slow
Diameter after 6 days (approx.)		8.5–9.0 cm	8.5–9.0 cm	1.7–2.0 cm
Hyphae		Aerial, surficial, submerged	Surficial, submerged	Surficial, submerged
Mycelium depth in agar		Shallow	Shallow	Shallow
Form of colony		Filamentous	Filamentous	Irregular
Colour of colony surface		White initially, becoming greyish olivaceous	White initially, then yellow, finally cinnamon brown	Pale yellow initially, then brownish-yellow
Colour of colony reverse		Yellowish brown to dark brown	Yellowish-brown	Reddish-yellow, becoming darker with age
Texture of colony surface		Cottony	Powdery	Granular with woolly edge
Elevation of colony		Crateriform	Raised	Flat
Margin of colony		Filiform	Filiform	Undulate
Microscopic characteristics*				
Spore view at		28 days	3 days	12 days
Hyphae		Septate	Septate	Septate, dark
Conidiophores		Large, oval shaped, brown, fragile with wavy ascomatal hairs; asci stalked, club shaped	Unbranched, relatively short, smooth, hyaline; conidial heads biseriate, covering the upper half of vesicle, compactly columnar	Branched, mostly bifurcated; conidiogenous cells discrete, subcylindrical
Conidia		Lemon shaped, olive-brown, single celled	Round shaped, hyaline, single celled	Straight or very-slightly curved, pale brown, multicellular
Molecular characteristics				
BLAST (ITS sequence)	Total score	887	1110	279
	Query cover/ Identity (%)	98/ 98.99	96/ 100	65/ 93.16
Bootstrap support value (%)		77	78	53
GenBank accession no.		OR338812	OR336320	OR335556

Table 2. Morphological and molecular characteristics of marine endophytic fungi associated with *Sargassum* Sp. *Colonies grown on potato dextrose agar (PDA) media at 28 ± 2 °C.

Chemical characterisation

The GC-MS analyses of seaweed extract from *Gracilaria* sp. (GE) and its associated endophytic fungal extracts (GE-1, GE-2, and GE-3) led to identifying 12, 13, 33, and 21 nos. of compounds, respectively. Similarly, GC-MS analyses on seaweed extract from *Sargassum* sp. (SE) and its associated endophytic fungal extracts (SE-1, SE-2, and SE-3) identified 12, 3, 15, and 17 nos. of compounds, respectively. Figure S1 (supplementary material) presents the distinct GC-MS chromatograms of the investigated extracts. The identified compounds with their retention time, molecular weight, molecular formula, and peak area percent are presented in (Tables 3 and 4). Based on the percentage, the most abundant component was Dodecanoic acid, 1,2,3-propanetriyl ester (**1**) in the seaweed extracts GE (92.10%) and SE (95.49%), and fungal extracts GE-1 (77.86%) and GE-3 (76.84%). The fungal extract GE-2 contained the following prominent bioactive components: Erucic acid (**2**, 11.71%), Hexanedioic acid, bis(2-ethylhexyl) ester (**3**, 7.28%), 26,27-Di(nor)-cholest-5, 7, 23-trien-22-ol, 3-methoxymethoxy- (**4**, 6.42%), Glutaric acid, dodec-2-en-1-yl pentafluorophenyl ester (**5**, 5.66%), and n-Hexadecanoic acid (**6**, 5.44%). The compound **6** was also present in the fungal extract GE-3 (9.95%). Other compounds identified abundantly were 1-(ethanesulfonyl)-2-(ethylsulfanyl)ethane (**7**, 99.52%) in the fungal extract SE-1, Methyl-4-deoxy-2-O-methyl-β-L-threo-hex-4-enopyranosid uronate (**8**, 26.43%), 3-(methylthio)propyl nonanoate (**9**, 25.63%), and Glycidyl palmitate (**10**, 6.68%) in the fungal extract SE-2, and once more, compound **10** (87.63%) in the fungal extract SE-3. The structures of these major bioactive chemical compounds of marine algal and fungal origin are presented in (Fig. 4).

Discussion

Bangladesh, a tropical country bordered by the Bay of Bengal to the south, boasts the extended coastline enriched with diverse seaweed resources. This presents a unique opportunity for more comprehensive investigations into the enormous possibilities of seaweed-associated endophytes, including fungi, bacteria, and other microbes. Marine microbes are prolific producers of bioactive molecules, such as antibiotics, cytotoxic agents, and antifungals, with applications in both pharmaceutical and agricultural fields[43]. Marine fungi remain relatively underexplored compared to terrestrial fungi, offering a vast reservoir of untapped biodiversity and bioactive potential[44]. This makes their study highly relevant for discovering novel compounds that are often absent or less abundant in other microbial sources. For example, certain exclusively fungal metabolites, such as cytochalasins[45], patulin[46], citrinin[47], sterigmatocystin[48], and gliotoxin[49], have been reported for their potential

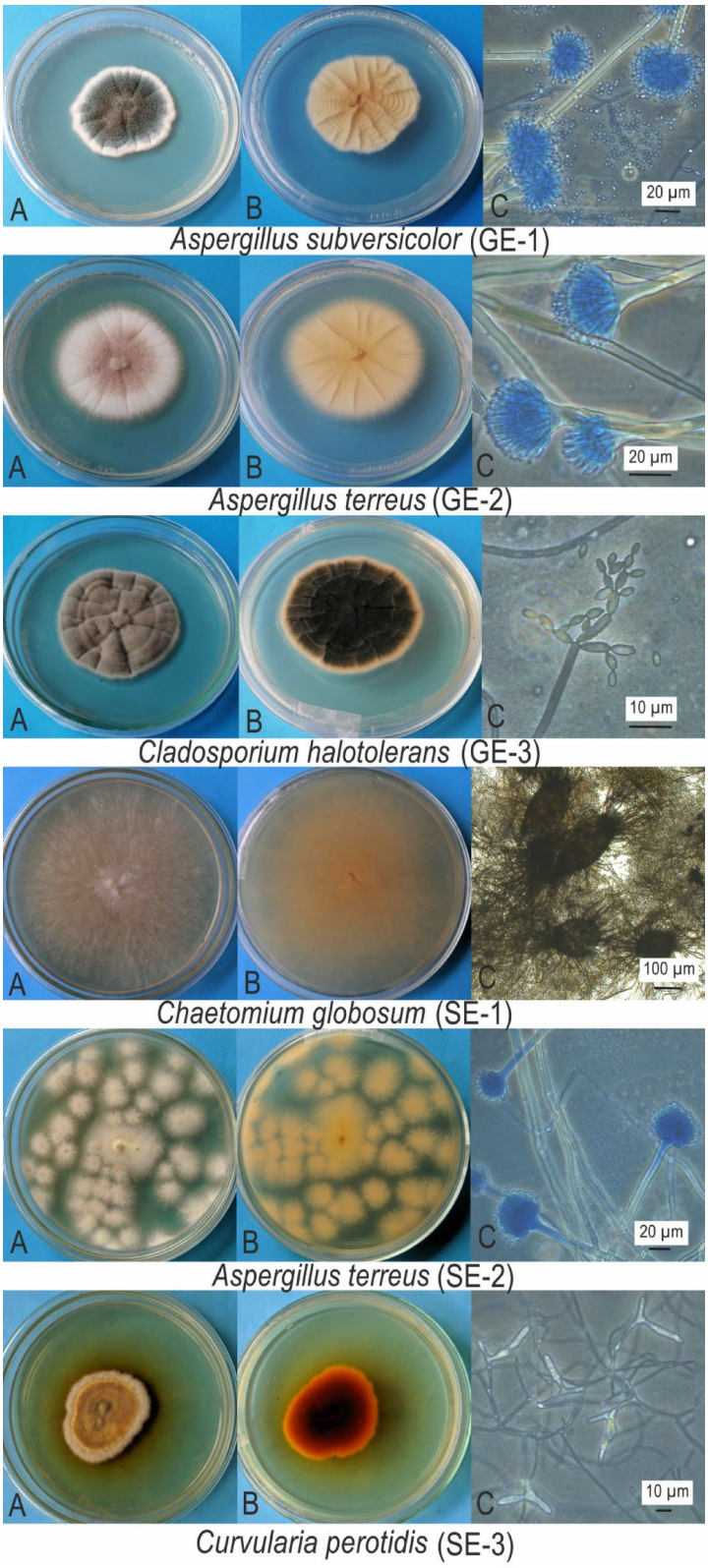

Fig. 1. Endophytic fungal isolates from *Gracilaria* sp. and *Sargassum* sp. (**A**) Colony surface. (**B**) Colony reverse, on potato dextrose agar media at 28 ± 2 °C. (**C**) Microscopic view. Codes in the parentheses represent internal strain nos. of the isolated endophytic fungi.

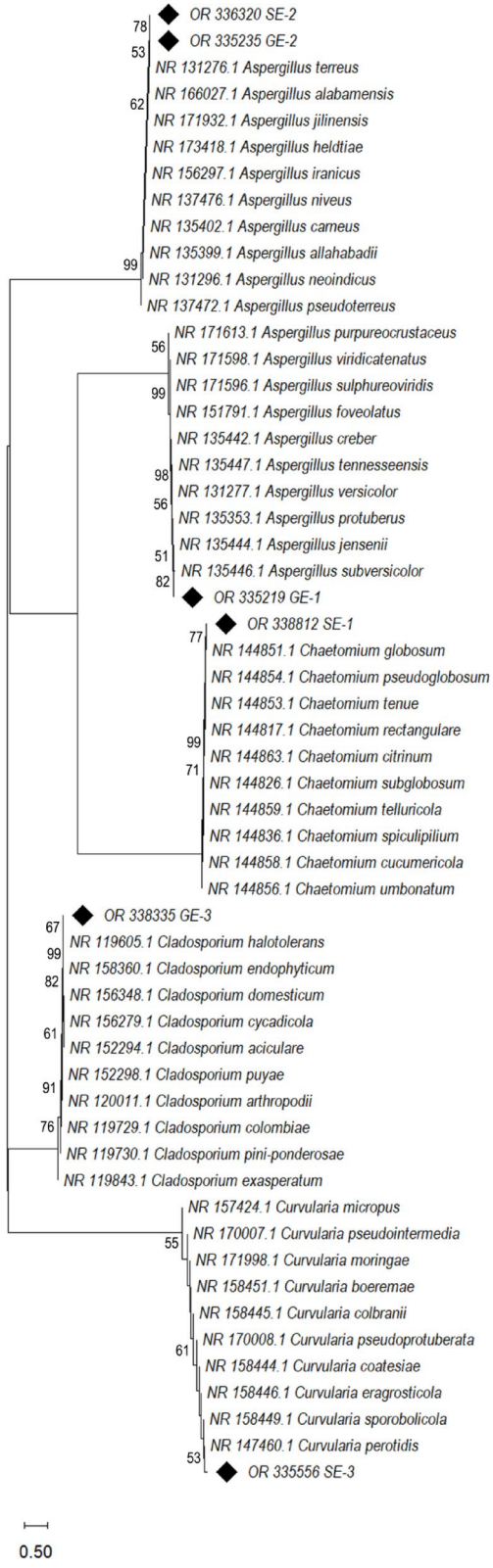

Fig. 2. Phylogenetic tree inferred from internal transcribed spacer sequences using Neighbor-Joining method. Codes on the labelled branches represent internal strain nos. of the isolated endophytic fungi from *Gracilaria* sp. and *Sargassum* sp.

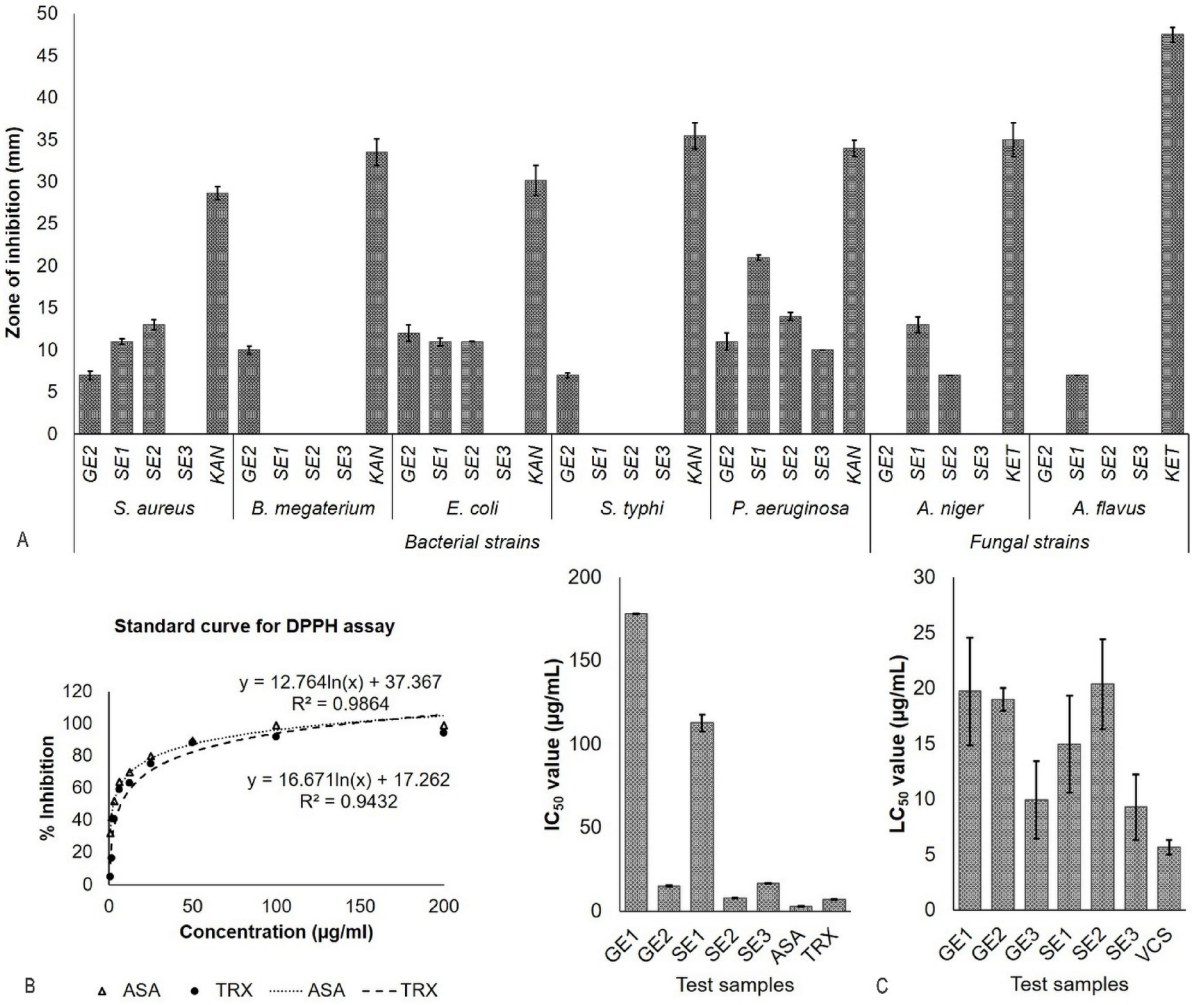

Fig. 3. Bioactivity screening of the marine endophytic fungi. (**A**) Antimicrobial activity, compared with standards kanamycin (KAN) and ketoconazole (KET). (**B**) DPPH radical scavenging activity, compared with standards ascorbic acid (ASA) and trolox (TRX). (**C**) Brine shrimp lethality bioassay, compared with standard vincristine sulfate (VCS). Values are expressed as mean ± SD, $n = 3$. Codes on the horizontal axis represent internal strain nos. of the endophytic fungal isolates.

pharmacological activities, including anticancer and immunosuppressive properties. Although several studies worldwide have demonstrated the great potential of marine endophytic fungi, there is insufficient information regarding the significance of seaweed-associated endophytic fungal flora in Bangladesh. A deeper understanding of this concept is required, as the bioactivity and chemical composition of fungal endophytes vary depending on geographic location and species[4,18]. Additionally, evaluating antimicrobial, antioxidant, and cytotoxic activities is a reliable measure of the ability of fungal endophytes to produce bioactive secondary metabolites. Thus, this type of research validates a seaweed species and its endophytic fungus as a leading source and eventually facilitates the discovery of novel bioactive compounds.

This study represents the preliminary evaluation of marine endophytic fungi, isolated for the first time, from two seaweeds, *Gracilaria* sp. and *Sargassum* sp., inhabited in the Bay of Bengal, Bangladesh. Among six fungal isolates in this study, *Aspergillus* was the dominant genus (internal strain nos. GE-1, GE-2, and SE-2), while the others belonged to the genera *Cladosporium*, *Chaetomium*, and *Curvularia* (internal strain nos. GE-3, SE-1, and SE-3, respectively) (Figs. 1 and 2; Tables 1 and 2). These genera are commonly detected across different indoor environments, terrestrial and marine plants[50] and are classified under the ascomycota division, a major dominant group within the fungi kingdom. Phylogenetic analysis illustrated the relationships of the fungal isolates with their genetically similar species. Each isolate was found to form a distinct monophyletic clade with its most closely related species. Taxonomically diversified endophytic fungi are widely found on seaweeds, sea sponges, sea snails, tunicates, and other sea organisms, playing vital roles in marine habitats[51]. A previous study reported the isolation of *Chaetomium globosum*, *Nigrospora magnoliae*, *Curvularia* sp., *Curvularia moringae*, *Aspergillus terreus*, and *Collariella* sp. from the green seaweed *Ulva* sp. collected from the coastal area of Bangladesh[27]. Lini et al. identified endophytic fungal isolates from marine weeds of Bangladesh, including a *Fusarium* sp. and a *Penicillium* sp. from red seaweed *Hypnea musciformis*, a *Fusarium* sp. from brown seaweed *S. crassifolium*,

Sample	Sl. no.	Retention time (min)	Identified compounds	Molecular weight (g/mol)	Molecular formula	Peak area (%)
GE	1	7.44	Dodecane, 1-fluoro	188	$C_{12}H_{25}F$	0.09
	2	22.04	Hentriacontane	436	$C_{31}H_{64}$	0.08
	3	24.22	(*Z*)-14-tricosenyl formate	366	$C_{24}H_{46}O_2$	0.06
	4	24.63	Diethyl phthalate	222	$C_{12}H_{14}O_4$	0.06
	5	25.42	Neophytadiene	278	$C_{20}H_{38}$	0.16
	6	26.04	Trans-1,3,3-trimethylbicyclo[3.1.0]hexane-1-carboxaldehyde	152	$C_{10}H_{16}O$	0.02
	7	26.39	Phytyl tetradecanoate	506	$C_{34}H_{66}O_2$	0.04
	8	26.55	4-methoxy-6-methyl-6,7-dihydro-4 h-furo[3,2-c]pyran	168	$C_9H_{12}O_3$	0.03
	9	28.26	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	270	$C_{17}H_{34}O_2$	0.07
	10	29.64	Dodecanoic acid	200	$C_{12}H_{24}O_2$	0.02
	11	29.78	Squalene	410	$C_{30}H_{50}$	2.11
	12	36.41	Dodecanoic acid, 1,2,3-propanetriyl ester	638	$C_{39}H_{74}O_6$	92.1
GE-1	1	3.09	Strychane, 1-acetyl-20.alpha.-hydroxy-16-methylene-	338	$C_{21}H_{26}O_2N_2$	0.01
	2	18.92	3,4-dihydroxyphenylglycol, 4tms derivative	458	$C_{20}H_{42}O_4Si_4$	0.01
	3	19.95	3-n-hexylthiolane, s,s-dioxide	204	$C_{10}H_{20}O_2S$	0.01
	4	21.06	Pentanedioic acid, (2,4-di-t-butylphenyl) mono-ester	320	$C_{19}H_{28}O_4$	0.01
	5	21.95	2,2,4-trimethyl-1,3-pentanediol diisobutyrate	286	$C_{16}H_{30}O_4$	0.03
	6	24.33	Chloroacetic acid, tetradecyl ester	290	$C_{16}H_{31}O_2Cl$	0.04
	7	24.63	Diethyl phthalate	222	$C_{12}H_{14}O_4$	0.04
	8	26.31	Methyl 8-methyl-nonanoate	186	$C_{11}H_{22}O_2$	0.02
	9	28.26	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	270	$C_{17}H_{34}O_2$	0.3
	10	29.64	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	0.44
	11	31.82	13-octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$	0.49
	12	32.02	Methyl 9-cis,11-trans-octadecadienoate	294	$C_{19}H_{34}O_2$	0.74
	13	36.76	Dodecanoic acid, 1,2,3-propanetriyl ester	638	$C_{39}H_{74}O_6$	77.86
GE-2	1	3.05	Isobutyl (3-(methylthio)propyl) carbonate	206	$C_9H_{18}O_3S$	0.02
	2	15.42	Ethyl (1r,4s)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl carbonate	226	$C_{13}H_{22}O_3$	1.30
	3	16.63	Cyclohexasiloxane, dodecamethyl	445	$C_{12}H_{36}O_6Si_6$	0.71
	4	17.28	Methyl 6-methylnicotinate	151	$C_8H_9NO_2$	0.55
	5	19.30	(3aR,4R,8R,8aS)-3a,4,7,8a-Tetramethyl-1,2,3,3a,4,5,8,8a-octahydro-4,8-methanoazulene	204	$C_{15}H_{24}$	0.75
	6	20.08	1-Dodecanamine, N,N-dimethyl-	213	$C_{14}H_{31}N$	0.01
	7	20.10	bicyclo[4.1.0]heptane-7-carboxylic acid-4-acetylphenyl ester	258	$C_{16}H_{17}O_3$	0.65
	8	20.43	1,3-Benzenediol, 4,6-dichloro-2-methyl-	192	$C_7H_6Cl_2O_2$	1.18
	9	23.33	2-Octanol	130	$C_8H_{18}O$	0.22
	10	24.81	Oxalic acid, neopentyl nonyl ester	286	$C_{16}H_{30}O_4$	0.95
	11	25.68	Phthalimidine	133	C_8H_7NO	0.54
	12	26.84	Heptasiloxane, hexadecamethyl-	533	$C_{16}H_{48}O_6Si_7$	0.37
	13	27.72	Dimethyl palmitamine	269	$C_{18}H_{39}N$	1.84
	14	29.29	1 H-Inden-1-ol, 2,3-dihydro-2-methyl-	148	$C_{10}H_{12}O$	0.69
	15	30.07	Benzoic acid, 2,4-dihydroxy-3,5,6-trimethyl-, methyl ester	210	$C_{14}H_{14}O_4$	1.33
	16	33.36	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	270	$C_{17}H_{34}O_2$	2.52
	17	34.25	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	5.44
	18	34.99	Octadecanoic acid, 17-methyl-, methyl ester	312	$C_{20}H_{40}O_2$	1.65
	19	37.28	Methyl 10-trans,12-cis-octadecadienoate	294	$C_{19}H_{34}O_2$	1.32
	20	37.42	n-Propyl 11-octadecenoate	324	$C_{21}H_{40}O_2$	2.08
	21	38.01	Heptadecanoic acid, 16-methyl-, methyl ester	298	$C_{19}H_{38}O_2$	0.68
	22	38.79	Octadecanoic acid	284	$C_{18}H_{36}O_2$	1.32
	23	41.65	Dibenzo[a, i]biphenylene, 5,6,6a,6b,7,8,12b,12c-octahydro-2,11-dimethoxy-	320	$C_{22}H_{24}O_2$	2.08
	24	43.72	Hexanedioic acid, bis(2-ethylhexyl) ester	370	$C_{22}H_{42}O_4$	7.28
	25	44.29	Ecgonine	185	$C_9H_{15}NO_3$	1.08
	26	45.2	Ethyl I-(2-formylethyl)-2-oxocyclooctane-l-carboxylate	254	$C_{14}H_{22}O_4$	2.07
	27	46.77	Bis(2-ethylhexyl) phthalate	390	$C_{24}H_{38}O_4$	1.90
	28	48.00	Hentriacontane	436	$C_{31}H_{64}$	0.46

Continued

Sample	Sl. no.	Retention time (min)	Identified compounds	Molecular weight (g/mol)	Molecular formula	Peak area (%)
	29	51.37	1-(3-Methylbutyl)-2,3,5-trimethylbenzene	190	$C_{14}H_{22}$	1.77
	30	52.47	Erucic acid	338	$C_{22}H_{42}O_2$	11.71
	31	53.77	13-Docosenamide, (*Z*)-	337	$C_{22}H_{43}NO$	2.78
	32	55.64	26,27-Di (nor)-cholest-5, 7, 23-trien-22-ol, 3-methoxymethoxy-	414	$C_{27}H_{42}O_3$	6.42
	33	56.95	Glutaric acid, dodec-2-en-1-yl pentafluorophenyl ester	464	$C_{23}H_{29}F_5O_4$	5.66
GE-3	1	3.44	Cyclohexylmethylsilane	128	$C_7H_{16}Si$	0.06
	2	9.65	Cis-2,4-dimethylthiane, S,S-dioxide	162	$C_7H_{14}O_2S$	0.06
	3	11.07	Diethylmalonic acid, monochloride, 4-methylcyclohexyl ester	274	$C_{14}H_{23}O_3Cl$	0.03
	4	12.13	Fumaric acid, di(3-methylbut-3-enyl) ester	252	$C_{14}H_{20}O_4$	0.04
	5	12.84	Tetrapentacontane	758	$C_{54}H_{110}$	0.01
	6	14.08	Diethylcyanamide	98	$C_5H_{10}N_2$	0.03
	7	15.19	1,1-dimethyl-1-silacyclobutane	100	$C_5H_{12}Si$	0.07
	8	16.85	1,4-benzodioxin, octahydro-2-methylene-, trans-	154	$C_9H_{14}O_2$	0.05
	9	17.46	Heptacosanoic acid, 25-methyl-, methyl ester	438	$C_{29}H_{58}O_2$	0.02
	10	18.07	Triacontane	422	$C_{30}H_{62}$	0.02
	11	18.92	Cyclooctasiloxane, hexadecamethyl	592	$C_{16}H_{48}O_8Si_8$	0.04
	12	19.88	Nonanoic acid, 9-oxo-, methyl ester	186	$C_{10}H_{18}O_3$	0.03
	13	21.93	2,2,4-trimethyl-1,3-pentanediol diisobutyrate	286	$C_{16}H_{30}O_4$	0.12
	14	24.22	Z, Z-6,28-heptatriactontadien-2-one	530	$C_{37}H_{70}O$	0.09
	15	24.62	Diethyl phthalate	222	$C_{12}H_{14}O_4$	0.05
	16	25.75	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	0.35
	17	28.26	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	270	$C_{17}H_{34}O_2$	1.42
	18	29.66	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	9.95
	19	31.82	13-octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$	2.06
	20	31.91	Heptadecanoic acid, 16-methyl-, methyl ester	298	$C_{19}H_{38}O_2$	0.01
	21	36.67	Dodecanoic acid, 1,2,3-propanetriyl ester	638	$C_{39}H_{74}O_6$	76.84

Table 3. Compounds identified in the seaweed *Gracilaria* Sp. (GE) and its associated endophytic fungi, GE-1, GE-2, and GE-3 by GC-MS analyses.

a *Penicillium* sp. from green seaweed *Dictyota dichotoma* and a *Aspergillus* sp. from green seaweed *Caulerpa peltate*[52].

Antimicrobial screening revealed that extracts of *Aspergillus terreus* (GE-2), *Chaetomium globosum* (SE-1), and *A. terreus* (SE-2) were more promising among all the evaluated extracts (Fig. 3A). Developing new antimicrobial agents is essential to combat the growing threat of drug-resistant microbes. It is quite natural that the crude extracts may exhibit lower antimicrobial activity compared to the standards. However, identifying and analyzing the specific metabolites responsible for this activity can lead to the discovery of novel and potent antimicrobial agents. For example, marine-derived *A. terreus* has been reported to produce the novel antibacterial metabolite butyrolactone J with a minimal inhibitory concentration of 12.5 µg/mL against *Staphylococcus aureus*[53].

The isolates GE-2 and SE-2, both identified as *A. terreus*, demonstrated antibacterial activity but did not show antifungal activity. Extract of GE-2 inhibited all five tested bacterial strains with zones of 7–12 mm, while extract of SE-2 inhibited three out of five strains with larger zones of 11–14 mm. Interestingly, another *A. terreus* strain isolated from a sea worm (*Spirorbis* sp.) showed no antibacterial activity, with no inhibition zones observed[54]. In contrast, *A. terreus* isolated from the leaf of the terrestrial plant *Psidium guajava* exhibited strong antibacterial activity, with inhibition zones ranging from 18 to 26 mm when tested with 10,000 µg of fungal extract[55].

On the other hand, extract of *C. globosum* (SE-1) inhibited the growth of three out of five tested bacterial strains, exhibiting the largest zone of inhibition (21 ± 0.3 mm) against the Gram-negative bacterial strain *Pseudomonas aeruginosa*. This finding aligns with the studies of Kamat et al.[56] and Noor et al.[27], who reported that marine green macroalgae-derived *C. globosum* produces diverse secondary metabolites exerting antimicrobial potential with inhibition zones of 13–27 mm. Goda et al.[57] also investigated the antibacterial potential of *C. globosum* strains isolated from medicinal terrestrial plants in Egypt, finding inhibition zones ranging from 10.4 to 25.4 mm with *E. coli* and *P. aeruginosa* being the most sensitive strains.

In the current study, extract of *Curvularia perotidis* (SE-3) was mildly active only against *P. aeruginosa*, which was the most susceptible bacterial strain (10–21 mm) to the tested extracts. In contrast, bacterial strains, *Bacillus megaterium* and *Salmonella typhi*, and fungal strains, *A. niger* and *(A) flavus* were less susceptible. Only the extract of isolate GE-2 (*A. terreus*) inhibited the growth of *(B) megaterium* and *S. typhi* mildly and insignificantly, respectively, compared to other isolates. Extracts of other isolates, *A. subversicolor* (GE-1) and

Sample	Sl. no.	Retention time (min)	Identified compounds	Molecular weight (g/mol)	Molecular formula	Peak area (%)
SE	1	7.44	Hexadecane	226	$C_{16}H_{34}$	0.06
	2	21.95	Cyclohexanecarboxamide, n-methallyl-	181	$C_{11}H_{19}NO$	0.03
	3	24.26	Tridecanoic acid, 12-methyl-, methyl ester	242	$C_{15}H_{30}O_2$	0.06
	4	24.64	Diethyl phthalate	222	$C_{12}H_{14}O_4$	0.04
	5	25.42	Neophytadiene	278	$C_{20}H_{38}$	0.12
	6	26.04	Trans, cis-1,8-dimethylspiro[4.5]decane	166	$C_{12}H_{22}$	0.05
	7	26.38	Trans-1,3,3-trimethylbicyclo[3.1.0]hexane-1-carboxaldehyde	152	$C_{10}H_{16}O$	0.07
	8	26.55	2-pentacosanone	366	$C_{25}H_{50}O$	0.04
	9	28.27	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	270	$C_{17}H_{34}O_2$	0.47
	10	29.63	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	1.35
	11	31.83	13-octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$	0.37
	12	36.39	Dodecanoic acid, 1,2,3-propanetriyl ester	638	$C_{39}H_{74}O_6$	95.49
SE-1	1	3.07	Isobutyl (3-(methylthio)propyl) carbonate	206	$C_9H_{18}O_3S$	0.02
	2	8.75	Benzene, 1-methyl-2-propyl	134	$C_{10}H_{14}$	0.28
	3	26.91	1-(ethanesulfonyl)-2-(ethylsulfanyl)ethane	182	$C_6H_{14}O_2S_2$	99.52
SE-2	1	3.08	Isobutyl (3-(methylthio)propyl) carbonate	206	$C_9H_{18}O_3S$	0.01
	2	3.09	Ether, bis[2-(ethylthio)ethyl]	194	$C_8H_{18}OS_2$	0.02
	3	4.81	1-(ethanesulfonyl)-2-(ethylsulfanyl)ethane	182	$C_6H_{14}O_2S_2$	1.11
	4	4.99	3-(methylthio)propyl nonanoate	246	$C_{13}H_{26}O_2S$	25.63
	5	5.24	Methyl-4-deoxy-2-O-methyl-β-L-threo-hex-4-enopyranosid uronate	204	$C_8H_{12}O_6$	26.43
	6	5.32	Propanoic acid, 2-mercapto-, ethyl ester	134	$C_5H_{10}O_2S$	0.02
	7	18.45	(3ar,4r,8r,8as)-3a,4,8a-trimethyl-7-methylenedecahydro-4,8-methanoaz	204	$C_{15}H_{24}$	0.09
	8	21.96	Isophytol	296	$C_{20}H_{40}O$	0.14
	9	28.24	Methyl 11-methyl-dodecanoate	228	$C_{14}H_{28}O_2$	0.7
	10	29.04	Benzoic acid, 2,4-dihydroxy-3,5,6-trimethyl-, methyl ester	210	$C_{14}H_{14}O_4$	0.6
	11	31.8	13-octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$	1.07
	12	32	Methyl 10-trans,12-cis-octadecadienoate	294	$C_{19}H_{34}O_2$	1.41
	13	33.66	Tetracosamethyl-cyclododecasiloxane	888	$C_{24}H_{72}O_{12}Si_{12}$	2.13
	14	35.82	Glycidyl palmitate	312	$C_{19}H_{36}O_3$	6.68
	15	39.58	Cyclononasiloxane, octadecamethyl	666	$C_{18}H_{54}O_9Si_9$	2.36
SE-3	1	3.08	Isobutyl (3-(methylthio)propyl) carbonate	206	$C_9H_{18}O_3S$	0.01
	2	7.59	Benzaldehyde	106	C_7H_6O	0.08
	3	10.91	Meso-hydrobenzoin	214	$C_{14}H_{14}O_2$	0.29
	4	16.99	1-cyclohexene-1-carboxaldehyde, 5,5-dimethyl-3-oxo	152	$C_9H_{12}O_2$	0.46
	5	17.45	Spiro[5.5]undecane	152	$C_{11}H_{20}$	0.39
	6	18.89	Cyclooctasiloxane, hexadecamethyl-	592	$C_{16}H_{48}O_8Si_8$	0.46
	7	22.01	Hentriacontane	436	$C_{31}H_{64}$	0.3
	8	22.77	4,4-dimethyl-2-propenylcyclopentanone	152	$C_{10}H_{16}O$	0.05
	9	24.15	Tetrapentacontane	758	$C_{54}H_{110}$	0.2
	10	24.61	Diethyl phthalate	222	$C_{12}H_{14}O_4$	0.13
	11	28.25	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	270	$C_{17}H_{34}O_2$	0.57
	12	31.8	13-octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$	0.43
	13	32	Cis, cis-1,9-dimethylspiro[5.5]undecane	180	$C_{13}H_{24}$	0.58
	14	33.14	Phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl ester	418	$C_{26}H_{42}O_4$	0.99
	15	35.82	Glycidyl palmitate	312	$C_{19}H_{36}O_3$	87.63
	16	37.81	Cyclononasiloxane, octadecamethyl	666	$C_{18}H_{54}O_9Si_9$	3.2
	17	39.74	Adipic acid, di(cis-non-3-enyl) ester	394	$C_{24}H_{42}O_4$	0.02

Table 4. Compounds identified in the seaweed *Sargassum* Sp. (SE) and its associated endophytic fungi, SE-1, SE-2, and SE-3 by GC-MS analyses.

Cladosporium halotolerans (GE-3) showed inactivity against all the test microorganisms. A zone of inhibition of ≤ 7 mm is considered inactive against microbial growth[58]. The highest antifungal activity with inhibition zone of 13 ± 0.9 mm was observed by the extract of *Chaetomium globosum* (SE-1) against *A. niger*. Other isolates were insignificant or inactive against the tested fungal strains. Goda et al.[57] also reported that three out of six *(C) globosum* isolates inhibited *Candida albicans* with zones of 11.3–25.6 mm.

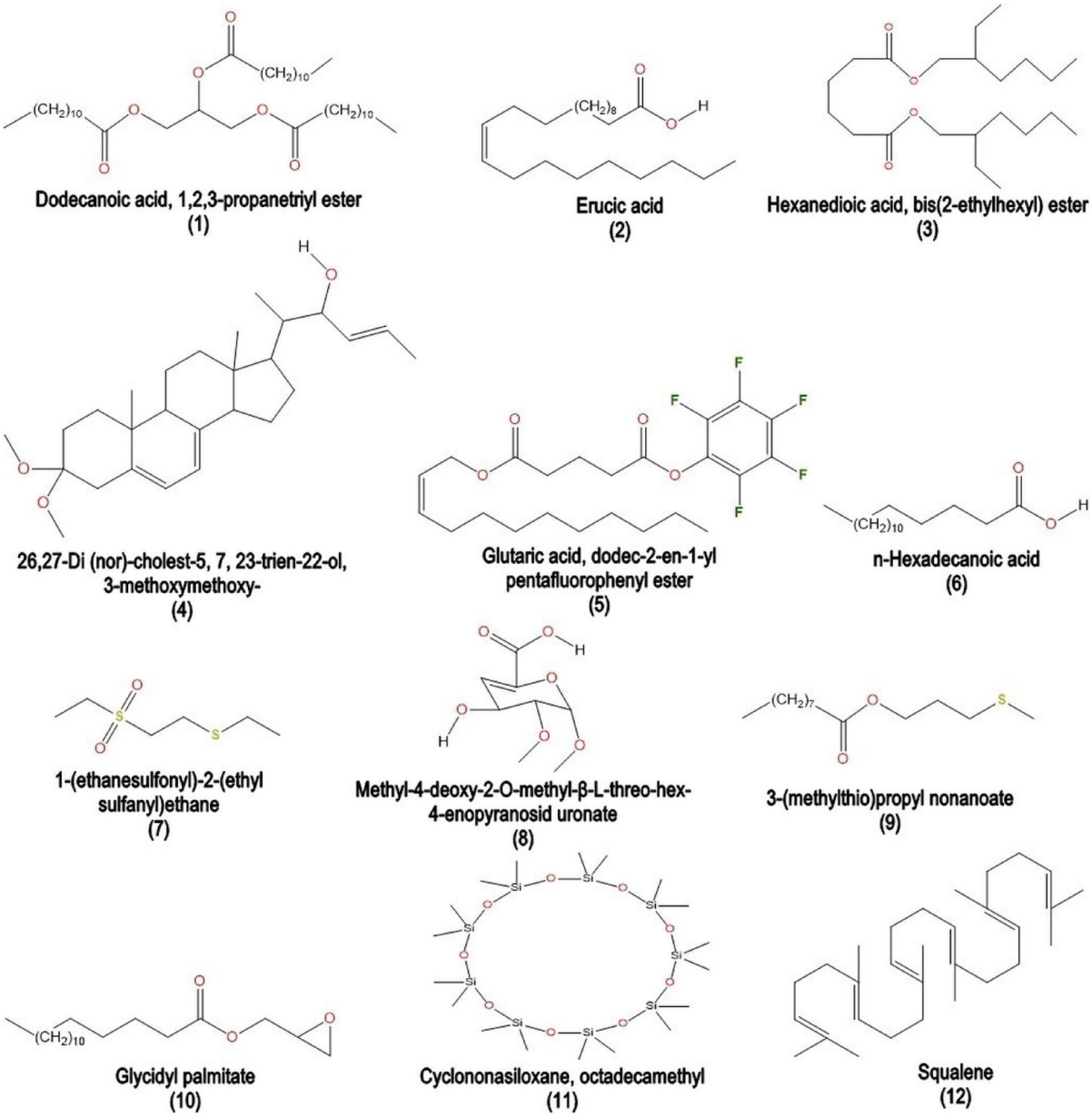

Fig. 4. Major bioactive chemical constituents of the seaweeds and their associated endophytic fungi by GC-MS analysis.

Most fungal extracts exhibited mild to strong antioxidant activity, with IC_{50} values ranging from 7.88 to 178.32 µg/mL (Fig. 3B). The strongest DPPH radical scavenging activity was observed by the extract of *A. terreus* (SE-2), with the lowest IC_{50} value of 7.88 ± 0.09 µg/mL among all the extracts tested[42]. However, the extracts of *A. terreus* (GE-2) and *Curvularia perotidis* (SE-3) showed strong antioxidant activity, with IC_{50} values of 15.08 ± 0.44 and 16.82 ± 0.11 µg/mL, respectively. The other extracts of *A. subversicolor* (GE-1) and *Chaetomium globosum* (SE-1) exhibited mild antioxidant activity, with IC_{50} values of 178.32 ± 0.28 and 112.87 ± 5.09 µg/mL, respectively. The findings presented in this study are supported by the concurrence of Ahamed and Murugan[59] and Lini et al.[52]. Their studies reported the antioxidant nature (IC_{50}, 17–20 µg/mL) of *Aspergillus* sp. derived from marine green macroalgae. Al Mousa et al.[60] also documented soil-derived *A. terreus* showing antioxidant properties with an IC_{50} value of 470 µg/mL. Segaran et al.[61] evaluated the antioxidant potential of *Curvularia* sp. isolated from the medicinal herb *Boerhaavia diffusa* L., finding only about 10% inhibition with ethyl acetate extract at 100 µg/mL concentration, while the highest inhibition (approximately 27%) was observed with butanol extract. In contrast, the ethyl acetate extract of *C. perotidis* (SE-3) in this study showed significantly higher inhibition (83.6%) at 100 µg/mL concentration.

The brine shrimp lethality bioassay serves as a preliminary screening tool for evaluating the cytotoxic activity of fungal crude extracts. Here, an LC_{50} value of ≤ 30 µg/mL is considered indicative of significant activity[34]. Thus, from this study, we can claim that all the fungal isolates have significant cytotoxicity, with LC_{50} values of

Biological activity	Fungal crude extract*					
	GE-1	GE-2	GE-3	SE-1	SE-2	SE-3
	Aspergillus subversicolor	Aspergillus terreus	Cladosporium halotolerans	Chaetomium globosum	Aspergillus terreus	Curvularia perotidis
Antibacterial activity						
Staphylococcus aureus	–	–	–	+	++	–
Bacillus megaterium	–	+	–	–	–	–
Escherichia coli	–	+	–	+	+	–
Salmonella typhi	–	–	–	–	–	–
Pseudomonas aeruginosa	–	+	–	+++	++	+
Antifungal activity						
Aspergillus niger	–	–	–	++	–	–
Aspergillus flavus	–	–	–	–	–	–
DPPH scavenging activity	+	+++	–	+	++++	+++
Brine shrimp lethality assay	+++	+++	++++	+++	+++	++++

Table 5. Overview of biological activities of the marine endophytic fungi. * '++++', '+++', '++' and '+' indicate very strong, strong, moderate and mild bioactivity, respectively, '–' means no activity.

≤ 20.39 ± 4.04 µg/mL. *Curvularia perotidis* (SE-3) and *Cladosporium halotolerans* (GE-3) exhibited the strongest activity (LC_{50}, 9.30 ± 2.96 µg/mL and 9.94 ± 3.49 µg/mL, respectively) among the samples tested (Fig. 3C). According to Noor et al.[27] and Vega-Portalatino et al.[62], seaweed-derived *Curvularia* sp. has a significant impact on cytotoxicity (LC_{50}, 16–18 µg/mL) and provides new insights into the prospects of optimizing its metabolites as anticancer compounds. Sandrawati et al.[63] also reported cytotoxic activity of *Cladosporium halotolerans* isolated from the marine sponge *Dactylospongia* sp., with an LC_{50} value of 64.7 µg/mL. These findings highlight the variability in bioactive potential of fungal extracts depending on their source and ecological niche. Table 5 summarizes the overall biological activities observed in this study. Thus, further studies are necessary to explore the biological potential of these extracts more intensely.

Several chemical compounds were identified from the experimental extracts by GC-MS analysis, most of which were different fatty acids or their esters. These identified compounds are thought to be accountable for the exerted bioactivity of the extracts. Previous studies have reported overwhelming pharmacological activities of these fatty acids and their esters, with their usage in cosmetics and biofuel production[64]. In this study, the most abundant metabolite of marine algal and fungal origin was Dodecanoic acid, 1,2,3-propanetriyl ester (**1**). Compound **1** is a lauric acid ester (also known as trilaurin) that has been reported for antioxidant, antibacterial, antiviral, hypocholesterolemic, antiarthritic, nematocidal, hepatoprotective and mosquito-repellent activities[65,66]. The fungal isolates GE-1 and GE-3 produced compound **1** in a significant quantity (77.86% and 76.84%, respectively), similar to the host seaweed (92.10%). A minor compound, Tetradecanoic acid, 10,13-dimethyl-, methyl ester, was also found in all the associated fungal extracts like the host *Gracilaria* sp., while the other metabolites were unique. The strong cytotoxic and mild antioxidant nature of *Aspergillus subversicolor* (GE-1) might be due to the abundance of compound **1**.

The fungal extract of isolate GE-2 contained Erucic acid (**2**, also called *Z*-13-docosenoic acid) in major quantity (11.71%), and its amide derivative, 13-Docosenamide, (*Z*)- (also called Erucamide), in minor quantity (2.78%). Compound **2** and its amide derivative have been reported for diverse beneficial effects, including antibacterial, antifungal, anti-inflammatory, neuroprotective, cytotoxic, and anticancer activities[67,68]. Other major bioactive compounds of fungal extract of GE-2 were Hexanedioic acid, bis(2-ethylhexyl) ester (**3**, 7.28%), 26,27-Di (nor)-cholest-5, 7, 23-trien-22-ol, 3-methoxymethoxy- (**4**, 6.42%), Glutaric acid, dodec-2-en-1-yl pentafluorophenyl ester (**5**, 5.66%), n-Hexadecanoic acid (**6**, 5.44%). Hexanedioic acid, bis(2-ethylhexyl) ester (**3**) showed antidiabetic, anti-inflammatory, and anticancer activity in the previous studies, and also has minor uses as a plasticizer[69]. Sekaran et al. evaluated the potential chemoprotective activity of 26,27-Di (nor)-cholest-5, 7, 23-trien-22-ol, 3-methoxymethoxy- (**4**) against hepatocellular carcinoma by In-silico study[70]. Glutaric acid, dodec-2-en-1-yl pentafluorophenyl ester (**5**) has no reported pharmacological activity. On the other hand, n-Hexadecanoic acid (**6**), also called palmitic acid, has been reported to have various bioactivities such as antioxidant, anti-inflammatory, antibacterial, anticancer, 5-alpha reductase inhibitor, hemolytic, antiandrogenic flavor, pesticide, nematicide, hypocholesterolemic, potent mosquito larvicide[71–75]. *A. terreus* (GE-2) revealed a potent antioxidant and cytotoxic nature with mild antibacterial activity (Table 5), possibly due to the existence of its aforementioned major components. Abdel-Wahab et al. reported 13-Docosenamide, (*Z*)- and palmitic acid (**6**) as major components of some marine microbes (including yeast and thraustochytid isolates)[76].

Similarly, the very strong antioxidant property, strong cytotoxicity, and mild to moderate antibacterial activity of the extract of *A. terreus* (SE-2) might refer to the contribution of its major constituents. 3-(methylthio)propyl nonanoate (**9**, 25.63%) is a nonanoic acid ester, one of the major compounds in the fungal extract SE-2. Nonanoic acid, also called pelargonic acid, has antifungal potential against *Candida albicans*[77] and cytotoxicity against the Vero cell line[78]. Nonanoic acid and some of its esters are also known as odour-active compounds[79]. Additionally, Methyl-4-deoxy-2-O-methyl-β-L-threo-hex-4-enopyranosid uronate (**8**, 26.43%) is an ester of hexenuronic acid (HexA) and possesses no known benefits[80]. HexA has been reported to have antioxidant property[81] and possibly

a hypoglycemic effect[82]. Glycidyl palmitate (**10**), another fatty acid ester, can be used to prepare lysophosphatidic acids, inhibiting apoptosis revealed in an earlier study[83].

Compound **10** was found to be a major component in the fungal extract of SE-3 (87.63%). Hashem et al.[84] and Pandurangan et al.[85] reported Glycidyl palmitate (**10**) as a fungal metabolite. Another bioactive compound of the isolate SE-3 was Cyclononasiloxane, octadecamethyl (**11**, 3.2%), a common fungal metabolite[5,86] that exhibited antimicrobial and cancer preventive activity[87,88]. These compounds might contribute to the very strong cytotoxicity, strong antioxidant property, and mild antibacterial activity of *Curvularia perotidis* (SE-3). A minor component, 13-octadecenoic acid, methyl ester, was also found in the fungal extracts SE-2 and SE-3, similar to the host *Sargassum* sp. The presence of 1-(ethanesulfonyl)-2-(ethylsulfanyl)ethane (**7**, 99.52%), a prevalent compound of the extract of *Chaetomium globosum* (SE-1), could be accountable for its strong cytotoxicity and mild antioxidant activity with mild to strong antimicrobial activity. A literature survey could not reveal any previous report of compound **7**, but its similar compounds are well-known as odorants[89]. The bioactive compounds **1** (76.84%) and **6** (9.95%) might be responsible for the very strong cytotoxicity exhibited by the extract of *Cladosporium halotolerans* (GE-3). Li et al.[90] and Wang et al.[91] revealed significant cytotoxicity of some pyrone derivatives isolated from the marine-derived fungus *C. halotolerans*.

The seaweed extract GE also contained a bioactive compound, squalene (**12**, 2.11%). Squalene (**12**) is a triterpene widely found in different seaweed species[6,92] and is recognised as a potent radical scavenger, especially in inhibiting lipid peroxidation. It is also indicated for anti-inflammatory activities[93], chemopreventive effects by reducing cancer risk[94], and significantly high UV protective effects with the least toxicity in cosmetics[95]. However, further chemical analyses are necessary to investigate the widespread metabolite profiling of each of these extracts.

This research focuses on isolating, identifying, and evaluating potential fungal endophytes associated with two seaweeds, *Gracilaria* sp. and *Sargassum* sp., collected from Bangladesh's Bay of Bengal. This study proves that fungal endophytes derived from these seaweeds are a valuable resource for biologically active compounds and may fulfill the emerging necessity for potent molecules in therapeutics.

Conclusion

Three fungal endophytes were isolated from the red seaweed *Gracilaria* sp. and another three from the brown seaweed *Sargassum* sp., inhabited in the Bay of Bengal of Bangladesh for the first time. The present study has disclosed the morphological and molecular characteristics of fungal endophytes. The isolated fungal endophytes revealed notable antimicrobial, antioxidant, and cytotoxic activities. GC-MS-based chemical analysis revealed the presence of 91 different compounds in total among the crude extracts of fungal isolates and their hosts. These identified compounds, especially the major ones, have been previously reported to possess various pharmacological activities, which could be responsible for the observed bioactivity in this study. Further studies should focus on isolating these bioactive compounds and their mechanistic analysis, providing scientific evidence of relevant bioactivity.

Data availability

The datasets generated during the current study are available in the NCBI GenBank repository, under accession numbers OR335219, OR335235, OR338335, OR338812, OR336320, and OR335556.

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Author contributions

All authors conceived the research idea. S.N. carried out the methodology and data analysis. S.R.R. and A.A.C. provided necessary resources and logistic support. M.H.S. and M.A.M. supervised the whole study. M.N.B. guided the isolation and identification of fungi. S.N. drafted the original manuscript. M.H.S. and M.A.M. reviewed and edited it. All authors read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

All the experimental procedures were conducted following the standard protocols.

Additional information

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