Identification and preliminary bioactivity screening of the marine endophytic fungus *Aspergillus terreus* strain BAWK-F6 derived from Algerian brown seaweed

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Abstract: Marine fungi are known for their ability to produce bioactive compounds with pharmaceutical potential. However, their growth in diverse and often extreme habitats makes laboratory cultivation difficult, thereby limiting research progress. The algicolous endophyte fungus *Aspergillus terreus* strain BAWK-F6 was isolated from brown seaweed *Dictyota dichotoma* and identified through molecular analyses. The fungal extract was evaluated for a range of biological activities. Molecular identification revealed a high genetic similarity with other *A. terreus* strains, and chemical analysis using gas chromatography mass spectrometry (GC-MS) identified 28 compounds. The extract demonstrated antioxidant activity, with IC_{50} values ranging from 1.98 to 2.32 mg/mL. Antimicrobial assays demonstrated that the extract effectively inhibited a broad spectrum of Gram-negative pathogens, with minimum inhibitory concentrations (MIC) ranging from 0.625 to 1.25 mg/mL. The extract also exhibited potent antibiofilm activity, especially against *Staphylococcus aureus*. It exhibited anti-inflammatory activity by inhibiting protein denaturation and stabilizing red blood cell membranes, as well as anti-urolithic potential by suppressing the formation of calcium phosphate crystals. These findings highlight the therapeutic promise of *Aspergillus terreus* BAWK-F6 as a source of bioactive compounds with potential applications in pharmacology and biotechnology.

Keywords: Algicolous endophyte, seaweed, anti-urolithic activity, bioactive compounds, pharmaceutical potential.

INTRODUCTION

Recent efforts to find novel biologically active molecules have increasingly focused on organisms derived from understudied environments. Oceans, covering about 67% of the Earth's surface, are ecologically important and highly biodiverse ecosystems that remain largely underexplored. Marine microorganisms are good sources of novel bioactive metabolites that may have significant applications in medicine and industry [1]. Marine ecosystems host a wide range of fungi, including parasites, saprobes, and endophytes. These fungal communities have been found in almost all marine habitats, from the water column and sediments to symbiotic associations with marine organisms such as sponges and algae [2,3]. Since the 1970s, interest in endophytes has grown, particularly with regard to their origin, biological diversity, interactions with host plants, and ecological roles. Particular emphasis has been placed on characterizing their bioactive compounds, as these microorganisms synthesize diverse natural metabolites with substantial pharmacological potential. Endophytes have attracted additional interest once it became clear that they can generate bioactive metabolites with structurally diverse molecules, many of which are difficult to reproduce through synthetic chemistry [4,5]. Abiotic physicochemical stress and biotic factors, such as intra- or interspecies interactions

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with hosts and other microorganisms, can influence marine symbiotic microorganisms. These influences equip them with unique metabolic pathways not typically observed in terrestrial taxa [6].

Fungal endophytes from marine environments constitute an important source of major compounds with strong biological potential [7]. Algae are considered the second most important source of marine fungi after mangrove swamps, with red and brown seaweeds typically yielding a broader diversity of endophytes than green seaweeds [8,9]. Marine algicolous fungi are marine fungi that live endophytically within macroalgae. They are notable for their ability to synthesize a wide range of bioactive secondary metabolites. These compounds are categorized according to their biological activities, including antioxidant, antibacterial, antifungal, and anticancer properties. Endophytic fungi, in general, possess the remarkable capacity to produce a comprehensive spectrum of bioactive metabolites with varied biological effects, making them valuable resources for pharmaceutical, medical, and biotechnological applications [10,11]. Marine *Aspergillus* have received attention because they produce bioactive compounds with antioxidant, antimicrobial, cytotoxic, anti-inflammatory, insecticidal, and antiviral properties. Different secondary metabolites, ranging from steroids, alkaloids, terpenoids, lactones, butenolides, and polyketones, have been recovered from these isolates [12]. *Aspergillus terreus* can be found in a range of highly challenging environments, including extreme habitats characterized by high salinity, high alkalinity, extreme temperatures, drought, and other severe abiotic conditions [13].

The present study reports on the isolation and bioactivity screening of the endophytic fungus *Aspergillus terreus* strain BAWK-F6, obtained from the brown seaweed *Dictyota dichotoma* collected from the Algerian coast. Although A. terreus has been previously reported from marine environments and is known to produce diverse secondary metabolites [6], this study provides new strain-level data from an Algerian algal host, including molecular identification and metabolite profiling. GC-MS analysis of the fungal extract revealed a chemical profile dominated by sesquiterpenes and related compounds. Biological assays demonstrated multiple activities, including antioxidant, antimicrobial, antibiofilm, anti-inflammatory, and anti-urolithic effects. Collectively, these findings highlight the therapeutic potential of marine-derived *A. terreus* and underscore the importance of algicolous fungi as valuable reservoirs of bioactive metabolites.

MATERIALS AND METHODS

Ethics statement

As this study did not include human participants or animal experiments, ethical approval was not necessary.

Seaweed sampling

The brown marine alga *Dictyota dichotoma* (Phaeophyceae) was collected in June 2024 from Kouali Beach, Tipaza, in north-central Algeria (36°35'27"N, 2°29'41"E). Healthy thalli were handpicked from intertidal rocks. Samples were rinsed on-site with ambient seawater to remove epiphytes and debris. Each specimen was placed in a sterile zip-lock bag with a small amount of seawater to maintain hydration and minimize microbial contamination. Samples were transported to the laboratory in an ice box and processed within one hour to isolate endophytic microorganisms.

Isolation of algicolous endophyte fungi

Surface sterilization followed the method of Zainee et al. [14], in which the seaweed specimen was sequentially immersed in 75% ethanol for 30 s, 5% sodium hypochlorite for 30 s, and 75% ethanol for 10 s. To show that the isolated microorganisms came from the internal tissue of the samples, fingerprints of the surface-sterilized sections were validated using agar plates, and incubated at 28°C. The final wash (0.1 mL) was inoculated onto culture media. Endophytic fungi were isolated from healthy thalli of the brown seaweed using the direct plating method [15]. Tissue sections of each algal specimen (approximately 5 mm^2) were cut with a flame-sterilized scalpel and gently placed on Wickerham medium [16] (3 g malt extract, 3 g yeast extract, 10 g glucose, 5 g peptone per 1000 mL natural seawater, 18 g agar, pH 7.8) supplemented with chloramphenicol (200 mg/L). The plates were incubated at 28°C for 7 days to observe mycelial growth at the margin of the algal segments. The hyphae of *A. terreus* were subcultured onto fresh PDA medium for purification.

Molecular identification and phylogenetic analyses

Total DNA was extracted from dry samples using a modified protocol based on Murray and Thompson [17]. Amplification of the ITS region of fungal rDNA was performed by PCR [18] in 35 cycles, with an annealing temperature of 54°C. Primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3'), ITS1 (5'-CCGTAGGTGAACCTGCGG-3'), and NLB4 (5'-GGTCCGTGTTTCAAGACGG-3'9) were used to target the region of interest. Amplification products were verified by 1% agarose gel electrophoresis, and amplicons were then sequenced using one or both PCR primers. The resulting sequences were then corrected to remove misreads detected in the chromatograms. Raw sequence data were processed with BioEdit v7.7.1 and then compared with sequences available in the rDNA/ITS database of GenBank (NCBI). Each isolate's nucleotide sequence was subjected to similarity analysis using the BLAST (Basic Local Alignment Search Tool) algorithm. Based on the total score, query coverage, and percent identity, the most relevant sequences were retained and grouped into distinct clades for the construction of the phylogenetic tree. The latter was generated according to the neighbor-joining method using the Tamura-Nei model implemented in the MEGA 12 software. Branch robustness was assessed by a bootstrap of 1,000 iterations, and the tree was scaled according to the average number of substitutions per site.

Fermentation and preparation of fungal crude extract

For the *A. terreus* BAWK-F6 isolate, 2 mL of activated fungal solution (10^8 spores/mL) was inoculated into a 500-mL Erlenmeyer flask with 200 mL of potato dextrose broth (PDB) medium. The culture was incubated in a rotary shaker at 150 rpm/min (New Brunswick™ Excella® E24, Germany) and 28°C for 10 days. Secondary metabolites were extracted according to Kjer et al. [19], with some modifications. To extract the metabolites released by the fungus into the culture medium, an equal volume of ethyl acetate (EtOAc) was added to the fungal cell-free supernatants (1:1, v/v). The mixture was kept at room temperature for 24 h and stirred at regular intervals. Then, the culture underwent ultrasonic treatment using an ultrasonic homogenizer (SMCLAB Quimica, China) at 40 kHz and 200 W for 10 min. The organic phase was subsequently evaporated using a rotary evaporator (EVA180-B, LBX, Spain), yielding a crude extract of the fungal metabolites. The dried extract was dissolved in dimethyl sulfoxide (DMSO), sterilized by filtration (0.22 µm filters), and stored at 4°C as a stock solution for subsequent biological activity assays.

Gas chromatography mass spectrometry (GC-MS) analysis

The sample was analyzed on a SHIMADZU GC-MS-QP2020 mass spectrometer equipped with an Rxi®-5ms fused-silica capillary column (Crossbond® 5% diphenyl/95% dimethylpolysiloxane; 30 m × 0.25 mm i.d.; 0.25 µm film thickness). This column is comparable to the HP-1ms, HP-1msUI, DB-1ms, DB-5ms, DB-1msUI, Ultra-1, VF-1ms, ZB-1, and ZB-1ms, and is considered equivalent to USP phases G1, G2, and G38. A 0.5-µL aliquot was injected in split mode (1:10). The injector temperature was set at 250°C, and the detector temperature was maintained at 220°C. The column temperature program was as follows: hold at 40°C for 3 min, increase to 220°C at a rate of 25°C/min, and hold at this temperature for 10 min. The carrier gas used was helium (purity 99.995%) with a constant flow rate of 1 mL/min. Mass spectrometry conditions were as follows: ionization energy 70 eV; ion source temperature 200°C; mass spectra acquired over the 45-600 m/z range.

Antioxidant activity

Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging assay was carried out according to Hussein et al. [20]. Two hundred µL of DPPH solution was mixed with 50 µL of the sample at different concentrations. The mixture was mixed and incubated for 15 min in the dark at room temperature. Ascorbic acid was used as the control. The absorbance of the resulting solution was measured at 517 nm using a microplate reader (SMCLAB Quimica, China). The following equation was used to calculate the percentage of DPPH radical scavenging activity:

$$Scavenging\ Activity\ (\%) = \left(\frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance}\right) \times 100$$

Results were expressed as IC_{50} values, representing the concentration of extract required to inhibit DPPH radical formation by 50% [21]. Each experiment was performed in triplicate.

ABTS⁺ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay

ABTS⁺ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity was assessed according to [22]. In a 96-well microplate, a total of 150 µL of ABTS⁺ solution was mixed with 50 µL of the fungal extract at varying concentrations, or with ascorbic acid as a positive control. After incubating the mixture at room temperature for 10 min, the absorbance was measured at 734 nm using a microplate reader (SMCLAB Quimica, China).

Hydroxyl radical scavenging assay

The reaction mixture contained 24 µL of ferrous sulfate ($FeSO_4$), 80 µL of salicylic acid ($C_7H_6O_3$), 50 µL of the fungal extract, and 20 µL of hydrogen peroxide (H_2O_2). It was kept in a water bath at 37°C for 30 min. Following the incubation period, the absorbance of the reaction mixture was determined with a microplate reader (SMCLAB Quimica, China) at 510 nm. Ascorbic acid was used as the control. The scavenging activity of the extract was calculated using the following formula:

$$Scavenging\ activity\ (\%) = \left(\frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance}\right) \times 100$$

Antimicrobial activity

Antibacterial activity was assessed using a panel of American Type Culture Collection (ATCC) type strains obtained from the Microbiology Laboratory of the Algerian Pasteur Institute: *Staphylococcus aureus* ATCC 23235, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 2453, and *Pseudomonas aeruginosa* ATCC 27853. Additionally, eight clinical bacterial isolates were provided by the Bacteriology Laboratory of Kolea Hospital (Tipaza, Algeria). Two phytopathogenic bacteria, *Pseudomonas savastanoi* and *Agrobacterium tumefaciens*, were supplied by the management and valorization of the agricultural and aquatic ecosystems laboratory. Antifungal activity was evaluated against one reference yeast strain, *Candida albicans* ATCC 10231, and four type cultures, *Aspergillus flavus* NRRL 3251, *Aspergillus ochraceus* ATCC 3174, *Aspergillus parasiticus* CBS 100926^T, and *Aspergillus carbonarius* M333. Three phytopathogenic fungi, *Aspergillus niger*, *Penicillium notatum*, and *Fusarium oxysporum,* were also included.

Agar well diffusion assay

The antimicrobial potential of the fungal extract was evaluated using the agar well diffusion method [23]. Suspensions of the tested bacterial and fungal strains were prepared under aseptic conditions. Mueller Hinton Agar (MHA) was used for bacterial strains, whereas PDA served as the culture medium for fungal isolates. Each plate was inoculated with 200 µL of the corresponding 0.5 McFarland microbial suspension and evenly spread over the agar surface using a sterile swab. Wells 6 mm in diameter were aseptically punched into the agar and filled with 50 µL of the fungal extract. The plates were then incubated at 37°C for 24 h for bacterial strains, and 25°C for 48 to 72 h for fungi. Experiments were conducted in triplicate, and the antimicrobial activity was determined by measuring the diameter of the inhibition zones (mm).

Determination of MIC and MBC/MFC

The minimum inhibitory concentration (MIC) of the fungal extract against the tested microorganisms was determined using the 96-well microplate broth dilution method [24,25], with minor modifications. Two-fold serial dilutions of the extract were prepared in PDB for fungi or MHB for bacteria, using sterile 96-well microplates. Each well was inoculated with 10 µL of a standardized bacterial suspension or 100 µL of a fungal spore suspension. Plates were prepared in triplicate and incubated at 37°C for 18-24 h for bacterial strains and at 25°C for 48-72 h for fungal isolates. Chloramphenicol (50 µg/mL) and nystatin (50 µg/mL) served as positive controls, while DMSO was used as the negative control. Following incubation, 20 µL of 0.01% resazurin solution was added to each well as a viability indicator. The MIC was defined as the lowest concentration of the extract that completely inhibited

visible microbial growth. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by subculturing 100 µL from wells showing no visible microbial growth onto agar plates. After 24-72 h of incubation, the lowest concentration that resulted in no microbial growth on the subculture was recorded as the MBC or MFC.

Anti-biofilm activity

The ability of bacterial isolates to form and resist biofilm inhibition was evaluated using the tryptic soy broth with glucose (TSBG) microplate method [26]. Freshly cultured bacteria were inoculated into TSB containing 2.5% glucose and incubated at 37°C for 24 h, then standardized at 0.5 McFarland. A volume of 150 µL from each suspension was dispensed into 96-well plates in triplicate, then incubated under the same conditions. After washing to remove free cells, biofilms were stained with 2.5% crystal violet for 30 min, rinsed, and decolorized with 96% ethanol. The absorbance was then measured at 590 nm. For the anti-biofilm assay [27], standardized bacterial suspensions were combined with an equal volume of the fungal extract (10 mg/mL) and incubated for 24 h at 37°C. The optical density was measured using a microplate reader (SMCLAB Quimica, China) at 590 nm, and the inhibition percentage was determined using the following equation:

$$\text{Inhibition (\%)} = \left(\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}}\right) \times 100$$

Anti-inflammatory activity, protein denaturation assay

A protein denaturation assay was conducted to assess the anti-inflammatory activity of the fungal extract [28]. The reaction mixture contained different concentrations of the extract, 900 µL of a 5% bovine serum albumin (BSA) solution or 400 µL of egg albumin (EA), and 2800 µL of phosphate-buffered saline (PBS). After incubation at 37°C for 15 min, the mixtures were heated at 65-70°C for 10 min, and the absorbance was measured at 660 nm. Diclofenac sodium (0.1 mg/mL) was used as the positive control, and distilled water served as the negative control. Each assay was conducted in triplicate. To calculate the percentage inhibition of protein denaturation, the following formula was applied:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Sample absorbance}}{\text{Negative control absorbance}}\right) \times 100$$

Human red blood cell (HRBC) membrane stabilization assay

The effect of the fungal extract on the HRBC membrane stabilization assay was assessed according to Kamal et al. [27]. Fresh human blood was used to isolate erythrocytes, which were washed with 50 mM PBS, and exposed to stress conditions that commonly cause membrane rupture, such as hypotonic or heat-induced hemolysis. In the hypotonic assay, the HRBC suspension was treated with the fungal extract in a hyposaline medium and incubated at 37°C, whereas in the heat-induced hemolysis test, HRBC suspensions were exposed to 54°C in a water bath. After incubation for 30 min and centrifugation at 1,118 ×g, the absorbance of the released hemoglobin was measured at 560 nm. Distilled water was used as the negative control and diclofenac sodium (0.1 mg/mL) as a positive control; the inhibition percentage of hemolysis by the fungal extract was calculated thus:

$$\text{Inhibition (\%)} = \left(\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}}\right) \times 100$$

Anti-urolithic activity

Anti-urolithic activity was evaluated using a nucleation assay [29,30] to assess inhibition of calcium phosphate (CaP) crystallization. Freshly prepared solutions of 500 µL calcium chloride ($CaCl_2$, 10 mM) and 500 µL disodium hydrogen phosphate (Na_2HPO_4, 10 mM) were used to prepare the reaction mixture containing 500 µL of physiological buffer NaCl (0.15 M, pH 6.5) and 500 µL of the extract solution (50-800 µg/mL). After gentle mixing and incubation at 37°C for 60 min, the turbidity resulting from calcium phosphate crystal formation was measured at 620 nm using a UV-Visible spectrophotometer (Jenway 6320D, UK). The percentage inhibition of nucleation was calculated as:

$$Inhibition\ (\%) = \left(\frac{ODc - ODs}{ODc}\right) \times 100$$

Where ODc and ODs represent the optical densities of the control and sample, respectively. Cystone (1 mg/mL) served as a positive control, while 1% DMSO served as the negative control. Furthermore, microscopic

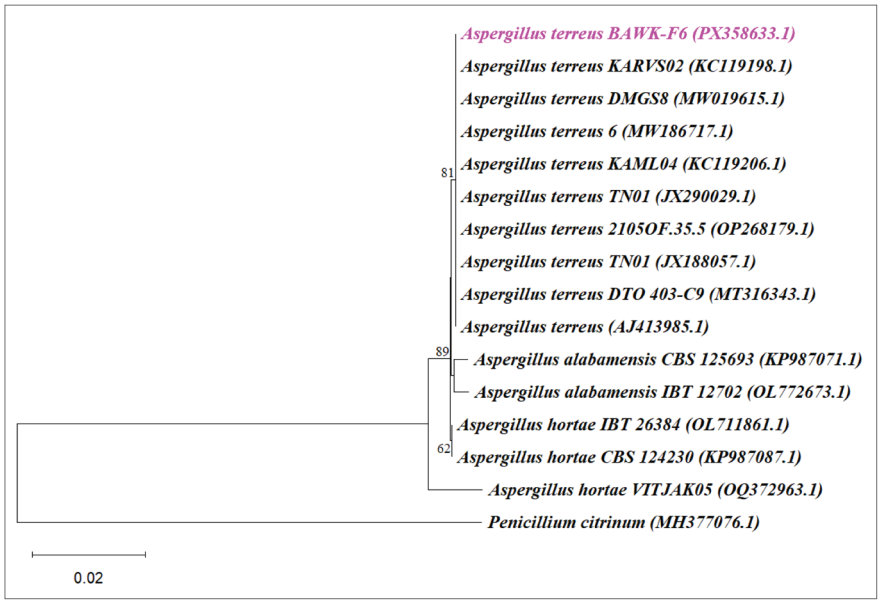

Fig. 1. Phylogenetic tree based on ITS rDNA sequences showing the relationship between *Aspergillus terreus* BAWK-F6 (PX358633.1) and closely related *Aspergillus* species.

observations of calcium phosphate crystallization were conducted in the presence and absence of the extract using a light microscope (Optika, Italy).

Statistical analysis

All statistics have been conducted using GraphPad Prism 10 (GraphPad Software, USA). One-way analysis of variance (ANOVA) and Duncan's multiple range test were used to compare mean values. Differences between the two groups were assessed using the Student's *t*-test. Statistical significance was considered at P <0.05 (*), P<0.005 (**), P<0.0005 (***), and P<0.00005 (****).

RESULTS

Molecular identification and phylogenetic analyses

The fungal isolate *Aspergillus terreus* BAWK-F6 was identified using a molecular biological approach involving DNA amplification and sequencing of the internal transcribed spacer (ITS) region. The obtained sequence was deposited in the GenBank database under accession number (PX358633.1). BLAST search results showed a 99.48% match with several *A. terreus* strains, such as *A. terreus* (KC119198.1) from seaweed, *A. terreus* (MW019615.1), *A. terreus* (MW186717.1) from marine soil, and *A. terreus* (JX290029.1). Most of these strains were found in marine or seaweed environments and form a well-supported group, as shown by the bootstrap values (Fig. 1). The phylogenetic relatedness of these marine and seaweed isolates highlights the genetic similarity of *A. terreus* in marine ecosystems. This group is clearly distinct from related species such as *A. alabamensis* and *A. hortae*. *Penicillium citrinum* (MH377076.1) was used as an outgroup to support the phylogenetic analysis and show the evolutionary differences between *Aspergillus* and other fungal genera.

Chemical composition identified by GC-MS analysis

GC-MS analysis was carried out to characterize the chemical constituents of the endophytic extract of *A. terreus* BAWK-F6. Twenty-eight compounds were identified from the *A. terreus* BAWK-F6 extract, and their retention times (RT), molecular formulas, molecular weights, and peak areas (%) are summarized in Table 1. The GC-MS chromatogram for *A. terreus* BAWK-F6 is shown in Supplementary Fig. S1. GC-MS analysis of the *A. terreus* BAWK-F6 extract identified a range of compounds, with retention times ranging from approximately 4.5 to 17.6 min and molecular weights between 136 and 310 g/mol. The chromatographic profile was predominantly composed of sesquiterpenes, mainly oxygenated sesquiterpene alcohols, and sesquiterpene hydrocarbons, with smaller proportions of alkanes, monoterpenes, and coumarin derivatives. The major constituents were sesquiterpene alcohols such as 2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl) propan-2-ol, and cyclohexanemethanol, 4-ethenyl-α, α,4-trimethyl-3-(1-methylethenyl)-, [1R-(1α,3α,4β)]. Other notable sesquiterpene hydrocarbons included β-elemene, γ-muurolene, and α-gurjunene, while minor compounds included eicosane and terpinelone.

Table 1. Chemical composition identified using GC-MS analysis

RT	Identified Compound	Compound Nature	Peak Area (%)	Formula	M.W. (g/mol)
10.784	2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl) propan-2-ol	Sesquiterpene alcohol	10.90	C15H26O	222.37
10.189	Cyclohexanemethanol, 4-ethenyl-α, α,4-trimethyl-3-(1-methylethenyl)-, [1R-(1α,3α,4β)]-	Sesquiterpene alcohol	10.37	C15H26O	222.37
11.040	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1α,4aβ,8aα)]-	Sesquiterpene alcohol	8.42	C15H24O	220.35
10.680	Agarospirol	Sesquiterpene alcohol	7.02	C15H26O	222.37
10.601	Selin-6-en-4α-ol	Sesquiterpene alcohol	6.68	C15H26O	222.37
7.680	Menth-2-en-1-ol (trans-, para-)	Monoterpene alcohol	5.12	C10H18O	154.25
10.847	Neointermedeol	Sesquiterpene alcohol	3.73	C15H26O	222.37
9.359	β-Elemene	Sesquiterpene hydrocarbon	3.25	C15H24	204.36
10.050	γ-Muurolene	Sesquiterpene hydrocarbon	3.22	C15H24	204.36
9.892	α-Gurjunene	Sesquiterpene hydrocarbon	2.77	C15H24	204.36
8.248	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, trans-	Monoterpenoid alcohol	2.96	C10H18O	154.25
10.650	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro-α, α,4a,8-tetramethyl-, (2R-cis)-	Sesquiterpenoid alcohol	1.98	C15H26O	222
10.305	Hexadecane	n-Alkane	1.95	C16H34	226.44
9.935	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta [1,3] cyclopropa[1,2] benzen-3-ol	Oxygenated sesquiterpene	1.85	C15H26O	222
17.608	Docosane	n-Alkane	1.59	C22H46	310.60
12.569	1H-2-Benzopyran-1-one, 3,4-dihydro-3,8-dihydroxy-3-methyl-, (-)-	Coumarin derivative	1.59	C10H10O4	194.18
9.288	Tetradecane	n-Alkane	1.37	C14H30	198.39
10.504	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-α, α,4a,8-tetramethyl-, (2α,4aα,8aα)-	Sesquiterpenoid alcohol	1.37	C15H26O	222
4.499	(R, R)-Butane-2,3-diol	Small aliphatic diol	1.22	C4H10O2	90.12
9.641	Cedrene	Sesquiterpene hydrocarbon	1.30	C15H24	204.36
9.288	Tricosane	n-Alkane	1.06	C23H48	324.64
9.821	α-Bisabolol	Sesquiterpene alcohol	0.98	C15H26O	222.37
10.390	Caryophyllene oxide	Oxygenated sesquiterpene	0.82	C15H24O	220.35
9.574	γ-Elemene	Sesquiterpene hydrocarbon	0.74	C15H24	204.36
9.745	Humulene epoxide I	Oxygenated sesquiterpene	0.62	C15H24O	220.35
12.951	Eicosane	n-Alkane	0.59	C20H42	282.55
9.984	α-Bulnesene	Sesquiterpene hydrocarbon	0.58	C15H24	204.36
6.747	Terpinolene	Monoterpene hydrocarbon	0.51	C10H16	136.24

Antioxidant activity

Table 1 presents the antioxidant activity of the *A. terreus* BAWK-F6 extract, which exhibited strong antioxidant capacity, as reflected by its low IC_{50} values across all three tested methods. Among the three assays, DPPH scavenging demonstrated the highest effectiveness with an IC_{50} of 1.98 mg/mL, followed by $ABTS^{+}$ (2.32 mg/mL), while hydroxyl radical scavenging exhibited the weakest activity. The IC_{50} value for ascorbic acid was in the range of 0.027-0.054 mg/mL. In the DPPH assay, the extract showed approximately 37-fold lower potency than ascorbic acid. Although less active than pure ascorbic acid, the *A. terreus* BAWK-F6 extract still exhibits substantial antioxidant potential as a natural fungal metabolite.

Antimicrobial activity

The antimicrobial screening of the *A. terreus* BAWK-F6 extract revealed a dose-dependent inhibitory effect against the tested microbial strains (Supplementary

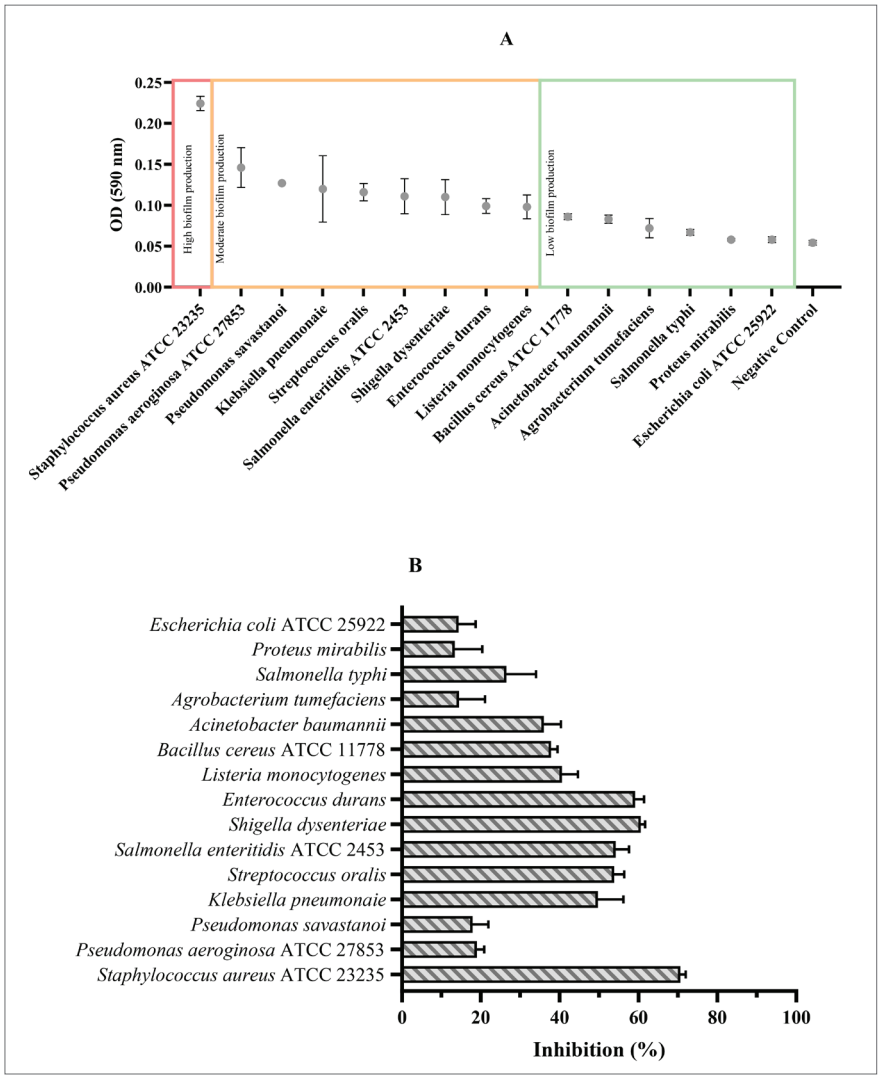

Fig. 2. Quantitative assessment of biofilm formation and inhibition by different bacterial strains. **A** – Biofilm-forming ability of the tested bacterial strains. **B** – Percentage of biofilm inhibition (%) exhibited by the *A. terreus* BAWK-F6 (against the corresponding bacterial strains

Table 2. IC_{50} antioxidant values/activities

IC_{50} values(mg/mL) of radical scavenging			
	DPPH radical	**Hydroxyl Radical**	**ABT^{S}+**
***A. terreus* BAWK-F6**	1.98±0.38***	4.62±0.14****	2.32±0.12****
Ascorbic acid	0.054± 0.008	0.043 ± 0.001	0.027±0.005

Fig. S2, S3). For antibacterial activity, the most sensitive strains were *S. enteritidis* ATCC 2453, *A. baumannii*, and *E. durans*, with inhibition zones of 35.16 mm, 17.32 mm, and 18.74 mm, respectively, followed by *K. pneumoniae* (16.35 mm) and *B. cereus* ATCC 11778 (15.15 mm). Moderate inhibition was recorded against *P. aeruginosa* ATCC 27853, *S. typhi*, and *S. aureus* ATCC 23235, with inhibition zones ranging from 11 to 13 mm, while *E. coli* ATCC 25922, and *L. monocytogenes* exhibited lower sensitivity. The extract had limited antibacterial efficacy against *P. savastanoi* and *A. tumefaciens*, producing relatively small inhibition zones (about 10 mm). The *A. terreus* BAWK-F6 extract demonstrated significant efficacy against specific Gram-negative pathogens.

The antifungal activity of the *A. terreus* BAWK-F6 extract increased significantly in most tested species. *C. albicans* ATCC 10231, *A. ochraceus* ATCC 3174, and *A. carbonarius* M333 showed moderate sensitivity and did not exhibit substantial increases in inhibition at higher concentrations. In contrast, *A. flavus* NRRL 3251, *A. niger*, *A. parasiticus* CBS 100926^{T}, *P. notatum*, and *F. equiseti* showed pronounced increases in inhibition zone diameter at 10 mg/mL, indicating strong susceptibility. The highest inhibition was observed in *A. niger* and *P. notatum*, with zones of 18.23-19.1 mm at 10 mg/mL.

The antimicrobial evaluation of the *A. terreus* BAWK-F6 extract demonstrated inhibitory activity against both Gram-positive and Gram-negative bacteria, as well as several fungal pathogens. The MIC ranged between 0.625 and 1.25 mg/mL, while the MBC and MFC varied between 1.25 to 5 mg/mL, depending on the strain (Table 3). Among bacterial strains, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 23235, and *S. enteritidis* ATCC 2453 were notably sensitive, with MIC values of 1.25 mg/mL and MBC values between 1.25 and 2.5 mg/mL. The extract demonstrated consistent antifungal activity against the fungal strains, with MICs ranging from 0.625 to 2.5 mg/mL. The lowest MICs were observed for *A. carbonarius* M333, *A. ochraceus* ATCC 3174, and *A. niger*, indicating strong activity against

Table 3. The MIC, MBC, and MFC of *A. terreus* BAWK-F6 extract

	Strains	*A. terreus* BAWK-F6 extract (mg/mL)		Chloramphenicol/Nystatin (mg/mL)	
		MIC	MBC/MFC	MIC	MBC/MFC
Bacterial strains	*Escherichia coli* ATCC 25922	1.25±0	2.5±0	0.006±0	0.012±0
	Salmonella enteritidis ATCC 2453	1.25±0	2.5±0	0.006±0	0.012±0
	Salmonella typhi	0.625±0	2.5±0	0.025±0	0.025±0
	Shigella dysenteriae	1.25±0	1.25±0	0.012±0	0.025±0
	Klebsiella pneumoniae	2.5±0	5±0	0.012±0	0.012±0
	Proteus mirabilis	1.25±0	2.5±0	0.05±0	0.05±0
	Acinetobacter baumannii	0.625±0	1.041±0.36	0.006±0	0.012±0
	Pseudomonas aeruginosa ATCC 27853	2.5±0	5±0	0.025±0	0.025±0
	Pseudomonas savastanoi	1.25±0	1.25±0	0.05±0	0.05±0
	Agrobacterium tumefaciens	1.25±0	2.5±0	0.05±0	0.05±0
	Staphylococcus aureus ATCC 23235	1.25±0	1.25±0	0.05±0	0.05±0
	Enterococcus durans	0.625±0	1.25±0	0.012±0	0.025±0
	Streptococcus oralis	1.25±0	1.25±0	0.025±0	0.025±0
	Bacillus cereus ATCC 11778	1.25±0	2.5±0	0.05±0	0.05±0
	Listeria monocytogenes	1.25±0	2.5±0	0.012±0	0.025±0
Fungal strains	*Candida albicans* ATCC 10231	1.25±0	2.5±0	0.025±0	0.05±0
	Aspergillus carbonarius M333	0.625±0	2.5±0	0.05±0	0.05±0
	Aspergillus ochraceus ATCC 3174	0.625±0	2.5±0	0.025±0	0.05±0
	Aspergillus flavus NRRL 3251	2.5±0	5±0	0.05±0	0.05±0
	Aspergillus parasiticus CBS 100926^T	2.5±0	2.5±0	0.05±0	0.05±0
	Aspergillus niger	0.625±0	2.5±0	0.05±0	0.05±0
	Penicillium chrysogenum	0.625±0	5±0	0.025±0	0.025±0
	Fusarium equiseti	2.5±0	5±0	0.025±0	0.05±0

MIC – minimal inhibitory concentration; MBC – minimal bactericidal concentration; MFC – minimal fungicidal concentration

toxin-producing *Aspergillus* species. In contrast, *A. flavus* NRRL 3251, *A. parasiticus* CBS 100926^T, and *F. equiseti* had higher MICs (2.5 mg/mL), reflecting moderate sensitivity.

Anti-biofilm activity

The ability of the tested bacterial strains to form biofilms and the inhibitory effect of the *A. terreus* BAWK-F6 extract were quantitatively evaluated (Fig. 2A). Among the isolates, *S. aureus* ATCC 23235 showed the highest biofilm-forming capacity, followed by *P. aeruginosa* ATCC 27853 (OD_{595} = 0.146), and *P. savastanoi*, classifying them as strong biofilm producers. Moderate biofilm formation was observed in *K. pneumoniae*, *S. oralis*, and *S. enteritidis* ATCC 2453 with OD_{595} ranging between 0.11 and 0.12. Other strains, including *E. coli* ATCC 25922, *P. mirabilis*, and *A. baumannii*, were weak biofilm producers ($OD_{595} \leq 0.10$). The antifouling potential of the *A. terreus* BAWK-F6 extract was further supported by its biofilm inhibition activity (Fig. 2B). The extract significantly inhibited biofilm formation, with the highest inhibition observed in *S. aureus* ATCC 23235 (70.6%), followed by *S. dysenteriae* (60.6%), *E. durans* (59.1%), *S. enteritidis* ATCC 2453 (54.3%), and *S. oralis* (53.8%). Moderate inhibition was found against *K. pneumoniae* (49.7%) and *L. monocytogenes* (40.6%), whereas lower inhibition was observed in *P. aeruginosa* ATCC 27853, *P. savastanoi*, *E. coli* ATCC 25922, and *P. mirabilis*.

Anti-inflammatory activity

The anti-inflammatory potential of the *A. terreus* BAWK-F6 extract was evaluated through protein denaturation and HRBC membrane stabilization assays (Fig. 3). Results showed that the fungal extract effectively inhibited the denaturation of both egg

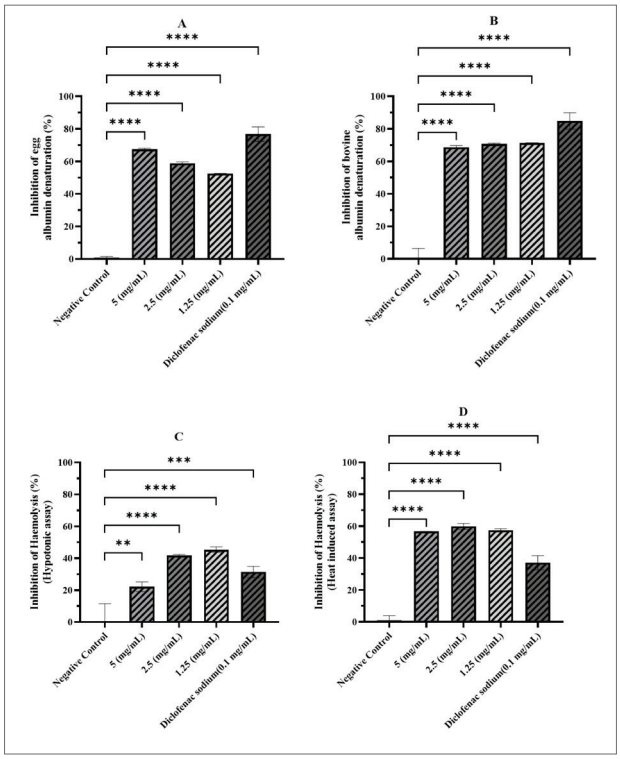

Fig. 3. Anti-inflammatory activity of the fungal extract evaluated by protein denaturation and HRBC membrane stabilization assays. **A** – Inhibition of egg albumin denaturation (%), **B** – inhibition of bovine serum albumin (BSA) denaturation (%), **C** – inhibition of hypotonic solution-induced hemolysis; **D** – inhibition of heat-induced hemolysis. The fungal extract was tested at different concentrations (1.25–5 mg/mL), and diclofenac sodium (0.1 mg/mL) served as the standard anti-inflammatory drug. Values are expressed as mean±SD (n=3). Statistical significance compared with the negative control: (*P<0.05, **P<0.005, ***P<0.0005, ****P<0.00005).

albumin (Fig. 3A) and bovine serum albumin (Fig. 3B), showing a dose-dependent effect. The highest inhibition was recorded at 5 mg/mL (67%) for egg albumin and at 1.25 mg/mL (71%) for bovine serum albumin. These effects were found to be comparable to those produced by diclofenac sodium at 0.1 mg/mL. The HRBC membrane stabilization assay showed that the fungal extract protected red blood cells from hemolysis caused by both hypotonic solutions (Fig. 3C) and heat (Fig. 3D). At 1.25 mg/mL, the extract reduced hemolysis by 45% under hypotonic stress, and at 2.5 mg/mL, it reduced hemolysis by 59% under heat-induced conditions. These findings confirm that the extract possesses significant anti-inflammatory activity, effectively stabilizing proteins and biological membranes against denaturation and lysis.

Anti-urolithic activity

The inhibitory effect of the *A. terreus* BAWK-F6 extract on calcium phosphate crystal formation was assessed microscopically and quantitatively (Fig. 4). Microscopic observations revealed clear differences in calcium phosphate crystallization patterns between extract-treated samples and controls. The positive control had almost no detectable crystals, confirming effective suppression of calcium phosphate crystal formation. In contrast, the negative control exhibited numerous, well-defined crystal characteristics. In the presence of the *A. terreus* BAWK-F6 extract (1.25-5) mg/mL, calcium phosphate crystals were visible as distinct crystals and aggregates of varying sizes. The lowest concentration (1.25 mg/mL) produced fewer and smaller crystals and aggregates, whereas higher concentrations showed more developed crystal structures (Fig. 4A). The extract demonstrated nucleation inhibition activity ranging from 36.45% to 55.64%. In contrast, the positive control showed the highest inhibition at 82.52%. Lower concentrations of the extract (1.25 mg/mL) exerted a more pronounced inhibitory effect on calcium phosphate nucleation (Fig. 4B). The results of the nucleation assay confirmed that the fungal extract has nucleation-preventing agents at low concentrations.

DISCUSSION

Marine filamentous fungi, especially those belonging to the genus *Aspergillus*, are increasingly recognized as promising producers of bioactive compounds with significant pharmacological properties [12]. *Aspergillus terreus*, in particular, has attracted considerable interest due to its capacity to biosynthesize a diverse range of metabolites, including phenolic derivatives, terpenoids, and polyketides, known for their antioxidant, antimicrobial, anti-inflammatory, and antibiofilm effects. In this study, the bioactive extract of marine-derived *A. terreus* BAWK-F6 was comprehensively evaluated for its chemical composition and multiple biological activities, including its anti-urolithic potential. These findings further support the potential of marine fungi as promising sources of novel natural therapeutic agents and sustainable biotechnological applications.

Molecular identification of the isolate by ITS rDNA sequencing confirmed that the fungal isolate belonged

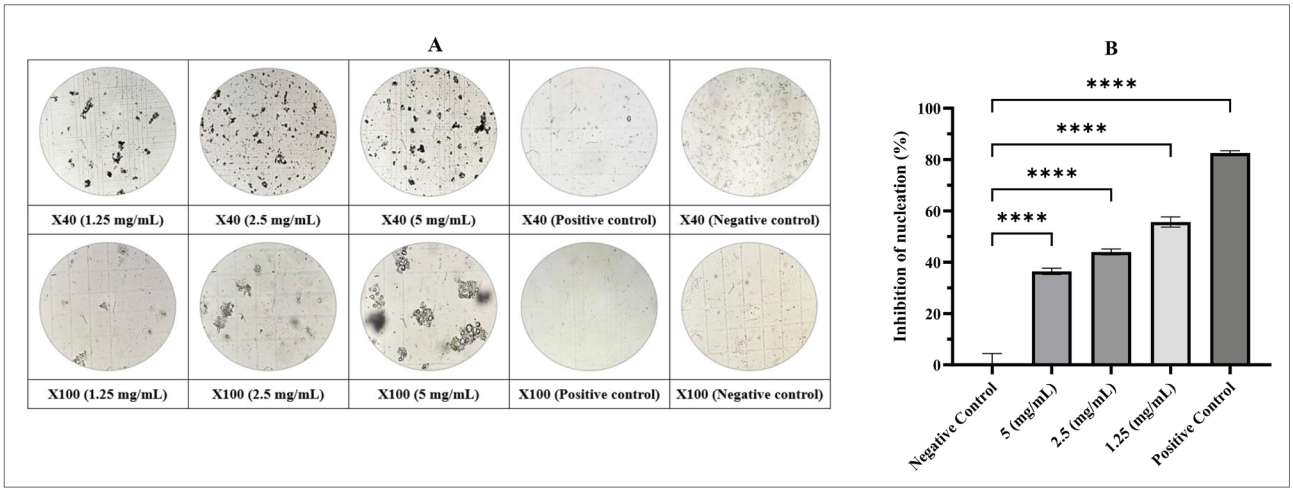

Fig. 4. Anti-urolithic activity of *Aspergillus terreus* BAWK-F6 extract evaluated by inhibition of calcium phosphate crystallization. **A** – Microscopic observation of calcium phosphate crystal formation at different concentrations of fungal extract. **B** – Quantitative evaluation of nucleation inhibition (%) of calcium phosphate crystal formation.

to *A. terreus*. BLAST analysis showed high similarity with reference *A. terreus* sequences in GenBank, supporting its taxonomic placement. The ITS region is widely recognized as the universal DNA barcode for fungi, providing reliable species-level identification within diverse fungal genera [29]. Phylogenetic analysis further supported this result, as *A. terreus* BAWK-F6 (PX358633.1) clustered with other *A. terreus* strains in a well-supported monophyletic clade with high bootstrap values, clearly distinct from related species such as *A. hortae* and *A. alabamensis*.

GC-MS analysis of the *A. terreus* BAWK-F6 extract identified a complex mixture of volatile and semi-volatile metabolites, with sesquiterpenes as the predominant components. Sesquiterpenoids represent an important class of secondary metabolites and are associated with a wide range of biological activities [31]. The predominance of oxygenated terpenoids is consistent with previous reports describing *A. terreus* as a prolific producer of terpenoid-based secondary metabolites with diverse biological functions [32,33]. These metabolites have many pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, and antibiofilm activities. These effects are often linked to hydroxyl, epoxy, or carbonyl groups, which can increase membrane permeability and redox activity. Marine-derived *A. terreus* strains share a similar chemical profile, with sesquiterpenes and phenolic derivatives linked to strong antioxidant and anti-inflammatory effects [34]. The presence of α-bisabolol and asarspidol in the extract further supports its observed bioactivity across assays, as these compounds are well documented for their radical-scavenging, membrane-stabilizing, and antimicrobial properties [35,36]. Additionally, several novel sesquiterpenes, including aspterrics A and B, have previously been isolated from a deep-sea *A. terreus* strain, confirming that this species is a prolific producer of structurally diverse sesquiterpenoids [37]. *A. terreus* BAWK-F6 and the *A. terreus* endophyte isolated from *Psidium guajava* exhibit both similarities and differences in their GC-MS chemical profiles. Despite originating from different ecological niches, both fungi produce bioactive compounds that reflect adaptations to their respective environments. The marine-derived *A. terreus* primarily produces sesquiterpenes, which constitute the largest and most diverse group of marine fungal terpenoids [38]. In contrast, the extract from *A. terreus* derived from the leaves of *Psidium guajava* contains compounds like 1,2-benzenedicarboxylic acid, di-iso-octyl ester, hexyl oxecan-2-one, and phenol, 3,5-dimethoxy-acetate, which are more phenolic and ester-based [39]. The endophytic fungus *Aspergillus terreus* var. *boedijnii* (Blochwitz), isolated from the marine red seaweed *Laurencia ceylanica* collected on the east coast of Sri Lanka, has been identified as a notable source of several sterols and triterpenes, including the particularly interesting phytosterol derivative glucopyranosyl-β-sitosterol [13]. The GC-MS profile confirms that *A. terreus* BAWK-F6 produces a

rich assemblage of bioactive terpenoids and associated metabolites, which likely contribute to its multifunctional biological profile.

The antioxidant activity of the *A. terreus* BAWK-F6 extract was demonstrated using $ABTS^{+}$, DPPH, and hydroxyl radical assays, each evaluating different aspects of electron-donating and radical-quenching capacity, consistent with earlier studies on *A. terreus* and other marine fungi [32]. The GC-MS profile, dominated by oxygenated sesquiterpenes and terpenoid alcohols, likely explains this activity through electron transfer, radical stabilization, and inhibition of lipid peroxidation, with compounds such as terpinolene, α-bisabolol, and α-gurjunene contributing to these effects [36]. Noor et al. [40] found that the *A. terreus* GE-2 strain, isolated from *Gracilaria sp*. (a red alga), showed strong antioxidant activity in the DPPH assay with an IC_{50} of 15.08 μg/mL, although this was slightly lower than that of the SE-2 strain from *Sargassum sp*. (IC_{50} = 7.88 μg/mL). Marine terpenoids are known for their antioxidant effects, which enhance endogenous antioxidant enzyme activities and reduce ROS-mediated cellular damage [41].

The *A. terreus* BAWK-F6 extract exhibited dose-dependent antibacterial activity, with the strongest inhibition observed at 10 mg/mL, particularly against *Salmonella enteritidis* ATCC 2453 (35.16 mm), while showing lower activity against pathogens such as *Escherichia coli* and *Pseudomonas savastanoi*. These findings agree with earlier studies showing that *A. terreus*, particularly strains from marine and endophytic sources, has strong antibacterial effects. This is mainly due to its diverse secondary metabolites, including terpenoids, phenolic compounds, and polyketides, which damage cell membranes and disrupt key enzyme functions [36]. The red macroalga *Hypnea musciformis* collected from the Kovalam coast, Trivandrum, Kerala, India, was found to harbor an endophytic fungus identified as *Aspergillus terreus* (MH469513). The crude extract of this fungus exhibited broad-spectrum antimicrobial activity when screened using the disk-diffusion method against bacterial and fungal pathogens. Among the 133 endophytic isolates obtained from eleven macroalgae, *A. terreus* was identified as one of the most potent strains [42]. The fungal strain *A. terreus* (2105OF.35.5), isolated from seaweed in the North Sea of Vietnam, was evaluated for its antibacterial properties as part of a broader investigation involving 61 marine fungal strains. The strain exhibited significant antibacterial activity, particularly against Gram-positive bacteria, with inhibition zones of 16 mm, 20 mm, and 20 mm observed for *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, and *Streptococcus faecalis* ATCC 19433, respectively. In addition, it displayed moderate inhibition against the Gram-negative bacterium *Klebsiella pneumoniae* ATCC 700603, with a 20-mm inhibition zone. However, no activity was recorded against *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, or *Salmonella typhimurium* ATCC 14028, highlighting a selective antibacterial effect, primarily targeting Gram-positive pathogens [43]. Monoterpenes and sesquiterpenes, as identified in the GC-MS analysis of *A. terreus* BAWK-F6 extract, are recognized for their potent antimicrobial properties against both antibiotic-susceptible and antibiotic-resistant bacteria. These compounds exert antimicrobial effects through multiple mechanisms, including inhibition of protein synthesis, disruption of microbial cell membranes, and interference with DNA replication [44]. The *A. terreus* extract obtained from an endophytic fungus isolated from *Moringa oleifera* exhibited notable antifungal potential against mucormycosis-causing fungi, specifically *Mucor racemosus*, *Syncephalastrum racemosum*, and *Rhizopus oryzae*. At a concentration of 10 mg/mL, the extract produced inhibition zones measuring 20 mm, 37 mm, and 18 mm, respectively. The MIC values were 0.3125 mg/mL for *M. racemosus*, 1.25 mg/mL for *R. oryzae*, and 2.5 mg/mL for *S. racemosum* [45]. In a study on *A. terreus*, an endophytic fungus isolated from *Cestrum parqui*, the fungus produced camptothecin, which demonstrated notable antifungal activity. The extracted camptothecin exhibited strong effects against *Candida* species, *A. flavus*, and *A. parasiticus*, with inhibition zones of 1.5 mm, 2.6 mm, and 0.6 mm, respectively [46]. In the study by Meenupriya et al. [47], the antibacterial activity of the *Aspergillus terreus* MP1 extract, isolated from a marine sponge, was evaluated against *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The extract had strong antimicrobial effects, with the largest inhibition zone (19 mm) observed against *P. aeruginosa*. The MIC values were 37.5 mg/mL for *P. aeruginosa* and 75 mg/mL for *S. aureus*, indicating greater activity against Gram-negative bacteria.

The antibiofilm assay revealed a strong anti-adhesive and biofilm-disruptive effect of *A. terreus* BAWK-F6. The extract showed the highest inhibition against *Staphylococcus aureus* ATCC 23235, *Shigella dysenteriae*, *Enterococcus durans*, *Salmonella enteritidis* ATCC 2453, and *Streptococcus oralis*. The results demonstrate that *A. terreus* BAWK-F6 produces bioactive metabolites that disrupt bacterial adhesion and biofilm formation, particularly in *S. aureus* and *S. dysenteriae* strains. These findings agree with previous studies showing that *Aspergillus* metabolites, especially sesquiterpenes, can inhibit bacterial adhesion, prevent biofilm maturation, and interfere with quorum-sensing pathways [48,49]. The GC-MS chemical composition of the extract further supports this mechanism, as oxygenated terpenoids and alcohols are recognized for their ability to penetrate bacterial membranes and destabilize the extracellular polymeric matrix, resulting in biofilm inhibition [50].

The discovery of new anti-inflammatory agents remains essential, and endophytic fungi are promising sources because they produce diverse stress-enhanced metabolites with the potential to complement or replace conventional drugs [51]. The *A. terreus* BAWK-F6 extract showed strong anti-inflammatory activity by preventing protein denaturation and stabilizing HRBC membranes. These findings agree with previous studies where *A. terreus* extracts or metabolites displayed anti-inflammatory activity via the inhibition of protein denaturation and membrane stabilization [52]. The chemical composition identified by GC-MS, dominated by sesquiterpenes, supports this biological behavior. These terpenoids are known to suppress inflammation by inhibiting COX and LOX pathways, reducing lysosomal enzyme leakage, and stabilizing cell membranes [53]. The secondary metabolites of the marine-derived fungus *Aspergillus terreus* PPS1 were investigated, leading to the isolation of seven undescribed metabolites, including alkaloids, peptides, and polyketides. The anti-inflammatory activity of those compounds was evaluated, specifically their ability to inhibit LPS-induced NO production in RAW264.7 macrophages [54].

Renal stone formation occurs through a series of physicochemical processes, including supersaturation, nucleation, growth, aggregation, and retention in kidney tubules. Nucleation represents the initial step in renal stone formation, during which the smallest structural unit of a crystal species is established [55,56]. The anti-urolithic potential of the fungal extract was evaluated through its ability to inhibit calcium phosphate crystal formation. The results of the nucleation assay confirmed that the *A. terreus* BAWK-F6 extract contained nucleation-preventing bioactive compounds. Lower concentrations were more effective, suggesting that certain active metabolites may function optimally at lower doses, possibly due to enhanced solubility or reduced molecular aggregation. The anti-urolithic effect observed is likely attributable to the terpenoid found in the GC-MS profile, which may interfere with nucleation, aggregation, and crystal-matrix interactions. Although no prior study has directly examined the anti-urolithic activity of *A. terreus* BAWK-F6, similar inhibitory effects of fungal metabolites on calcium oxalate and phosphate crystallization have been demonstrated *in vitro*, where natural extracts significantly reduced the nucleation and aggregation of urinary crystals [57,58]. Moreover, sesquiterpenes such as α-bisabolol, present in the extract, are known for their nephroprotective effects, including the prevention of crystal growth and aggregation [59]. A previous study [60] reported that the ethyl acetate extract of *Curcuma zedoaria* is particularly rich in terpenoids. The study confirmed both the strong anti-urolithic activity and the nephroprotective properties of the rhizome extract. The results indicate that *A. terreus* BAWK-F6 possesses significant anti-urolithic potential by inhibiting crystal nucleation and aggregation, supporting its potential use as a natural preventive agent against urinary stone formation.

CONCLUSIONS

The algicolous endophyte *Aspergillus terreus* BAWK-F6, isolated from the brown seaweed *Dictyota dichotoma*, shows considerable potential as a source of therapeutically valuable bioactive compounds. Its phylogenetic relatedness to other marine fungal strains underscores the significance of marine environments as reservoirs of bioactive filamentous fungi. The extract, dominated by sesquiterpenes, exhibited notable antioxidant, antibiofilm, anti-inflammatory, and broad-spectrum antimicrobial activities. Its anti-urolithic activity further suggests potential for preventing kidney stone formation. Additional studies are needed to fully characterize

the active molecules and elucidate their mechanisms of action. Overall, the marine-derived endophyte *A. terreus* BAWK-F6 represents a promising candidate for the development of natural pharmaceutical products.

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Data availability: The data supporting this article are available in the online dataset: https://www.serbiosoc.org.rs/NewUploads/Uploads/Fodili%20et%20al_Dataset.xlsx

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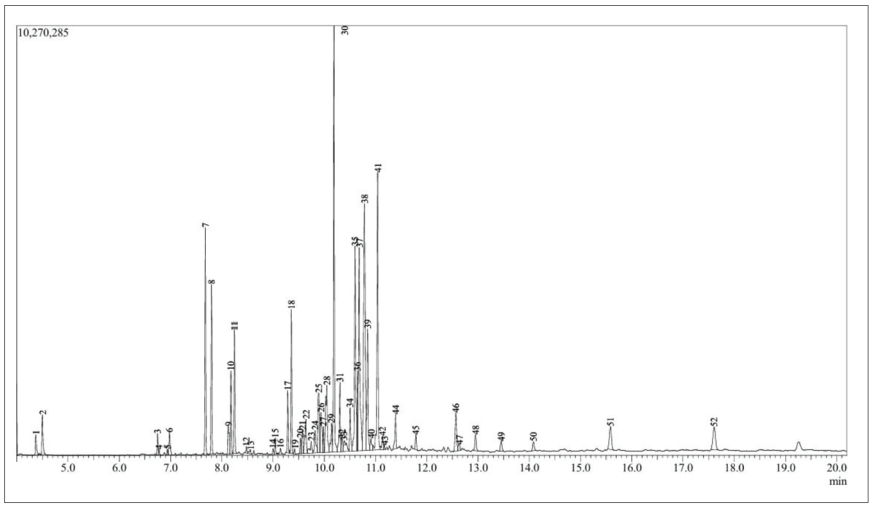
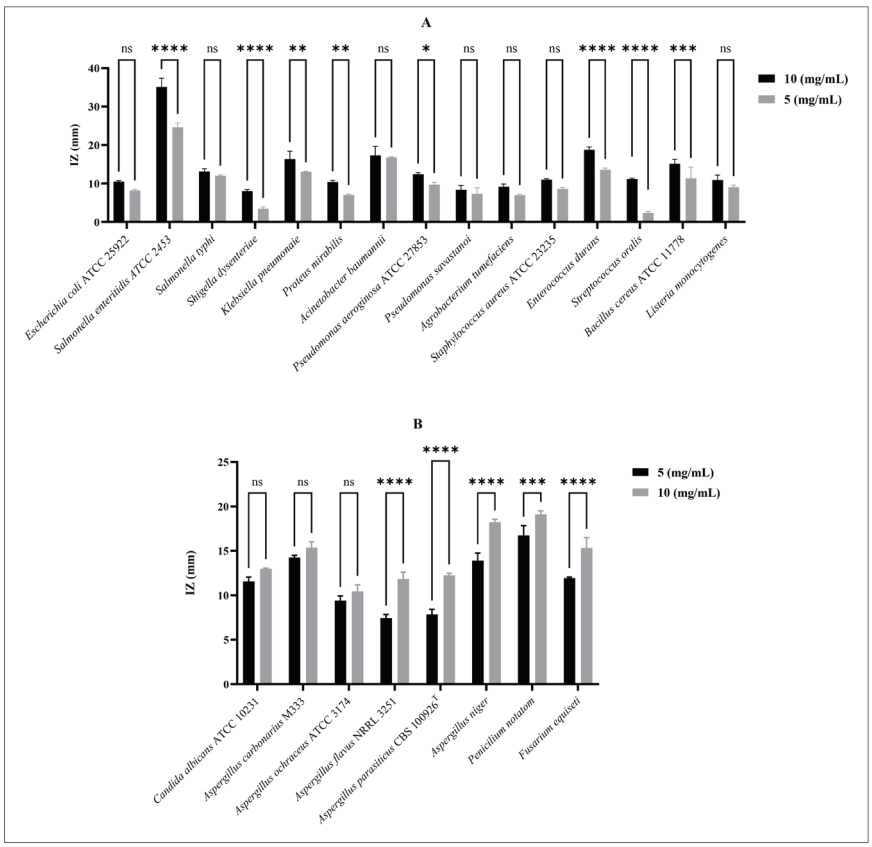
SUPPLEMENTARY MATERIAL



Supplementary Fig. S1. GC-MS chromatogram of volatile compounds from *Aspergillus terreus* BAWK-F6 extract.



Supplementary Fig. S2. Well diffusion assay against microbial strains at two concentrations 5 (mg/mL) and 10 (mg/mL).