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Antimicrobial and Cytotoxic Activities Screening of Marine Invertebrate-Derived Fungi Extract from West Sumatera, Indonesia

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Abstract

BACKGROUND: The coral reef on Mandeh Island, West Sumatra, Indonesia, consists of an abundant source of sponge and soft coral. Secondary metabolites of marine-derived fungi isolated from the sponge and soft coral possess numerous biological activities.

AIM: This study collected, identified, and screened marine-derived fungi isolated from marine invertebrates for antibacterial and cytotoxic bioactivities.

MATERIALS AND METHODS: The marine invertebrates used are sponges; *Xestospongia testudinaria* and *Placortis communis*) and soft corals (*Sarcophyton elegan* and *Subergorgia suberosa*). The EtOAc extracts were analyzed for antimicrobial and cytotoxic activities using the diffusion agar method and brine shrimps lethality test.

RESULTS: After cultivating on rice medium, the EtOAc extracts of 22 isolated fungi showed potent antimicrobial activity with an inhibitory zone of 15.9 mm against *Staphylococcus aureus* (XT2 extract), and *Pseudomonas aeruginosa* of 26.7 mm (XT6 extract), and *Candida albicans* of 29 mm (SE5 extract). XT6 extract showed the potential cytotoxic activity with an LC_{50} value of 100 μg/ml.

CONCLUSION: The ability of the marine-derived fungi to produce bioactive compounds is promising potential as a source of antimicrobial and cytotoxic compounds.

Introduction

Marine-derived fungi are considered a prolific source of unique bioactive compounds. These microbes are known as symbionts of marine invertebrates, which of the richest source of secondary metabolites. Marine invertebrates include sponges, soft coral, and jellyfish harbor microorganisms within their tissues (either intracellular or extracellular space) [1].

Marine-derived fungi present enzymes with alkaline and cold-activity characteristics, and salinity is considered an essential condition in screening and production processes. The adaptability of fungi originating from the sea to extreme conditions with marine conditions affects the ability to produce secondary metabolites [2]. Salinity, high pressure, low temperature, oligotrophic conditions, extreme pH, highly variable mineral content in seawater, and lighting conditions can affect enzymes produced by marine microorganisms [3]. Marine invertebrate-derived fungi produce secondary metabolites with unique structures and bioactivity. Bioactive compounds, polyketides, alkaloids, peptides, lactones, terpenoids, and steroids are the most commonly found in fungi. They exhibit bioactivity, such as anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, antibiotic, and cytotoxic [4].

Marine-derived fungus *Aspergillus unguis* and *Penicillium citrinum* isolated from *Callyspongia* sp. have cytotoxic and antibacterial activity [5]. Marine sponge-derived fungus *Aspergillus ochraceus* isolated from *Acanthostrongylophora ingens* has antibacterial activity against *Vibrio cholerae inaba, Enterococcus faecalis* ATCC 29252, *MDRPA, Pseudomonas aeruginosa, MRSA*, *Staphylococcus aureus* ATCC 25923, and *M. tuberculosis* H37Rv [6]. *Trichoderma harzianum* from brown algae *Padina* sp. has antibacterial bioactivity [7]. Marine-derived fungi from marine invertebrates can produce secondary metabolites similar to their host organisms [8]. This study isolates marine fungi from marine invertebrates and tests their antimicrobial and cytotoxic activities.

Materials and Methods

Sample preparation

Marine sponges and soft corals were collected at Mandeh island, West Sumatran, Indonesia. The samples were put in plastic bags containing sterile seawater and then stored in ice and delivered to the laboratory. The marine sponges were identified as *Xestospongia testudinaria* and *Placortis communis* by Dr. Nicole J. De Voogd at the Natural Biodiversity Center, Netherland. At the same time, soft corals were identified as *Sarcophyton elegan* and *Subergorgia suberosa* in the Ecology Laboratory of Andalas University, Padang, Indonesia.

Isolation, cultivation, and extraction marine-derived fungi

Isolation and cultivation of marine-derived fungi were done as the previous method [9], [10].

Screening for antimicrobial activity

The antimicrobial activity from ethyl acetate (EtOAc) extracts of marine invertebrate-derived fungi was tested against *S. aureus*, *P. aeruginosa*, and *C. albicans* using the diffusion agar method. Briefly, sterilized disks with fungal crude extract were placed on nutrient agar/Sabouraud dextrose agar plates with the test organisms and incubated at 37°C for 24 h. The presence of a clearance zone around the disk was used as an indicator of antimicrobial bioactivity. Chloramphenicol disc (30 μg) and nystatin disc (10 μg) are positive control, while DMSO is negative, respectively. The zone of inhibition was measured in mm [11]. The diameter of the inhibition zone is used to categorize the strength of antibacterial activity according to Davis and Stout (1971) as follows: Very strong (≥20 mm), strong (10–20 mm), moderate (5–<10 mm), and weak (≤5 mm).

Screening for cytotoxic activity

Artemia salina eggs were incubated using 500 ml of seawater at a temperature of 27 ± 2°C for 48 h. Cytotoxic tests were carried out at concentrations of 1000 μg/ml, 100 μg/ml, and 10 μg/ml, and each concentration was repeated 3 times. The negative control is DMSO 50 μl [12]. Based on the results, some fungi extracts exhibited cytotoxic activity.

Phytochemical screening

Phytochemical screening for all EtOAc extracts was conducted based on the previous research methods [7]. This method aims for the qualitative identification of phenolics, alkaloids, steroids, and terpenoid compounds in EtOAc extracts of marine invertebrate-derived fungi.

Fungal identification

Macroscopic, microscopic, and molecular identification

Fungal identification was carried out only on fungi that had interesting antibacterial and cytotoxic activities. The fungal identification was done as the previous method [5].

Results

Antibacterial activity

In the present study, 22 marine invertebrates-derived fungi were isolated from two marine sponges and two soft corals on Mandeh Island, West Sumatera, Indonesia. The highest fungi number was found in sponges, followed by soft coral. The fungi obtained from the sponge *X. testudinaria* and *P. communis* were XT1, XT2, XT3, XT4, XT5, XT6, XT7, PC1, PC2, PC3, PC4, and PC5, respectively. While, fungi obtained from the soft coral *S. elegan* and *S. suberosa* were SE1, SE2, SE3, SE4, SE5, ES1, ES2, ES3, ES4, and ES5, respectively.

The antimicrobial activity of fungi extract was done using a method of agar diffusion assay against pathogenic bacteria such as *S. aureus*, *P. aeruginosa*, and *C. albicans* (Table 1). The results showed that 91% of the marine-derived fungi extracts (20 extracts from 22) presented activity against at least one bacterial strain and 86.3% were active against pathogenic fungus. Against the Gram-negative bacteria *P. aeruginosa*, all extracts from 22 extracts (100%) displayed activity, and three extracts (13.6%) showed interesting activity with

Table 1: Antibacterial activity of EtOAc extracts marine invertebrate derived fungi

Marine invertebrates	Fungi code	Diameter of inhibition zone (mm ± SD)		
		S. aureus	*P. aeruginosa*	*C. albicans*
X. testudinaria	XT1	8.1 ± 0.36	8.3 ±0.56	7.5 ± 0.31
	XT2	15.9 ± 0.51	13.5 ± 0.06	9.0 ± 0.14
	XT3	7.09 ± 0.25	11.6± 0.45	8.5 ± 0.28
	XT4	9.2 ± 0.25	21.5 ± 0.52	8.1 ± 0.35
	XT5	7.5 ± 0.43	9.5 ± 0.26	8.1 ± 0.23
	XT6	15.2 ± 0.36	26.7± 0.32	18.0 ± 0.32
	XT7	8.0 ± 0.46	16.5 ± 0.55	9.6 ± 0.19
P. communis	PC1	11.4 ± 0.20	9.2 ± 0.26	12.2± 0.69
	PC2	9.5 ± 0.85	8.6 ± 0.43	9.1 ± 0.52
	PC3	8.8 ± 0.55	8.9 ± 0.10	10.5 ± 0.36
	PC4	11.3 ± 0.35	22.2 ± 0.56	20.1 ± 0.45
	PC5	13.5 ± 0.46	19.0 ± 0.50	20.4 ± 0.50
S. elegan	SE1	12.5 ± 0.53	13.5 ± 0.30	15.9 ± 0.09
	SE2	22.2 ± 2.17	23.1 ± 0.17	19.4 ± 0.40
	SE3	14.2 ± 0.10	18.5 ± 0.10	21.0 ± 0.65
	SE4	12.5 ± 0.35	19.0 ± 0.16	20.0 ± 0.52
	SE5	11.2 ± 0.25	23.5 ± 0.26	29.0 ± 1.24
S. suberosa	ES1	10.5 ± 0.40	12.0 ± 0.47	7.5 ± 0.40
	ES2	13.5 ± 0.61	14.1 ± 0.15	8.5 ± 0.10
	ES3	10.1 ± 0.56	11.1 ± 0.26	7.6 ± 0.36
	ES4	9.5 ± 0.47	10.5 ± 0.46	9.5 ± 0.41
	ES5	12.5 ± 0.30	15.5 ± 0.46	14.1 ± 0.13

SD: Standard deviation, *X. testudinaria: Xestospongia testudinaria, P. communis: Placortis communis, S. elegan: Sarcophyton elegan, S. suberosa: Subergorgia suberosa.*

inhibition zone >20 mm: Marine sponge derived fungus extract of *X. testudinaria* (XT6) and soft coral derived fungus extracts of *S. elegan* and *S. suberosa* (SE2 and SE5). Against the Gram-positive bacteria *S. aureus*, 20 extracts from 22 extracts (91%) displayed activity and two extracts (9.1%) showed good activity with inhibition zone >15 mm: Marine sponge derived fungus extract of *X. testudinaria* (XT6) and soft coral derived fungus extracts of *S. elegan* (SE2). According to Table 1, from 22 extracts analyzed, 19 extracts showed activity against yeast *C. albicans* (66.6%) and six extracts (27.27%) displayed interesting activity with inhibition zone >15 mm: Marine sponge derived fungus extract of *X. testudinaria* (XT6) and *P. communis* (PC4, PC5), soft coral derived fungus extracts of *S. elegan* (SE3, SE4, SE5).

Cytotoxic activity

The cytotoxic activity of fungi extract was done using a method of brine shrimps lethality test (Figure 1). According to Meyer, extract with LC_{50} values below 1000 μg/ml is classified as toxic, and if greater than 1000 μg/ml is classified as non-toxic [12]. Based on the results, some fungi extracts exhibited cytotoxic activity. The results showed that 70% of the marine-derived fungi extracts (14 extracts from 22) presented toxic effect to *A. salina*. Fungi extracts of *X. testudinaria* (XT6) showed LC_{50} values of 100 μg/ml (Figure 1).

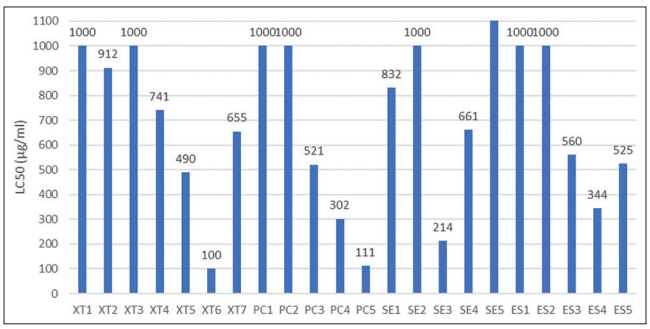


Figure 1: The cytotoxic activity of EtOAc extracts of marine invertebrate derived fungi

Phytochemical screening

Phytochemical screening of fungus extracts (XT2, XT6, PC3, PC5, SE2, SE5, ES2, and ES5) was done to identify the presence of alkaloid and phenolic compounds. The antibacterial and cytotoxic activities are influenced by the content of secondary metabolites produced by fungi originating from the marine sponges and soft corals, so further research is needed.

Molecular identification

Based on screening results of antimicrobial and cytotoxic activity, the fungus isolates ES5 and XT6 had a high potential to produce antimicrobial and cytotoxic agents. The fungus ES5 was colony-like white cotton, but the white color will turn into a dark gray color after several days. Microscopic observations of its hyphae were broad without insulated, "finger-shaped" sporangia (microsporangia), forming around the vesicles and forming a spherical pattern (Figure 2). Molecular identification showed that SE5 is 99% identic with the *Syncephalastrum racemosum* strain AUMC 7965.

The results of the sequencing were analyzed using BLAST contained in the NCBI. The phylogenetic tree analysis using MEGA X software with the Neighbor-Joining method. The results of molecular identification of the fungus *S. racemosum* have 70% homolog with *S. racemosum* strain NRRL 2496 (Figure 3).

Fungus XT6 is a green colony, and uneven edges are white. The surface is uneven with stripes (Figure 4). The molecular identification showed that XT6 had 99% identity with the *P. citrinum* strain 132.

The results of the sequencing were analyzed using BLAST contained in the NCBI. The phylogenetic tree analysis using MEGA X software with the Neighbor-Joining method. The results of molecular identification of the fungus *P. citrinum* have 39 % homolog with *P. citrinum* XQ3-1 (Figure 5).

Discussion

Based on the previous research, repeatedly the marine-derived fungi showed antimicrobial properties [5], [10]. In addition, several marine sponge-derived fungi originating from Mandeh Island, West Sumatra, Indonesia, also exhibited antimicrobial activity. *Penicillium* sp. and *Aspergillus niger* isolated from *N. chaliniformis* AR-01 are a potential secondary metabolite producer as anticancer and antibacterial [11]. Marine-derived fungus *P. citrinum* Dc04 isolated from *Dactylospongia* sp. can produce antibacterial compounds [10]. Endophytic fungus NT3 from *Padina* sp. has the potential to produce metabolites that have bioactivity as an antimicrobial against micro-pathogenic *S. aureus*, *E. coli*, and *C. albicans* [7].

Cytotoxic compounds are toxic compounds characterized by cell death after exposure. Cytotoxic compounds must have selective activity against normal cells and cancer cells, having a mechanism of cell death in the form of apoptosis [13]. However, not all cytotoxic compounds can be used as anticancer. Some secondary metabolites from marine sponge-derived fungus *Penicillium* sp. have cytotoxic activity with an LC_{50} of 16.79 μg/ml [11]. *Aspergillus versicolor* isolated from *Neopetrosia* sp. has an LC_{50} of 32 μg/ml [14], and meroterpenoids isolated from *Alternaria* sp. have displayed an LC_{50} of 39 μM [15].

Molecular identification showed that SE5 is 99% identic with the *S. racemosum* strain AUMC

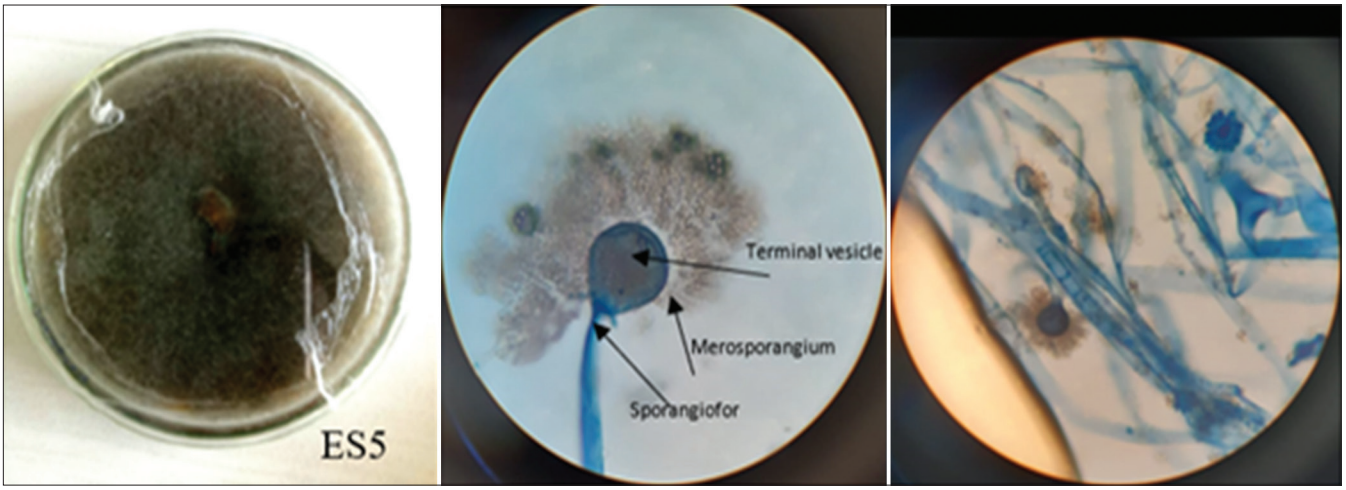


Figure 2: The macroscopic and microscopic observations of fungus ES5 (Syncephalastrum racemosum)

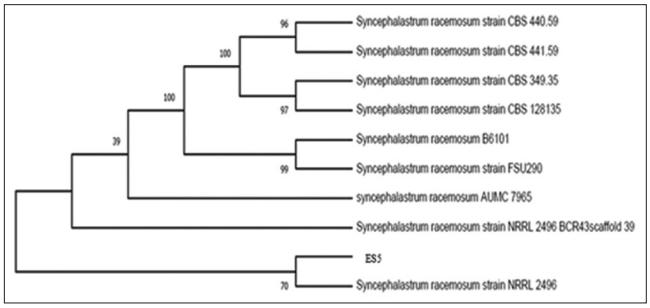


Figure 3: Neighbor-joining phylogenetic tree of potential fungal isolates from Syncephalastrum racemosum

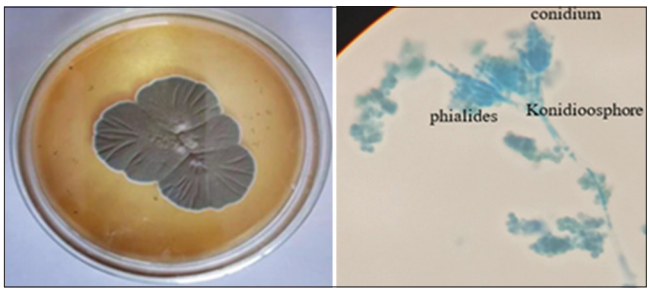


Figure 4: The macroscopic and microscopic observations of fungus XT6 (Penicillium citrinum)

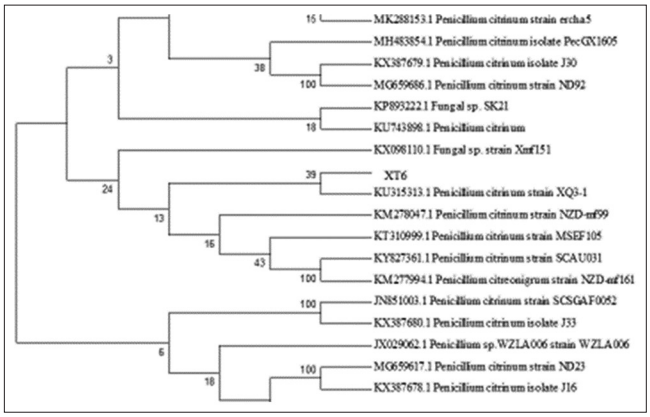


Figure 5: Neighbor-joining phylogenetic tree of potential fungal isolates from Penicillium citrinum

7965. *S. racemosum* is a soil fungus commonly found in subtropical and tropical areas. This fungus has been reported due to its bioactivity, such as lipolytic [16], anti-HCV [17], antidiabetic [18], cytotoxic [19], emulsifier [20], antifungal, and anti-proliferation [21]. Secondary metabolites of *S. racemosum* which have been reported are (3R, 5S)-5-hydroxy-de-O-methyllasiodiplodin, 6-oxo-de-O-methyllasiodiplodin, de-O-methyllasiodiplodin, lasiodiplodin, ergosterol, and ergosterol peroxide [19], [22] (Figure 6).

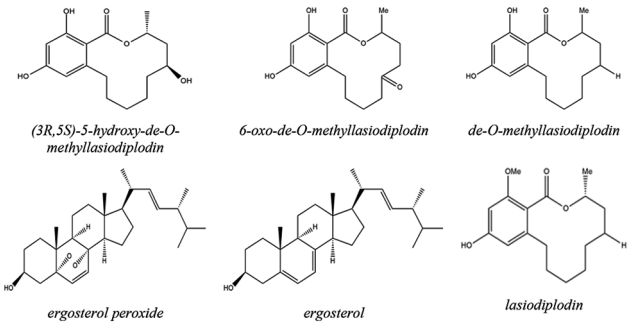
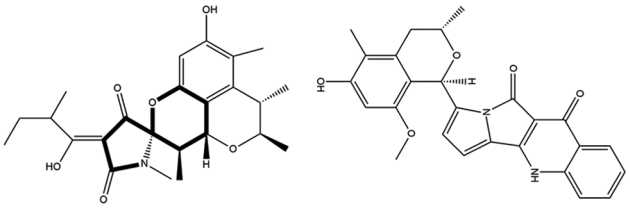


Figure 6: Secondary metabolites of Syncephalastrum racemosum [19], [22]

P. citrinum is a marine-derived fungus widely isolated from sponges, algae, and marine sediments. This fungus exhibited cytotoxic activity on several cancer cells such as histiocytic lymphoma U937 cells [23], L5178Y lymphoma cells [24], A-375 melanoma [25], MCF-7, HeLa and A549 cells [26], MOLT4 2.97 cells, and HL-60 cells. The bioactive metabolite of *P. citrinum* with anti-proliferative activity on several tumor cells, such as A-375, SPC-A1, and HGC-27 is penicitrinine A [25], and citriquinochroman has cytotoxic activity against L5178Y lymphoma cells [27] (Figure 7).

The fungus strain ES5 and XT6 will be subjected to detailed research for the isolation of biologically active molecules along with the search for new compounds. The report of biological activities of marine invertebrates-derived fungus of West Sumatera,

Indonesia is a potential source of a great variety of antibiotics worthy of further research because there is not any study of the antimicrobial activity of associated fungi from this area. This study was the first step to discovering a new antibiotic and anti-cancer [28].



Penicitrinine A (26) *Citriquinochroman* (28)

Figure 7: Chemical structure of secondary metabolites Penicillium citrinum

Conclusions

Marine invertebrates of Mandeh Island are rich in derived-fungi biodiversity, as explored in the present study. These marine-derived fungi isolated from sponges and soft corals have the potential to produce compounds that have antimicrobial and cytotoxic activities. Further studies are needed to isolate and identify the structure of pure compounds from the potential derived-fungal extracts.

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