



Antimicrobial and cytotoxic activities of marine sponge-derived fungal extracts isolated from *Dactylosporgia* sp.

Neny Sandrawati^{1,2}, Shuci Permata Hati², Fujia Yunita², Andani Eka Putra¹, Friardi Ismed², Trina Ekawati Talle³, Triana Hertiani⁴, Dian Handayani^{2*}

¹Department of Biomedical, Faculty of Medicine, Andalas University, 25163 Padang, Indonesia.

²Laboratory of Sumatran Biota / Faculty of Pharmacy, Andalas University, Kampus Limau Manis, 25163 Padang, Indonesia.

³Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Kampus UNSRAT Kleak, 95115 Manado, Indonesia.

⁴Faculty of Pharmacy, Gadjah Mada University, Sekip Utara, Yogyakarta, Indonesia.

ARTICLE INFO

Received on: 29/10/2019
Accepted on: 30/01/2020
Available online: 04/04/2020

Key words:

Marine sponge, *Dactylosporgia* sp., marine-derived fungi, antibacterial activity, cytotoxic activity, methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Pseudomonas aeruginosa* (MDRPA).

ABSTRACT

This study is the first report on the antimicrobial and cytotoxic activities of fungal extracts isolated from marine sponge *Dactylosporgia* sp., which is collected from Mandeh Island, West Sumatra, Indonesia. The isolation of fungal was conducted using dilution method with Sabouraud Dextrose Agar + chloramphenicol (0.05%) as a medium. The pure isolated fungal was cultivated on rice medium at temperature 25°C–27°C and then extracted using ethyl acetate solvent. The ethyl acetate extract of each isolated fungal was tested for antimicrobial and cytotoxic activities. Nine fungal strains have been isolated from this sponge. Two ethyl acetate extracts of fungal strains (Dc03 and Dc04) were categorized as having strong inhibition against the growth of *Staphylococcus aureus*, *Escherichia coli*, methicillin-resistant *S. aureus*, and multidrug-resistant *Pseudomonas aeruginosa* in a concentration of 5% with zone inhibition in range of 12.31 ± 0.54–16.14 ± 0.75 mm. The cytotoxic activity screening of the ethyl acetate extracts of fungal strains was done by using the brine shrimp lethality test. Four fungal strains had LC₅₀ below 80 µg/ml (Dc03, Dc04, Dc05, and Dc08) and were further tested with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay on T47D cell line. These selected fungi were identified molecularly as *Cladosporium halotolerans* MN859971, *Penicillium citrinum* MN859968, *Aspergillus versicolor* MN859970, and *Aspergillus sydowii* MN859969, respectively. The results suggest that these fungal strains are quite rich in the production of bioactive compounds that are very effective as antibacterial and cytotoxic agents.

INTRODUCTION

Marine-derived fungi have attracted much attention in recent years due to the production of structurally unique pharmacological properties (Blunt *et al.*, 2007; 2015). Fungi have proven as promising sources of new compounds compared to other microbial sources such as bacterial isolates from the sea (Gamal-Eldeen *et al.*, 2009). The first report of bioactive compounds from marine-derived fungi was cephalosporin C,

which was isolated from *Acremonium chrysogenum* that associated with a sewage outlet in the Mediterranean Sea close to the Sardinia Island. Next, cephalosporin C was developed as a lead compound of modern cephalosporin antibiotics for the treatment of numerous infections (Abraham and Loder, 1972).

Many researchers, nowadays, have developed the study which is focused on the biological activity of marine-derived compounds. Screening for antimicrobial and cytotoxic activity of marine-derived compounds is becoming more popular due to the search for novel antibiotic and antitumor agents. Hu *et al.* (2015) reported, in their study, that of 4,196 biologically active compounds isolated fungi from a marine organism, 2,225 had anticancer activities (56%) and 521 had antibacterial activities (13%).

Some symbiotic fungi of marine sponge in West Sumatra, Indonesia, had been reported to produce bioactive

*Corresponding Author
Dian Handayani, Laboratory of Sumatran Biota / Faculty of Pharmacy,
Andalas University, Kampus Limau Manis, 25163 Padang, Indonesia.
E-mail: dianhandayani@phar.unand.ac.id

compounds with various biological activities. Fungi associated with marine sponge *Haliclona fascigera* were reported active toward pathogenic bacteria such as *Staphylococcus aureus* and *Candida albicans* (Handayani *et al.*, 2015a; 2015b). Marine sponge *Acanthrongylophora ingens* and *Neopetrosia chaliniformis* AR-01-derived fungi collected from Mandeh Island, West Sumatra, Indonesia, showed an inhibitory activity against *Bacillus subtilis*, *Staphylococcus epidermidis*, *Salmonella typhi*, and *Escherichia coli*. The screening for cytotoxic activity using brine shrimp lethality test (BSLT) method against brine shrimp larvae also showed a significant LC₅₀ value (Handayani and Aminah, 2017a; Handayani and Artasasta, 2017b).

In the study of marine-derived microbial in West Sumatra, Indonesia, we had reported the potential marine sponge-derived bacteria (Handayani *et al.*, 2015a; 2015b; 2016; Sandrawati *et al.*, 2019), marine sponge-derived fungi (Handayani *et al.*, 2018a; 2018b; 2019b), and algae-derived fungi (Handayani *et al.*, 2019a) which displayed interesting biological activities. In this present study, we described the isolation of fungi associated with marine sponge *Dactylosporgia* sp. which was cultivated on a rice medium. We also evaluate the antimicrobial and cytotoxic activities of its EtOAc extracts and the identification of fungi by microscopic and molecular determination.

MATERIALS AND METHODS

Sample material

Sponge sample code DH3 was collected from the Mandeh Island, West Sumatra, in a depth of ±10 m. It was surface sterilized by sterile seawater three times and stored in a plastic bag. The sample was placed in an icebox and transferred to the laboratory immediately for the isolation of symbiotic fungi.

Identification of sponge

Sponge identification was conducted by Dr. Nicole J. De Voogd at the Natural Biodiversity Center, the Netherland. The sponge code DH3 was identified as *Dactylosporgia* sp.

Isolation, cultivation, and extraction of symbiotic fungi

The method of isolation and cultivation of symbiotic fungi was carried out in the previous research (Kjer *et al.*, 2010). The symbiotic fungi were macerated with ethyl acetate in the ratio 1:1 with three repetitions. The resulting extract was then evaporated *in vacuo* with a rotary evaporator and used for the antimicrobial and cytotoxic assays.

Screening for antimicrobial activity

The EtOAc extract of fungus was tested using pathogenic microbial as follows: *S. aureus* ATCC25923, *E. coli* ATCC25922, *C. albicans*, and clinical isolate methicillin-resistant *Staphylococcus aureus* (MRSA) which were obtained from Cipto Mangunkusumo Hospital, Jakarta, Indonesia, and multidrug-resistant *Pseudomonas aeruginosa* (MDRPA) was from M. Djamil Hospital, West Sumatera, Indonesia.

DISK DIFFUSION METHOD

The EtOAc extract of fungus was prepared for the concentration of 1, 3, and 5 mg/ml in dimethyl sulfoxide (DMSO) solvent. About 10 µl of each EtOAc extract was pipetted onto a

6-mm sterile paper disk (Advantec®). Each of the disk was put on the surface of the Sabouraud Dextrose Agar (Merck®) medium containing 0.7 McFarland of fungi suspension and on the surface of Natrium Agar (Merck®) contained 0.5 McFarland of bacterial suspension. Chloramphenicol (Oxoid®) in a concentration of 30 µg/ml was used as a positive control for antibacteria and nystatin (Oxoid®) in a concentration of 100 unit/disk as antifungi. The paper disk was also inoculated with 100 µl DMSO as a negative control. Each treatment consisted of three replicates.

The plates were incubated overnight at 37°C for bacteria and at 25°C–27°C for fungi. The antimicrobial screening was conducted based on the method described previously (Handayani *et al.*, 2015a; 2017a; 2017b; 2018a; 2018b). The clear zones in the media were an indication of antimicrobial activities. The diameter of the inhibition zones was measured, and the standard deviations were also determined. The strain that showed inhibition zone >10 mm was selected for phytochemical test, microscopic examination, and molecular identification.

Screening for Toxicity and cytotoxic activity

A preliminary study for toxicity activity was conducted with the BSLT method which as written in the previous research report (Handayani *et al.*, 2018a; 2018b; Meyer *et al.*, 1982). The present cytotoxic assay was performed by MTT assay using T47D (human ductal breast epithelial tumor) cell lines using four potential fungi. Fungal extracts were made in various concentrations of 1,000, 500, 250, 125, 62.5, and 31.25 ppm. Value Ln concentration is regressed against cell viability. Regression equations were then used to calculate the IC₅₀ value.

Phytochemical test

The phytochemical examination was carried out for the EtOAc extract of symbiotic fungi that had the clear zone >10 mm as the standard methods described previously (Handayani *et al.*, 2019a; 2019b; Tiwari *et al.*, 2011).

Microscopic examination

The potential fungi with cytotoxic activity were examined under a microscope with a magnification of ×2000. The specimen was put on object glass and dripped with lactophenol and mixed. The specimen was covered with cover glass. The morphology of the specimen was observed, and the result was compared with literature.

Molecular identification

The procedure for DNA extraction was performed according to the modification method of Atashpaz *et al.* (2010). Four endophytic fungal strains with the highest antimicrobial and cytotoxic activities were identified using the internal transcribed spacer (ITS) region. The Polymerase Chain Reaction (PCR) reaction contained KOD FX Neo (Toyobo, Tokyo, Japan) with ITS1 and ITS4 primers, and the amplification was carried out using the method described by Ferrer *et al.* (2001).

The PCR products were sent to the first BASE, Malaysia, for sequencing. The sequences were subjected to pairwise sequence alignment using the BLAST (Basic Local Alignment Search Tool) program on NCBI (National Center for Biotechnology Information) and MycoBank database. The phylogenetic tree was constructed using the neighbor-joining

method, and p-distance model was set with a bootstrap value of 1,000 replication integrated into MEGA 7.0 software (Kumar *et al.*, 2016).

RESULTS AND DISCUSSION

Nine fungal isolates (designated as Dc01 to Dc09) from sponge *Dactylospongia* sp. were able to be isolated. The ethyl acetate extract of each fungal isolates was tested for antimicrobial activities. Four fungal isolates showed good antibacterial activity in various concentrations against *S. aureus* and *E. coli* (Table 1). Davis and Stout (1971) classified the strength of inhibition into four categories based on the diameter of inhibition as follows: very strong (≥ 20 mm), strong (10–20 mm), medium (5–10 mm), and weak (≤ 5 mm). The fungal extracts of Dc03, Dc04, and Dc08 in the concentration of 5% showed strong inhibition against *S. aureus* and *E. coli*. The isolates Dc03 and Dc04 were selected for further test against resistant bacteria. The isolate Dc04 showed the biggest inhibition zone for MRSA with a diameter of 15.50 ± 0.54 mm. The potential fungi were next subjected to determine the phytochemical constituents and identification of species.

Cytotoxic activity screening results are listed in Fig. 1. If the LC_{50} value is below 1,000 $\mu\text{g/ml}$, then the sample is classified as toxic, and if it is greater than 1,000 $\mu\text{g/ml}$, it is classified as nontoxic (Meyer *et al.*, 1982). In this present study, all fungal extracts were toxic and, therefore, have the potential to produce cytotoxic compounds. The five fungal isolates with LC_{50} value below 80 $\mu\text{g/ml}$ were selected further to determine their active compounds and species identification.

Further testing on the cytotoxic activity was conducted by MTT assay using the T47D cell lines. The first step was the determination of the viability percentage of cancer cells in the culture medium after the addition of fungal extracts with a concentration of 100 $\mu\text{g/ml}$. Data obtained were analyzed by a linear regression equation to obtain IC_{50} value. The IC_{50} extract value of Dc03, Dc04, Dc05, and Dc08 against T47D cell line difference was in the range of 225.75–1,760.98 ppm (Table 2). The potential fungal extracts did not show good IC_{50} value. It

might be due to the cytotoxic activity of fungal extracts selective to another human cancer cell lines.

The results of the screening of antimicrobial and cytotoxic activity showed that the isolates such as Dc03, Dc04, Dc05, and Dc08 had a high potential as antibacterial and cytotoxic agents. The chemical constituent analysis showed that most of the fungal extracts contained terpenoid, steroid, and flavonoid but did not contain alkaloid, phenol, and saponin (Table 3).

The morphological observations showed that isolate Dc03 was greenish-brown, the surface was centered to the middle, and the bottom was black with white flat edges (Fig. 2). The microscopic identification showed that this fungus had round conidia bulkhead along the conidiophores (Fig. 2). The examination of macroscopic and microscopic observation was concordance with literature that Dc03 was identified as *Cladosporium halotolerans* MN859971. This result was confirmed by molecular identification conducted at Indonesian Center for Biodiversity and Biotechnology. A BLAST search in NCBI-GenBank and MycoBank showed that isolate Dc03 had 100% identity with *C. halotolerans* MN859971. The phylogenetic tree showed that Dc03 was clustered together with *C. halotolerans* strain DTO127E8 (Fig. 3). The study about secondary metabolites from *C. halotolerans* is still limited. Pang *et al.* (2017) reported that *Cladosporisteroid* A, B, and C and *Cerevisterol* compounds isolated from *Cladosporium* sp. which was derived from marine sponge *Callyspongia* sp. showed antiviral and cytotoxic activities (Pang *et al.*, 2017).

The fungus Dc04 strain was brownish-green, the surface was stripped to the center, and the bottom was yellow-white and white flat edges (Fig. 2). In the microscopic observation, Dc04 had conidiophores that are not broad but branched, phialide, and with round conidia (Fig. 2). The isolate Dc04 was identified molecularly as *Penicillium citrinum* MN859968. On the phylogenetic tree, the isolate Dc04 resided in the same with *P. citrinum* strain ercha 16 (Fig. 3). The secondary metabolites isolated from the marine-derived fungi from the genus *Penicillium* produced various bioactive compounds with wide biological activities, such as

Table 1. Antimicrobial activity of EtOAc extracts of marine-derived fungi from *Dactylospongia* sp.

Fungi code	Zone of inhibition (mm) \pm standard deviation (SD)										
	<i>E. coli</i>			<i>S. aureus</i>			<i>C. albicans</i>			<i>MRSA</i>	<i>MDRPA</i>
	5%	3%	1%	5%	3%	1%	5%	3%	1%	5%	5%
Dc01	8.51 \pm 0.40	6.43 \pm 0.05	-	-	-	-	-	-	-	n	n
Dc02	-	-	-	-	-	-	-	-	-	n	n
Dc03	12.74 \pm 0.45	11.28 \pm 0.10	9.56 \pm 0.36	11.84 \pm 0.10	11.28 \pm 0.10	9.39 \pm 0.14	-	-	-	10.88 \pm 1.25	9.42 \pm 1.59
Dc04	15.50 \pm 0.30	14.21 \pm 0.25	11.08 \pm 0.30	16.14 \pm 0.75	12.75 \pm 0.27	10.30 \pm 0.24	-	-	-	15.50 \pm 0.54	8.33 \pm 0.29
Dc05	10.41 \pm 0.42	8.01 \pm 0.43	6.21 \pm 0.14	6.37 \pm 0.42	-	-	-	-	-	n	n
Dc06	6.53 \pm 0.39	-	-	-	-	-	-	-	-	n	n
Dc07	6.50 \pm 0.23	-	-	8.70 \pm 0.35	6.42 \pm 0.28	-	-	-	-	n	n
Dc08	12.31 \pm 0.54	10.55 \pm 0.40	7.53 \pm 0.29	14.25 \pm 0.10	9.46 \pm 0.29	7.19 \pm 0.13	-	-	-	n	n
Dc09	6.15 \pm 0.11	-	-	8.61 \pm 0.21	7.10 \pm 0.05	-	-	-	-	n	n
Positive control		22.15			22.35			22.15		24	8.75
Negative control		-			-			-		-	-

- = no inhibition
n = not tested

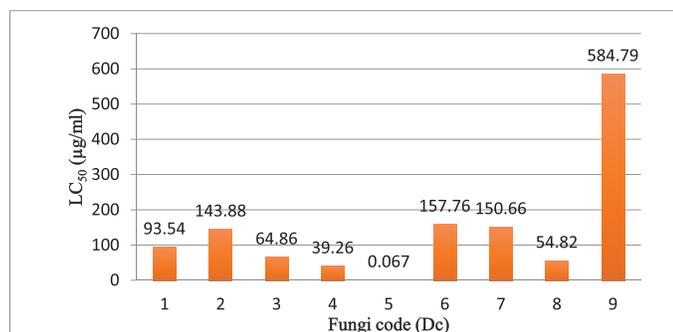


Figure 1. The cytotoxic activity of EtOAc extracts of marine-derived fungi from *Dactylosporgia* sp.

Table 2. The IC₅₀ value of selected fungal extracts on T47D cancer cell line.

No.	Code of Extract	The IC ₅₀ value of T47D cell line (ppm)
1	Dc03	225.75
2	Dc04	640.12
3	Dc05	1,760.98
4	Dc08	456.75
5	Doxorubicin	10.05

Table 3. The chemical constituents of EtOAc extracts of marine-derived fungi from *Dactylosporgia* sp.

Fungi code	Chemical constituents					
	Alkaloid	Flavonoid	Terpenoid	Steroid	Phenolic	Saponin
Dc01	-	-	-	+	-	-
Dc03	-	+	-	+	-	-
Dc04	-	+	+	-	-	-
Dc05	-	-	+	+	-	-
Dc08	-	-	+	+	-	-

Note: - = no reaction

antifouling polyketides (Bao *et al.*, 2013), antibacterial communal A (Wang *et al.*, 2012), cytotoxic penicoline (Shao *et al.*, 2010), and many others. Some bioactive compounds from *P. citrinum* that had been reported included citrinin (Subramani *et al.*, 2013), isocyclocitrinol A and 22-acetylisocyclocitrinol A (Amagata *et al.*, 2003), and γ -sitosterol, stigmasterol, pentadecanoic acid, and 14-methyl-methyl ester-17-Pentatriacontene (Binh *et al.*, 2018). The genera of *Penicillium* are not only marine borne but also soil borne as well. Even though their terrestrial counterparts have been the subject of chemical screening for at least five decades, marine-derived *Penicillium* sp. continues to yield a broad of new secondary metabolites.

The next potential fungi were Dc05 which had yellowish-green, hairy surface, white, and flat edges' colony (Fig. 2). Dc05 had round conidia, conidiophores, phialide, and vesicles (Fig. 2). The macroscopic and microscopic determination allowed Dc05 as *Aspergillus versicolor* MN859970. Molecular identification showed that Dc05 had an identity 100% with *A. versicolor* strain upm1 on BLAST analysis (Fig. 3). The isolate Dc05 had the highest cytotoxic activity with the LC₅₀ value of 0.067 μ g/ml. Many secondary metabolites have been isolated from *A. versicolor* with various biological activities, such as

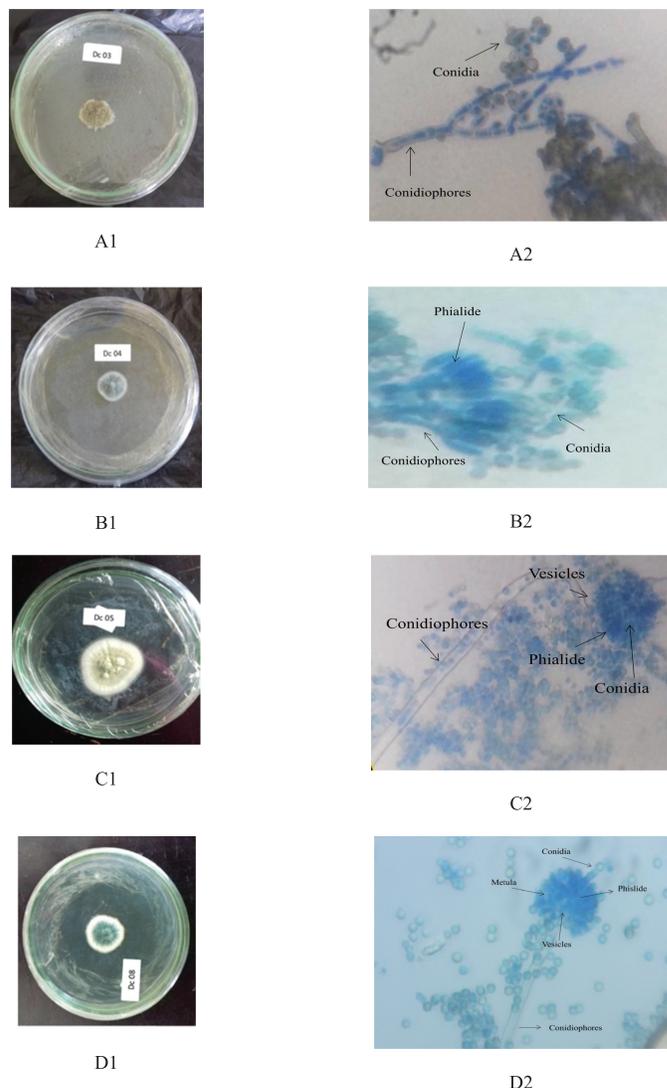


Figure 2. The macroscopic (left side) and microscopic (right side) observations of the fungi. A1 and A2 are the isolate Dc03 (*C. halotolerans* MN859971), B1 and B2 are the isolate Dc04 (*P. citrinum* MN859968), C1 and C3 are the isolate Dc05 (*A. versicolor* MN859970), and D1 and D2 are the isolate Dc08 (*A. sydowii* MN859969).

5-methoxydihydrosterigmatocystin as antimicrobial agent (Song *et al.*, 2014), aspergoterpenins A-B with antimicrobial and cytotoxic activity (Guo *et al.*, 2018), 1'-O-ethyl-6,8-di-O-methylaverantin as antibacterial agent (Zhang *et al.*, 2012), and novel anthraquinone [2-(dimethoxymethyl)-1-hydroxyanthracene-9,10-dione] which exhibited strong inhibitory activities against MRSA ATCC43300 and MRSA CGMCC 1.12409 (with MIC (minimum inhibitory concentration) values of 3.9 and 7.8 μ g/ml, respectively) and moderate activities against tested strains of *Vibrio* (with MIC values ranging from 15.6 to 62.5 μ g/ml) (Wang *et al.*, 2018). This fungus can also be found in a plant, soil, marine habitat, and air (Ebada *et al.*, 2018).

The macroscopic characterization showed that the isolate Dc08 colony was solid green with hairy surface and white-flat edges (Fig. 2). This fungus had round conidia, conidiophores, vesicles, phialide, and metula (Fig. 2). Molecular identification

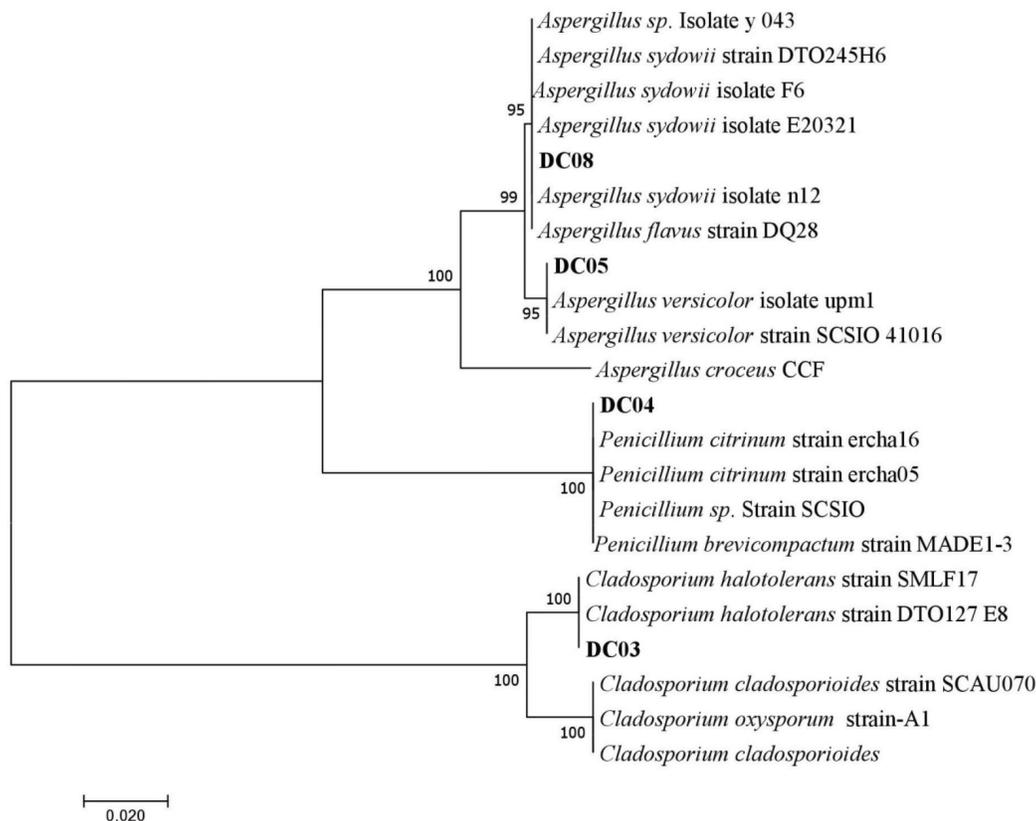


Figure 3. Neighbor-joining phylogenetic tree of potential fungal isolates from *Dactylosporgia* sp.

showed that Dc08 had 100% identity with *Aspergillus sydowii* strain E20321 ITS and identified as *A. sydowii* MN859970 (Fig. 3). Gao *et al.* (2017) reported about bioactive compounds from *A. sydowii*, a fungal strain obtained from the Marine Culture Collection of China. Lumichrome, N-acetyltyramine, 3-(2-hydroxypropyl)-4-(hexa-2E,4E-dien-6-yl)furan-2(5H)-one, 1,4-dioxa-9,12-diazacyclohexadecane-5,8,13,16-tetraone, and cycloerodiol were succeed isolated from the EtOAc extract of this fungal and displayed moderate antibacterial activity with an MIC value between 6.3 and 25.0 μ M.

The bioactivities of secondary metabolites from marine fungi reveal interesting pharmacological activities, and the fungi from the genera *Penicillium* and *Aspergillus* were the major object in this field. Most of the published work on secondary metabolites of marine fungi has focused on these genera due to the ability of these fungi to produce the new compounds (Imhoff, 2016).

CONCLUSION

Marine-derived fungi have already provided a variety of pharmacologically active novel secondary metabolites and thus represent a valuable resource for structurally unique and interesting biological properties. In this study, we had succeeded in the isolation of nine fungi-derived marine sponge *Dactylosporgia* sp. and screened for their antimicrobial and cytotoxic activities. In further research, it is challenging to isolate the bioactive compounds from the potential marine-derived fungi, especially for *P. citrinum* Dc04 for the discovery of new drug candidates.

ACKNOWLEDGMENT

This work was financially supported by the Ministry of Research and Technology, Indonesia in the project “Master Program of Education Leading to Doctoral Degree for Excellent Graduate research”. under Contract 163/SP2H/LT/DRPM/2019.

The authors would like to thank Dr. Nicole J. De Voogd from Natural Biodiversity Center, the Netherlands, for her kind assistance in the identification of the marine sponge.

CONFLICT OF INTERESTS

Authors declare that they do not have any conflicts of interest.

REFERENCES

- Abraham EP, Loder PB. In: Flynn EH (ed.). Cephalosporins and penicillins; chemistry and biology. Academic Press, New York, NY, pp 1–26, 1972.
- Amagata T, Amagata A, Tenney K, Valeriote FA, Lobkovsky E, Clardy J, Crews P. Unusual C25 steroids produced by a sponge-derived *Penicillium citrinum*. Org Lett, 2003; 5(23):4393–6.
- Atashpaz S, Khani S, Barzegari A, Barar J, Vahed S, Azarbaijani R, Omidi Y. A robust universal method for extraction of genomic DNA from bacterial species. Microbiology, 2010; 79(4):538–42.
- Bao J, Sun YL, Zhang XY, Han Z, Gao HC, He F, Qian PY, Qi SH. Antifouling and antibacterial polyketides from marine gorgonian coral-associated fungus *Penicillium* sp. SCSGAF 0023. J Antibiot 2013, 66:219–23.
- Binh NT, Lam PVH, Diep CN. Bioactive compounds from marine fungus *Penicillium citrinum* strain nd7c by gas chromatography-mass spectrometry. Pharm Chem J, 2018; 5(1):211–24.

- Blunt JW, Copp BR, Hu W, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. *Nat Prod Rep*, 2007; 24:31–86.
- Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR. Marine natural products. *Nat Prod Rep*, 2015; 32:116–211.
- Davis WW, Stout TR. Disc plate method of microbiological antibiotic assay. *J Microbiol*, 1971; 22(4):659–65.
- Ebada SS, El-Neketi M, Ebrahim W, Mándi A, Kurtán T, Kalscheuer R. Phytochemistry letters cytotoxic secondary metabolites from the endophytic fungus *Aspergillus versicolor* ku258497. *Phytochem Lett*, 2018; 24:88–93.
- Ferrer C, Colom F, Frases S, Mulet E, Abad JL, Alió JL. Detection and identification of fungal pathogens by PCR and by ITS2 and 5.8S ribosomal DNA typing in ocular infections. *J Clin Microb*, 2001; 39(8):2873–9.
- Gamal-Eldeen AM, Abdel-Lateff A, Okino, T. Modulation of carcinogen metabolizing enzymes by chromanone A; a new chromone derivative from algicolous marine fungus *Penicillium* sp. *Environ Toxicol Pharmacol*, 2009; 28:317–22.
- Gao T, Cao F, Yu H, Zhu H. Secondary metabolites from the marine fungus *Aspergillus sydowii*. *Chem Nat Compd*, 2017; 53(6).
- Guo Z-Y, Tan M-H, Liu C-X, Lv M-M, Deng Z CF. Aspergoterpenins a-d: four new antimicrobial endophytic fungus *Aspergillus versicolor*. *Molecules*, 2018; 23(1291):1–10.
- Handayani D, Ahdinur RF, Rustini R. Antimicrobial activity of endophytic fungi from marine sponge *Haliclona fascigera*. *J App Pharm Sci*, 2015a; 5(10):154–6.
- Handayani D, Aminah I. Antibacterial and cytotoxic activities of ethyl acetate extract of symbiotic fungi from West Sumatra marine sponge *Acanthoryngylophoraingens*. *J App Pharm Sci*, 2017a; 7(2):237–40.
- Handayani D, Ananda N, Artasasta MA, Rustini R, Fadriyanti O, Tallei TE. Antimicrobial activity screening of endophytic fungi extracts isolated from brown algae *Padina* sp. *J App Pharm Sci*, 2019a; 9(3):9–13.
- Handayani D, Artasasta MA. Antibacterial and cytotoxic activities screening of symbiotic fungi extract isolated from marine sponge *Neopetrosiachaliniformis* AR-01. *J App Pharm Sci*, 2017b; 7(5):66–9.
- Handayani D, Murniati M, Rustini R. *In vitro* inhibitory activity of ethyl acetate extract of symbiotic bacteria isolated from the marine sponge *Haliclona fascigera* against *Multi-drug Resistant Organism* (MDRO). *J App Pharm Sci*, 2016; 6(11):218–22.
- Handayani D, Rasyid W, Rustini ZE, Hertiani T. Cytotoxic activity screening of fungal extracts derived from the West Sumatran marine sponge *Haliclona fascigera* to several human cell lines: HeLa, WiDr, T47D, and Vero. *J App Pharm Sci*, 2018a; 8(1):55–8.
- Handayani D, Rivai H, Mulyana R, Suharti N, Rasyid R, Hertiani T. Antimicrobial and cytotoxic activities of endophytic fungi isolated from mangrove plant *Sonneratia alba* Sm. *J App Pharm Sci*, 2018b; 8(2):49–53.
- Handayani D, Sandrawati N, Nestianda O, Ruslan R, Fajrina A, Tallei TE. Cytotoxic and antimicrobial activities of ethyl acetate extract of mangrove plant *Scyphiphora hydrophyllacea* C. F. Gaertn-Associated fungi. *J Appl Pharm Sci*, 2019b; 9(6):75–9.
- Handayani D, Sandrawaty N, Murniati M, Regina R. Screening of endophytic bacteria isolated from marine sponge *Haliclona fascigera* for inhibition against clinical isolates of methicillin-resistant staphylococcus aureus (MRSA). *J App Pharm Sci*, 2015b; 5(9):139–42.
- Hu Y, Chen J, Hu G, Yu J, Zhu X, Lin Y, Chen S, Yuan J. Statistical research on the bioactivity of new marine natural products discovered during the 28 years from 1985 to 2012. *Mar Drugs*, 2015; 13:202–21.
- Imhoff JF. Natural products from marine fungi—still an underrepresented resource. *Mar drugs*, 2016; 14(16):19.
- Kjer J, Debbab A, Aly AH, Proksch P. Methods for isolation of marine-derived endophytic fungi and their bioactive secondary products. *Nat Protoc*, 2010; 5(3):479–90.
- Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*, 2016; 33(7):1870–4.
- Meyer BN, Ferrign RN, Putnam JE, Jacobson JE, Nicholas DE, McLaughlin JL, Brineshrimp: a convenient general bioassay for active plant constituents. *Planta Med*, 1982, 45:31–4.
- Pang X, Lin X, Wang J, Liang R, Tian Y, Salendra L. Three new highly oxygenated sterols and one new dihydroisocoumarin from the marine sponge derived fungus *Cladosporium* sp. scsio41007. *Steroids*, 2017; 30(40):1–6.
- Sandrawati N, Pariatno R, Suharti N, Handayani D. *In vitro* cytotoxic activity assay of bacteria extract derived marine sponge *Haliclona fascigera* toward HeLa, WiDr, T47D, and Vero cell line. *J Appl Pharm Sci*, 2019; 9(08):66–70.
- Shao CL, Wang CY, Gu YC, Wei MY, Pan JH, Deng DS, She ZG, Lin YC. Penicnoline, a new pyrrolyl4 quinolinone alkaloid with an unprecedented ring system from an endophytic fungus *Penicillium* sp. *Bioorg Med Chem Lett*, 2010; 20:3284–6.
- Song F, Ren B, Chen C, Yu K, Liu XZY. Three new sterigmatocystin analogues from marine-derived fungus *Aspergillus versicolor* mf359. *Appl Microbiol Biotechnol*, 2014; 98:3753–8.
- Subramani R, Kumar R, Prasad P, Aalbersberg W. Cytotoxic and antibacterial substances against multi-drug resistant pathogens from marine sponge symbiont: citrinin, a secondary metabolite of *Penicillium* sp. *Asian Pac J Trop Biomed*, 2013; 3(4):291–6.
- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction : a review. *Int Pharm Sci*, 2011; 1(1):98–106.
- Wang JF, Liu PP, Wang Y, Wang H, Li J, Zhuang YB, Zhu WM. Antimicrobial aromatic polyketides from gorgonian-associated fungus, *Penicillium commune* 518. *Chin J Chem*, 2012; 30:1236–42.
- Wang W, Chen R, Luo Z, Wang WCJ. Antimicrobial activity and molecular docking studies of a novel anthraquinone from a marine-derived fungus *Aspergillus versicolor*. *Nat. Prod Res*, 2018; 6419(12):558–63.
- Zhang Y, Li XM, Wang BG. Anthraquinone derivatives produced by marine-derived fungus *Aspergillus versicolor* EN-7. *Biosci Biotechnol Biochem*, 2012; 76:1774–6.

How to cite this article:

Sandrawati N, Hati SP, Yunita F, Putra AE, Ismed F, Tallei TE, Hertiani T, Handayani D. Antimicrobial and cytotoxic activities of marine sponge-derived fungal extracts isolated from *Dactylospongia* sp. *J Appl Pharm Sci*, 2020; 10(04): 028–033.