Available online at website: www.bbp4b.litbang.kkp.go.id/squalen-bulletin

Squalen Bulletin of Marine & Fisheries Postharvest & Biotechnology, 8 (2), 2013, 47-56

Screening of Antitumor Bioactivity of Fungi Associated with Macro Algae and Sponge from Indrayanti Beach, Jogjakarta

Penapisan Bioaktivitas Antitumor Kapang yang Berasosiasi dengan Makroalga dan Spons dari Pantai Indrayanti, Jogjakarta

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Article history:
Received: 12 December 2012; revised: 1 Juni 2013; accepted: 1 Juni 2013

ABSTRACT

This research was aimed to isolate marine derived-fungi which associated with macro algae and sponge from Indrayanti Beach, Jogjakarta and to screen the antitumor (T47D and HeLa) bioactivity of the fungi extracts. Three solid media of MEA (malt extract agar), GPY (glucose peptone yeast) and MFM (minimal fungi medium) were used as isolation medium. Each of the pure fungi isolates was then cultivated in 100 ml of liquid medium for 4 weeks at room temperature (27-28°C) in static conditions. The antitumor activity of the fungi extracts were tested against breast tumor cells (T47D) and cervical cancer cells (HeLa) using Thiazolyl Blue Tetrazolium Blue (MTT) assay method. A number of 21 isolates of fungi were isolated from 4 macro algae and 1 sponge samples. The identification of fungi isolate was conducted using combination of molecular approach (ITS1-5.8S-ITS4 DNA regions) and macro-micro morphological characteristics. Among those 21 marine fungi species isolated, MFGK-21 extract showed the best anti-servical tumor (HeLa) with an IC_{50} value of 240.1 µg/ml and MFGK-27 extract showed the best anti-breast tumor (T47D) with an IC_{50} value of 59.6 µg/ml. The MFGK-21 fungi isolate was identified as *Penicillium steckii*, while the MFGK-27 fungi isolate was identified as *Aspergillus sydowii.*

Keywords: marine derived-fungi, antitumor, *Aspergillus sydowii, Penicillium steckii,* Indrayanti Beach

ABSTRAK

Penelitian ini bertujuan untuk mengisolasi kapang yang berasosiasi dengan makroalga dan spons dari Pantai Indrayanti, Jogjakarta dan untuk menapis bioaktivitas ekstrak kapang tersebut sebagai antitumor payudara (T47D) dan serviks (HeLa). Tiga jenis media digunakan sebagai media isolasi, yaitu MEA (*malt extract agar*), GPY (*glucose peptone yeast*) dan MFM (*minimal fungi medium*). Masing-masing isolat kapang murni kemudian dikultivasi dalam media cair volume 100 ml selama 4 minggu pada suhu kamar (27-28°C) dalam kondisi statis. Aktivitas antitumor dari ekstrak kapang diuji menggunakan metode *Thiazolyl Blue Tetrazolium Blue* (MTT) assay. Sejumlah 21 isolat kapang telah diisolasi dari 4 sampel makroalga dan 1 sampel spons. Identifikasi isolat kapang dilakukan dengan menggunakan kombinasi pendekatan molekuler (ITS1-5.8S-ITS4) dan karakteristik morfologi makro-mikro. Di antara 21 spesies kapang yang diisolasi, ekstrak MFGK-21 menunjukkan aktivitas antitumor HeLa terbaik dengan nilai IC_{50} 240,1 µg/ml dan ekstrak MFGK-27 menunjukkan aktivitas antitumor T47D terbaik dengan nilai IC_{50} dari 59,6 µg/ml. Isolat kapang MFGK-21 diidentifikasi sebagai *Penicillium steckii*, sedangkan isolat kapang MFGK-27 diidentifikasi sebagai *Aspergillus sydowii*.

Kata Kunci: kapang laut, antitumor, *Aspergillus sydowii, Penicillium steckii,* Pantai Indrayanti

Permalink/DOI: http://dx.doi.org/10.15578/squalen.v8i2.25

1. Introduction

The 2008 global cancer statistics (GLOBOCAN) released by the International Agency for Research on Cancer-WHO provides statistical information on new cancer cases and cancer deaths in the world. Based on that resource, 56% of new cancer cases and 63% of cancer deaths in the world are occurred in developing countries (Anon., 2012). In Indonesia, the incidence of breast cancer in 2008 reaches 39,831 cases (13.6%) and causes the highest mortality of 20,052 cases (9.3%). In 5-years prevalence, breast cancer in Indonesia will approximately reach 139,012 cases. In addition to breast cancer, another significant cause of death is cervical cancer. In 2008, the new incidence of cervical cancer in Indonesia reaches 13,762 cases, with the death rate caused by this cancer reaches 7,493 cases. That significance number of mortality caused by cancer shows that many cancer therapies that exist today have not been effective. Therefore,

finding new drugs to treat severe human diseases such as cancer becomes an important goal for pharmaceutical industry (Bhatnagar & Kim, 2010). As natural products in general play an important role in the drugs development, intensive search for novel natural products is getting intensive. In addition to that, the hit rates number in the screening of marine natural products for drugs leads makes the search for new marine bioactive compounds is highly attractive.

Marine natural products researches in the last decades of the last century reveal numerous novel compounds with interesting bioactivities. However, the application or developments of those compounds into drugs are hampered by material supply problems (Mayer et al., 2010; Fajarningsih, 2012). Mariculture problems of most of marine macro organisms made them difficult to produce a sufficient amount of active substances for further studies (Mayer et al., 2010). As a case example, to obtain 1.4 g of Bryostatin-1, an anticancer drug candidate currently in clinical studies, approximately of 1 metric ton *Bugula neritina* should be extracted (Proksch et al., 2006; Kollar et al., 2013). Numerous data show that marine bioactive compounds are produced either by the symbiont or by the association between the host and the symbiont (Kornprobst, 2010; Thomas et al., 2010). Marine microbes, especially fungi, are important source to obtain new compounds with potential pharmacological activity (Kjer et al., 2010; Thomas et al., 2010). During the last decade, at least 273 new chemical structures have been isolated from marine fungi (Bugni & Ireland, 2004). Marine fungi have proved to be a promising source in the search for anticancer compounds, antibacterial, antiviral, anti-inflammatory and many other bioactive compounds (Bhadury et al., 2006). In addition to its promising bioactivity, marine microorganisms can be cultured using traditional culturing method to produce bioactive secondary metabolites, hence the material supply problem can be avoided (Fajarningsih, 2012). Those reasons made marine derived-fungi comes into focus of marine natural products research in the last few decades.

Various studies have proved the hypothesis that the production of secondary metabolites by an organism was not random, but is strongly influenced by ecological niche (specific habitats) (Gloer, 1997 in Holler et al., 2000). For this reason, many researchers in the world are becoming more interested in exploring marine organisms than terrestrial organisms that have long been explored in attempt to obtain new bioactive compounds (Holler et al., 2000; Schulz et al., 2008). Microorganisms, including fungi, are greatly distributed in all available niches in the world, from hydrothermal vents to the polar ice. Among those niches, marine biota is a particular attractive niche for many microorganisms to live as a symbiont. Many new promising bioactive compounds have been isolated from marine derived-fungi. Sorbicillactone A is an alkaloid compound produced by marine fungi *Penicillium chrysogenum* that was previously isolated from marine sponge *Ircinia fasciculate*. Sorbicillactone A shows selective bioactivity against leukemia cells and shows ability to protect human T cells against the cytopathic effects of HIV-1 (Bhatnagar & Kim, 2010). Yu et al. (2008) isolated cyclodepsipeptides scopulariade A (1) and B (2) compounds from marine derived fungi *Scopulariopsis brevicaulis.* Few years ago they filed the patent application of "the production and the use of antitumoral cyclodepsipeptides", and recently the patent has been granted with patent no of EP2229401 (EP Patent No. 2229401, 2013).

Several papers also report some potent bioactive compounds isolated from Indonesian marine derived fungi. Fungi *Emericella nidulans* which associated with ascidian *Aplidium longithorax* collected from Wakatobi waters, Indonesia, produces bioactive compound Emestrin diketopiperazine (Emestrin A). The Emestrin A show strong antitumor activity through apoptosis induction mechanism (Nursid et al., 2011a, b). Tarman et al. (2011) reported that the ethyl acetate crude extract of algicolous fungus (KT31) isolated from Indonesian red macro algae *Kappahycus alvarezii* shows a potent cytotoxicity against bladder carcinoma cell lines with an IC_{50} value of 1.5 µg/ml. Yet, research on bioprospecting of marine-derived fungi associated with Indonesian marine biota is still very limited. On the other hand, Indonesia is blessed to possess a mega-biodiversity in the world, especially marine biodiversity. Those marine biodiversity is an asset to be utilized as research material in biodiscovery research. Up till now, there is no research paper reporting biodiscovery of marine derived fungi activity in Indrayanti Beach, Jogjakarta. Thus, this research was aimed to isolate marine derived-fungi which associated with all macro algae and sponge collected from Indrayanti Beach, Jogjakarta and to screen the biological activity of the fungi extracts as antitumor cervix (HeLa) and breast (T47D).

2. Material and Methods

2.1. Sampling of Macro algae and Sponge

We collected all macroalgae and sponge species found in Indrayanti Beach, Jogjakarta. A total of 10 macro algae samples and 1 sponge sample were collected . All the samples were cleaned using sterile sea water, then were put into a sterile plastic container, labeled and stored in a cool box containing ice to be brought to the Biotechnology Laboratory of the Research and Development Center for Marine and

Fisheries Product Processing and Biotechnology (RDCMFPPB), Jakarta.

2.2. Isolation of Fungi Associated with Macro Algae and Sponge Samples

Marine-derived fungi isolation was conducted using three types of isolation medium, ie. MEA medium (0.3% malt extract, 0.3% yeast extract, 0.5% peptone, 1.5% agar), MFM (0.02% yeast extract, 0.1% soluble starch, 2% agar), and GPY (0.1% glucose monohydrate, 0.05% soybean peptone, 0.01% yeast extract, 1.5% agar). The entire medium were dissolved in artificial sea water (ASW), and then sterilized at temperature of 121 °C for 15 minutes. After sterilization, the media was aseptically poured into petri dish and allowed to become solid.

Ten macro algae samples (code GK-01-11; GK-02-11; GK-03-11; GK-04-11; GK-05-11; GK-06-11; GK-07-11; GK-08-11; GK-10-11; GK-11-11) and 1 sponge sample (code GK-09-11) were aseptically cut into small pieces (thinly sliced using a scalpel). Five pieces of each samples were then inoculated in three different mediums that were prepared (MEA, MFM, and GPY) and incubated for 3-5 days at room temperature (±27 °C). Each different fungi strain that grew on the samples was then isolated to a new petri dish containing the same isolation medium until the pure fungi strain was obtained.

2.3. Cultivation of The Pure Fungi Strain

Each of pure fungi strains obtained in the isolation step was then cultivated in liquid medium similar to its solid isolation medium, i.e. the MEB (0.3% malt extract, 0.3% yeast extract, 0.5% peptone), GPY (0.1% glucose monohydrate, 0.05% soybean peptone, 0.01% yeast extract) or MFM (0.02% yeast extract, 0.1% soluble starch). For this purpose, each strain that covers the surface of the inoculated petri dish was aseptically cut into small pieces of 1x1 cm^2. These pieces were then aseptically transferred into a flask containing 10 ml of the sterilized liquid medium and incubated at room temperature for 3 days. For larger-scale fermentation, each pure fungal strain that grew on the 10 ml of liquid medium was aseptically transferred into a flask containing 100 ml of sterilized liquid medium and cultivated at room temperature for 4-5 weeks under static conditions.

2.4. Extraction of the Fungi Cultures

The Extraction of the fungi cultures was conducted using Kjer et al. (2010) method with modification. The cultivation of the pure fungi strains were ended by adding 200 ml of EtoAc solvent to the culture flask and macerated overnight. The fungi cells were then disrupted using sonicator (Sonics vibra cell model CV-33) at amplitude of 81% for 5 minutes. The mixture was then filtered using filter paper and the mycelium residue was discarded. The culture filtrate was then transferred into a separation funnel to separate the EtoAc and H_2O phases. The aqueous phase was reextracted twice using 200 ml of EtoAc. The EtoAc phase was then collected and evaporated using rotary evaporator (Buchi) to obtain the ethyl acetate crude extract of each fungal strain.

2.5. In Vitro Antitumor Assay

In vitro antitumor assay was performed using MTT assay method according to Zachary (2003) with modification. T47D breast tumor cell lines and HeLa cervical cancer cell lines were cultured in RPMI 1640 medium containing 10% Fetal Bovine Serum (FBS), 0.5% fungizone and 2% Penicillin-Streptomycin. T47D and HeLa cells were plated at 10,000 cells per well and incubated at 37 °C with 5% of CO_2 flow for 24 hours. After that, each fungi crude extract and doxorubicyn as positive control was tested in a single concentration of 30 µg /ml (dissolved in RPMI medium) for 24 hours. Each sample was tested in three replicates. The cell-growth medium was removed from each well before the extracts were plated into the wells containing T47D or HeLa cells attached. Three kinds of controls were made, i.e. control of tumor cells, control of medium (medium without tumor cells) and control of samples (samples without tumor cells). After 24 hours treatment of the fungi crude extracts, the solution was removed from each well. After that, 100 µl of MTT reagent (500 µg/ml) was added into each wells and incubated for 4 hours in CO_2 incubator until purple precipitate was visible. As much as 100 µl of sodium dodecyl sulfate (SDS) 10% was added into each well, and incubated at room temperature (±27 °C) in the dark for 12 hours. After incubation, the absorbance of each well was measured by DYNEX micro-plate reader at wavelength of 570 nm.

The percentage of cell death was calculated using the formula:

$$\% \text{ Mortality} = \frac{(A - D) - (B - C)}{(A - D)} x100\%$$

Description: A=absorbance of tumor cell control, B=absorbance of the sample, C= absorbance of sample control, D=absorbance of medium control.

Fungi crude extract that showed the best antitumor activity againts T47D (MFGK-27) and HeLa (MFGK-21) was then tested in serial concentration of 12.5, 25, 50, 100, and 200 µg/ml. The Inhibition $Concentration_{50}$ (IC_{50}) value was then calculated using probit analysis.

2.6. Identification of the Fungal Species

Identification of fungi isolates was conducted using combination of molecular approach and morphological characteristics.

Molecular Approach

Genomic DNA of the fungi was extracted using DNeasy plant mini kit (Qiagen). Universal fungi primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') were employed to amplify the ITS1-5.8S-ITS4 DNA regions of the fungi isolates. PCR reactions were carried out in 50 µl reaction, as follows: 25 µl of PCR master mix 2x (Promega), 5 µl of Primer ITS1 (10 µM), 5 µl of Primer ITS4 (10 µM), 10 µl DNA template and 5 µl ultra purified water. The thermal cycler (Biometra) was set up as follows: initial denaturation 94 °C for 2 minutes, 35 cycles of: 94 °C for 1 minute, 60 °C for 1 minute, and 72 °C for 1 minute; a final step of 5 minutes at 72 °C completed the protocol. The PCR products were then sent to the 1st BASE (Malaysia) for DNA clean up, followed by DNA sequencing. Sequence similarity searches of the ITS1-5.8S-ITS4 DNA sequence were performed against DNA sequence database maintained by the National Center for Biotechnology Information using BLAST algorithm *(http://blast.ncbi.nlm.nih.gov/Blast.cgi).*

Morphological Characteristic

The top BLAST sequence match results were confirmed with the morphological characteristics of the fungi isolates. The fungi isolates were inoculated on MEA and CYA medium (0.1 % of K_2HPO_4, 0.5% of yeast extract, 3% of sucrose, 1% of Czapek concentrate, and 1.5% of agar bacteriological) and the growth characteristics were examined (colonies diameter, colonies' surface and reverse color). The microscope slide of the fungi mycelium was prepared and the micro-morphological characteristics of the fungi mycelium and sporulating structures were then examined using microscope.

3. Result and Discussion

Ten macro algae samples of *Caetomorpha crassa* (GK.01.11), *Ulva reticulata* (GK.02.11), *Sargassum* sp. (GK.03.11), *Acantophora* sp. (GK.04.11), *Sargassum* sp. (GK.05.11), *Codium* sp. (GK.06.11), *Rhodymenia* sp. (GK.07.11), *Ulva fasciata* (GK.08.11), *Galaxaura* sp. (GK.10.11), *Padina* sp. (GK.11.11) and 1 unidentified sponge sample (GK.09.11) were collected from tidal zone of Indrayanti Beach, Jogjakarta. However, fungi isolates only grew in 4 of those macro algae samples (Figure 1). A total of 17 fungi isolates were isolated from 4 macro algae samples and 4 fungi isolates were isolated from 1 sponge sample (Table 1.). Sponge is a filter-feeding organisms that obtaining food by pumping its surrounding seawater. Sponge can pump up to 24,000 liters of seawater through it's per 1-kg body per day (Gao et al., 2008). According to Gao et al. (2008), microbial populations comprise as much as 40% of the volume of sponge tissue, and every ml of sponge tissue constitute more than 10^9 cells of microbial densities which is several order of magnitude higher than the microbial densities of water.

Three different types of medium (MEA, GPY, MFM) were used to isolate the fungi. The uses of those different mediums were intended to get more types of fungi isolates. Eight fungi isolates were isolated using MEA medium, twelve isolates were isolated using GPY medium and only four isolates were isolated using MFM, a medium with the poorest nutritional level compared to the other two mediums.

3.1. Bioactivity of Fungi Extracts

Antitumor Bioactivity

The antitumor activity of the fungi extracts were tested against HeLa and T47D tumor cell lines using MTT assay method. Percent mortality of HeLa and T47D tumor cell lines after treated with the ethyl acetate crude extracts of the marine fungi derived from macro algae and sponge collected from Indrayanti Beach were presented in Figure 2.

Figure 1. Macro algae and sponge collected from tidal zone of Indrayanti Beach, Jogjakarta from which 21 fungi isolates were isolated.

Table 1. Marine-derived fungi isolated from macro algae and sponge from Indrayanti Beach, Jogjakarta

No	Host	Type of Host	Isolation Medium	Fungi Isolates
1	GK.01.11	*Chaetomorpha crassa* (macro algae)	MFM	MFGK 01
2	GK.09b.11	Unidentified Sponge	MFM	MFGK 03
3	GK.09b.11	Unidentified Sponge	MFM	MFGK 04
4	GK.01.11	*Chaetomorpha crassa* (macro algae)	GPY	MFGK 06
5	GK.05.11	*Sargassum* sp.	GPY	MFGK 08
6	GK.06.11	*Codium* sp. (macro algae)	GPY	MFGK 09
7	GK.06.11	*Codium* sp. (macro algae)	GPY	MFGK 10
8	GK.09b.11	Unidentified Sponge	GPY	MFGK 11
9	GK.01.11	*Chaetomorpha crassa* (macro algae)	MEA	MFGK 14
10	GK.01.11	*Chaetomorpha crassa* (macro algae)	MEA	MFGK 15
11	GK.01.11	*Chaetomorpha crassa* (macro algae)	MEA	MFGK 16
12	GK.04.11	*Acantophora* sp.	MEA	MFGK 18
13	GK.06.11	*Codium* sp. (macro algae)	MEA	MFGK 19
14	GK.06.11	*Codium* sp. (macro algae)	MEA	MFGK 20
15	GK.06.11	*Codium* sp. (macro algae)	MEA	MFGK 21
16	GK.09b.11	Unidentified Sponge	MEA	MFGK 23
17	GK.06.11	*Codium* sp. (macro algae)	GPY	MFGK 25
18	GK.01.11	*Chaetomorpha crassa* (macro algae)	GPY	MFGK 26
19	GK.01.11	*Chaetomorpha crassa* (macro algae)	GPY	MFGK 27
20	GK.01.11	*Chaetomorpha crassa* (macro algae)	GPY	MFGK 28
21	GK.01.11	*Chaetomorpha crassa* (macro algae)	GPY	MFGK 29

It can be seen in Figure 2. that MFGK 21-crude extract showed the best antitumor HeLa (26.15 %) and MFGK 27-crude extract showed the best antitumor T47D (37.3%) among 21 crude extracts of fungi isolates tested. Carballo et al. (2002) categorized a sample as antitumor active if at concentration of 30 µg/ml the crude extract was able to cause ≥ 50% mortality of the tumor cells tested. Based on that criteria, among 21 crude extracts tested, there is no extract of the fungi tested that can be categorized as an active sample. However, the fungi extracts were tested as crude extract which likely is a mixture of various kinds of secondary metabolites produced by the fungi, as well as some impurities such as salts and proteins from the growth medium. The extract impurities might lowered the concentration of the cytotoxic compound tested causing the false negative result in the bioactivity screening platform. Nursid et al. (2011c) reported that 30 µg/ml of *Emericella nidulans* crude extract tested against T47D cells only caused 35.4% cells mortality. However, when later they isolated the antitumor active compounds from the *E. nidulans* crude extract, they were able to isolate a very active diketopiperazine cytotoxic compound, Emestrin (Nursid et al., 2011b).

To confirm the antitumor bioactivity of the MFGK-21 and MFGK-27 crude extracts, we tested the extracts in serial concentrations. The results were presented in Figure 3 and 4. The IC_{50} value (calculated using probit analysis) of the MFGK-21 extract againts HeLa cell lines was 240.1 µg/ml, while the IC_{50} value of the MFGK-27 extract againts T47D cell lines was 59.6 µg/ml. Thus, the MFGK-21 extract is apparently not active againts HeLa cell line, while the MFGK-27 extract showed a moderate cytotoxic activity against T47D cell lines.

MTT assay is one of the most rapid, sensitive and widely used assay to study cell proliferation and cytotoxicity. The assay is dependent on the ability of viable cells to metabolise a water soluble tetrazolium salt MTT into water-insoluble formazan (Twentyman & Luscombe, 1987; Zachary, 2003). The amount of formazan produced will be proportional to the number of living cells in the MTT assay. Therefore, it is

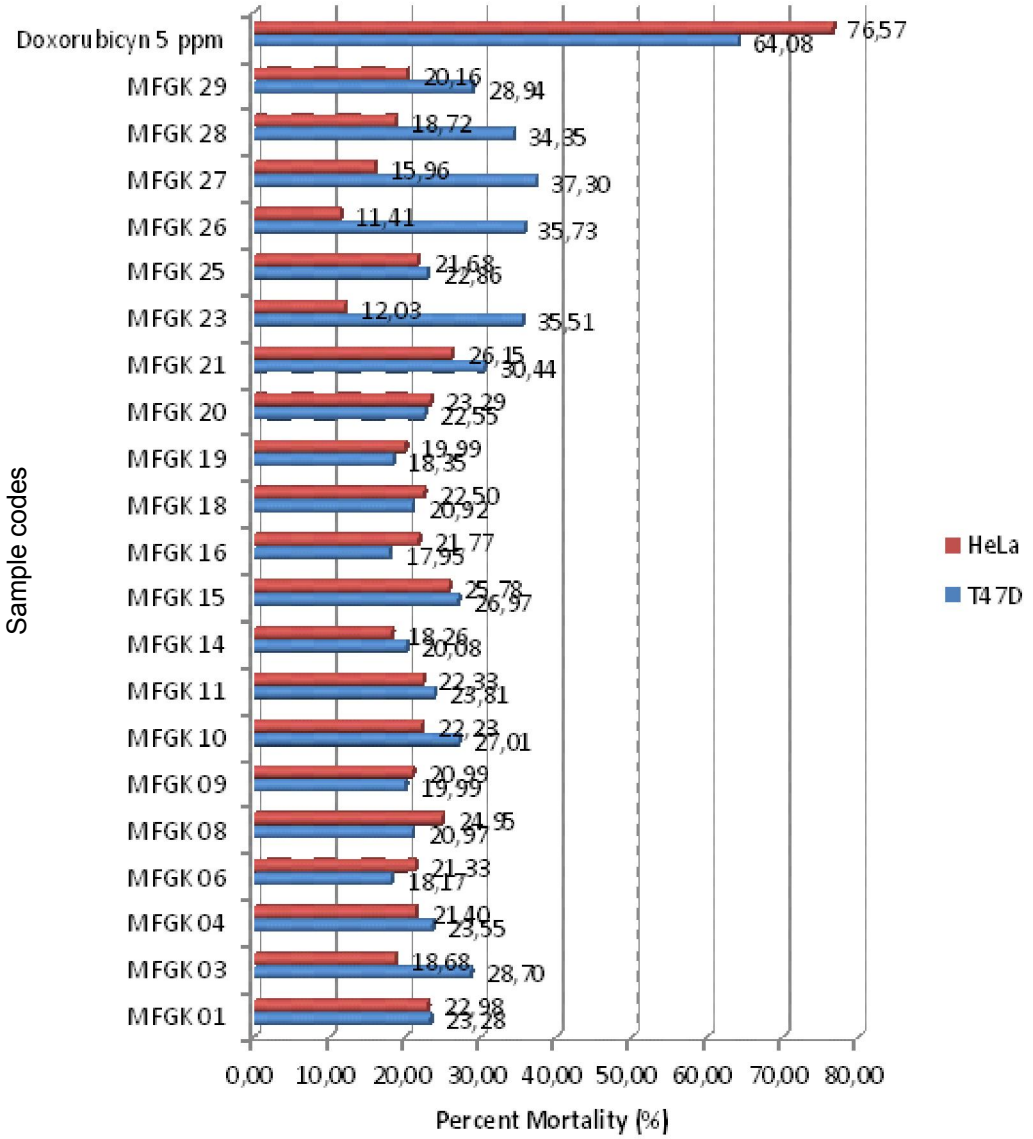

Figure 2. Percent mortality (%) of HeLa and T47D tumor cell lines after treated with 30 µg/ml of ethyl acetate crude extracts of marine fungi isolates derived from macro algae and sponges collected from Indrayanti Beach, Jogjakarta. Data represents means of two assays, each assay of 3 replicates.

important to confirm the cells mortality percentage data with the morphology of the treated cells in every MTT assay. The morphology of T47D cells that was treated with the MFGK-27 extract was showed in Figure 5. It can be seen in figure 4, MFGK-27 extract treatment caused cells mortality. The higher the extract concentration the more the cells mortality number. At concentration of 200 µg/ml, all of the cells were mortalized.

3.2. Identification of The Fungi Species

Three fungi species with the best bioactivity in each assay (MFGK-21, MFGK-27 and MFGK-03) was identified using combination of molecular and morphological characteristics approach. The sequence similarity searches of the ITS1-5.8S-ITS4 DNA sequence were performed against DNA sequence database maintained by the National Center for Biotechnology Information using BLAST algorithm. The BLAST result showed that MFGK-21 had a 99% similarity with *Penicillium steckii.* However, since one of the main disadvantages of public database is the high number of misidentified strains in the database (Samson et al., 2010) the BLAST result should be confirmed with the morphological characteristic data.

The macro-micro morphological characteristic of the MFGK-21 is presented in Figure 6. According to Houbraken et al. (2010), the conidia color of *P. Steckii*

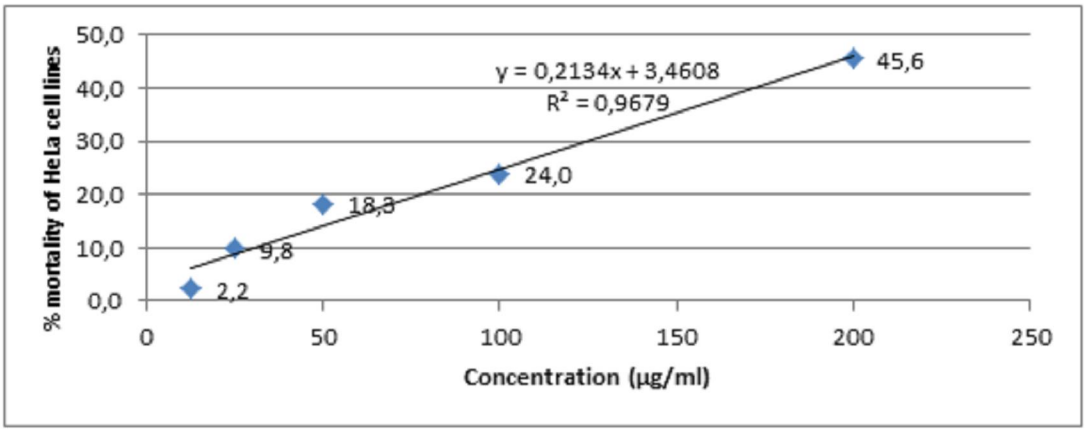

Figure 3. Mortality of HeLa cell lines treated with serial concentration of the MFGK-21 crude extract.

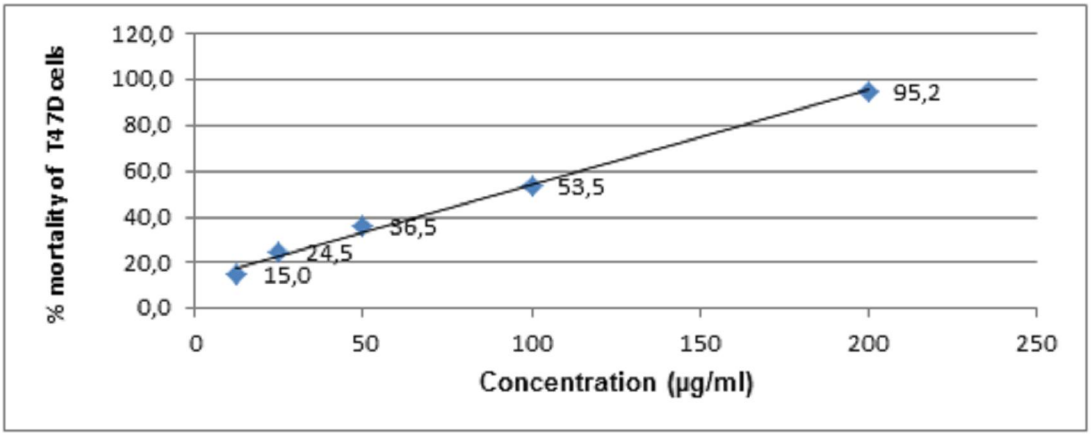

Figure 4. Mortality of T47D cell lines treated with serial concentration of the MFGK-27 crude extract.

on MEA is grey or dull green and reverse color on CYA medium is creme-brown; the shape and ornamentation of conidia is broadly ellipsoidal (oval), in some strains is slightly fusiform, smooth. The fungi has biverticillate conidiophores (two branched point) bearing 6-10 phialides (Samson et al., 2010). Based on that characteristics, MFGK-21 fungi isolate is macro-micro morphologically confirmed as *Penicillium steckii.*

Based on BLAST result, DNA sequence of MFGK-27 fungi isolate has 99% similarity with DNA sequence of *Aspergillus versicolor* and *Aspergillus sydowii.* The macro-micro morphological characteristic of the MFGK-27 fungi isolate was presented in Figure 7. *A. versicolor* and *A. sydowii* were closely related, thus both fungi have similar physiological properties (Pitt & Hocking, 2009; Samson et al., 2010). Both fungi have velvety colony. The colony color at first were white, then changing to yellow, orange-yellow to yellow-green, and often intermixed with flesh of pink color. The reverse colony color on CYA medium mostly in reddish brown (Samson et al., 2010). According to Samson et al. (2010), what differentiate *A. sydowii* from *A. versicolor* is that it has blue green conidia, rough walled conidia and small reduced conidial heads. As can be see in Figure 7, the MFGK-27 fungi colony consisting of blue green conidiphores (Fig.7A). Thus, MFGK-27 fungi isolate is more likely a *A. sydowii.* Moreover, *A. sydowii* was known as a halo tolerant fungi species and was a common fungi pathogen species of sea fan corals (Alker et al., 2001).

Hong et al. (2012) filed a patent of "Marine fungus *Aspergillus sydowii* and application thereof to preparation of anti-tumor medicines" and the patent (CN 201210574332) was published at May, 2013 . Their invention includes anti tumor activity of *Aspergillus sydowii* metabolite. The *A. sydowii* metabolite has high anti tumor activity on HepG2, PC12 and U937 cells with IC_{50} value of 62.63 µg/ml, 165.83 µg/ml and 42.83 µg/ml respectively.

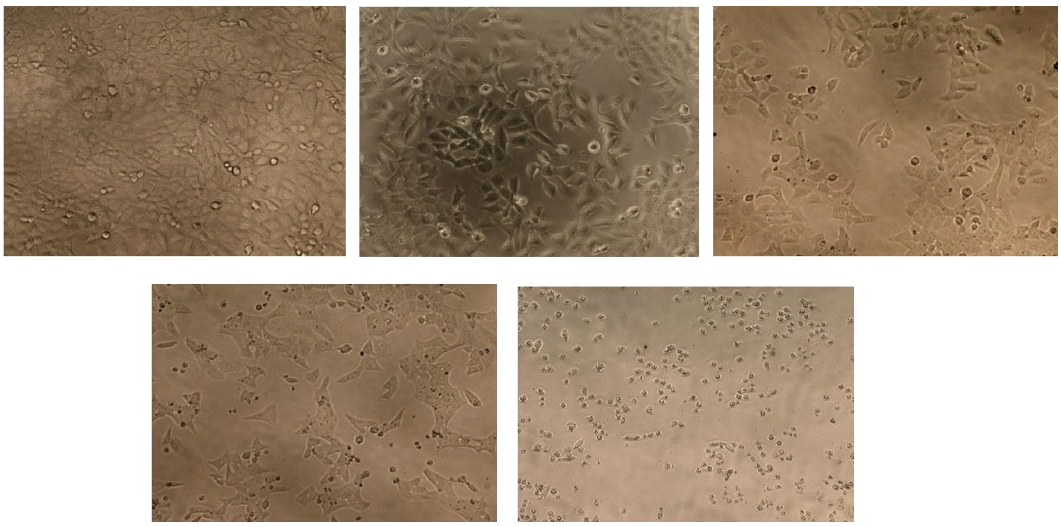

Figure 5. Morphology of T47D cells treated with MFGK-27 fungi crude extracts A. Cell control (untreated); B. 25 μg/ml; C. 50 μg/ml; D. 100 μg/ml; E. 200 μg/ml.

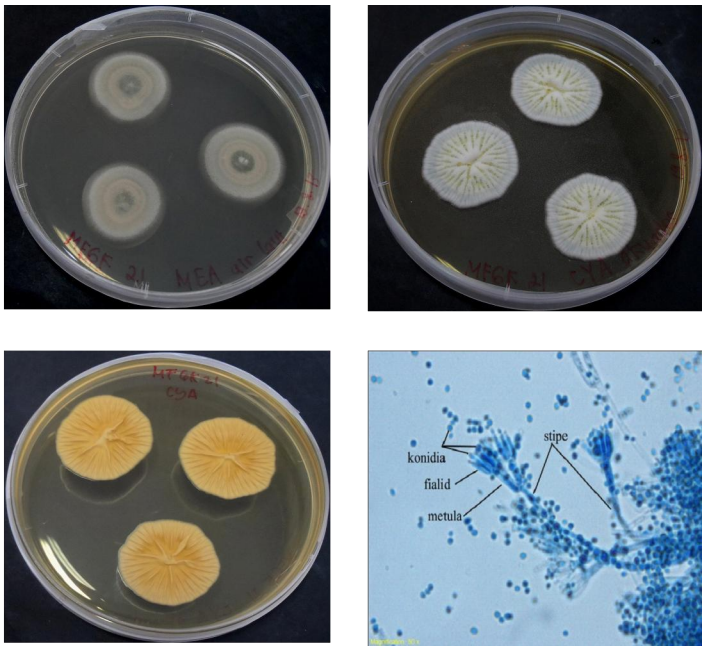

Figure 6. Macro-micro morphology of MFGK-21 fungi at : A. MEA medium; B. CYA medium; C. CYA medium-reverse; D. Conidiophores and conidia at 400 x manification.

4. Conclusion

Among 21 marine fungi species isolated from *Sargassum* sp., *Codium* sp., *Chaetomorpha crassa,* Acantophora sp. GK.04.11 algae and 1 unidentified GK.09b.11 sponge samples from Indrayanti Beach-Jogjakarta, based on MTT assay, MFGK-21 extract had the best anti-servical tumor (HeLa) and MFGK-27 extract had the best anti-breast tumor (T47D) with IC_{50} value of 240.1 μg/ml and 59.6 μg/ml respectively. Based on molecular approach (ITS1-5.8S-ITS4 DNA regions) and macro-micro morphological characteristics, the MFGK-21 fungi isolate was identified as *Penicillium stekii*, while the MFGK-27 fungi isolate was identified as *Aspergillus sydowii.*

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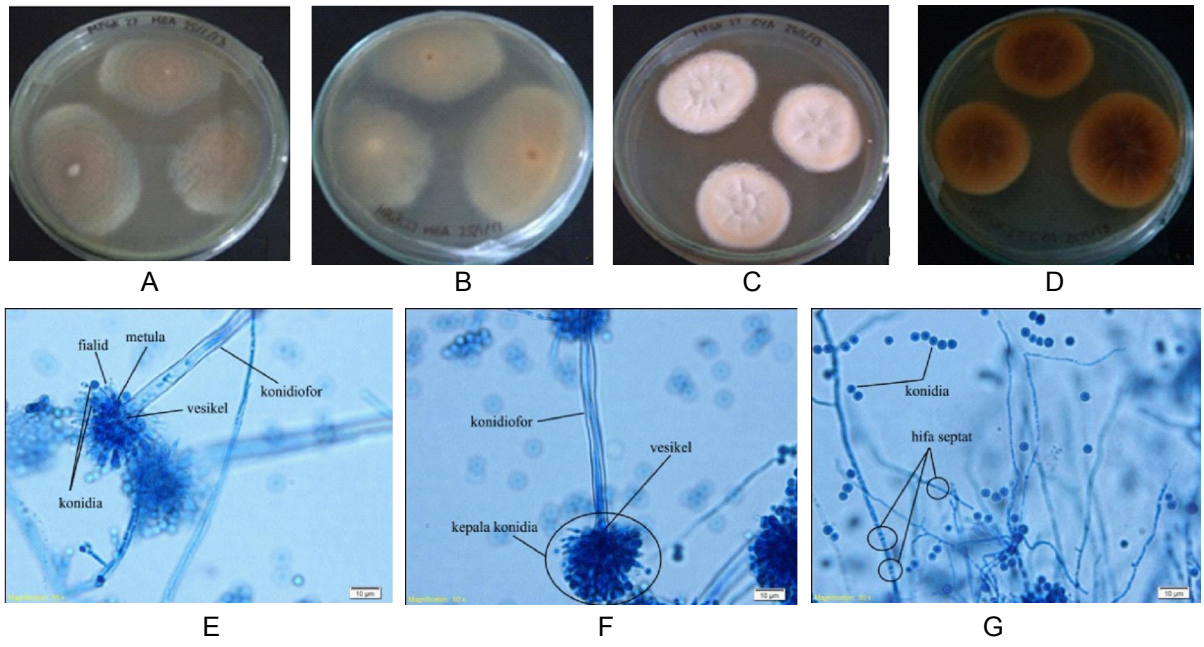

Figure 7. Macro-micro morphology of MFGK-27 fungi at : A. MEA medium (10 days); B. MEA medium-reverse; C. CYA medium-10 days; D. CYA medium-reverse; E-G: conidiophores & conidia at 400 x magnifications.

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