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Antimicrobial and Cytotoxic Activities Screening of Symbiotic Fungi Extract Isolated from Marine Sponge *Xestospongia testudinaria* DD-01

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ABSTRACT

Research of antimicrobial activity and cytotoxic activity from isolates symbiotic fungi from marine sponge *Xestospongia testudinaria* has be done. Xestopongia testudinaria collected from Mande, South Pesisir, West Sumatera. Isolation symbiotic fungi using sabouraud dextrose agar (SDA) as growth medium. Seven isolates symbiotic fungi strains were obtained from this sponge *Xestospongia testudinaria*. Symbiotic fungi isolate was cultivated using rice medium for 40 day. The cultivation yield was extracted using an ethyl acetate, from the extraction result obtained the weight ethyl acetate extract which varied that 320-756 mg.The ethyl acetate extracts were analyzed for antimicrobial and cytotoxic activities by using diffusion agar method and BSLT. The research revealed 71.42% of the total extract had antimicrobial activity against pathogenic bacteria and fungal such as, *Staphylococcus aureus*, *Pseudmonas aeruginosa* and *Candida albicans* and 71% were cytotoxic activity with *Artemia salina* L. This selected fungal isolates were first macroscopically and microscopically characterized and later molecularly identified as *Penicillium citrinum*. This research concluded that the sponge *Xestospongia testudinaria* symbiotic fungi can be developed as a new source of antibiotic and anticancer compounds.

KEY WORDS: Marine sponge, symbiotic fungi, *Xestospongia testudinaria*, antimicrobial activity, cytotoxic activity.

1. INTRODUCTION

Indonesia country is the largest archipelagic country in the world with two-thirds of its territory being an ocean that is part of the Indonesian waters. The Indopasific region, which is partly center in Eastern Indonesia and the Philippines, the center of the world's largest marine biodiversity. These marine biota resources are potential assets that can be used into various products, ranging from food products to medicinal products (Van Soest, 2008). Various types of secondary metabolites with various bioactives have been found in these marine biota. Potential marine resources trigger in an effort to find new drugs to deal with various diseases (Lee, 2001).

Sponge is a porous animal that includes filter feeders which are animal have a way of eating by filtering seawater containing food through pores (ostium) (Lee, 2001). *Xestospongia testudinaria* (Desmospongia, phylum porifera) known as sponge barrels are members of a large and common coral reef community at more than 10 meters deep. The sponge *Xestospongia testudinaria* has been recognized as a rich source of chemicals including alkaloids, quinones, terpenoids, sterols and fatty acids (Zhou, 2010). The metabolites contained in sponges are strongly related to metabolites synthesized by symbiotic microorganisms, and among the symbiotic microorganisms, symbionous fungi from sponges are the best producers of bioactive compounds (Bhadury, 2006).

Symbolic fungi have high biological activity. Several studies on microbial symbionts, especially fungi, indicate that symbionous fungi have biological activity as antimicrobial, anticancer, antimalarial, antifungal, anti-inflammatory, antiviral, antiprotozoa, immunosuppressive, cytotoxic and antioxidant (Strobel & Daisy, 2003; Subramani, 2013; Singh, 2014; Handayani & Aminah, 2017). The search for sources of bioactive compounds using symbioticfungi is better, because the short symbion life cycle and the compounds produced can be produced on a large scale. Where symbiotic fungi can produce secondary metabolite such as alkaloids, steroids, terpenoids, flavonoids, quinones and phenols (Tan & Zou, 2001).

Sponge *Xestospongia vanilla* founded triterpenoid glycoside compounds (Edrada, 1996), four new bioactive compounds menzamine type alkaloids from sponge *Xestopongia ashmorica (*Edrada, 1996), seven tricyclic angular compounds chrom derivatives of sponge *Xestospongia exigua* which have antibacterial (Lin, 2003), xestodecalacton compounds but the bioactivity is unknown (Eddrada, 2002) and succeeded in isolating estre sterol and xestosterol compounds from sponge *Xestospongia testudinaria* which have anticancer activity (El-Gamal, 2016).

Symbolic fungi extract from marine sponge *Neopetrosia chaliniformis* AR-01 has antibacterial and cytotoxic activity (Handayani & Artasastra, 2017). Ethyl acetate extract of simbiotic fungi from marine sponge *Acanthrongylophora ingens* also has antibacterial and anti cancer activity (Handayani & Aminah, 2017). Cytotoxic activity on marine sponges of *Xestospongia testudinaria* by testing on human cervical cancer cells with EC_{50} 0.67 mm (Quah, 2017). *Actinomycetes* associated with marine sponge *Xestospongia* sp show activity as antimicrobials (Kim, 2006; Montolvo, 2005; Zhang, 2006) and *Aspergillus aculeatus lizuka* symbiotic from marine sponge *Xestospongia testudinaria* have activity as α-glucoside inhibitors (Ingavat, 2009).

2. MATERIAL AND METHODS

Sponge material: Sponge *Xestospongia testudinaria* is taken from the Mandeh island, West Sumatra at a depth 10-15 meters. Sponge rinsed with 70% ethanol for surface sterylation then rinsed with sterile sea water to stop sterilization process, then disinfect it in a sterile plastic container and bring it to the laboratory. The sponge was identified by Dr. Nicole J. De Voogd, Natural Biodiversity Center, Netherlands. A voucher specimen (DD-01) has been preserved at the Marine Reference Collection, Laboratory of Biota Sumatera.

Isolation Symbiotic Fungi From Sponge *Xestospongia testudinaria:* Isolation symbiotic fungi begins with sterilization on the surface sample. Sponge *Xestospongia testudinaria* rinsed with sterile sea water. And 10 grams of sponge is mashed into an erlemeyer and add 100 ml sterile sea water. Dilute to 10^{-6}, and as much as 1 ml of suspension solution sample is poured on Sabouraud dextrose agar (SDA) media, incubate 3-5 days. Colonies that have different shapes and colors with other colonies are considered different isolates.

Cultivation Of Isolate Fungi In Medium Of Rice: Pure isolate symbiotic fungi obtained at the purification stage are then cultured on rice as a medium, and incubated at room temperature for 4-6 weeks until the volume of rice in erlenmeyer is overgrown with the fungi (Kjer, 2010).

Extraction Of Secoundary Metabolites From Fungi Isolate: Cultures of isolates symbiotic fungi after cultivation were then macerated with 100 ml ethyl acetate for 24 hours with 3 times repetition. Extract ethyl acetate symbiotic fungi was separated from culture medium by filtering using filter paper. Ethyl acetate solvent was then evaporated (*in vacuo)* using rotary evaporator until a thick ethyl acetate extract was obtained (Xiaoling, 2010). Ethyl acetate extract was then tested for antimicrobial activity and cytotoxic test.

Scrining Of Antibacterial Activity: Screening of antimicrobial activity from ethyl acetate extract symbiotic fungi was tested against *Staphylococcus aureus*, *Psudomonas aeruginosa* and *Candida albicans* using the paper disk method. One pieces of sterile paper disk 6 mm was soaked in each of Ethyl acetate extract (50 mg/ml in DMSO). Paper disks were also inoculated with DMSO (negative control) and Choramphenicol as positive control for bacterial and nystatn as positive control for fungi (*Candida albicans*). Antagonist activity was detected after incubation for 1 day (24 h) at 30 ^{o}C. The existence of the clear zone in the media was considered as indicator for antibacterial activity. The zone of inhibition was measured and expressed in millimeters. Strain that showed maximum inhibition was selected to phytochemical test.

Scrining Of Cytotoxic Activity: Brine shrimp lethality test with *Artemia salina* eggs were hatched in 500 mL of filtered seawater under constant aeration for 2 day (48 h) at (27±2) ^{o}C. After hatching, active nauplii free from egg shells were collected and used for the assay. Five hundred, fifteen and five microliters of all fungi isolate were added in well plate at 1000 ppm, 100 ppm and 10 ppm concentration in triplicate. Fiftteen microliters were added 50 µl of DMSO and until 5000 µl of seawater containing ten nauplii, while placed in the respective well and maintained at room temperature for 24 h. Filtered seawater was used as negative control. The LC_{50} value was calculated using curva method based on probit analysis (Xiaoling, 2010).

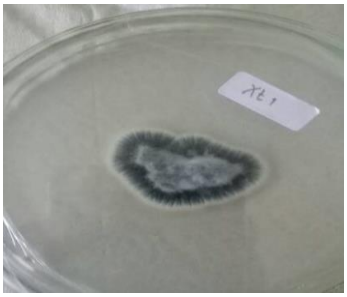
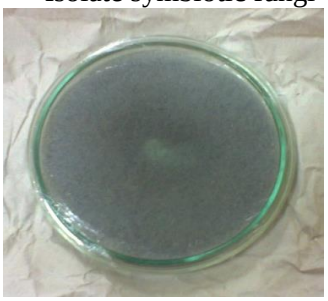
Metabolites Secunder Test: Phytochemical examinations were carried out for all the ethyl acetat extracts of symbiotic fungi as per the standard methods (Meyer, 1982). Phenolic, alkaloid, steroid and terpenoid test were performed to know secondary metabolite constituent by this method.

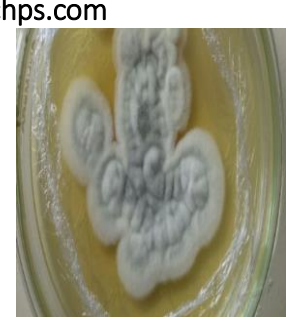
3. RESULT AND DISCUSSION

Identification of sponge was confirmed ad *Xestospongia testudinaria* based on spicules morphology. In taxonomy, this sponge including Demospongiae class, part of family Petrosiidae. In this stufy, 7 symbiotic fungi were iolated from marine sponge *Xestospongia testudinaria*.

Table.1. isolate symbiotic fungi from sponge *Xestospongia testudinaria*

Isolate symbiotic fungi


Antimicrobial activity test result are listed in Table II in this study, 7 fungi extract showed inhibitory activity against pathogenic microbial test with diameter of inhibition zone more that 10 mm. The highest antimicrobial activity result shown by extract of Xt 6 against bacterial pathogen of *Staphylococcus aureus*, *Psudomonas aeruginosa* and *Candida albicans* (inhibition zone of 15 mm, 26.5 mm and 18 mm).

The results of screening of antimicrobial activity in the seven extract ethyl acetate isolates symbiotic fungi extract showed that 71.42% had antimicrobial activity against pathogenic microbes of *Staphylococcus aureus*, *Psudomonas aeruginosa and Candida albicans*.

Table.2. Antimicrobial activity of symbiotic fungi extract with pathogenic microbial

Fungi Extract	Zone of Inhibition (mm)		
	Gram positive	Gram negative	Fungi
	S. aureus	*p. aeruginosa*	*C. albicans*
Xt1	8	8	7.5
Xt2	16	13.5	9
Xt3	7	11.5	8.5
Xt4	9.5	21.5	8
Xt5	7.5	9.5	8
Xt6	15	26.5	18
Xt7	8	16.5	9.5
Chloramphenicol 0.03%	36	15	-
Nystatin 2%	-	-	0

Based on the result cytotoxic activity test screening, five of seven extract ethyl acetate isolate symbiotic fungi from sponge *Xestospongia testudinaria* showed cytotoxic activity. The cytotoxic isolate were Xt2, Xt4, Xt5, Xt6, Xt7. The most cytotoxic activity of total isolates was Xt6 with LC_{50} of 100 ppm.

Table.3. LC_{50} value of symbiotic fungi extract

No	Fungi extract	LC_{50}(ppm)
1	Xt1	1552.96
2	Xt2	912.01
3	Xt3	1256.02
4	Xt4	741.31
5	Xt5	489.77
6	Xt5	100
7	Xt7	654.63

Analysis of the chemical reaction of ethyl acetate extraxt of symbiotic fungi isolates from *Xestospongia testudinaria* was conducted to determine the constituens of secondary metabolites. In this study, phenolic, alkaloid, terpenoid and steroid were tested using appropriate reagents. Based on the result of phytochemical examination, must fungi extract contain alkaloid, phenolic. Based on previous studies most drug compounds, especially those isolated from fungi, have semi-polar to non-polar properties, but those that are non-polar are very rarely found (Gritter, 1991).

Table.4. Phytochemical constituent of ethy acetate extract of symbiotic fungi

Fungi extract	Chemical constituent			
	Phenolic	**Alkaloid**	**Steroid**	**Terpenoid**
Xt1	-	-	-	+
Xt2	+	+	-	-
Xt3	-	-	-	+
Xt4	-	+	-	-
Xt5	+	+	-	-
Xt6	+	+	-	-
Xt7	-	+	-	-

"+" indicates positive reaction, "-" indicates negative reaction

The result of screening of antimicrobal and cytotoxic activity showed that isolate Xt6 is the most active as an antimicrobial agent. Based on identification macroscopically, fungal colonies are blue grey green to dark green, colony diameter 7 dayd 27-33 mm, difusible pigments yellow. Conidiophores arising from mycelium mat, predominant symmetrically biverticillate, terverticillate structures abundantly produced in fresh isolates; stipes smooth. From the macroscopic and microscopic observation, Xt6 was identified as *Penicillium citrinum*.

The mechanism of action of alkaloids as antibacterial is related to the high quarteneric aromatic compounds of alkaloids such as *barberine* and *harmane* which have a contribution to form *inter-khelate* with DNA, whereas phenol compounds can cause protein denaturation through an adsorption process involving hydrogen bonds. At low levels, a protein-phenol complex is formed with a weak bond and immediately decomposes, followed by penetration of phenol into the cell and causes precipitation and protein *denaturation*. At high levels, phenols cause coagulation of proteins and membrane cells will undergo *lysis* and alter the permeability membrane cell of bacterial (Soekardjo & Siswandono, 2000).

In the cell cycle process, alkaloids will bind to *tubulin* in the form of microtubules. The binding of *tubulin* to the alkaloids results in protein polymerization into microtubules which will be inhibited and cause mitotic spindle formation to be inhibited and the cell cycle will stop at metaphase. Because there is no proliferasion of cell, the cell will experience apoptosis or cell death (Wickremasinghe, 1999). Phenolic compounds can induce apoptosis due to DNA fragmentation by reactive oxygen compounds such as hydroxyl radicals. The induction of apoptosis passes through the p53 pathway. DNA damage can be caused by phenolic interactions with the DNA topoisomerase enzyme (Wickremasinghe & Hoffbrand, 1999).

4. CONCLUSION

Ethyl acetate extract symbiotic fungi from sponge *Xestospongia testudinari* had antimicrobial activity with a inhibition zone 26.5 mm and cytotoxic activity with a LC50 100 ppm. Best activity simbiotic fungi with code Xt6 and secondary metabolites is alkaloids and phenolics

5. ACKNOWLEDGEMENT

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