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introduction

Sponges are currently interesting for research because they contain microorganisms that have not been identified from the marine environment and have the potential as new chemicals (Mahfur *et al* . 2022). Sponges contain compounds such as alkaloids, phenols, terpenoids, flavonoids, porphyrins, cyclic aliphatic peroxides, and sterols. The compounds contained in the sponge have biological activities such as antiviral, antifungal, antibacterial, antimalarial, anti-inflammatory, and *neuro-suppressive.* (Apriyandi *et al* . 2019) .

The use of sponges as an active substance is constrained in a limited amount if used continuously it can disrupt the ecosystem of the sponge. This can be prevented by isolating sea sponges in the form of symbiont fungi. Fungi that have symbionts with sponges can produce the same compounds as sponges because genetic transfer occurs from the sponge to the fungal body. Fungal symbiosis with sponges can occur in the sponge body cells' cytoplasm (Rau *et al* . 2018).

Research conducted by Setyowati *et al*. (2018) Sponge symbiont fungus of the *Stylissa* species sponge *flabelliformis*, namely the fungi species *Aspergillus fumigatus, Aspergillus sp, Trichoderma reesei, Aspergillus flavus, Aspergillus nomius*, and *penicillium sp* have antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria. Similar research by Handayani *et al*. (2017) The sponge symbiont fungus *Neopetrosia chaliniformis* has antibacterial activity due to the bioactive steroid and terpenoid compounds it contains.

Almaqtri, (2019) stated that *Aspergillus* fungus is a source of secondary metabolites because it contains compounds (3-Methylbutyl)-butyrolactone, stigmasta-5,7,22-trien-3-β-ol, stigmast-4-ene-3- one, [4-(2-methoxyphenyl)-1-piperazinyl(1-methyl-1H-indole-3-yl)-methanone, α-amylases these compounds have antibacterial, anticancer, antioxidant, antifungal activity.

This study aims to determine the type of fungus that has symbiont with the sponge *Rhabdastrella sp*, the compounds contained in the sponge symbiont fungus, and to see the antibacterial activity and the MIC value of the sponge symbiont fungus *Rhabdastrella sp*.

Materials and Methodology

Sampling and Cultivation of Sponge Symbion Fungus

Sponge samples were obtained from Gili Layar Island, Sekotong, West Lombok, and West Nusa Tenggara at a depth of ± 12 meters below sea level using scuba diving. Sponges were identified at the Gajah Mada University Laboratory, Yogyakarta. Sponges of *Rhabdastrella sp* were sterilized with 70% v/v ethanol for 30 seconds, followed by three subsequent items of washing with sterile seawater, the sponges were cut into small pieces and cultivated on Sabouraud Dextrose Agar Saline with the addition of 1 mL ciprofloxacin, put into the incubator at a temperature of 25˚C for 14 days (Setyowati *et al*. 2018).

Purification and Identification of Sponge Symbion Fungus

Purification is done by taking the dominant fungal colonies growing on the media. The purification process was carried out based on the morphological appearance of each different colony (Hidayat *et al*. 2021). This process is carried out by taking one more dominant isolate in growing on the media and planting it in new media until a pure isolate is obtained (Setyowati *et al*. 2017).

Identification was carried out by observing the sponge symbiont fungi macroscopically, microscopically, and molecularly based on partial locus genetic analysis Internal Transcribed Spacer (ITS). Molecular identification begins with DNA extraction from a 7-day-old fungus with Zymo Research Extractor. DNA amplification was carried out using the polymerase chain reaction (PCR) thermal cycler method with ITS 1 and ITS4 as DNA regions (Setyowati *et al*. 2018).

PCR product quality was seen using electrophoresis on 1% agarose. PCR visualization results were analyzed using PT 1st Base Laboratories Sdn Bhd, Malaysia. Jakarta Genetics for sequencing. DNA sequences were analyzed for homology using the Basic Local Alignment Search Tool BLAST) (www.ncbi.nlm.nih.gov). Phylogenetic trees were analyzed using MEGA 7.0 software, while statistical analysis used the Neighbor-Joining method with 1000 bootstrap replication (Larasati *et al*. 2021).

Fermentation and Extraction of Fungal Secondary Metabolites

Fermentation was carried out by inoculating pure fungal on an SDB medium and incubating at 25°C for 10 days. The fermented sponge symbiont fungus was separated between the supernatant and mycelia. The supernatant obtained was 90 mL and extracted with a 1:1 ratio of ethyl acetate. The ethyl acetate extract obtained was concentrated with a rotary evaporator at 60˚C (Setyowati *et al*. 2017).

Identification of Metabolite Compound Profiles

Identification of secondary metabolites using the TLC test with the mobile phase of chloroform: methanol 9:1 (Ambarwati *et al*. 2015). The results of the TLC analysis were seen in visible light, and UV light 254 and 366 nm. Then

it is sprayed with a reagent to determine whether there are groups of secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenols contained in the extract (Raihan *et al*. 2020). Identification of compounds by GC-MS (Shimadzu GCMS-QP2010SE), by injection of polar, semi-polar, and non-polar fractions in GC-MS with GC-MSQP-2010 Ultra running conditions was carried out at a column oven temperature of 50˚C and an injection temperature of 250˚C The carrier gas used is helium with a pressure of 100 kPa and a flow rate of 89.3 mL/minute (Kanjana *et al*. 2019).

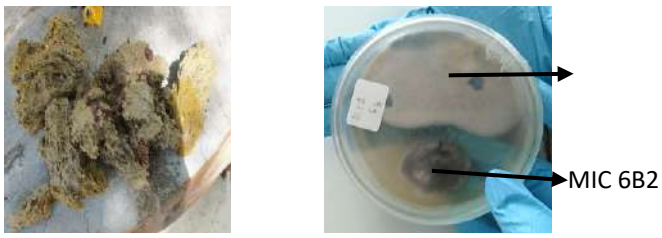
Antibacterial Activity Test

The antibacterial method used well diffusion with three bacteria *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, and *Streptococcus pyogenes*. Performed with extract concentrations of 50,000 ppm, 25,000 ppm, 12,500 ppm, and 6,250 ppm). positive control (ciprofloxacin) and negative control (ethyl acetate). Incubate the petri dish at 37˚C for 1x24 hours. After incubation, the clear zone was measured from each test and analyzed with SPSS 22 (Marpaung *et al*. 2019).

Result and Discussion

Sponge Symbion Fungus Cultivation

The isolate results showed the presence of fungal growth. *Rhabdastrella sp* sponges obtained 2 fungus isolates white (MIC 6B1) and green (MIC 6B2) characteristics because sea sponges are filter feeders so they are used as symbionts for marine microorganisms, one of which is a fungus. The symbiotic relationship between fungi and sponges is a symbiotic mutualism in which genetic transfer occurs from the body of the sponge to the body of the fungus (Gaino *et al*. 2014).



(a) (b)

Figure 1: (a) Spons *Rhabdastrella sp*, (b) Sponge symbiont fungus *Rhabdastrella sp*

Purification and Identification of Sponge Symbion Fungus

The purification process aims to obtain pure MIC 6B1 isolate based on its morphology (Setyowati *et al*. 2017). The sponge symbiont fungus used for purification is a fungus that is 14 days old because the 14th day is in the logarithmic/exponential phase range, where fungal cell division occurs very quickly and constantly (Rendowaty *et al*. 2017). Purification was carried out on the dominant fungus growing, namely MIC6B1 with white mycelium. Purification results obtained green fungus with round colonies.

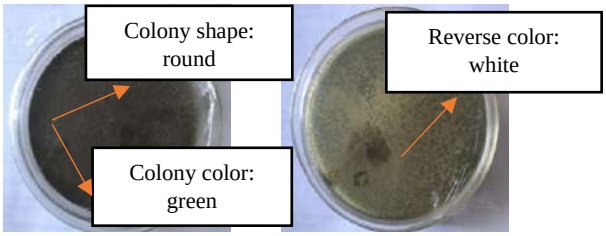


Figure 2: Results of MIC 6B1 purification

The results of macroscopic identification of the colonies were round, green in color with white reverse colony color, and textured like cotton. Previous research conducted by Manuel *et al*. (2021) *Aspergillus sp* isolates showed the growth of white cotton colonies which turned green over time.

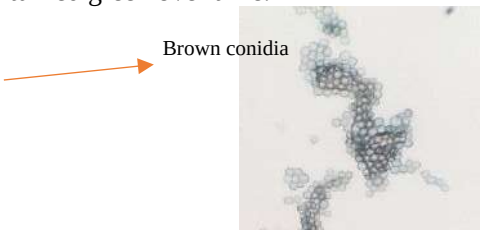


Figure 3: Results of microscopic identification

The results of microscopic identification showed the presence of round and pale brown conidia. According to research by Ayob *et al*. (2018), The fungus *Aspergillus sp* has pale brown radiating conidia consisting of catenate conidia.
The results of molecular identification electrophoretic analysis showed that the fungal ITS region sequence was successfully amplified with ITS1-ITS4 primer pairs. The visualization results are shown in Figure 3 which shows the electrophoretic band of MIC 6B1 fungus DNA parallels to the marker band. The results of amplification of the ITS region sequence with ITS1-ITS4 primer pairs on MIC 6B1 isolates between 500-600 bp. According to Alshehri *et al*. (2020), The ITS region of the phylum *Ascomycetes* is 500-600 bp long.

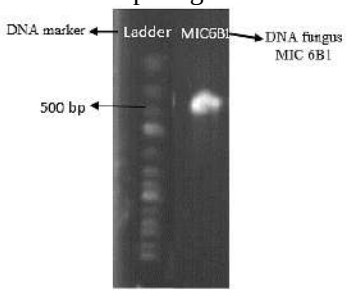


Figure 4: Visualization of PCR results

Sequencing with BLAST analysis on NCBI aims to determine the level of similarity of isolates compared to isolates in GenBank data. The samples were analyzed based on the similarity of the composition of the nucleotide acids with a certain base length. The results of the combined sequences obtained a sequence length of 1061 bp. The sequenced ITS results were sent to 1st Base Laboratories Sdn Bhd, Malaysia via PT. Genetics Science Jakarta to do sequencing to find out the homology using the Basic Local Alignment Search (www.ncbi.nlm.nih.gov).
The results of the analysis based on the ITS area showed that the isolate MIC 6B1 had a 74% similarity with *Aspergillus calidoustus*. The max identity value indicates the percentage similarity of the sample nucleotide sequence to the species sequence in the BLAST database. A max identity value above 97% indicates that the isolate has a high degree of homology and is the same species. Meanwhile, the max identity value >97% indicates that the isolates belong to the same genus. The max identity value of MIC 6B1 isolate was 93.80%, it was stated that MIC 6B1 is a new species from the same genus as *Aspergillus calidoutus*, namely *Aspergillus sp*.
DNA sequences from BLAST analysis were reconstructed by phylogenetic trees using neighbor-joining and bootstrap methods and analyzed using software 7.0.

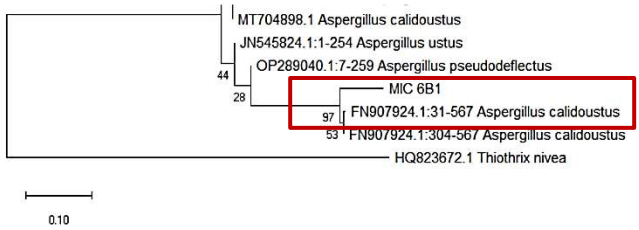


Figure 5: Phylogenetic tree of MIC 6B1 sample with Neighbor-Joining ree method, 1000 bootstrap, and Kimura-2 parameter model

The results of the phylogenetic tree analysis showed that the MIC 6B1 isolate has a close distance between MIC 6B1 and the fungus of the genus *Aspergillus calidoustus* FN907924. 1:31-567 with a bootstrap value of 97%.
This indicates that MIC 6B1 isolate is only 97% related to *Aspergillus calidoustus*. The max identity value of MIC 6B1 isolate was 93.80%, it was stated that MIC 6B1 is a new species from the same genus as *Aspergillus calidoutus*, namely *Aspergillus sp*.

Identification of Metabolite Compound Profiles

TLC profile results with chloroform: methanol 9:1 mobile phase at 254 and 366 nm UV light (Ambarwati *et al* . 2015). Then it is sprayed with reagents to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenols (Raihan *et al*. 2020). The results of the KLT profile can be seen in Figure 6.

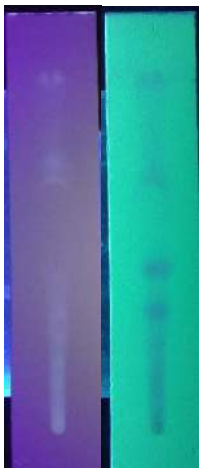


Figure 6: Profile of secondary metabolites

The results obtained on the identification of the ethyl acetate extract of the fungus *Aspergillus sp.* qualitatively can be seen in Table 1.

Table 1: Results of identification of compounds with TLC

Compound Class	Information
Alkaloids	Positive
Flavonoids	Positive
Terpenoids	Positive
Phenolic	Positive

Based on the results in the table above, shows that the ethyl acetate extract of the *Aspergillus sp* fungus contains flavonoids, terpenoids, phenolics, and alkaloids. Research by Tanod *et al*. (2020), stated that the fungus *Aspergillus sp* sponge symbiont produces alkaloid, terpenoid, and phenolic compounds which produce antibacterial activity. Identified the active compound of the ethyl acetate extract quantitatively using the GC-MS instrument to determine the specific compound content in the ethyl acetate extract of the fungus *Aspergillus sp*. The chromatogram results of the ethyl acetate extract of the fungus *Aspergillus sp* can be seen in Figure 7.

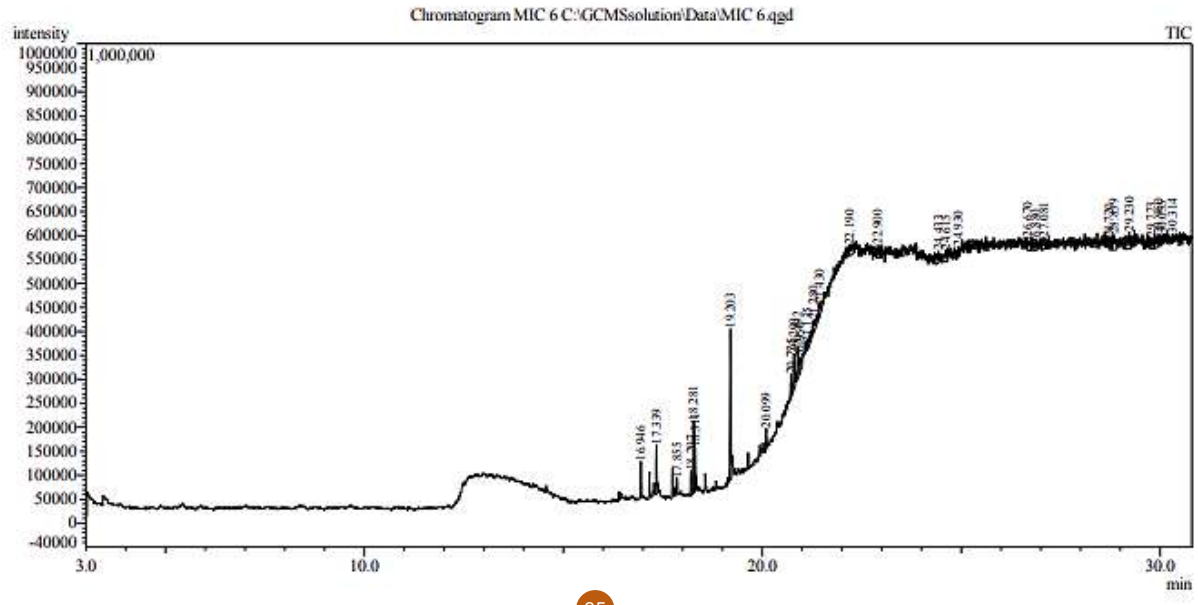


Figure 7: GC-MS chromatogram of *Aspergillus sp. ethyl acetate extract*

Table 2: Result of compound identification with GC-MS

GC-MS results	
% Area	Compound
13.50	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-
5.05	silicone grease
5.03	2,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane
4.02	1,4-diaza-2,5-dioxobicyclo[4.3.0]nonane
4.01	3-benzyl-6-isobutyl-2,5-dioxo-piperazi
3.93	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate
3.92	1,1,3,3,5,5,7,7,9,9-Decamethyl-Pentasiloxane
3.84	2,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane
3.61	Cyclobuta[1,2:3,4]dicyclooctene, hexadecahydro-, (6a.alpha.,6b.alpha.,12a.alpha.,12b.alpha.)- (CAS) Tricyclo[8.6.0.0(2,9)]Hexadecan
3,24	silicone grease

Antibacterial activity test

Antibacterial activity testing was carried out to determine the potential of the ethyl acetate extract of the fungus *Aspergillus sp* in inhibiting bacterial growth. The test was carried out on the gram-negative bacteria *Escherichia coli* ATCC25922, and the gram-positive bacteria *Staphylococcus aureus* ATCC25923 and *Streptococcus pyogenes*, so that the spectrum could be determined. The results of the antibacterial activity test of the good diffusion method can be seen in Figure 8.

The ethyl acetate extract of the fungus *Aspergillus sp* can inhibit the growth of bacteria which is characterized by the formation of clear zones around the wells. The diameter of the inhibition zone was measured after 24 hours of incubation.

Table 3: Results of measuring the diameter of the inhibition zone

No.	Bacteria	Extract concentration (ppm)	$\bar{x}$ inhibition zone(mm)
1	*Escherichia coli*	50,000	16.77 ± 1.73
		25,000	12.99 ± 3.35
		12,500	7.81 ± 3.36
		6,250	6.94 ± 1.27
2	*Staphylococcus aureus*	50,000	7.00 ± 0.41
		25,000	5.90 ± 0.55
		12,500	5.50 ± 1.07
		6,250	4.50±0.88
3	*Streptococcus pyogenes*	50,000	6.41 ± 0.84
		25,000	5.42 ± 0.76
		12,500	4.48 ± 0.67
		6,250	4.41 ± 0.78

Based on the results of testing the antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes* in Table 3, the antibacterial activity of *Aspergillus sp* fungus extract against *Escherichia coli* at a concentration of 6,250 ppm has an inhibition zone diameter of 6.94 mm. Whereas *Staphylococcus aureus* and *Streptococcus pyogenes* have an average diameter of the inhibition zone at a concentration of 6,250 ppm, namely 4.50 mm and 4.41 mm. The test results showed that the inhibition of ethyl acetate extract on *Escherichia coli bacteria* was greatest compared to *Staphylococcus aureus* and *Streptococcus pyogenes.* This can be seen in Figure 9.

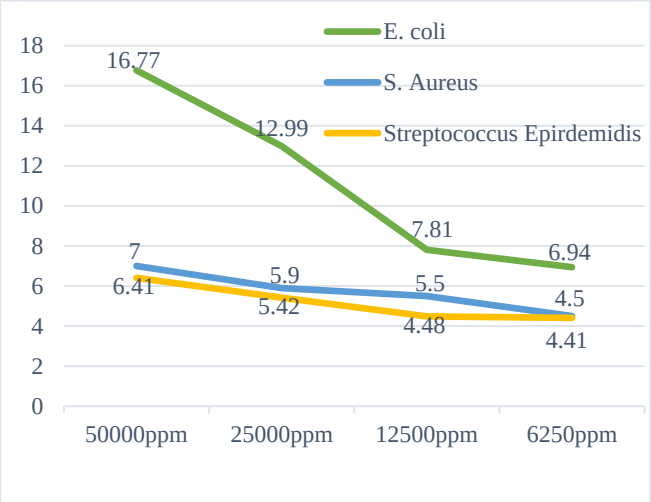


Figure 8: Comparison graph of the diameter of the inhibition zone

The ethyl acetate extract of the fungus *Aspergillus sp* is known to contain organic compounds based on the identification of secondary metabolite profiles. These compounds have chemical components that contain nitrogen atoms in their structure, making it possible that the compounds obtained are classified as weak basic alkaloids that have antibacterial activity (Verawati *et al*. 2019). The natural alkaloid antibacterial mechanism shows that alkaloids can disrupt bacterial cell membranes, affect DNA function, and inhibit protein synthesis. Thus, natural alkaloids are potentially active against various bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes* (Yan *et al*. 2021). Flavonoids have an antibacterial mechanism, namely by inhibiting nucleic acid synthesis, inhibiting the function of the cytoplasmic membrane, and inhibiting the energy metabolism of bacterial cells (Xie *et al*. 2014). The antibacterial mechanism of terpenoid compounds is by destroying the bacterial cell membrane which will affect the basic properties of bacteria and as an inhibitor of protein synthesis which can achieve an antibacterial effect by blocking every process of the protein synthesis pathway (Huang *et al*. 2022). The antibacterial mechanism of phenolic compounds by modifying the permeability of cell membranes, changes in various intracellular functions induced by the binding of phenolic compounds to enzymes, thus, phenolic compounds can increase their antimicrobial activity by supporting their interactions with cell membranes (Bouarab *et al*. 2019).

Conclusion

The fungus *Aspergillus sp* (MIC 6B1) which is in symbiont with the sponge *Rhabdastrella sp* from Gili Layar Island, Sekotong Lombok Barat produces secondary metabolites in the form of alkaloids, flavonoids, terpenoids, and phenolics as well as organic bioactive compounds which play a role in inhibiting bacterial growth, due to their antibacterial activity. The antibacterial activity of *Aspergillus sp* fungus extract against *Escherichia coli* bacteria has an average diameter of the inhibition zone at a concentration of 6,250 ppm which is 6.94 mm which indicates a larger inhibition zone than *Staphylococcus aureus* and *Streptococcus pyogenes* which have an average inhibition zone diameter of 4.50 mm and 4.41 mm.

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