

ANTIBACTERIAL ACTIVITY AS INHIBITORS PATHOGEN BACTERIAL ON POND SHRIMP OF EXTRACT MARINE BIOTA COLLECTED FROM MASPARI ISLAND, SOUTH SUMATERA, INDONESIA

AKTIVITAS ANTIBAKTERI SEBAGAI INHIBITOR BAKTERI PATOGEN PADA UDANG TAMBAK DARI EKSTRAK BIOTA LAUT YANG DIKUMPULKAN DARI PULAU MASPARI, SELATAN SUMATERA, INDONESIA

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ABSTRACT

Marine biota has variety of bioactive compound that is potential to be an antibacterial for shrimp ponds diseases. Some of marine biota has potential as antibacterial i.e. soft coral *Sarcophyton* sp., Sponge *Aaptos* sp., seaweeds *Sargassum* sp. and *Halimeda* sp. and mangroves *Avicennia* sp. and *Rhizophora* sp. species. This study is purposed to find the most potential marine biota as antibacterial. The research method was as follows; sampling, sample identification, extraction and antibacterial activity test. The results is soft coral and sponge extract *Sarcophyton* sp. has bioactivity against of shrimp bacteria pathogen such as *Vibrio* spp2. bacteria for around 6.3±0.1 mm, while *Aaptos* sp. has bioactivity against *Vibrio* spp1. bacteria for around 7.9±0.1 mm, *Vibrio* spp2. bacteria for around 7.2±0.1 to 7.9±0.1 mm, *Vibrio* spp6. bacteria for around 7.5±0.2 mm, *Escherichia coli* for around 7.2±0.1 mm and *Staphylococcus aureus* for around 15.9±0.2 mm. Seaweed extract activity found only of *Sargassum* sp. which has antibacterial activity against for around 7.1±0.0 mm for *Vibrio* spp6. and mangrove species activity has *Rhizophora* sp. extract which has bioactivity against *Vibrio* spp4. were 7.3±0.1 mm and *E. coli* bacteria were 6.7±0.1 mm. The most potential marine biota as antibacterial is showed on sponge *Aaptos* sp.. with an inhibitory zone for around 15.9±0.2 mm (for *S. aureus* bacteria).

Keywords: bioactive compound, mangrove, Maspari island, seaweeds, soft corals, sponge

ABSTRAK

Biota laut memiliki beragam senyawa bioaktif yang berpotensi untuk dijadikan antibakteri pada penyakit udang tambak. Beberapa biota yang memiliki potensi antibakteri yaitu spesies karang lunak *Sarcophyton* sp., sponge *Aaptos* sp., rumput laut *Sargassum* sp. dan *Halimeda* sp. dan mangrove *Avicennia* sp. dan *Rhizophora* sp. Penelitian ini bertujuan untuk menemukan biota laut yang paling berpotensi sebagai antibakteri. Metode yang dilakukan meliputi pengambilan sampel, identifikasi sampel, ekstraksi dan uji aktifitas antibakteri. Hasil yang ditemukan adalah bahwa ekstrak karang lunak *Sarcophyton* sp. memiliki bioaktifitas terhadap bakteri penyakit udang *Vibrio* spp2. berkisar 6,3±0, mm, sedangkan *Aaptos* sp. memiliki bioaktifitas terhadap bakteri *Vibrio* spp1. berkisar 7.9±0,1 mm, *Vibrio* spp2. berkisar 7,2± 0,1 to 7,9 ±0,1 mm, *Vibrio* spp6. berkisar 7,5±0,2 mm, *E. coli* berkisar 7,2±0,1 mm dan *S. aureus* berkisar 15,9±0,2 mm. Aktivitas antibakteri ekstrak rumput laut ditemukan hanya pada *Sargassum* sp. yang memiliki aktifitas antibakteri sekitar 7,1±0,0 mm terhadap bakteri *Vibrio* spp6. dan ekstrak mangrove *Rhizopora* sp. memiliki bioaktifitas terhadap bakteri *Vibrio* spp4. sekitar 7,3±0,1 mm dan *E. coli* sekitar 6,7±0,1 mm. Biota laut yang paling berpotensi sebagai antibakteri adalah sponge *Aaptos* sp. dengan zona hambat berkisar 15,9±0,2 mm (untuk bakteri *S. aureus*).

Kata kunci: karang lunak, mangrove, pulau Maspari, rumput laut, senyawa bioaktif, sponge

I. INTRODUCTION

Coastal oceans have different types of biota to producing bioactive compounds to defend themselves from predatory attacks (Mayer *et al.*, 2003; Sivaramakrishnan *et al.*, 2017; McClintock *et al.*, 2010). Some biota can produce bioactive compounds for antibacterial such as soft corals (Afifi *et al.*, 2016; Putra *et al.*, 2017; Rozirwan *et al.*, 2015), sponge (Mohamad *et al.*, 2009; Graça *et al.*, 2015), seaweed (El-Shouny *et al.*, 2017; Águila-Ramírez *et al.*, 2017; Rajkumar *et al.*, 2018) and mangrove (Usman, 2018; Behbahani *et al.*, 2018).

Soft coral and sponge groups such as from the genus Sarcophyton and Aaptos have potential as antibacterial. This Sarcophyton has shown resistance to pathogenic bacteria such as *S. aureus*, *Bacillus subtilis*, *E. coli*, *Vibrio cholera*, *Staphylococcus epidermidis*, *S. Staphylococcus pneumonia*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter* sp. (Al-Footy *et al.*, 2015; Gomaa *et al.*, 2016). While the genus of Aaptos has resistance with bacteria *Pseudomonas fluorescens*, *Klebsiella oxytoca*, *Serratia marcescens*, *Salmonella enterica*, *Bacillus cereus* and *S. aureus* (Beesoo *et al.*, 2017).

Seaweeds from the Genus Halimeda and Sargassum have also been known to have potential as an antibacterial. The species of *Halimeda* sp. have shown resistance to *E. coli*, *Shigella dysenteriae*, *B. subtilis*, *Aeromonas hydrophyla*, *S. aureus* and *S. pneumoniae* (Sivaramakrishnan *et al.*, 2017; Basir *et al.*, 2017). While *Sargassum* sp. has resistance against *Propionibacterium acnes*, *S. epidermidis*, *Pseudomonas aeruginosa*, *E. coli* and *S. aureus* (Kim *et al.*, 2016; Huang *et al.*, 2018).

Mangrove is a coastal plant that has various types and has the potential for antibacterial. Several types of mangroves that have been known to produce bioactive compounds for antibacterial are *Rhizophora* sp. and *Avicennia* sp. Species *Rhizophora* sp.

is known to produce antibacterial compounds for fish and human pathogenic bacteria such as *Proteus* sp., *P. florescence*, *Flavobacterium* sp., *K. oxytoca*, *V. parahaemolyticus*, *P. aeruginosa*, *B. subtilis*, *K. pneumonia*, *S. aureus* and *E. coli* (Umashankari *et al.*, 2012; Arunprabu *et al.*, 2016; Powar and Gaikwad, 2013; Joel and Bhimba, 2010). While *Avicennia* sp are known to have resistance to bacteria such as *Enterococcus faecium*, *S. pneumoniae*, *K. pneumoniae*, *S. aureus*, *Vibrio harveyi*, *Shigella flexneri* and *S. aureus* (Behbahani *et al.*, 2018; Thatoi *et al.*, 2016).

In addition, antibacterials from the marine biota above are reported to be also used to treat some diseases in pond shrimp caused by pathogenic bacteria. Common shrimp diseases such as *Vannamei* sp. are White Spot Syndrome (BWSS), Taura Syndrome Virus (TSV), Fouling Disease (FD), Black Gill Disease (BGD), and Infectious Hypodermal Hematopoietic Necrosis Virus (IHHNV). Some cases prove that this disease cannot be handled effectively (Andriyanto *et al.*, 2014).

Some studies on marine biota that makes antibacterial to shrimp bacteria such as seaweed *Ulva* sp. and marine sponge *Dendrilla* sp. as antibacterial to bacteria from shrimp (*V. fischeri*, *E. coli*, *P. aeruginosa*, *A. hydrophila* and *M. luteus*) showed that *Ulva* sp. can be used as prophylactic drugs and *Dendrilla* sp. can be used as a therapeutic agent (Yatip *et al.*, 2018). *Acanthus ilicifolius* is also used as an antibacterial for shrimp and fish pathogenic bacteria (*A. hydrophila*, *V. harveyi* and *E. coli*) showing that the extract is effective for antibacterial (Rao *et al.*, 2015). Therefore, this study was conducted to obtain and determine the most potential marine biota as antibacterial.

II. MATERIALS AND METHODS

2.1. Sampling Site

The study was conducted on September 2017 to January 2018. Marine

biota samples were taken from Maspari island, South Sumatra, Indonesia (Figure 1). Maspari is a very small island with a size of approximately 3 km² that is not inhabited by humans. This island is located in the southern part of Bangka Strait which belongs to the Ogan Komering Ilir district, South Sumatra. Marine biota on this island are very diverse, where coral is found in many fringing reefs at the depth <5 m with a maximum depth waters about 15 m. For seaweeds is found in shallow waters <2 m by the coast with sandy and partially rocky substrate, while mangrove plants found in the north and south of the island.

2.2. Samples Collection

The marine biota samples collected from Maspari island were one species of soft coral *Sarcophyton* sp. and one sponge *Aaptos* sp. two species of seaweed *Sargassum* sp. and *Halimeda* sp., and two species of mangroves *Avicennia* sp. and *Rhizophora* sp., 250 (g) respectively (Figure 2A-F). Samples are inserted into plastic bags, labeled and stored in a cold box. then carried in the laboratory for macerations.

2.3. Sample Extraction

Marine biota samples were cleansed by dH₂O waters, weighed 250 g and smoothly cut. The sample was macerated using an Ethyl Acetate (EtOAc) solvent at a ratio of 1:4 (w/v) for 48 hours at room temperature, and each was repeated three times. After that, maceration continued with Methanol (MeOH) solvent. The extraction was carried out using rotary evaporation drying at 60°C until obtaining a crude extract.

2.4. Antibacterial Activity Test

Bacteria isolates used for the test were six isolates of *Vibrio* sp. (with code *V. spp1* to *spp6*), isolates were isolated from *Vannamei* sp. shrimp ponds at shrimp farming location in Sungsang, South Sumatra. In addition, the performance is also tested on the bacteria *S. aureus* and *E. coli* using Nutrient Broth (NB) and Nutrient Agar (NA) growing media. Inhibition test is done with agar diffusion (10.000 ppm for extract concentration). The extracts were tested using paper disks and solvents as controls with repeated three times (Rozirwan *et al.*, 2015).



Figure 1. Sampling location of marine biota.

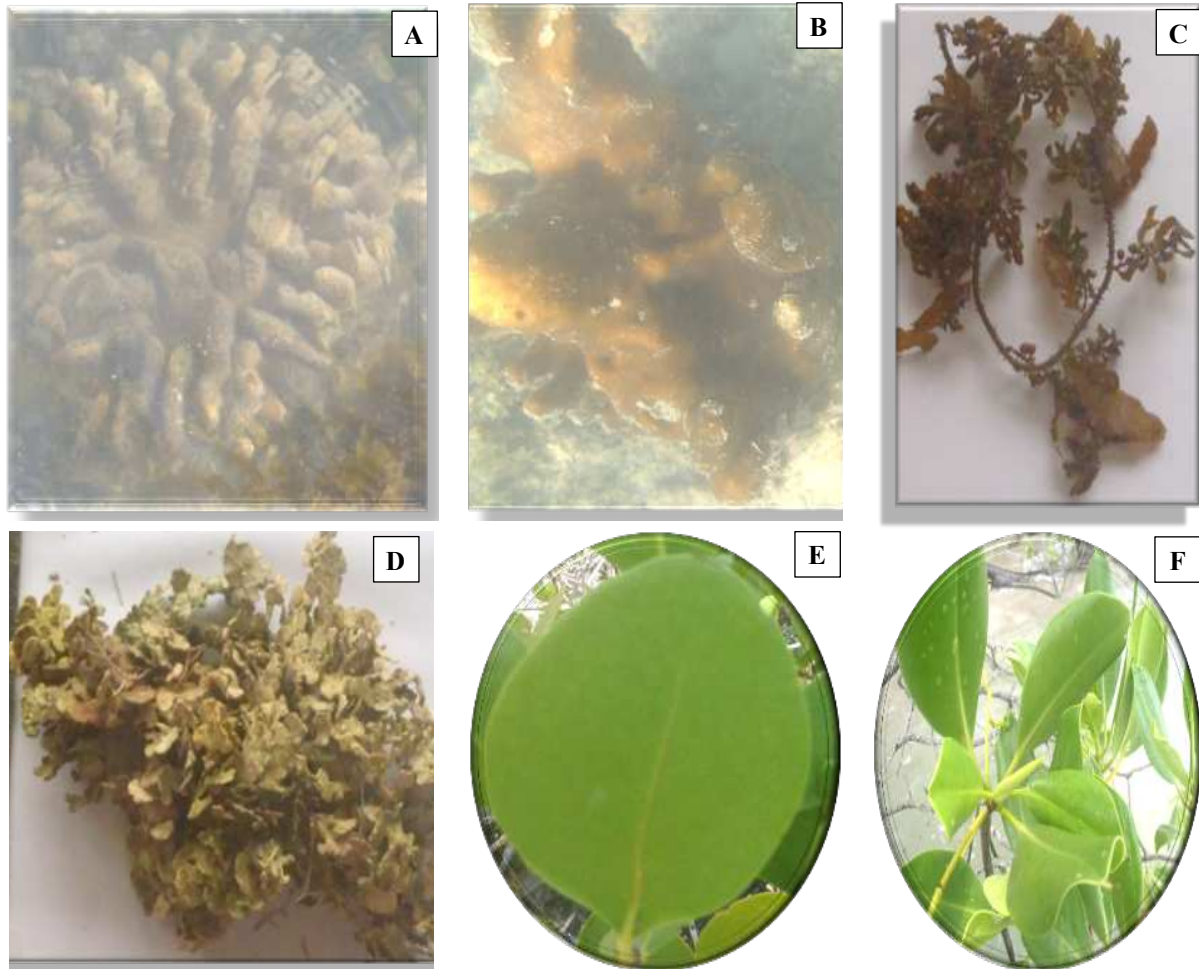


Figure 2. Marine biota samples of Maspari island. A) *Sarcophyton* sp.; B) *Aaptos* sp.; C) *Sargassum* sp.; D) *Halimeda* sp.; E) *Avicennia* sp.; F) *Rhizophora* sp.

III. RESULTS AND DISCUSSION

3.1.1. Extracts Biomass

Six samples of marine biota extracted by biomass were varied in each solvent. The

biomass extract from the methanol solvent was obtained vacant on the mangrove samples (Table 1).

Table 1. The extract biomass of marine biota.

No.	Sample (Weight 250 g)	Samples Code	Extract Biomass (g/1L)		Extract (%)	
			in EtOAc	in MeOH	in EtOAc	in MeOH
1	<i>Sarcophyton</i> sp.	SC	0.34	11.93	0.14	4.77
2	<i>Aaptos</i> sp.	AT	11.95	2.26	4.78	0.90
3	<i>Sargassum</i> sp.	SG	0.80	1.81	0.32	0.72
4	<i>Halimeda</i> sp.	HM	0.61	6.14	0.24	2.46
5	<i>Avicennia</i> sp.	AN	2.95	-	1.18	-
6	<i>Rhizophora</i> sp.	RH	5.75	-	2.30	-

Note: EtOAc = ethyl acetate solvent, MeOH = methanol solvent

Table 1 showed that the extract had high weight in ethyl acetate solvent, excepted species of soft coral *Sarcophyton* sp. and two species of seaweed (*Halimeda* sp. and *Sargassum* sp.). This showed that bioactive compounds had dominated semi polar, particularly of mangrove species (*Avicennia* sp. and *Rhizophora* sp.), and no found extracts of methanol solvent.

3.1.2. Antibacterial Activities

Antibacterial activities had shown species of soft corals (*Sarcophyton* sp.) and sponge (*Aaptos* sp.), seaweed (*Sargassum* sp.) and mangrove (*Rhizophora* sp.). Inhibition zone is shaped by extract in ethyl acetate solvent with 10.000 ppm concentrations (Table 2).

Based on Table 2, the soft coral samples of *Aaptos* sp. (ATE) showed the highest inhibit zone to *S. aureus* with a diameter of about 15.9 ± 0.2 mm. The lowest inhibition zone showed in *Sarcophyton* sp. extract against *Vibrio* spp2 bacteria with diameter of 6.3 ± 0.1 mm. The inhibitory zone

had shown from 5 extracts to 8 isolates was 7.9 ± 0.1 mm (for ATE / *Vibrio* spp2). 7.8 ± 0.1 mm (for ATM / *Vibrio* spp1). 7.5 ± 0.2 mm (for ATE / *Vibrio* spp6). 7.3 ± 0.1 mm (for ATE / *E. coli* and ATM / *Vibrio* sp2). 7.1 ± 0.0 mm (for SGE / *Vibrio* spp6) and 6.7 ± 0.1 mm for RHE / *Vibrio* spp4, 7.2 ± 0.1 mm (for RHE / *E. coli*) (Fig. 1B-G). The most active antibacterial activity was shown by the soft coral extract of *Aaptos* sp., which was able to inhibit all isolates except *Vibrio* sp3.

Marine and coastal biota were marine natural products because they have a wide variety of bioactive compounds, which one of its many potentials has been reported as antibacterial (McClintock *et al.* 2010). This study is purposed to see the potential of bioactive compounds in some marine biota collected from Maspari island such as soft coral (*Sarcophyton* sp.), sponge (*Aaptos* sp.), seaweed (*Sargassum* sp. and *Halimeda* sp.) and mangroves (*Rhizophora* sp. and *Avicennia* sp.) as antibacterial (*Vibrio* spp1 to 6, *E. coli* and *S. aureus*).

Table 2. Inhibition zone of marine biota extracts as against bacterial pathogen isolates of pond shrimp.

Isolate Bacterial	Inhibition Zone of Marine Biota Extract (mm)				
	ATE	ATM	SCE	SGE	RHE
<i>Vibrio</i> spp1	-	7.8 ± 0.1	-	-	-
<i>Vibrio</i> spp2	7.9 ± 0.1	7.2 ± 0.1	6.3 ± 0.1	-	-
<i>Vibrio</i> spp3	-	-	-	-	-
<i>Vibrio</i> spp4	-	-	-	-	7.3 ± 0.1
<i>Vibrio</i> spp5	-	-	-	-	-
<i>Vibrio</i> spp6	7.5 ± 0.2	-	-	7.1 ± 0.0	-
<i>E. coli</i>	7.2 ± 0.1	-	-	-	6.7 ± 0.1
<i>S. aureus</i>	15.9 ± 0.2	-	-	-	-

Note: ATE= extract of *Aaptos* sp. in EtOAc solvent; ATM= extract of *Aaptos* sp. in MeOH solvent; SCE= extract of *Sarcophyton* sp. in EtOAc solvent; SGE= extract of *Sargassum* sp. in EtOAc solvent; RHE= extract of *Rhizophora* sp. in EtOAc solvent; for extracts of *Avicennia* sp. (ANM) and *Halimeda* sp. (HMM) in MeOH solvent showed inactivate.

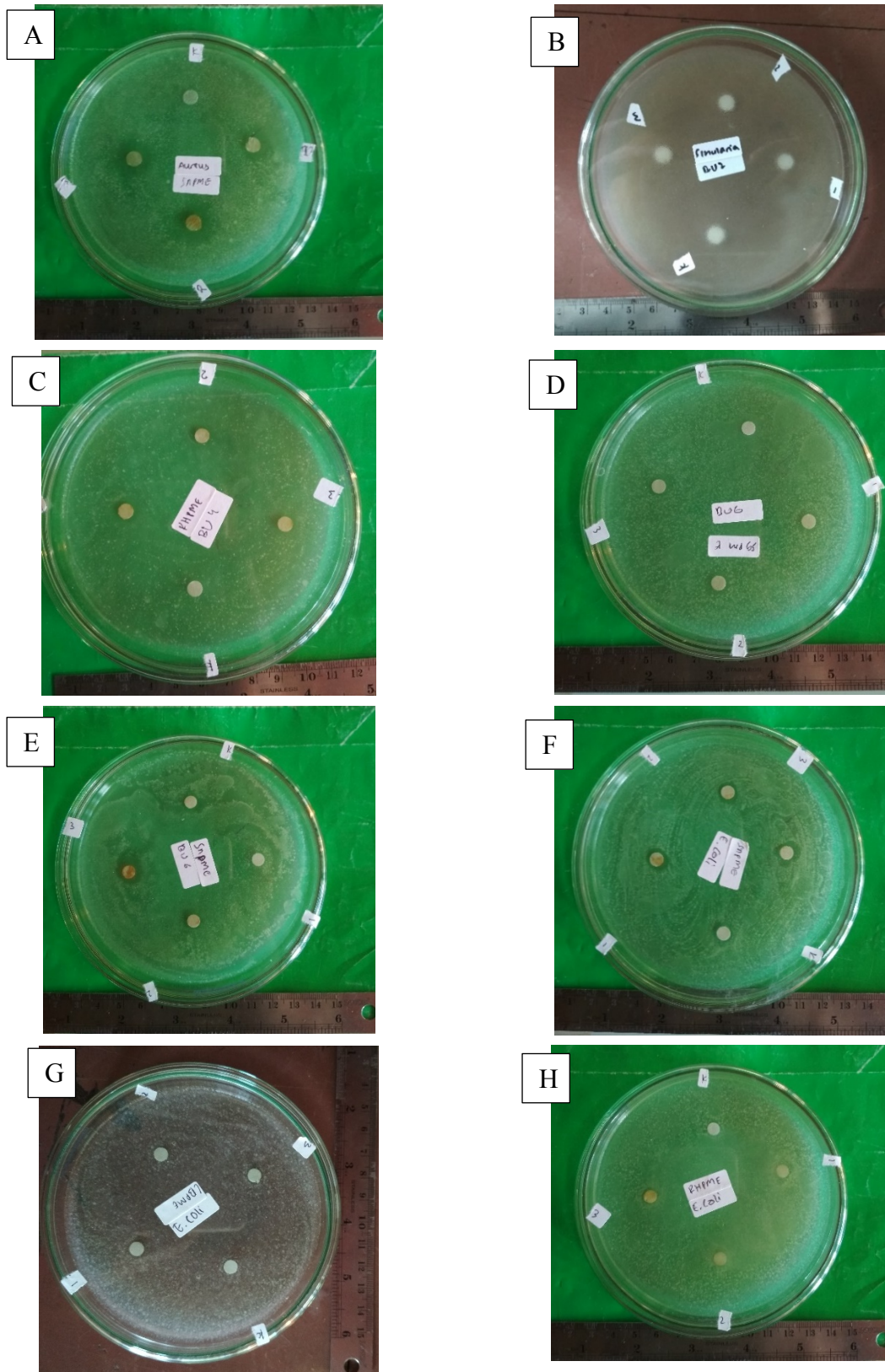


Figure 2. Antibacterial activities of marine biota extracts in EtOAc solvent. A) ATE (*S. aureus*); B) ATE (*Vibrio* sp2); C) ATE (*Vibrio* sp6); D) RHE (*Vibrio* sp4); E) ATE (*E. coli*); F) SGE (*Vibrio* sp6); G) SCE (*Vibrio* sp2); H) RHE (*E. coli*).

Soft coral extracts in *Sarcophyton* sp. and sponge *Aaptos* sp. species have antibacterial activity. In *Sarcophyton* sp. extract had antibacterial activity against *Vibrio* spp2 bacteria, while *Aaptos* sp. extract against *Vibrio* spp1,2,6, *E. coli* and *S. aureus*. The antibacterial activity of the *Aaptos* sp. extract was found to be better than *Sarcophyton* sp. This is probably caused by *Aaptos* sp. that produce Aaptamine alkaloids bioactive compounds (Shubina *et al.*, 2009), while *Sarcophyton* sp. produce terpenoid bioactive compounds (Al-Footy *et al.*, 2015; Cheng *et al.*, 2015). Antibacterial activity found in also *Aaptos* sp. (Beesoo *et al.*, 2017; Murniasih and Bayu, 2016; Rosmiati *et al.*, 2014). *Sarcophyton* sp. is similar, although its antibacterial activity is shown only with one isolate (*Vibrio* spp2) but indicates its antibacterial potential (Gomaa *et al.*, 2016; Afifi *et al.*, 2016; Rozirwan *et al.*, 2015). The extract *Aaptos* sp. in Ethyl Acetate solvent was shown that it has more diverse antibacterial activity, where the extract in Ethyl Acetate solvent was more effective in inhibiting compared to methanol solvents.

Antibacterial activity in seaweed extract is found only in *Sargassum* sp. species, which has formed an inhibitory zone against the *Vibrio* spp6 isolate. Antibacterial activity on *Sargassum* sp. showed better than other species of seaweed (Kim *et al.*, 2016). In mangrove extract *Rhizophora* sp. has antibacterial activity against isolate *Vibrio* spp4 and *E. coli*. The *Rhizophora* sp. species was also more active as a bacterial inhibitor (Arunprabu *et al.*, 2016; Ernawati, 2016; Powar and Gaikwad, 2013; Umashankari *et al.*, 2012).

The potential of bioactive compounds can also be done with the exploration of microbial symbionts. Symbian microbes can be expected to produce bioactive compounds that are almost identical to their inhibitors. Antibacterial activity of microbial symbionts from marine biota has been done in many researches such as marine biota *Sarcophyton*

sp. (ElAhwany *et al.*, 2015), *Aaptos* sp. (He *et al.*, 2017; Santos-Gandelman *et al.*, 2014), *Sargassum* sp. (Susilowati *et al.*, 2015) and *Rhizophora* sp. (Deivanai *et al.*, 2014). For all samples of marine biota extract collected from Maspari Island, it was found that sponge extract of *Aaptos* sp. species showed the best antibacterial activity as inhibitor of shrimp pond bacteria and also pathogen bacteria.

The seaweed *Halimeda* sp. and mangrove *Avicennia* sp. showed no antibacterial activity, whereas the antibacterial activity of these species has been widely reported in *Halimeda* sp. (Basir *et al.*, 2017; Sivaramakrishnan *et al.*, 2017; Indira *et al.*, 2013), and *Avicennia* sp. (Dhayanithi *et al.*, 2012; Joel and Bhimba, 2010; Raju and Sreeramulu, 2017). The loss of antibacterial activity is suspected because the concentration is too small that the inhibitory zone is not formed and may also be due to resistance to other bacteria.

IV. CONCLUSIONS

There were four from six marine biotas collected from Maspari island found for having antibacterial activity for pond shrimp disease i.e. *Sarcophyton* sp. and *Aaptos* sp. species (soft coral and sponge), *Sargassum* sp. (seaweed) and *Rhizophora* sp. (mangrove). The best antibacterial activity was found in sponge extract of *Aaptos* sp. for around 7.9 ± 0.1 mm and 15.9 ± 0.2 mm for *Vibrio* spp2. and *S. aureus* bacteria, where both acted as inhibition of bacteria isolate of pond shrimp and bacterial pathogen.

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