

COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF *Sinularia*, *Sarcophyton*, AND *Lobophytum* FROM TULAMBEN, BALI

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ABSTRACT

Softcoral has a potency as the new source of bioactive compound. This research aims to study antibacterial activity from several softcorals. Samples were identified as *Sinularia*, *Sarcophyton* and *Lobophytum* taken from Tulamben, Bali. Samples were extracted by maceration technique with methanol (70, 80, and 96%) and dried by rotary evaporation. Antibacterial activity of the extracts was tested by agar diffusion method to pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. Antibacterial activity results were: *Sarcophyton* showed highest activity, followed by *Lobophytum* and *Sinularia*. The most inhibited bacterium was *P. aeruginosa*. Methanol 80 and 90% showed similar results, while 70% resulted below them. Minimum inhibitory concentration (MIC) of *Sarcophyton* (96% methanol extract) to *P. aeruginosa* was 4% v/v.

Key words: Softcoral, antibacterial, *Sinularia*, *Sarcophyton*, *Lobophytum*

INTRODUCTION

The search for new bioactive compound which has antibiotic activity has been done in order to cope with microbial resistance to antibiotic used in medical practices (Setyaningsih et al., 2012). On the other hand, Indonesia has a big potency to find such a compound. Soft coral has no cnidocyte, instead they use spicules and bioactive compounds to protect their existence in their habitat. Bioactive compounds such as prostaglandin, cembrenediterpenoid, sesquiterpenoid, and sterol were used by softcoral as defence mechanisms (Sammarco & Coll, 1992). Some of those compounds were thought to have cytotoxic, antitumor, anticancer, and antibacterial activities (Setyaningsih et al., 2012). Softcoral were known to contain inhibitory protease compound to pathogenic bacteria (Nurhayati et al., 2010). There were two genera of softcoral belong to family Alcyonidae that have high antibiotic activity, namely *Sarcophyton* (Nurhayati et al., 2010) and *Sinularia* (Nurhayati et al., 2010; Setyaningsih et al., 2012). The aim of this present research was to compare the antibacterial activity of the two genera with *Lobophytum*, which was belonged to the same family.

MATERIALS & METHODS

Samples were taken by scuba diving gear from Tulamben, Bali. As much as 300 gram softcoral of each genus were cut, placed it in the mesh bag, and brought to surface. Samples were soaked in sterile seawater and were put into cool box with ice pack, then transported to Yogyakarta. Samples were identified based on morphology and growth structure following the work of Manuputty (2002) and Cesnales (2012). Samples were extracted utilizing the work of Nurhayati et al. (2010) such as: 100 gram sample was rinsed, cut into 1 cm block, blended with 200 ml methanol (70, 80, and 96%). Crude extracts of the samples were mac-

erated and placed on the shaker for 24 hours at 29 °C. The extracts were then filtered with Whatman No. 1 filter paper. Filtrates were evaporated and dried utilizing rotary vacuum evaporator at 60 °C. The extracts were tested its antibacterial activity by measuring the inhibition zone using agar diffusion method with No. 4 perforator. Methanol (96%) and ampicillin 500 mg 10% v/v were utilized as control (Setyaningsih *et al.*, 2012). Zone of inhibitions were analyzed using ImageJ® program (Collins, 2007). MIC was done to sample which has highest antibacterial activity with concentrations as follows: 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10% v/v (Mazola *et al.*, 2009). Data were analyzed using Anova, followed by DMRT (Prasad, 2012) and were calculated utilizing SPSS version 17.

RESULTS & DISCUSSION

Soft coral samples were identified as *Sinularia*, *Sarcophyton* dan *Lobophytum* following the work of Manuputty (2002) and Cesnales (2012). Zone of inhibition of the three softcorals were shown in the Table 1.

Table 1. Inhibition zone (in mm²) of the soft coral samples to four pathogenic bacteria

Extract Samples	Conc. (%)	Bacteria				Extract Aver.	Concentration aver.	
		<i>E.coli</i>	<i>P. aeruginosa</i>	<i>S.aureus</i>	<i>S. pyogenes</i>		Conc.	Aver.
<i>Sinularia</i>	70	36,00 ^{abcd}	48,40 ^{bcde}	1,64 ^a	1,86 ^a	38,36 ^a	70%	32,31 ^a
	80	94,60 ^{fg}	109,00 ^{gh}	10,00 ^{ab}	16,40 ^{abc}			
	96	29,20 ^{abcd}	52,60 ^{bcde}	43,20 ^{abcde}	21,00 ^{abcd}			
<i>Sarcophyton</i>	70	13,00 ^{ab}	118,00 ^{gh}	12,20 ^{ab}	31,60 ^{abcd}	59,93 ^b	80%	53,9 ^b
	80	27,40 ^{abcd}	143,40 ^h	31,40 ^{abcd}	27,60 ^{abcd}			
	96	10,80 ^{ab}	221,20 ⁱ	29,80 ^{abcd}	52,80 ^{bcde}			
<i>Lobophytum</i>	70	37,00 ^{abcd}	57,80 ^{cdef}	8,60 ^{ab}	25,20 ^{abcd}	42,75 ^a	96%	54,83 ^b
	80	82,20 ^{efg}	63,80 ^{def}	14,80 ^{abc}	26,20 ^{abcd}			
	96	22,40 ^{abcd}	96,20 ^{fg}	38,00 ^{abcd}	40,80 ^{abcd}			
Bacteria Aver.		39,17 ^b	101,15 ^c	20,88 ^a	26,84 ^a			

Note: numbers in the same columns with same alphabets show no significant difference at P<0.05

The results showed that extract methanol 96% of *Sarcophyton* to *P. aeruginosa* was highest among others (Figure 1) and differ from Nurhayati *et al.* (2010) and Setyaningsih *et al.* (2012), i.e. *Sinularia* was the highest. The difference was probably due to the lack of identification of Indonesian softcorals at the species level (Nurhayati *et al.*, 2010; Sulistiyani *et al.*, 2010; Setyaningsih *et al.*, 2012). Extract methanol 96% *Sarcophyton* was also shown inhibition activity to Gram positive bacterium such as *S. pyogenes*. Hence, the extract can be considered as broad spectrum (Taskin *et al.*, 2007). MIC of this extract was at 4% v/v.

Compared to ampicilline, antibacterial activity of the softcoral extracts were relatively low. The results were caused by the condition of the extracts which were crude. The concentrations of the extracts were lower since lyophilization was not applied to the samples. The bioactive compound of soft coral which has antibacterial activity was terpen and sterol (D'Auria, 1992). These two compounds were known to be able to interact with bacterial cell membrane and disrupt the normal physiological function of the cell membrane (Trombetta *et al.*, 2005). Sinularosides, for instance, was modified sterol (added with glycoside from saponin), that behave as surfactant and was able to react with phospholipid membrane and caused lysis of bacterial cells (Sun *et al.*, 2012).



Figure 1. Inhibition zones of softcoral extracts to *P. aeruginosa* Note: upper left *Lobophytum*, upper right *Sarcophyton*, below *Sinularia*

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REFERENCES

- Cesnales, A. 2012. Dichotomous key for soft coral identification. <http://www.utahreefs.com/articles/Dichotomous%20key%20for%20soft%20corals.pdf>. accessed 1 Oktober 2012.
- Collins, T.J. 2007. Image J for microscopy. *BioTechniques* 43 (1): 25–30.
- D’Auria, M.V., L. Minale, and R. Riccio. 1993. Polyoxygenated steroids of marine origin. *Chemistry* 93(5): 1839–1895.
- Manuputty, A.E.W. 2002. *Karang lunak (Soft Coral) Perairan Indonesia*. Puslitbang Oseanologi-LIPI, Jakarta.
- Mazzola, P.G., A.F. Jozala, L.L. Novaes, P. Moriel, and T.V. Penna. 2009. Minimal inhibitory concentration (MIC) determination of disinfectant and/or sterilizing agents. *Brazilian Journal of Pharmaceutical Sciences* 45(2): 242-248.
- Nurhayati, T., M. Fikri, and Desniar. 2010. Aktivitas Inhibitor Protease dari Ekstrak Karang Lunak Asal Perairan Pulau Panggang Kepulauan Seribu. *ILMU KELAUTAN* 15 (2): 59-65.
- Prasad, R. 2012. *Basic Statistical Techniques: Multiple Comparison Procedure*. IASRI, New Delhi.
- Sammarco, P.W. and J.C. Coll. 1992. Chemical adaptations in the Octocorallia: Evolutionary consideration. *Marine Ecology Progress Series* 8 8:93-104.
- Setyaningsih, Nurhayati, Nugraha, and Gunawan. 2012. Comparative evaluation of the antibacterial activity of soft corals collected from the water of Panggang Island, Kepulauan Seribu. *Pharmacie Globale (IJCP)* 6 (03): 1-3.
- Sulistiyani, N.S.A., Radjasa, O.K., Sabdon, A., and Khoeri, M.M. 2010. Antibacterial Activities of Bacterial Symbionts of Soft Coral *Sinularia sp.* Against Tuberculosis Bacteria. *Journal of Coastal Development* 14(1):45-50.
- Sun, P., L.Y. Meng, H. Tang, B.S. Liu, and L. Li. 2012. Sinularosides A and B, Bioactive 9,11-Secosteroidal Glycosides from the South China Sea Soft Coral *Sinularia humilis* Ofwegen. *Journal of Natural Product* 75 (9):1656–1659.

- Taskin, E., M. Ozturk, E. Taskin, and O. Kurt. 2007. Antibacteria activities of some marine algae from the Aegean Sea (Turkey). *African Journal of Biotechnology* 6(24): 2746-2751
- Trombetta, D., F. Castelli, M.G. Sarpietro, and V. Venuti. 2005. Mechanisms of Antibacterial Action of Three Monoterpenes. *Antimicrobial Agents Chemotherapy* 49(6): 2474–2478.