METHANOL EXTRACT DERIVED FROM NIAS'S AND MENTAWAI'S SPONGES AND SOFT CORALS AS ANTI ANGIOGENESIS

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ABSTRACT

The exploration of anti angiogenesis potency from Mentawai and Nias's sponges and soft corals was studied. The anti angiogenesis test was conducted based on the population of endothelia cell line (CPAE, ATCC CCL). From 201 samples collected only two samples namely methanol extracts of soft coral GSO6 from Mentawai and sponge no. 18 from Nias. and their fractions were tested for anti angiogenesis. Fractionation for the two samples were done using silica gel column chromatography and gave 33 and 32 fractions respectively. The results indicated that methanol crude extract of sponge no. 18 was the active one. The fraction no. 16 extract 18 had 56 ppm of IC_{50} and IC_{50} of fraction no 6 GSO6 was 144 ppm. Further studied of active compounds for their pharmaceutical potency still are needed.

Keywords: sponges, soft coral, anti angiogenesis, screening, Nias and Mentawai islands

INTRODUCTION

Indonesia is megadiversity country in the world that produce many resources of active compounds for medicine. The use of drug substances derived from plants, fungi, bacteria, and marine organisms has a long tradition in medicine. Together with their derivatives, and synthetic compounds deduced from natural product precursors, they represent a major part of today's pharmaceutical market. Used in ancient oriental medicine as a source of bioactive compounds, sea cucumbers, sea stars and sea urchins are now used for the extraction and purification of cytotoxic,

haemolytic, antiviral, antifungal, antifouling, antimicrobial and even anti-tumoural activities (Zhang L and Demain A L. 2005). In addition, of the five extant classes, sea urchins and sea cucumbers are important economic resources for current fishery and aquaculture. Indonesian sea also produce marine biodiversity such as sponges and soft corals as medicine resources. Many sponges and soft corals contain active compounds which have role as anti bacteria, antifungal, anti tumor, etc. Active compound produced by sponges and soft corals is one of alternative for exploration new medicine resources. In Indonesia, Nias and Mentawai are places which produce potential sponges and soft corals as medicine resources. Samples from Nias and Mentawai islands may have many chemical compounds as anti angiogenesis. Recently, research regarding exploration of potency of extract and fraction of soft coral and sponges derived from Nias and Mentawai islands as anti angiogenesis. The purpose of the research is to explore the potency of extract and fraction of soft coral and sponges derived from Nias and Mentawai islands as anti angiogenesis.

MATERIALS AND METHOD

Materials

Variety of soft corals dan sponges derived from Nias and Mentawai islands.

Method

Extraction and Fractionation

The samples obtained were as many as 201 samples. Overall of the samples were taken a little and added methanol to make specimens. From 201 samples, 100 samples were selected to be extracted. From 100 samples, 90 samples extracted with methanol and 10 samples extracted with methanol, chloroform and hexane. Crude extracts produced were then tested for toxicity by using brine shrimp lethality test (BSLT) method (Mayer *et al* 1982). Crude extracts with the best LC_{50} values were then tested for anti angiogenesis activity. The anti angiogenesis test was conducted based on the population of endothelia cell line (CPAE, ATCC CCL). Crude extracts with the best anti angiogenesis activity, next, were separated (fractionation) using sephacore-silica gel column of HPLC. From this process, it would be produced some fractions which would be tested again their anti angiogenesis activity, so that would be obtained active fraction as anti angiogenesis.

Fractionation of 18 M (Haliclona sp) and GSO-6 (Labophytum sp) Extract. Ten grams of methanol crude extract were extracted by chloroform, then would be obtained methanol fraction and chloroform fraction. Each fraction was tested qualitatively to determine the secondary metabolite

content. After that, methanol fraction was separated again by sephacore column of HPLC, and obtained some fractions. Each fraction then was assayed to get the fractions being anti angiogenesis active.

RESULTS AND DISCUSSION

Extraction

Nine selected samples were extracted using methanol. Samples' weight, extracts' weight, and their yields could be seen in Table 1 (Nias island) and Table 2 (Mentawai island).

Figure 1. Sponge sample extracts from Nias dan Mentawai Islands.

Table 1. Sample extracts from Nias Island

No	Solvent	Sample Code	Wet Sample Weight (g)	Filtrate Volume (mL)	Extract Weight (g)	Yield (%)
1	methanol	3	9900	20.000	1184,8	12.09
2	methanol	11	2100	9.000	115,29	5.49
3	methanol	18	2200	8.500	106,7	4.85
4	methanol	22	3475	9.500	72,98	2.10

Table 2. Sample extracts from Mentawai Island

No	Solvent	Sample Code	Wet Sample Weight (g)	Filtrate Volume (mL)	Extract Weight (g)	Yield (%)
1	methanol	GSO-06	810	4.500	31,19	3,85
2	methanol	TPB-04	1230	7.150	37,88	3,08
3	methanol	TPB-12	900	5.050	22,59	2,51
4	methanol	TPP-20	800	5.500	9,76	1,22
5	methanol	TLP-14	950	6.000	28,12	2,96

Secondary Metabolites Assay of Extracts

All obtained extracts were assayed for secondary metabolites contained qualitatively. The results were shown in Table 3.

Tabel 3. Secondary metabolite of all extracts examined qualitatively

Sample	Alkaloid			Flavonoid	Steroid	Terpenoid	Tanin	Saponin
	Dragendorf	Wagner	Mayer					
TPP 20	-	-	-	+	-	-	-	-
18	-	-	-	+	+	-	-	-
11	-	-	-	+	+	-	-	-
GSO6	-	-	-	+++	-	-	-	++
TPB12	-	-	-	-	+	-	-	+
3	-	-	-	+	-	+	-	+
TPB 4	-	-	-	+	++	-	-	++
TLP 14	-	-	-	+	+	-	-	-
22	-	-	-	+	-	-	-	+++++

From Table 3, it was noted that the extract of TPP 20 was only detected the presence of flavonoid, 18 and 11 M extracts contained flavonoids and steroids, GSO 6 extract contained a lot of flavonoids and also saponins, TPB 12 extract contained steroids and saponins, 3M extract contained terpenoids and saponins, TPB extract contained flavonoids, steroids, and saponins, TLP 4 extract contained flavonoids and steroids, and 22 M extract contained flavonoids and saponins

Fractionation of Extract Derived from Sample of Nias Island

Obtained extracts then were separated to get purer fractions. The compounds contained from these fractions were expected to have specific bioactivity. The extracts were fractionated using sephacore-silica gel HPLC column. The fractionation condition was different for each extract, depended on the extract being separated.

18 M sample. Ten grams methanol crude extracts of 18 M sample were partitioned using chloroform as solvent. Chlororform fraction obtained was 0.35 g. Methanol fraction of this partition still had quite a lot of weight, was equal to 9.65 grams. The high of methanol fraction weight was not caused by a lot of active compound, because this fraction contained most of salts.

The methanol fraction contained alkaloids, flavonoids, steroids, and saponins, while the chloroform fraction only contained flavonoids. The methanol fractions was fractionated using sephacore with silica gel as

stationary phase and methanol then chloroform as mobile phase. The fractions resulted were 32 fractions with different weight for each fraction (Table 4). From these results, it was selected 14th, 16th, and 31st fractions to be assayed their anti angiogenesis activity.

Table 4. Fractionation of 18 M sample using sephacore

Fraction	Weight (g)	Yield (%)
1	0.0102	1.276116602
2	0.0159	1.989240586
3	0.0215	2.689853622
4	0.0009	0.112598524
5	0.0016	0.200175153
6	TU	TU
7	0.0004	0.050043788
8	0.0008	0.100087577
9	0.0442	5.529838609
10	TU	TU
11	TU	TU
12	TU	TU
13	0.0018	0.225197047
14	0.1101	13.77455273
15	0.0215	2.689853622
16	0.0736	9.20805705

Fraction	Weight (g)	Yield (%)
17	0.0245	3.065182034
18	0.0012	0.150131365
19	TU	TU
20	0.0035	0.437883148
21	0.0243	3.04016014
22	0.0165	2.064306268
23	0.0071	0.888277243
24	TU	TU
25	0.152	19.01663956
26	TU	TU
27	0.075	9.383210309
28	0.0088	1.100963343
29	0.0114	1.426247967
30	0.055	6.881020893
31	0.1175	14.70036282
32	TU	TU

Fractionation of Extract Derived from Sample of Mentawai Island

GSO 6 Sample. Ten grams methanol crude extracts of GSO 6 sample were partitioned using chloroform as solvent. The chlororform fraction obtained was 2.15 g. Methanol fraction of partition still had quite a lot of weight, was equal to 7.85 grams. As well as methanol fraction of 18 M sample from Nias Islands, the high of GSO 6 methanol fraction weight was not caused by a lot of active compound, because this fraction also contained most of salts.

The methanol fraction from this partition was not detected its secondary metabollites at all, while the chlorform fraction contained flavonoids, steroids, and saponins. The chloroform fraction was fractionated using sephacore with silica gel as stationary phase and methanol then chloroform as mobile phase. This process resulted 33 fractions with different weight for each fraction (Table 5). For ant angiogenesis assay, it was chosen fraction 6th, 8th, 17th, and 18th.

Figure 2. GSO 6 sample fractionation using sephacore.

Tabel 5. Fractionation of GSO 6 sample using sephacore

Fraction	Weight (g)	Yield (%)
1	0.0102	1.276116602
2	0.0159	1.989240586
3	0.0215	2.689853622
4	0.0009	0.112598524
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33	TU	TU

LC_{50} Value Determination by Brine Shrimp Lethality Test (BSLT) Method

BSLT is *in vivo* toxicity assay against microorganism (brine shrimp larvae). LC_{50} value describes compounds' citotoxicity level which can kill 50% of larvae population. Statistical analysis was done due to data obtained, ie relationship between death larvae and fractions concentration using probit analysis. LC_{50} value could be seen in Table 6.

All of tested sample gave positive result but none of them were toxic. The values were various between 35 to 1893 ppm. The fractions that had LC_{50} value smaller than 1000 ppm were considered as biologically active fractions or toxic against *A. salina*, while higher than 1000 ppm were considered as not toxic. While for pure compound, it was considered as biologically active if its LC_{50} value was smaller than 200 ppm (Anderson *et al.* 1991, Mayer *et al.* 1982). If tested fractions were considered as pure compounds, then the fractions which biologically active were 6th, 16 th, and 23rd fraction of TLP 14 sample, 6th fraction of GSO 6, and 5th fraction of 22M. 3,5-dibromo-2-(2',4'-dibromophenoxy)phenol had LC_{50} value of BSLT equal to 8.66 ppm based on Handayani *et al* (1997).

Tabel 6. LC_{50} value of separated fractions

No	Sample	LC_{50} (ppm)
1	TLP 14-6	111.52
2	TLP 14-16	86.70
3	TLP 14-18	1241.38
4	TLP 14-21	403.50
5	TLP 14-23	194.41
6	TLP 14-28	425.04
7	TLP 14-31	237.66
8	GSO 6-3	1052.52
9	GSO 6-5	565.38
10	GSO 6-6	35.65
11	GSO 6-8	820.63
12	GSO 6-10	490.34
13	GSO 6-17	1892.88
14	GSO 6-18	492.43
15	GSO 6-19	322.75
16	22M-5	88.55
17	22M-7	982.55
18	22M-8	523.96
19	22M, 16,17,18	1551.27

Anti Angiogenesis Assay of Selected Extracts and Fractions

Anti angiogenesis assay was carried out due to only selected extracts and fractions. The result was shown on Figure 3. Extract of 18M, 16th fraction of 18, and 8th fraction of GSO6 could kill almost all of cells. Meanwhile, TLP 10 crude extract and 18M could not kill even a single extract although cell population after 4th day of analysis was not as much as control. IC_{50} value of 16th fraction of 18M was 56 ppm, while 6th fraction of GSO6 was 144 ppm.

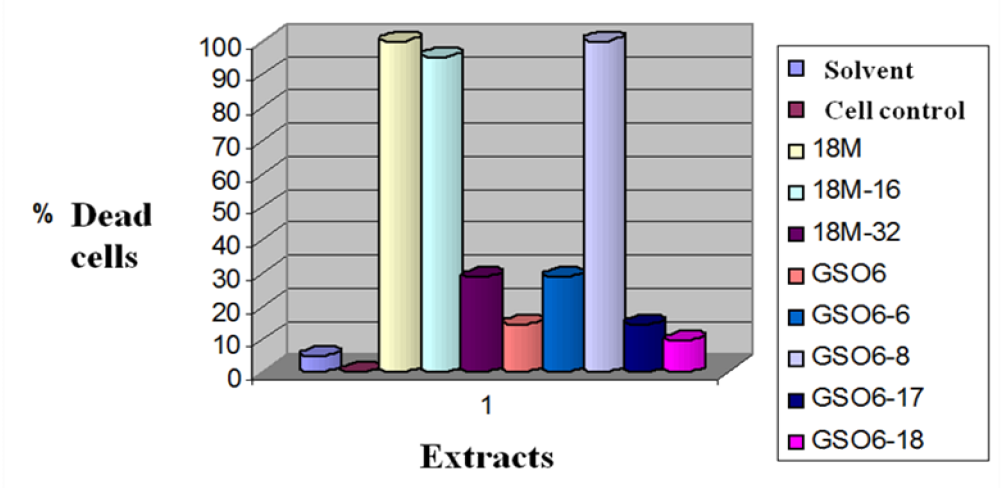

Figure 3. Anti angiogenesis assay result.

CONCLUSIONS

In conclusion, 18 M sample contained flavonoids and steroids and GSO-6 sample contained flavonoids and saponins. BSLT analysis indicated that TLP 14 fraction 6th, 14th, and 23th, GSO-6 fraction 6th, and 22M fraction 5th had high toxicity. Active fraction which had potency as anti angiogenesis were fraction 16th of 18M extract with IC-50 of 56 ppm and fraction 6th of GSO6 extract with IC-50 of 144 ppm.

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