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A Preliminary Study of Bioactivity and Identification of Secondary Metabolite Functional Groups in Extracts of *Agelas nakamurai* Hoshino Sponge from Spermonde Archipelago, Indonesia

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ABSTRAK

Uji pendahuluan bioaktivitas dan identifikasi gugus fungsi metabolit sekunder ekstrak dalam metanol, n-heksan and metilen klorida dari spons *Agelas nakamurai* Hoshino, asal pulau Kapoposang, kepulauan Spermonde telah dilakukan. Juga test bioaktivitas dilakukan menggunakan *Artemia salina* (LC50) untuk mendapatkan konsentrasi letalnya. Hasilnya LC50 adalah 187.932 ppm yang menunjukkan potensi ekstrak sebagai obat anti kanker. Identifikasi komponen dengan reagensia spesifik seperti Lieberman-Burchard, Mayer, Wagner, Dragendorff dan Salkowski ditambah hasil analisis FTIR menunjukkan kandungan komponen ekstrak seperti alkaloid, steroid and terpenoid. Karakteristik pita serapan alkaloid adalah for alkaloid adalah stretching NH at 3600 to 3400 cm^{-1} dan stretching pendukung C-N at 1250 to 1930 cm^{-1}. Komponen spesifik untuk *Agelas nakamurai* adalah substitusi bromo dalam alkaloid yang ditandai oleh munculnya pita serapan C-Br medium pada 760 to 745 cm^{-1}.

Kata kunci : *Agelas nakamurai*, metanol, bioaktivitas, komponen, Spermonde

I. Introduction

Research on the sponge *Agelas nakamurai* have been carried out in various countries. Specifically, genus of Agelas sponge contain Oroidin (Figure 1) and related compounds like Sceptrin (Figure 2) found for the first time on Australian Agelas sponge (Hao et.al., 2001)

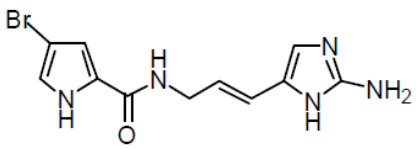


Figure 1. Oroidin compound

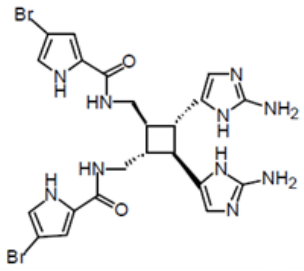


Figure 2. Oroidin compound

Another article show that on the polar methanol fraction in *Agelas* sp. sponges was found Slagenine A, B, C, *Agelasidine* B and C from origin Okinawa waters (Dembitsky and Tolstikov, 2003; Tsuda et al., 1999; Bhakuni, 2005). Meanwhile, *Longamide* C, *Mukanadine* C, *Agelasine* D, *Ageloxime* D, and *Hymenidine* have been isolated from *Agelas sp.* sponge in Menjangan Island (Hertiani *et. al.*, 2010), ().

On *Agelas nakamurai* Hoshino sponge from Papua New Guinean also have been isolated diterpene compound possessing a 9-methyladenium moiety and two new bromopyrrole alkaloids. The compounds showed no inhibitory activity against HIV-1 and no antimicrobial activity against the Gram-negative bacterium *Escherichia coli* and *Pseudomonas seruginosa* (Tetsuo Iwagawa, et.al., 1998). From the same species, an ageladine with the structure of 4-(4,5-dibromo-1H-pyrrol-2-yl)]-1H-imidazo [4,5-c]pyridine-2-amine (Figure 3) has activity as antiangiogenic (Fujita et.al., 2003)

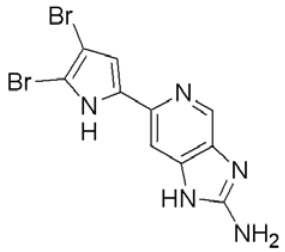


Figure 3. Ageladine compound

The all compounds from *Agelas* sp in different places show that *Agelas* sponge have diversity of secondary metabolite. Therefore, preliminary study to *Agelas nakamurai* is need to conducted to find new compounds have different activity.

Materials and Methods

2.1. Material and Apparatus

FTIR (IRPrestige-21 Shimadzu), Analitic (Mettler AE 100), and rotary evaporator (Heidolph 4000).

Reagents: methanol grade, and n-heksan grade, Methylene Chloride grade. Salkowski reagent, Mayer reagent, Wagner reagent, and Dragendorff reagent.

2.2. Sampling and species identification

Sponge of *Agelas nakamurai* Hoshino was collected from a depth 8 m by hand in Kapoposang Island, Spermonde Archipelago, South of Sulawesi, Indonesia on 15 August 2012. Sponge taxonomy identified in Research Center for Oceanography LIPI Jakarta. The Taxonomy data as follows : Phylum Porifera, Class Demospongiae, Order Agelasida, Family Agelasidae, Genus Agelas, Species *Agelas nakamurai*.

The morphology of *Agelas nakamurai* is a form massive or thickly encrusting with a irregular surface like in figure 4. The body colour is red brick in dry state. Its consistency is compressible and the skeleton consist of spicules-enclosed ramifying and acanthostyles 190-250 µm x 8-12 µm like in figure 5. A voucher code of specimen was SPV01/09/13 and its has been deposited in Research Center for Oceanography (RCO) LIPI Jakarta, Indonesia 14430.

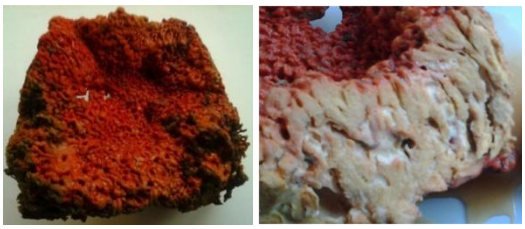
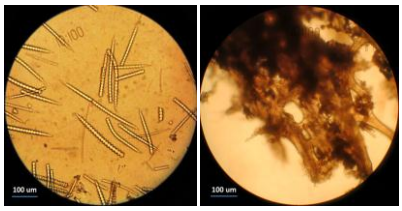


Figure 4. Morphology *Agelas nakamurai* Hoshino



a b

Figure 5. Skeleton (a) and specule (b) of *Agelas nakamurai*

2.3.Microscale extraction

Extraction was done in micro scale which 36 g of sponge *Agelas nakamurai* Hoshino macerated using methanol. The maceration results filtered and evaporated using an evaporator to obtain 52.2 mg methanol extract. Then the methanol extract partitioned with n-hexane and methylene chloride, respectively obtained 10.6 mg and 17.4 mg and remain 21,1 mg a residue.

2.4. Specific test and identification of secondary metabolite group

Specific test carried out on methanol extract of the sponge *Agelas nakamurai* Hoshino. The test using specific reagent as follows: Lieberman-Burchard (LB) for steroids, Salkowski for terpenoids, Wagner, Mayer and Dragendorff for alkaloids. Meanwhile, FTIR instrument need to support prediction of secondary metabolites groups in specific test. FTIR analysis was done to methanol, n-hexane, methylene chloride (MTC) and residue.

2.5. Bioactivity test

Secondary metabolites are often toxic to shrimp larvae *Artemia salina*. Lethality test in vivo to shrimp *Artemia salina* larvae used as a screening to get the data as a measurement of toxicity LC50 sample . The method has been developed for the natural product to monitor cytotoxicity natural product compounds (Meyer et al, 1982; McLaughlin and Rogers, 1998). In this method, the sample was made in a concentration of 1000, 100 and 10 ppm in triplo and emulsificated with DMSO. The solution was tested against 10 *Artemia salina*. *Artemia salina* mortality datas were analyzed using Probit Analysis

3. Results and Discussion

3.1. Specific test and cytotoxicity

Specific test results to the group of secondary metabolites methanol extracts showed that methanol extract containing steroid compound, terpenoid and alkaloids (Table 1). The presence of alkaloids, steroid, terpenoid in the extracts of the sponge structure bromopyrrol *Agelas nakamurai* has been published in several journals. Cytotoxicity test results showed that the methanol extract methanol extract has potential as an anticancer *Agelas nakamurai* with LC50 values below 500 ppm is 187.932 ppm.

Table 1. Results of specific test group of secondary metabolites

Sample	Reagent	Colours	Level
KP-01 Sample	LB	Green	+++
	Salkowsy	Red-brown	+
	Mayer	Yellow-white	+
	Wagner	brown	+
	Dragendorff	red	+++

3.1.FTIR Analysis

FTIR analysis conducted on extracts of the methanol extract (KP-01 samples), n-hexane (KP-01 Hex), methylene chloride extract (KP-01 MTC) and the residue (KP-01 residues). The FTIR analysis only provides information and prediction of functional groups of secondary metabolites.

3.2.1. FTIR Analysis of KP-01 Methanol Extract

Absorption pattern FTIR for the KP-01 samples undergo overlapping, especially peaks around 1000 ppm, 1230 ppm, 1600 ppm and above 3000 ppm (Figure 6). This is shows complexity of secondary metabolites containing in extract. Indications overlapping absorptions seen in shoulder formation on peaks area mentioned above.

However, some absorption clearly shows the presence of major functional groups stretching including methyl absorption ($-CH_3$), methylene ($-CH_2$) in the range of 2800-2930 cm^{-1} , metin (=CH) at 3200 cm^{-1} (shoulder shape), carbonyl (C=O) about 1735 cm^{-1} in shoulder form, ethylene (C=C) at 1645.20 cm^{-1}, (C-N) at 1220.73 cm^{-1}, (C-O) at 1050 cm^{-1}, C-Br at 744.02 cm^{-1}, bending NH about 1610 cm^{-1} but NH stretching absorptions above 3200 cm^{-1} has not seen (overlapping), the CH_3 and CH_2 bending at 1450.20 and 1404.10 cm^{-1}.

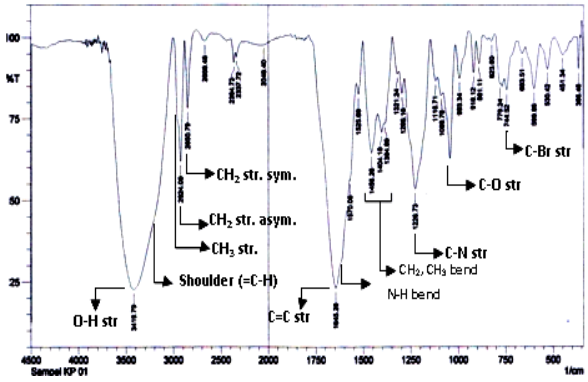


Figure 6. FTIR Spectrum of Sample KP-01

3.2.2. FTIR Analysis of KP-01 N-Hexane Extract

Spectrum pattern of FTIR on KP-01 n-hexane (Figure 7) more simple if compare to spectrum KP-01 sample. Sorption at 3419.79 cm^{-1} is OH str. And supported by C-O str at 1043.49 cm^{-1}. Weak band sorption above 3000 cm^{-1} is =CH str., 2924.09 cm^{-1}, 2852.72, are CH_3 and CH_2 str., sorption at 1739.79 cm^{-1} (weak) is C=O, sorption at 1651.07 cm^{-1} (w) is C=C, 1043.49 cm^{-1} (C-O). Weak sorption Serapan at 721,30 cm^{-1} indicated the compound contain 4 or more methylene (-CH_2-) functional groups in bending rocking.

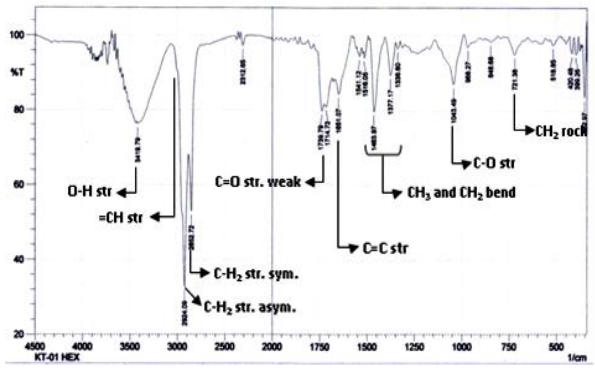


Figure 7. FTIR Spectrum of KP-01 Hexane

3.2.3. FTIR Analysis of KP-01 MTC Extract

On FTIR spectrum of KP-01 MTC (Figure 8), peaks shoulder have separated and appear clearly. Strecthing sorption of amina secunder NH at 3421.72 cm^{-1} still overlapping to streching OH. NH bend. (strong) at 1610.50 cm^{-1} has separated as shoulder. C-N at 1299.29 cm^{-1} and weak-medium sorption at 765.00 cm^{-1} specify for stretching C-Br str.

All sorption fot KP-01 MTC indicated that extract contain alkaloid compound and suspected bromo alkaloid and related compounds.

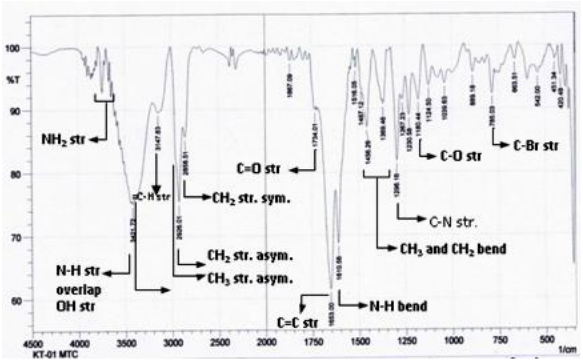


Figure 8. FTIR Spectrum of KP-01 MTC

3.2.1. FTIR Analysis of KP-01 Residue

Characteristic band sorption for alkaloid and C-Br str. 750.31 cm^{-1} at still appear on FTIR spectrum of KP-01 residue. Stretching (C=C) strong at 1643.35 cm^{-1}, NH bend. Still overlapping with C=C, and stretching C-O at 1047.35 cm^{-1}. Sorption OH seem broad effected hydrogen bond on polar fraction. Base on the data, residue still contain alkaloid compounds.

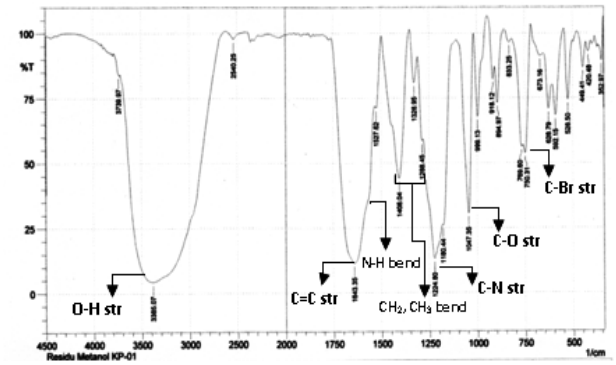


Figure 9. FTIR Spectrum of KP-01 residu

4. Conclusion

1. Combination of FTIR analysis, using specific test reagent and reference about *Agelas nakamurai* can help to predict secondary metabolite groups extract and fraction.
2. Polar fraction of *Agelas nakamurai* sponge contain bromo-substituted alkaloid compounds which was supported by band sorption of C-N str, NH str. NH bend. bend. and C-Br str. at 1250 to 1930 cm^{-1}, 3600 to 3400 cm^{-1}, 1610.50 cm^{-1}, and 760 to 745 cm^{-1} respectively.
3. Methanol extract of *Agelas nakamurai* sponge is potential as anticancer with LC50 187.932 ppm.

5. Acknowledgment

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