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Isolation and Identification of Alkaloid Compound of Marine Sponge Cinachyrella sp. (Family Tetillidae)

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ABSTRACT

Marine sponge *Cinachyrella* sp. produces many kinds of secondary metabolites. Isolation of active compounds used bioassay guided isolation method. Based on the analysis of spectroscopic IR, UV, NMR data and structural confirmation analysis using EI-MS, it could be proposed that chemical structure of isolate F3.2.1 belongs to *cinachyramine* compound derivative, with molecular formula of $C_{10}H_{13}N_3O$ and structural name of 1,4,9-*triazatricyclo*[7,3,1,0]*trideca*-3,5(13),10-*trien*-8-*ol.*

Keyword: *Cinachyrella* sp., *bioassay guided isolation*, 1,4,9-*triazatricyclo*[7,3,1,0]*trideca*-3,5(13),10-*trien*-8-*ol.*

INTRODUCTION

Sponge is a member of Metazoa, which has successfully thorough million years of evolution. It is widely distributed on earth, both at the salt water and fresh water areas[1]. Sponge is a sessile animal – filter feeder with specific physiological strategy, reproduction, and effective defensive mechanism against bacteria, fungi, viruses, and predator animals [2], spatial competition with other organisms [3] and sea-based pathogens[4]. The sponge can produce various kinds of secondary metabolite [5] and have pharmacological potentials[6]. It lives in coral habitat, which can produce highly toxic secondary metabolite as the consequence of extreme water environmental pressure, competition, self-defense against nudibranch, sponge-eating gastropods, and carnivorous fishes [7].

Research for the natural ingredients of sponge began to increase since Bergmann and Feeney successfully isolated arabino-nucleoside from the sponge *Tectitethya crypta* in 1951. The efforts for finding and developing medicine from marine sponge began in 1970s[8]. Sponge is the source of huge secondary metabolite, with novel and unique structure, which is not found in terrestrial organisms[9]. The sponge produces about 20.000 types of active compounds. It has wide-ranging chemical diversity, which includes sterol, terpenoid, isoprenoid, nonisoprenoid, quinone, brominates, heterocyclic nitrogen sulphur[10], amino acid, porphyrin and peroxide. Sponge-derived compounds also have varied functional groups (OH, OCH_3, OAc, OSO_3, Na^+)[11]. Any change in the functional group is potential to modify the polarity of each component in a radical way. Oncological studies found that compounds from the marine sponge could interact with the essential components in the cellular cycles, enzymes, and other targets[12]. The problems proposed in this research are related to the structure and identity of the active compound isolates of marine sponge *Cinachyrella* sp.

MATERIAL AND METHODS

Sampling of marine sponge *Cinachyrella* sp.

The sponge *Cinachyrella* sp. was taken from Kukup Beach, Kemadang Village, Tanjungsari Subdistrict, Gunung Kidul Regency, DIY at an intertidal area by means of direct collection. Sponge samples were taken from various microhabitats. Then, the samples were put into plastic bags and stored in icebox under a temperature of 5°C until the extraction time.

Extraction of Marine Sponge *Cinachyrella* sp.

Sponge extraction was conducted by modifying the method proposed by Isnansetyo (2001) [13]. The sponge mass was cleaned and polluting particles were desiccated into the size of smaller than 2-3 mm, based on the method proposed by Orhan (2012). The sponge mass was macerated using 96% ethanol for 2x24 hours, while being stirred occasionally to prevent saturation. After being let for 2 nights, the sponge mass was filtered using a vacuum filter [14]. The resulting filtration was collected (filtrate 1), while the sponge mass was re-extracted using ethanol in the same volume, let for 2x24 hours, and then filtered to generate filtrate 2. The extraction process was repeated once more to generate filtrate 3. All of the filtrates

were evaporated by using a rotary vacuum to generate thick extract.

Trituration of the extract of marine sponge *Cinachyrella* sp.

The ethanolic extract was partitioned using ethyl acetate in a funnel flask. The extract suspension was homogenated for about 15 minutes. The suspension was let to form 2 layers, then separated using a isolation funnel, to generate ethyl acetate soluble filtrate and ethyl acetate non-soluble filtrate. Each of the filtrates was thickened.

Fractionation of EA of marine sponge *Cinachyrella* sp.

EA was fractionated using vacuum-liquid column chromatograph with stationery-phase silica-gel and mobile phase *n*-hexane; *n*-hexane : ethyl acetate (9;1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9 v/v); ethyl acetate; *n*-hexane : chloroform =1:1v/v); methanol. The resulting fraction was evaporated and then identified with thin-layer chromatograph (TLC). Fractions with similar TLC profiles were united. The united fraction was used as a sample of activity assay. The major fraction was derived and then isolated using preparative TLC (PTLC).

Isolation of the extract fraction of marine sponge *Cinachyrella* sp.

Isolation was conducted using preparative thin-layer chromatograph (TLC) with *n*-hexane-acetone = 7:3 v/v as eluent. The isolates that indicated 1 spot was proceeded for purification using 2-dimensional TLC, with the first eluent (*n*-hexane-acetone = 7:3 v/v) and the second eluent (chloroform-methanol = 6:4 v/v).

Purification Test of Active Compounds of marine sponge *Cinachyrella* sp.

Purity of the isolation was based upon TLC and high-performance liquid chromatography (HPLC). In this research, HPLC used the instruments of LC-MS Shimadzu, column ODS 20 X 150 mm, *photo diode array* (PDA) detector, elusion system 20%H_2O and 90% acetonitrile in a gradient way.

Spectroscopic Data Measurement

Spectroscopic data, which were measured, included UV=Vis spectrum, FT-IR spectrum, ^{1}H NMR, ^{13}C NMR spectra, and two-dimensional NMR. The measurement was conducted by using the following instrument, namely JEOL JNM ECA 500Mhz. Mass spectral measurement was conducted by using LC-ESI-MS (Waters) as instrument, in addition to SunFire column 4.6x150 mm, isocratic condition of H2O+ formic acid: acetonitrile 45/55 v/v, 0.7 ml of water/minutes and injection volume of 10 µL.

RESULTS AND DISCUSSION

Fifteen kilograms of newly harvested *Cinachyrella* sp. was used for the research, and 448,31g (2,988%) of ethanolic extract was produced. There was a significant loss on drying, namely, more than 50% (Wright, 1998). The trituration generated 46 grams (10.26%) of the extract and 262,9 g (58,64%) of ethyl-acetate non-soluble fractionation. Isolation was conducted on major fraction F3 using preparative TLC method (Figure 1).

Isolates are clear non-colored noodle crystals, which a melting point of 121^{0}C. The isolate is soluble in metahnol:chlorofrom =1:1 but insoluble in water. Chromatographic profile of isolates F3.2.1 revealed purified isolates in HRf value of 70. Under blue visible light, UV 254 nm was brick red and UV 366 was fluorescent. The isolates softens at UV 254 nm. Detection using sulphate serium under heating showed brown color.

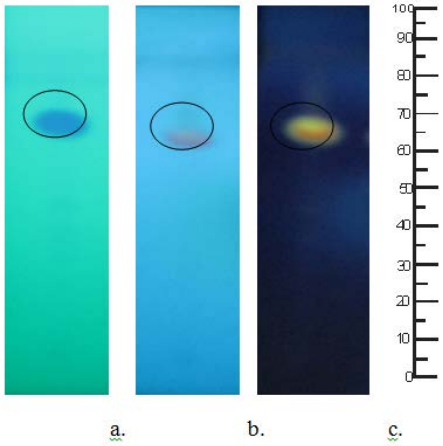

Figure 1 Chromatographic profile of active isolates F3.2.1 of marine spone *Cinachyrella* sp. Spot detector = CeSO4. Mobile phase = n-hexane:ethyl acetate = 7:3 $^v/_v$. Stationary phase = Silica Gel GF_{254}. a. Visible light b. UV 254, c. UV 366

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Interpretation of UV spectrum of the active compound F3.2.1 of the marine sponge *Cinachyrella* sp. (in metahnol:chlorofrom =1:1) showed absorption at 200,50; 210,50; 219,50 dan maximum (λ_{max}) of 251 nm (Figure 4.21). This shows that the isolates has chromophoric diena group[15]. Spectroscopic measurement of IR spectrum showed some functional groups, which are visible from the absorption tape. The infra red spectrum with wide absorption peak (v_{max}) of 3405 cm^{-1} (weak) showed hydroxyl group (OH) strengthened by absorption of 1058 cm^{-1} for CO. Absorption peak of 2976 cm^{-1} showed phynylic but not aromatic binds.

Data for the spectral analysis of proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C-NMR) were collected by dissolving the samples into *deuterated-methanol* (CD_3OD) (0,5 mL) (0,5 mL) in the tube NMR (5 mm). The spectra were recorded using spectrometer Jeol-450 (^{1}H at 450 MHz and ^{13}C at 125 MHz). Isolates F3.2.1 in the spectrum proton nuclear magnetic resosnance (^{1}H-NMR) (Figure 4.23) showed the types and number of proton. Methyl protin appeared in the chemical shift area δ_H 0,95 and 2,00 ppm. Metin proton was found in the chemical shifts of δ_H 1,00; 1,01; 3,30; 3,32; 3,43 and 4,30 ppm while methylene proton was not found in DEPT measurement.

The spectrum in the isolate F3.2.1 revealed 10 types of carbon atoms. The type of carbon was analyzed further using DEPT (*Distortionless Enhancemen by Polarization Transfer)* technique, that the numbe of types of –CH_3, -CH_2, -CH and –C (quartenary carbon) of the isolate F3.2.1 could be determined. DEPT spectral data for the isolate F3.2.1. showed 2 methyl carbons CH_3 and 6 metin carbons –CH. Methyl carbon appeared at the chemical shift area of δ_H 13,77 and 19,81 ppm.

Metin carbon appeared at the chemical shift area of δ_H 18,20; 19,20; 68,70; 70,39; 70,79 ; 74,79 and 74,25 ppm.

Confirmation of the data in the 2 dimensional NMR spectrum was conducted using ^{1}H-^{1}H COSY (*Correlation Spectroscopy*). Data in the spectrym COSY for isolate F3.2.1 showed that protin in methyl group (H-1) was related with metin proton (H-1) and there is a relationship between proton of methylen group (H-1') and proton of the nearby metin group (H-4') and (H-5').

Analysis of mass spectroscopy (MS) was conducted using Impact Electron (IE) pattern. Data of the spectrum EI-MS for the isolate F3.2.1 shoed ion molecule at the molecular weight (BM) of 191 m/z based on the assumed mocular weight. The peaks of 191 and 169 m/z were the fragments of mother ion molecules for the isolate F3.2.1 of the marine sponge *Cinachyrella* sp.

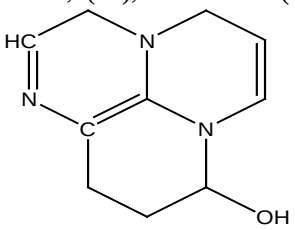
All of the UV and IR measurements and chemical shift of ^{13}C-NMR and ^{1}H NMR, types and number of carbons of the isolate F3.2.1. in CD_3OD are displayed in the Table 4.10. As a comparison for the molecular structure assumption for the active isolates F3.2.1 of marine sponge *Cinachyrella* sp, library study revealed some structures and compound molecular formula of the sponge genus *Cinachyrella* (Table 1).

Table 1 Measurement of IV, IR, chemical shifts 13C-NMR and 1H NMR, types and number of carbons of the isolate F3.2.1 in CD3OD and molecular weight of (BM) 191,182 m/z

No	UV (nm)	IR Absorption (cm^{-1})	^{1}H NMR	Multiplicity	^{13}C NMR	Carbon type
1	200,50	3405 (Hydroxy OH group)	0,95	d	13,77	CH_3
2	210,50	1058 (Hydroxy OH group)	1,00	d	18,20	CH
3	219,50	1718 CO	1,01	k	19,20	CH
4	251	2934 (CH group of CH_3)	2,00	s	19,80	CH_3
5		2976 (phynylic but not aromatic C bind)	3,30	k	68,70	CH
6			3,32	k	70,39	CH
7			3,43	s	70,79	CH
8			4,30	s	74,79	CH
9					74,20	CH
10					170	C=O

Note : s=singlet; k=quartet; d=duplet

Based the analysis of IR, UV, and NMR spectroscopic data and structural comfirmation analysis using EL-MS, it could be proposed that chemical structure of the isolate F3.2.1 belongs to the cinachyramine derivative group with molecular formula of C10H13N3O and structural name of 1,4,9-triazatricyclo [7,3,1,0]trideca-3,5(13),10-trien-8-ol (Figure 2).



$C_{10}H_{13}N_3O$
Exact Mass: 191.11
Mol. Wt.: 191.23
m/e: 191.11 (100.0%), 192.11 (11.0%), 192.10 (1.1%)
C, 62.81; H, 6.85; N, 21.97; O, 8.37

1,4,9-triazatricyclo[7,3,1,0]trideca-3,5(13),10-trien-8-ol
Figure 2 Molecular struture of isolate F3.2.1

Cinachyramine Trifluoroacetatic salt in the sponge *Cinachyrella* sp. Collected in Okinawan, Japan, is a novel alkaloid with phenanthren and aminal unit if C_9. The molecular structure was $C_{10}H_{18}N_4$ with 1*N*-methyl, 7-methylene, and 1-metin. *Cinachyramine* Trifluoroacetate in acid condition was not stable[16]. resulted data should be well presented by the form of schemes, figures, graphs, tables, reactions and equations. These items should be numbered clearly.

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