Research Article

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BIOACTIVITY A POLY HYDROXYL ISOCOPALANE FROM *CALLYSPONGIA* SP. AS ANTIBACTERIAL RESISTANT *ESCHERICHIA COLI*

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ABSTRACT:

Bioactivity research from Indonesian's marine sponge Callyspongia sp. has been done. Bioactive metabolite diterpene was isolated based on bioassay guided fractionation with several steps chromatography. The interpretation LCMS-ESI data showed 412.576 m/z with molecular formula $C_{24}H_{44}O_5$. Interpretation with FTIR data showed bioactive metabolite has eter C-O functional group at 1261 cm^{-1} and hydroxyl functional group O-H at 3419 cm^{-1}. Interpretation with ^{1}H NMR and ^{13}C NMR indicated that metabolite active compound as a poly hydroxyl isocopalane. The antibacterial activity of isolate compound has been conducted against Escherichia coli resistant. The result antibacterial activity showed that isolate compound activity is strong at concentration 0,06□M with inhibition zone 28 mm (dia). Furthermore, diameter ratio of inhibition zone against chloramphenicol at 0,09□M (dia. 9 mm) showed the significant different. The result showed that poly hydroxyl isocopalane has a potential activity against Escherichia coli which resistant to chloramphenicol.

KEYWORDS:

Bioactivity; marine sponge; antibacterial resistant; Escherichia coli.

INTRODUCTION

Escherichia coliis one of the most common causes of morbidity and mortality humans in the world[1]. 8.6 million deaths from Escherichia coli was estimated that each year[2]. The search for a new drug with the development of screening methods has been done to fight the bacteria, especially bacteria resistant.[3-5]

In recent years the chemical research focused on the sponge and has been widely reported to be used in the pharmaceutical. Some research sponge containing diterpene compound has various activity such as antibacterial.[6-9]

Study on antibacterial compound resistant limited. In this paper has done the isolation of compounds from marine sponges and tested the bioactivity against resistant bacteria Escherichia coli that are resistant to chloramphenicol.

MATERIAL AND METHODS

Chemicals and reagents

n-Heksana, n-Butanol, DCM, $CHCl_3$, EtOH, MeOH, H_2O, NP-SiO_2, RP-C18, cerium sulfate reagent, ninhydrin reagent.

Sponge specimen

The sponge taken in Sabang, Indonesia. Deposit sponge taken, stored and analyzed in UPT LaboratoriumTerpadudan Sentra InovasiTeknologi (Indonesia).

Mycobacterial specimen

Mycobacterial resistant Escherichia coli taken from RSUD Abdul Moeloek's patient and cultured in UPT LaboratoriumTerpadudan Sentra Inovasi Teknologi (Indonesia).

Mycobacterial Growth Media

Strainof mycobacterial resistant Escherichia coliwere growninNutrient Agarmedium (NA) at temperature 37°C.[10]

Figure 1. Callyspongia sp.

General Procedure:

Extraction and Isolation

Crude Sponge (15 gr) was partitioned using *n*-Heksana : H_2O (1:1 v/v). With the result thatobtain *n*-Heksana extract (0,18 gr) and polar extract (14,82 gr). Furthermore polar extract was partitioned again using DCM (1:1 v/v) and evaporated. The result obtain DCM extract (0,62 gr) and polar extract (14,2 gr). Polar extract (14,2 gr) fractionated using RP-C18 column chromatography and eluted using H_2O and MeOH (H_2O and MeOH 100 %).

The fractionation result from MeOH extract (2,85 gr) fractionated again using NP-SiO_2 column chromatography and eluted using $CHCl_3$: EtOH gradient start from 8:2, 7:3, 5:5, 3:7, and EtOH 100%. $CHCl_3$:EtOH gradient fraction (5:5) set as the target compound and tested bioactivity against mycobacterial resistant *Escherichia coli*.

Antibacterial Screening

Antibacterial test using diffusion method with *Nutrient Agar* medium (NA) was added to mycobacterial resistant *Escherichia coli*, added 2% DMSO as a negative control, the active compound and chloramphenicol as a comparison.[10]

Detection Methods

Data from MS-ESI were measured using LCMS-ESI Mariner. Spectrum data from functional group were measured using FTIR Shimadzu Prestige 21.Spectrum data from ^1H dan^{13}C NMR were measured using Jeol 500 MHz (CD_3OD; H_2O).

RESULTS AND DISCUSSION

Identification of Structure

A poly hydroxyl isocopalane compound known to have the molecular formula is $C_{24}H_{44}O_5$ that determined by LCMS-ESI with molecular weight is $[M+H]^+$ 412.576 m/z and this compound has *Double Bond Equivalent* (DBE) value is 3. Result from FTIR spectrum showed functional groups from C–O ether at 1261 cm^{-1}, C–H methyl CH_3at 2854 cm^{-1}, C–H methylene CH_2at 2925 cm^{-1} and O–H at 3419 cm^{-1}.

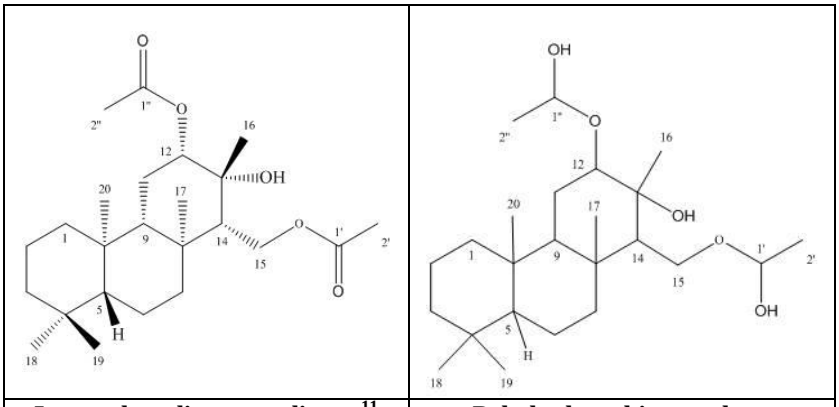
^1H NMR spectrum (Table 1) showed seven methyl CH_3 signals (δ_H = 0.82, 0.84, 0.85, 1.12, 1.15, 1.17, 1.49 ppm). Proton signals for CH-OH hydroxyl functional groups (δ_H = 4.74, 4.81 ppm) as secondary alcohol). According to data obtained, this compound indicate as labdanediterpene skeleton as has been reported.[11-12]

^{13}C NMR spectrum (Table 1) showed seven methyl CH_3 signals (δ_C = 12.6, 21.9, 25.2, 27.8, 29.3, 43.9, 45.4 ppm), seven aliphatic methylene CH_2 signals (δ_C = 15.4, 19.6, 25.2, 36.7, 40.1, 41.2, 57.7 ppm), one oxygenated quaternary carbon signal (δ_C = 75.7 ppm), two quartenary carbon signals (δ_C= 34.1, 37.7 ppm) which indicated the presence of hydroxyl groups (δ_C = 43.9, 45.4 ppm).[11-12] Based on interpretation data and reference, this compound as a poly hydroxyl isocopalane compare with reference structure (Figure 2).[11]

Table 1.^{1}H NMR (500 MHz, CD_3OD; H_2O) and ^{13}C NMR (500 MHz, CD_3OD)

Position	Reference*	V11B30	Reference*	V11B30	Reference*	V11B30
	δ_C		δ_H		Mult. (*J*), int	
1	40.5 (t)	36.7 (t)	1.57	1.56	m, 1H	m, 1H
			0.69	1.3	m, 1H	m, 1H
2	18.9 (t)	19.6 (t)	1.32	1.46	m, 2H	m, 1H
				1.53		
3	42.3 (t)	41.2 (t)	1.09	1.06	m, 1H	m, 1H
			1.34	1.29	m, 1H	m, 1H
4	33.5 (s)	34.1 (s)				
5	57.2 (d)	55.9 (d)	0.73	0.69	m, 1H	m, 1H
6	18.7 (t)	15.08 (t)	1.48	1.62	m, 1H	m, 1H
			1.5	1.64	m, 1H	m, 1H
7	39.1 (t)	40.1 (t)	1.3	1.33	m, 1H	m, 1H
			1.53	1.56	m, 1H	m, 1H
8	38.7 (s)	35.2 (s)				
9	52.6 (d)	45.5 (d)	1.35	1.36	m, 1H	m, 1H
10	38.0 (s)	37.7 (s)				
11	23.7 (t)	25.2 (t)	1.76	1.67	m, 1H	m, 1H
			1.8	1.82	m, 1H	m, 1H
12	78.3 (d)	78.8 (d)	5.01	3.05	m, 1H	m, 1H
13	74.6 (s)	75.7 (s)				
14	58.0 (d)	57.4 (d)	1.44	1.46	m, 1H	m, 1H
15	62.8 (t)	57.7 (t)	4.27	2.71	m, 1H	m, 1H
16	27.2 (q)	27.8 (q)	1.12	1.12	s, 3H	s, 3H
17	26.1 (q)	25.2 (q)	1.41	1.49	s, 3H	s, 3H
18	21.7 (q)	21.9 (q)	0.80	0.82	s, 3H	s, 3H
19	33.6 (q)	29.3 (q)	0.83	0.85	s, 3H	s, 3H
20	16.6 (q)	12.6 (q)	0.82	0.84	s, 3H	s, 3H
1'	169.7 (s)	45.4 (d)		4.74		m, 1H
2'	20.6 (q)	15.4 (q)	1.72	1.17	s, 3H	s, 3H
1"	169.3 (s)	43.9 (d)		4.81		m, 1H
2"	20.6(q)	15.4 (q)	1.65	1.15	s, 3H	s, 3H

*** ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data (C_6D_6).[11]**



Isocopalanediterpene diester[11] | **Poly hydroxyl isocopalane**

Figure 2.Poly hydroxyl isocopalane compare with reference structure

Bioassay

A Poly hydroxyl Isocopalane compound containing bioactivity as antibacterial resistant for gram negative mycobacterial (mycobacterial *Escherichia coli* resistant) (Figure 3). The compound exhibit biological activity at concentration 0,06µM (Table 2).

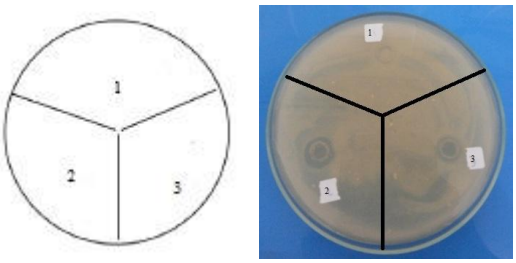


Figure 3. Bioactivity of poly hydroxyl isocopalane

Table 2. Inhibition zone data

Nama Sampel	Concentration	Inhibition zone (mm)
1. DMSO 2 %	-	-
2. Poly hydroxyl isocopalane	25 µg/mL	28
3. Chloramphenicol	30 µg/mL	9

CONCLUSIONS

Test antibacterial activity against resistant *Escherichia coli* showed activity at concentration 0,06µM with inhibition zone 28 mm (dia). The result indicate that poly hydroxyl isocopalane have activity against Escherichia coli resistant to chloramphenicol with inhibition zone diameter ratio of the chloramphenicol at concentration 0,09 µM with inhibition zone 9 mm (dia) showed a significant difference.

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