

**ANTIFUNGAL POTENTIAL TEST OF GLYCOSIDE COMPOUND FROM
ROOT WOOD OF *Pterospermum subpeltatum* C. B. ROB**

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Abstract

A steroidal compound, 3-O- β -glucopyranosyl- β -cytosterol is one of steroidal from chloroform fraction of root wood of *Pterospermum subpeltatum* C. B. Rob (Sterculiaceae). The structure of compound was determined based on IR, ¹H, dan ¹³C-NMR spectra. This compound active against *Artemia salina* with the toxicity of LC₅₀ 160,93 μ g/mL and it also active as antifungal against *Candida albicans* with inhibition diameter 11,0 mm

Keywords: 3-O- β -glukopiranosil- β -sitosterol, steroid, *Pterospermum subpeltatum*, antifungal

1. Introduction

Poor sanitary conditions and weather are often characterized by very high temperatures and humidity can cause infections in wounds. It generally occurs in tropical developing countries. synthetic antibiotic therapy is not always possible due to high cost. To solves this problem people use plants that grow in their area as a traditional medicine, even without scientific support.

Indonesian traditional medicines from plants have been widely used as a form of treatment. Through intensive research has been known the bioactive compounds, which are secondary metabolites compound in these plants. For example, derivatives compounds of cassane furano diterpen and norcassane from the seeds of Bagore (*Caesalpinia crista*, Linn) as an antimalaria. These bioactive compound can be further developed through the modification of the molecular groups or bonds contained in the compound become more active or more secure and can be used as precursor in guiding us to find new drugs (Attamimi, 2004).

Chemical investigation of the cinchona tree was first reported by French researchers Caventou and Pelletier (Cragg, in Ahmad, 2007) and further investigations have been found more than 30 classes of chemical compounds different alkaloids including quinine and quinidine, each of which has been used for the treatment of malaria disease and cardiac disorders (Ahmad, 2007). The results of the investigation consistent with the use of cinchona bark *Cinchona sp.* by people in the Amazon, South America for the treatment of malaria.

In Indonesia, the development of drugs from natural ingredients have great potential because of the tropical climate. Tropical plants are believed have the ability to manipulate diverse chemical compounds which have various interesting bioactivity. The ability of one caused by a self-defense mechanism against the environment. In general, these plants live under the harsh environmental conditions both climatic factors and disorders of herbivore, insects, and diseases. Tropical plants can produce natural chemical compounds that potentially as an insecticide and

anti-fungi. As an example, *Sterculia africana* containing of antifungal compounds (Hamza et al., 2005), *Azardirachta indica* as insecticides (Ahmad, 2007).

One of Indonesian tropical plant is Sterculiaceae, which is one of a quite large family, consisting of 70 genus and about 1500 species (Gressier et al., 2008). The genus *Pterospermum* also included in the family Sterculiaceae are efficacious as medicinal plants such as the bark of *P. javanicum* to treat dysentery, toothache, boils, and sprains.

Leaves of *P. diversifolium* used to reduce itchy and skin medicine. The roots of this plant are used as fish poison (Ogata et al., 1995). According to Heyne (1987), leaves of *P. acerifolium* also used as an itchy medicine in Central Sulawesi.

Camporese (2003) reported on the antibacterial activity of hexane extract and methanol extract of stem bark of *Guazuma ulmifolia* (Sterculiaceae). n-hexane extract can inhibit the growth of *E. coli*, while the methanol extract inhibited the growth of *Pseudomonas aeruginosa*. Reid et al. (2005) studied the antibacterial activity of various extracts of *Cola greenway* and reported that the ethyl acetate fraction actively inhibit the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus*.

In this study reported the bioactivity, isolation, and structural determination of 3-O- β -glucopyranosyl- β -cytosterol from chloroform fraction of *Pterospermum subpeltatum* root wood. The molecular structure of the compound was determined based on the interpretation of spectroscopic data which include IR, ^1H , dan ^{13}C -NMR spectra.

2. Research Method

2.1. General

The melting point was determined with Fisher John melting point apparatus. The infrared spectra obtained with Perkin Elmer FTIR spectrophotometer, while ^1H (500 MHz) and ^{13}C (125 MHz) NMR obtained with Jeol spectrophotometer.

2.2. Materials

The material used is the root wood of *Pterospermum subpeltatum* C. B. Rob, obtained from Mamuju, West Sulawesi and had been determined in Herbarium Bogoriene, Biological Research and Development Center, LIPI Bogor.

2.3. Isolation and Purification

2.3.1. Extraction and Isolation

10 kg of dry weight root wood *Pterospermum subpeltatum* C. B. Rob mashed then maceration with methanol for 1 x 24 hours for several times. Maserat obtained was evaporated until 3 L of thick maserat obtained with dry weight of 1,047 kg. The maserat further partitioned with solvents. Chloroform fraction (35.7 kg) was fractionated by vacuum column chromatography (VCC) with eluent n-hexane, ethyl acetate: n-hexane, ethyl acetate, acetone: ethyl acetate, acetone, and methanol, with increasing its polarity.

Merger fractions obtained were monitored by TLC yielded eight major fractions. Fraction B7 generating 25 g cream-colored floured compound, the test results showed a steroid group with melting point 284-285°C

2.3.2. Biological Test

Toxicity tests performed using baby shrimp *Artemia salina* corresponding with the method of Meyer et al, and anti-microbial testing done by using Nutrient Agar method.

3. Result and Discussion

Compound 1 obtained as cream-colored powder, with melting point 284 - 285°C. IR (KBr) showed an absorption band in 3377 cm^{-1} wavelength indicated the existence of free OH group supported by the peak at 1070 cm^{-1} for the stretching of C – O. Other absorption at 2954 and 2931 cm^{-1} for aliphatic C- H that supported by peaks at 1463 cm^{-1} (CH_2) and 1367 cm^{-1} (CH_3).

Analysis of ^{13}C -NMR spectroscopy data, showed 35 signals with the degree of protonation determined through DEPT-135 experiments. Twenty nine carbon signals that consist of 6 methyl at δ 11,7 (C-18), 18,9 (C-19), 18,6 (C-21), 19,8 (C-26), 19,1 (C-27), and 11,8 (C-29) ppm; 11 methylen at δ 36,8 (C-1), 29,3 (C-2), 28,6 (C-4), 31,4 (C-7), 20,6 (C-11), 39,2 (C-12), 23,9 (C-15), 27,8 (C-16), 33,3 (C-22), 25,4(C-23), and 22,6 (C-28) ppm; 9 methyne at δ 76,9 (C-3), 121,3 (C-6), 31,4 (C-8), 49,6 (C-9), 56,2 (C-14), 55,4 (C-17), 35,5 (C-20), 45,1 (C-24), and 28,7 (C-25) ppm, and 3 quaternary carbon at δ 140,4 (C-5) 36,2 (C-10), and 41,9 (C-13) ppm, form a steroid skeleton steroid types cytosterol. While 6 other carbon signals consisting of 5 methyne at δ 100,8 (C-1), 73,5 (C-2), 76,8 (C-3), 70,1 (C-4), and 76,8 (C-5) ppm; and 1 methylen at 61,1 ppm (C-6), form a monosaccharide skeleton types glucopyranoside.

Analysis of ^1H -NMR spectroscopy data, compound 1 showed some peculiar signals, those are at δ 5,32 ppm (H-6, 1H, brd, J=5,0 Hz) that showed an alkene proton are influenced by two vicinal protons with each position at σ 1,93 ppm (H-7 $_{\alpha}$, 1H, m) and δ 1,52 ppm (H-7 $_{\beta}$, 1H, m) at δ 3,46 pp (H-3, 1H, tt), J=6,7 and 11,6 Hz) that showed the methyne proton binding the oxy group, and at the δ 0,65 ppm (H-18, 3H, s) and δ 0,92 ppm (H-19, 3H, s) each showed methyl proton that bound to a quaternary carbon. The proton signals indicate that steroid skeleton substituted by two methyl groups and one oxy group. At the aliphatic region there are also two groups of signals, which are the group which indicated an alkane unit, at δ 1,29 ppm (H-20, 1H, m); δ 0,89 ppm (H-21, 3H, d, J=6,8 Hz); δ 1,32 pm (H-22 $_{\alpha}$, 1H, m), and δ 0,97 (H-22 $_{\beta}$, 1H, m); δ 1,13 ppm (H-23, 2H, m); δ 0,93 ppm (H-24, 1H, m); δ 1,62 ppm (H-25, 1H, okt, J=6,8 Hz); δ ,81 ppm (H-26, 3H, d, J=6,8 Hz); δ 0,79 ppm (H-27, 3H, d, J=6,8 Hz); δ 1,23 ppm (H-28, 2H, m); and δ 0,82 pp (H-29, 3H, t, j=6,7 Hz), and signals that indicate a unit of glucopyranosyl, include at δ 4,21 ppp (H-1, 1H, d, J=8,0 Hz); δ 2,88 ppm (H-2, 1H, td, J=8,0 and 5,0 Hz), δ 3,11 ppm (H-3, 1H, td, J=8,5 and 3,7 Hz), δ 3,0ppm (H-4, 1H, td, J=8,5 and 5,0 Hz), δ 3,06 ppm (H-5, 1H, ddd, J=9,5; 6,0 and 1,8 Hz), δ 3,64 ppm (H-6 $_{\alpha}$, 1H, dd, J10,5 and 6,0 Hz) and 3,39 ppm (H-6 $_{\beta}$, 1H, m). The relation of bonding in the structure was evidenced by long distance correlation of ^1H - ^{13}C from HMBC spectrum. HMBC spectrum showed a long distance correlation between the signal of proton and the peculiar carbon, that is at δ 4,21 ppm (H-1') with carbon at δ 76,7 ppm (C-3); δ 0,65 ppm Me-18) with δ 36,2 ppm (C-10); δ 0,92 ppm (Me-19) with δ 41,9 ppm (C-13); δ 0,89 ppm (Me-21) with δ 55,4 ppm (C-17); and δ 0,81 ppm (Me-26) and δ 0,27 ppm (Me-27) with δ 28,7 ppm (C-25), this indicates that the position of O-piranosil group at C-3 and each successive methyl carbon at position C-18, C-19, C-21, C-26 and C-27. HMBC correlations of compound 1 is shown in Figure 1. Data of ^1H and ^{13}C NMR spectroscopy (1D and 2D) of compound 1 is shown in Table 1. Based on analysis data above, compound 1 can be summarized as 3-O- β -glucopyranosyl- β -cytosterol. NMR data of compound 1 has similarities with derivative stigmasterol compounds previously reported (Alam M. S., 1995).

Toxicity test against baby shrimp *A. salina* show the value of $\text{LC}_{50} = 16,93 \mu\text{g/mL}$ and active against fungi *C. albicans* with inhibition diameter 11 mm

Table 1. ^1H , ^{13}C , and 2D NMR Spectrum of Compound 1

No.	δ_{H} (multi, J in Hz)	δ_{C}	δ_{C} (lit)	COSY H \leftrightarrow H	HMBC C \leftrightarrow H
1	1,78 (1H, <i>m</i>) 0,99 (1H, <i>m</i>)	36,8	37,1	2	18
2	1,80 (1H, <i>m</i>) 1,51 (1H, <i>m</i>)	29,3	28,2	1,3	-
3	3,46 (1H, <i>tt</i> , $J = 6,75$ & $11,65$ Hz)	76,7	79,8	2,4	1'
4	3,46 (1H, <i>br d</i> , $J = 3,05$ & $10,55$ Hz) 3,46 (1H, <i>br t</i> , $J = 11,60$ Hz)	38,3	39,7	3	-
5	-	140,3	140,4	-	18
6	5,32 (1H, <i>br d</i> , 5,0 Hz)	121,1	121,8	7	-
7	1,93 (1H, <i>m</i>) 1,52 (1H, <i>m</i>)	31,4	31,8	6, 8	-
8	1,41 (1H, <i>m</i>)	31,4	31,8	7, 9,	-
9	0,86 (1H, <i>m</i>)	49,6	50,1	14	18
10	-	36,2	36,6	8, 11	18
11	1,38 (1H, <i>m</i>)	20,6	21,0	-	-
12	1,97 (1H, <i>m</i>) 1,16 (1H, <i>m</i>)	39,2	39,7	9, 12 11	19
13	-	41,9	42,2	-	19
14	0,98 (1H, <i>m</i>)	56,2	56,7	-	19
15	1,50 (1H, <i>m</i>) 1,02 (1H, <i>m</i>)	23,9	24,1	8, 15 14,16	-
16	1,79 (1H, <i>m</i>) 1,29 (1H, <i>m</i>)	27,8	28,2	15, 17	-
17	1,05 (1H, <i>m</i>)	55,4	56,0	-	19,21
18	0,65 (3H, <i>s</i>)	11,7	11,7	16, 20	1, 5, 9, 10
18	0,92 (3H, <i>s</i>)	18,9	19,2	-	12, 13, 14, 17,
20	1,29 (1H, <i>m</i>)	35,5	36,0	-	20
21	0,89 (3H, <i>d</i> , $J = 6,75$ Hz)	18,6	18,7	17, 21,	19, 21
22	1,32 (1H, <i>m</i>) 0,97 (1H, <i>m</i>)	33,3	33,8	22 20	17, 20, 22 21
23	1,13 (2H, <i>m</i>)	25,4	26,0	20, 23	-
24	0,93 (1H, <i>m</i>)	45,1	45,7	-	-
25	1,62 (1H, <i>okt.</i> , 6,75 Hz)	28,7	29,1	22, 24	29
26	0,81 (3H, <i>d</i> , $J = 6,75$ Hz)	19,8	19,7	23, 25,	26, 27
27	0,79 (3H, <i>d</i> , $J = 6,75$ Hz)	19,1	18,9	28	25, 27
28	1,23 (2H, <i>m</i>)	22,6	23,0	24, 8,	25, 26
29	0,82 (3H, <i>t</i> , $J = 6,75$ Hz)	11,8	11,9	27	29
1'	4,21 (1H, <i>d</i> , 8,0 Hz)	100,6	-	25	24, 28
2'	2,88 (1H, <i>td</i> , 8,0 & 5,0 Hz)	73,3	-	25	3
3'	3,11 (1H, <i>td</i> , 8,5 & 3,7 Hz)	76,6	-	24, 29	-
4'	3,00 (1H, <i>td</i> , 8,5 & 5,0 Hz)	69,9	-	28	-
5'	3,06 (1H, <i>ddd</i> , 9,5; 6,0 & 1,8 Hz)	76,6	-	2'	-
6'	3,64 (1H, <i>br dd</i> , 10,5 & 6,0 Hz) 3,39 (1H,*)	60,9	-	1', 3' 2', 4' 3', 5' 4', 6' 5'	- - - -

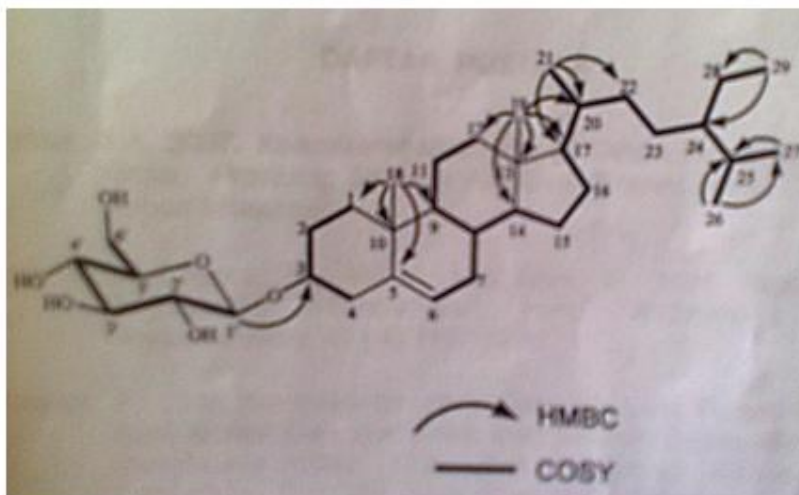


Figure 1. 3-O-β-glucopyranosyl-β-cytosterol

4. Conclusion and Suggestion

The compound of 3-O-β-glucopyranosyl-β-cytosterol have been found for the first time from *P. subpeltatum* and potential as an antifungal with the inhibition diameter 11 mm against *Candida albicans* and also active against *Artemia salina* LC₅₀: 160,93 μg/mL. Clinical trials of compounds found in plants such *P. subpeltatum* based activities, to be developed and can be biosynthesized.

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