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Test of Dihidroisocoumarin Activity Against Murine Leukemia Cells P-388 from the Stem Bark Extract of *Shorea Singkawang* (Miq) .Miq

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***Abstract*— Has succeeded in isoalsi elegat acid compounds from the stem bark of dichloromethane fraction Shorea Singkawang (Miq) .Miq and identified as dihidroisocoumarin or bergenin, based on the data of UV spectroscopy, IR, GC-MS and ^{1} H NMR and ^{13} C NMR, and. This compound is a derivative elegat acid that was first discovered in this plant, bioassay cytotoxic activity using P-388 cells. With the value of IC_{50} = 25, 33 lm / mL.**

***Keywords*— Shorea Singkawang (Miq) .Miq; elagat acid, dihidroisocoumarin; Murin leukemia cells P-388; IC 50**

I. INTRODUCTION

Dipterocarpaceae is relatively large family of plants comprising 16 genera and 600 species, timber producers have high economic value and is known by the name of "meranti, keruing or camphor". Meranti wood and wood keruing for example, is a kind of high-quality timber, because it is resistant to termites or other insects [7]. The three main genera of the dipterocarp family: Shorea (Meranti) is the largest genus has about 165 species, Hopea (merawan) has 100 species, dipterocarpacus (keruing) has 75 species, in Indonesia there are 79 types of shorea these species and 56 species of the spread in Borne. In addition, 27 species of which are endemic to Indonesia, on the island of Sumatra about 20 species, for there are two types of Jambi Province shorea is endemic to the eastern part of the Sumatran Sumatrana shorea (yellow meranti) and Shorea singkawang (Miq) .Miq (red meranti), Shorea or meranti wood species there are divided white meranti, yellow meranti and red meranti. Chemical content of plants is very diverse covering dipterocapaceae phenolic groups, such as oligostilbenoid, flavonoids, phenyl propanoid and penolat acid derivatives, as well as non-phenolic groups namely steroids, terpenoids and triterpenoids [8]. Some compounds of this shorea plant known to have activity as an antifungal [3], anti-HIV [2], antibacterial [20], antiinflammatory [14], antitumor [13][23], and as 5α-reductase inhibitors [12]. Phytochemical research has been done on Shorea singkwang (Miq) .Miq indicates that the main chemical constituents of this species is the phenolic, flavonoids, coumarins, steroids, terpenoids, and saponins In this article reported the results of an insulating compound coumarin class of dichloromethane extract of the stem bark of Shorea singkawang (Miq) .Miq namely dihidroisocoumarin alegat acid derivative known as bergenin. Additionally discovered also cytotoxic properties of these compounds against murine leukemia cells P-388. Phytochemical studies and cytotoxic compounds dihidroisocoumarin from plants Shorea singkawang (Miq) .Miq is the result of research that has not been previously reported.

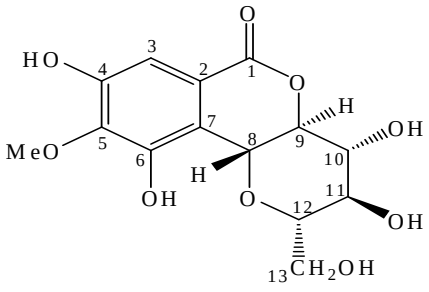

Fig.1. Dihidroisokumarin

II. EXPERIMENT

The melting point is done with mico asparatus melting point, UV and IR spectrum measured respectively with Pharmaspee 1700 spectrophotometer (Shimadzu), an infrared spectrophotometer (Spectrum One FT-IR) spectrum JEOL 125 MHz ^{1}H NMR and ^{13}C NMR JEOL 500 MHz using TMS as standard, vacuum liquid chromatography

column diameter 3 cm and height 20 cm, Chamber, distillation equipment, analytical TLC using Kieselgel 60 GF254 TLC plates of 0.25 mm (Merck) were used solvents are distilled all of the technical quality.

A. Plant materials

Plant materials such as bark of Shorea singkawang (Miq) .Miq, plant material derived from the community garden village Rantau Panjang subdistrict Seling Marangin Jambi district. Specimens of this plant has been inspected and stored in the Herbarium of Biological Science, University of Andalas

B. Extraction and isolation.

Stem bark of Shorea singkawang (Miq) .Miq been refined (6.0 kg) .Miq macerated with methanol (20 l) 3 x 24 hours. After the solvent was evaporated at low pressure extracts obtained as much as 380 gr thickened, red-brown colored residue form. Then 250 grams of condensed methanol extract successively performed fractionation using an organic solvent n-hexane, dichloromethane and ethyl acetate and evaporated the solvent n-hexane fraction obtained 15 g, dichloromethane fraction (DCM) 6 g and 8 g of ethyl acetate fraction, faction residual 216.8 gr.

Vacuum liquid chromatography on 4 g fraction of dichloromethane done gradually using silica gel G 60 GF 254 as the stationary phase and eluent a mixture of chloroform, n-hexane, using the gradient eluent (n-hexane, a mixture of n-hexane-chloroform = 9: 1- 8: 2 and 7: 3 and 6: 4, 5: 5 and methanol) are performed in a gradient. Based on the results of monitoring by thin layer chromatography (TLC) 81 vials can be combined into 5 sub-fractions combined consecutive F1 vial 1-4), 5-6 (F2) (F3), 7-14 (F3), 15-2 (F4) and 28-81 (F5)., F3 (vials 7-14) in back by gravity column chromatography (KKG) (silica gel 60 -230 mesh) eluted in isocratic (mixture of n-hexane -kloroform = 8: 2) resulted in two fractions F1 and F2, F2 fraction was purified by recrystallization techniques obtained white amorphous compound 1 (20 mg).

Compound (1), white amorphous, mp: 256-258 OC, UV (MeOH) λ_{ma} fractionation x 223, 274, 320 nm, (MeOH + NaOH); IR (KBr) ν_{max} 3427, 2360, 1708, 1465, 1382, 1063, 1053, and 1022; 1H NMR (500 MHz) seen in Table 1; 13 C NMR (125 MHz, CDCl3) see table 2.

III. DISCUSSION

Compound 1 was obtained in the form of a white amorphous with mp 256-258 OC. IR Spectrum UV dn spekrum UV (MeOH) showed absorptions at λ_{max} benzoyl system (log) at λ_{max} 223, 274, and 320 nm, showing characteristic coumarin group [7], while the IR spectrum (KBr) showed the presence of bands of absorption for a hydroxyl group (3427 cm^{-1}) aliphatic CH (2934, 2360 cm^{-1}), C = O terkelasi carbonyl group (1708 cm-1), aromatic (1465, 1382 cm-1), and CO (1063, 1053 and 1022 cm-1). UV and IR spectrum is characteristic for elegat acids, with a molecular formula of C_{14} H_{16} O_9 [7].

1H NMR analysis of the data shows that there is a set of signals and six aliphatic protons at δ oksikarbon 3, 54 (1H, dd, J = 8.7 and 9.5 Hz-H11), 4.20 (1H, dd, J = 6, 9 and 11, 8 Hz, H13eq), 4.07 (dd, J = 8.7 and 9.5 Hz 10) 5.71 (1H, d, J = 104, H-8), 4.72 (1H , dd, J = 9.5 and 10.4, H-9), 3.73 (1H, m, H-12), 3.95 (1H, DDJ 6.9 dn = 11.8, H-13 ax), 4.19 (1H, s, OCH3), which is typical of a system glukopiranosil [7], ^{1}HNMR spectrum of compound 1 showed a singlet for the aromatic protons at δ 7.61 (1H, s, H-3), and a singlet for the methoxy group at 4.19 (1H, s, OCH_3), and aromatic syste substitution ms at positions 1,3,4,5, and 6, and the aromatic protons bound in the second. 1H NMR Based on this analysis and is shown in figure 1, it can be concluded that compound 1 is dihidroisocoumarin with molecular formula C_{14} H_{16} O_9. To prove the ^{13}C NMR spectrum of existence of four carbon atoms aksimetin signal at -10 C (76, 0), C-12 (83.4), C-8 (74.30 and C-9 (81.5) and signal oksimetilen carbon atom C-13 (62.8) [7] .

Further prove the structure of compound 1 was obtained ^{1}H NMR data comparison and ^{13} CNMR dihidroisocoumarin compounds similar to previously reported data for compounds dihidroisocoumarin [7] can thus be concluded that the compound known as dihidroisokumarin bergenin, which is the first discovery of Shorea singkawang (Miq).Miq.

In the cytotoxicity test using murine cells leukimi P-388 were cultured according to the protocol as described previously, the compounds showed dihidroisocoumarin IC50=25,33µm / mL and toxicity tests using fry shrimp Artemia sativa, following the way [22]. Dihidroisocoumarin compounds showed him LC50=66,68µm / mL, but it has been reported previously that dihidroisocoumarin compounds also found in, among others, on Shorea stenoptera [7] Shorea seminis [1] and Shorea robusta [12] is anti -HIV and antihepatotoksik , Shorea sumatrana Lyn is antibacterial [26]

TABLE I
^{1}H-NMR CHEMICAL SHIFT OF THE ISOLATED COMPOUND WITH REFERENCE DIHIDROKISOCOUMARIN

position	δ, ppm	Description	Amount H
H-3	7,61	Singlet	1H
H-8	5,71	d, J = 10,4	1H
H-9	4,72	dd, J = 9,5 dan 10,4	1H
H-10	4,07	dd, J = 8,7 dan 9,5	1H
H-11	3,54	dd, J = 8,7 dan 9,5	1H
H-12	3,73	multiplet	1H
$H\text{-}13_{(eq)}$	4, 20	dd, J = 1,9 dan 11,8	1H
$H\text{-}13_{(ax)}$	3,95	dd, J = 6,9 dan 11,8	1H
OCH_3	4,19	singlet	3H (OMe)

TABLE III
^{1}H-NMR SPECTRA AND ^{13}C NMR OF COMPOUND 1 IN ACETONE-D6 AND ^{1}H AND ^{13}C NMR DIHIDROISOCOUMARIN REFERENCE (H.EIUS.H ET AL., 2003)

No	Compounds 1		Dihidroisocoumarin References	
	^{13} CNMR ^{1}	^{1}H NMR	^{13} CNMR ^{1}	^{1}H NMR
1	166,2	-	165,7	-
2	120,0	-	119,4	-
3	110,4	7,61 (s)	111,1	7,09 (s)
4	153,1	-	152,4	-
5	142,0	-	142,3	-

6	149,8	-	149,5	-
7	117,0	-	117,3	-
8	74,3	5,71 d	74,3	4,96(d)
9	81,5	4,72 dd	81,4	4,06 (dd)
10	76,0	4,07 dd	75,6	3,81(dd)
11	71,3	3,54 (dd)	71,9	3,43 (dd)
12	83,4	3,72 (m)	83,1	3,66 (m)
13	62,8	4,20 eq dd 3,95 ax dd	62,7	4,03 eq, (dd) 3,69ax (dd)
OMe	60,9	4,19 (s)	60,9	3,90

Based on the spectrum analysis of ^{1}H NMR and ^{13}CNMR, DEPT experiment, this compound and compare the spectrum with ^{1}H NMR and ^{13}C NMR spectrum of this compound by ^{1}H NMR and ^{13}CNMR compounds reported [7][21] in table 3. These compounds have been found previously by Naseer [21] from the stem bark of Hopea Sangal, but did not report its activity, whereas in 2005, the Judge also been reported that these compounds are found from the bark of Dipterocarpus Retusus Blume, these compounds have weak activity against cell cytotoxicity murine leukemia P-388 and weak cytotoxicity against Artemia salina shrimp fry.

Cytotoxic testing using murine leukemia cells were cultured P-388 protocol over the plate, dihidroisocoumarin compounds showed values of IC50 =25.33 lm / mL, whereas sitoksisitas test using Artemia salina shrimp fry, brine shrimp lethality using a bioassay method [22] with the values of LC 50=66.68 lm / mL.

IV. CONCLUSIONS

One dihidroisocoumarin compounds have been isolated for the first time from the bark of Shorea singkawang (Miq) .Miq. The test compounds against murine leukemia cells P-388, showed weak cytotoxic activity with IC50 values 25, 33 lm / mL, and activities with Artemia salina showed values of LC 50 =66.8 lm / mL .

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