DITERPENOID COMPOUNS FROM LEAF OF SUREN (*Toona sureni*) AND TOXICITY AGAINTS BRINE SHRIMP

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Abstracts

Indonesia is second country with the biggest of biological variety in the world. These natural resources are the main source to get some secondary metabolism compounds which theirs utility have been known specially in health and agricultural fields. Besides of theirs utility, the variety of theirs structures also become an interesting matter for the researchers to keep doing isolation and elucidation of secondary metabolism compounds. Toona sureni is one of potential plant to find some secondary metabolism compounds. This plant which include to family of Meliaceae, was known its activity as insectiside, repellent, antiplasmodial, and antidiarhoea. Through of extraction and isolation method, with so many technic of chromatography, had been got a compound of 3,7,11,15-tetramethyl-2-hexadeken-1-ol (phytol) that include of acyclic diterpene from n-hexane extract of T. sureni leaves. Determinig of its structure was done by spectroscopy method, Infrared (IR) and Nuclear Magnetic Resonance (NMR). Toxicity assay of Brine Shrimp Lethality Test (BSLT) used larvae of Artemia salina as bioindicator showed that phytol was include to good toxic compound with the LC_{50} value is 16.64 ppm.

Keywords : Secondary metabolism, Toona sureni, Diterpene, BSLT, LC_{50}

Introduction

Trees of family Meliaceae was used to live in the tropic and subtropic region. Literatures mentioned that family Meliaceae rich of active compounds that can be used in medicinal and agricultural field. One species of the family Meliaceae which traditionally used to be medicine is *Toona sureni*. Its stem bark and roots frequently used for diarrhea, its leaves has antibiotic, antiplasmodium, and antifeedant effect, while its fruit as atsiri oil source (Djam'an, 2002). Chemical active compound report of *T. sureni* informed that leaf of this species contains of triterpenoid, limonoid, and carotenoid compounds (Cuong *et al*., 2007; Kraus & Kypke, 1979; Nurdin, 2000; Salome, 1999). While its stem bark was reported contains of triterpenoid compounds also which has toxic activity toward *Artemia salina* leach (Hudri, 2008; Hardianto, 2009).

The report about *T. sureni* active compounds content was not much publicated, whereas its utility by people traditionally has been done a lot, especially its leaves for medicinal. That is why needed some more experiment further to know others active compounds from leaves part which suitable with its utility traditionally.

In this research, toxic activity assay that used to know toxicity level of the compound which content in the leaves of *T. sureni* was Brine Shrimp Lethality Test (BSLT) method. BSLT methode has much gaining, several are fast, easy in the practice, relative economically, and simple that made this method in general often used for first screening deciding toxicity of the compound.

Materials and Methods

Plant material and tools

Sample of *T. sureni* leaves were taken from Arboretum, Departemen of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University.

Tools : maseration vessel, distillation apparatus, evaporator R114 Buchi with *vacuum system Buchii* B169, silika gel GF_{254}, analytical balance, chamber, column chromatography, sprayer, UV detector Vilber Lourmat λ 254 and 365 nm, infra red FT-IR *Prestige* 21, Spectrometer ^{1}H-NMR and ^{13}C-NMR JEOL system 500 MHz and 125 MHz, Brine Shrimp Lethality Test (BSLT) kit.

Extraction and isolation

Four kilograms of *T. sureni* leaves were dried at the open room. Dried sample then extracted using maseration method with *n*-hexane, ethyl acetat, and methanol, as long as 5x24 hours for each. Extract of each fraction was assayed of its toxicity towards *A. salina* leach. Extract *n*-hexane was separated by column vacuum liquid chromatography, silica gel G 60 as stationary phase and *n*-hexane-ethyl acetat (gradient 10%) as mobile phase. The fractions which have same spot were joined then yield 7 fractions as the results (F1-F7).

F3 was separated by column vacuum liquid chromatography, silica gel G 60 as stationary phase and *n*-hexane-ethyl acetat (gradient 5%) as mobile phase. The fractions which have same spot were joined then yield 8 fractions as the results (F3A-F3H).

F3D was separated by gravitation column chromatography, silica gel G 60 as stationary phase and *n*-hexane-chloroform (6:4) as mobile phase. The fractions which have same spot were joined then yield 5 fractions as the results (F3A-F3H). F3D2 which has target compound on it then was separated by reverse method (C18) and methanol-water (7:3-10:0, gradient 1%) as eluent. From result of thin layer chromatography, known that F3D2C fraction given single spot which it has 25.4 mg weight and yellow oilic formed.

The active compound structure was determined by infra red spectrophotometer, ^{1}H-NMR, ^{13}C-NMR, and DEPT 135^{o} technique. Then toxicity of the compound was assayed by BSLT method.

Results and Discussions

Crude extract of *T. sureni* leaves from each solvent were : methanol 109.13 g; ethyl acetat 46.97 g; dan *n*-hexane 17.29 g. Toxicity of every extract was assayed using BSLT to determine which extract with highest toxic activity will be choice to separate then. Comparing data was death percent value of *A. salina* leach in variable concentration 1000, 500, and 100 ppm from each extract.

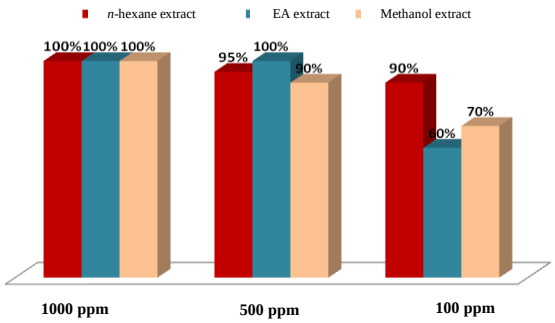

Figure 1. BSLT results of *n*-hexane, ethyl acetat, and methanol extract of *T. sureni* leaves

From the graph can be known that *n*-hexane extract gives highest death percent value in many variable concentration.

Spectroscopy analysis

Infra red spectrum of isolate F3D2C showed strong absorption in the wave number of 3328 cm^{-1} which indicate strecthing of O-H group. This is clarified with primary alcohol strecth of C-O group in the finger print region 1031 cm^{-1}. Strecthing of C-H sp^{3} alifatic group at the wave number region of 2849 and 2981 cm^{-1} estimated from asymetric and symetric strecthing of C-H group. Olefinic group was showed by absorption at the wave number 3017 cm^{-1} which indicate C-H sp^{2} vibration and absorption at 1466 cm^{-1} which indicate vibration of C=C olefinic group. *Gem*-dimethyl group was showed by vibration at the wave number 1386 cm^{-1}.

Based on ^{13}C-NMR spectrum, can be known that isolate F3D2C has 20 carbon atom. Oxigenated carbon was showed by signal at δ_C 59.6 ppm. Two carbons sp^{2} in the isolate F3D2C structure looked at chemical shift 123.3 and 140.4 ppm. It can be conclused that structure has one double bond group.

Data from ^{13}C-NMR spectrum was clarified by using DEPT (Distortionless Enhancement by Polarization Transfer) 135° technique. The spectrum given information that in the structure there are 5 methyl carbon atom at δ_C 16.4; 19.9; 19.9; 22.9; and 22.8 ppm. Beside that, there are signals of 10 methylene carbon at δ_C 24.7; 24.9; 25.3; 36.8; 37.4; 37.5; 37.6; 39.6; 40.0; and 59.4 ppm. Signal methylene carbon atom at δ_C 59.4 ppm bounded to an oxygen atom that from IR spectrum known as hydroxil group. Signals of 4 methyn carbon atom showed at δ_C 28.1; 32.8; 32.9; and 123.3 ppm. Also one quartenary carbon atom can be seen from the signal at δ_C 140.4 ppm, which it's not appeared on DEPT 135° spectrum.

Based on analysis of ^{1}H-NMR spectrum, there were 39 signal protons within isolate F3D2C, which consist of 15 protons sp^{3} at δ 0.84-1.98 ppm. These proton sp^{3} also consists of 5 methyl group, 10 methylene group, and 4 methyn group, adequate with analysis results of DEPT 135° spectrum. A methylene group bounding oxygen atom showed by the signal at δ_H 4.14 ppm (2H, *d*, *J* =4.5 Hz). Proton signal at δ_H 5.40 ppm (1H, *t*, *J*= 4.5 Hz) is from an sp^{2} proton bound to olefinic carbon. The same value of coupling constant between oxygenated methylene group at δ_H 4.14 ppm with methyn group at δ_H 5.40 ppm as much as 4.5 Hz showed that each of them so closed with range 3 bounding (^{3}J). This gives conclusion that hydroxil group is primary alcohol group.

Signals at range 0.83–0.88 ppm of isolate F3D2C ^{1}H-NMR spectrum are overlap, then make it's hard to interpretated. Closer chemical shift difference among alifatic proton group make the signals overlap to each others. The intensity which has 12 protons, estimated that the signals are 4 methyl which have same chemical environment. Two from the methyl is suitable with IR spectrum, forming a *gem*-dimethyl group. The signals at δ_H 1.03–1.43 ppm with the intensity 18 protons were estimated from 9 methylene groups.

Data analysis ^{13}C-NMR and ^{1}H-NMR spectrum of isolate F3D2C compared with diterpene compound from literature that has been found in *Toona*, they have very same chemical shift value. Diterpene compound which is compared is phytol that isolated from *T. sinensis* by Luo *et al.* (2000).

Manitto (1992) explained that plant which has closed taxonomy relationship, like one family or genous, will contain almost same secondary metabolite compound or even really same. That's

why closed relationship between *T. sinensis* and *T. sureni* make it probably to find the same compound, because secondary metabolism itself is expression of genetic.

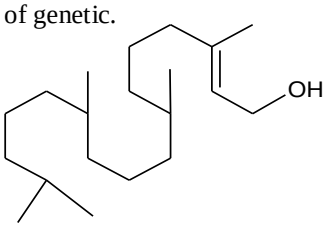

Figure 2. Phytol structure, an acyclic diterpen

Toxicity assay result of isolate F3D2C towards *A. salina* leach is showed by Table 1.

Table 1. Number of alive and dead *A. salina* to variable concentration of isolate F3D2C

Concentration / ppm	Total *A. salina*	Dead *A. salina*			Alive *A. salina*	
		A	B	Average	A	B
Control	10	1	0	1	9	10
1000	10	10	10	10	0	0
500	10	10	8	9	0	2
250	10	8	8	8	2	2
125	10	6	6	6	4	4
62	10	4	5	5	6	5
31	10	7	3	5	3	7
16	10	3	9	6	7	1
8	10	7	7	7	3	3

Guerrero (2004) said, that the compound can be told has activity if its LC_{50} value under 100 ppm. Using software *EPA probit analysis program ver. 1.5*, LC_{50} value of isolate F3D2C is 16.64 ppm, that can be said if the isolate includes of the active toxic compound. Several of researchers have been reported that phytol compound has antifertility and anticancer activity towards human lymphoid cell cancer (Herlina dkk., 2006; Komiya & Hibasami, 2001).

Conclusions

From *n*-hexane extract of 4 kg *T. sureni* leaves has been isolated F3D2C compound as much as 25.4 mg and has yellowish oilic liquid formed. Based on analysis of IR, ^{1}H-NMR, ^{13}C-NMR, DEPT 135° spectrum and its reference literature, could be estimated that F3D2C isolate was phytol (3,7,11,15-tetramethyl-2-hexadeken-1-ol), an acyclic diterpene compound which has molecular formula $C_{20}H_{40}O$. F3D2C isolate then assayed by Brine Shrimp Lethality Test and given LC_{50} value 16.64 ppm.

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