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Isolation of Triterpenoid from Katemas (*Euphorbia geniculata* Ortega) Stem Extracted using Methanol and Its Toxicity Test

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Abstract

Katemas (*Euphorbia geniculata* Ortega) known as (Mexican) Fireplants, Painted Euphorbia, Japanese poinsettia is a plant belong to Euphorbiaceae or spurge family. Traditionally, this plant is used as furgative and treat dysentry. The aims of the research were to evaluate the toxicity and to isolate one of the triterpenoid extracted using methanol from the stem of this plant. The toxicity was evaluated by mortality test against armyworm larvae (*Spodoptera litura*). This assay was obtained by Methanol extraction at various concentrations (0, 1, 2, 3, 4 and 5%). The isolation process was conducted by series of work steps such as extraction, fractionation and purification. Structure elucidation was determined by spectroscopy techniques. Toxicity assay exhibited highest mortality at 5 % concentration with LC_{50} 3,92%. Spectroscopy data analysis was able to identified a type of pentacyclic triterpenoid compound namely lupeol acetate.

Keywords: Euphorbia geniculata Ortega, toxicity, lupeol acetate

Abstrak (Indonesian)

Ketemas dikenal sebagai, tumbuhan api (Meksiko), *Painted Euphorbia*, *Japanese poinsettia* merupakan tumbuhan yang masuk ke dalam famili Euphorbiaceae. Secara tradiosional, tumbuhan ini digunakan untuk menyembuhkan penyakit disentri. Tujuan penelitian ini adalah untuk menguji toksisitas dan mengisolasi triterpenoid yang terekstrak dari batang tumbuhan ketemas menggunakan metanol. Uji toksisitas dilakukan dengan tes mortalitas terhadap ulat grayak (*Spodoptera litura*). Pengujian ini diperoleh dari ekstraksi metanol pada beragam konsentrasi (0, 1, 2, 3, 4 and 5%). Proses isolasi dilakukan serangkaian prosedur yang meliputi ekstraksi, fraksinasi, dan purifikasi. Elusidasi struktur dilakukan dengan menggunakan teknik spektroskopi. Uji toksisitas menunjukkan nilai mortalitas tertinggi pada konsentrasi 5% dengan LC_{50} 3,92%. Data analisis spektroskopi dapat mengidentifikasi tipe senyawa pentasiklik yang disebut lupeol asetat.

Keywords: Euphorbia geniculata Ortega, toxicity, lupeol acetate

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INTRODUCTION

Euphorbia is the largest genus in the plant family of Euphorbiaceae, comprising about 2000 species, five of them can be found in Indonesia. The plant specifically produced a milky latex which causes skin's burn and could be toxic and carcinogenic [1,2]. *Euphorbia* genus is known to contain a wide variety of terpenoids. In addition to mono-, sesqui-, and diterpenes, another triterpenoid skeleton also has been found in Euphorbia. Many of these compounds have been investigated for their toxicity or their potential therapeutic activity, and some have been used as medicines since ancient times [3].

Plant can be used not only for medicines but also for pesticidal purpose. The toxicity properties shown by some plant species is a potential biopesticides or natural pesticides. Some of *Euphorbia* have been reported on their toxicities. *E.kamerunica* have an acute toxicity on the fingerlings of *Oreochromis niloticus* [4] while *E. royleana* exhibit toxicity on catfish (*Heteropneustes fossillis*) [5]. In addition, several diterpenoid and triterpenoid compounds from this plant have bioactivity as molluscicidal and antifeedant [3].

Katemas (*Euphorbia geniculata* Ortega) is more widely known by *Euphorbia heterophylla*. This plant is an annual weed plant which can grow fast and disrupt the crops [6]. *E. heterophylla* is originated from tropical and subtropical regions of America but it is known distributed throughout tropical Africa, Asia and the pasific in at least 65 countries including Indonesia [5]. The application of this plant for medicines are known as furgatives, antigonoreal, migrain and wart cures [8]. Phytochemical study of *E. heterophylla* carried out in Nigeria showed that it contains flavonoid, tannins, saponin, glycoside, steroid and triterpenoid [6]. The plant has biological activities including anticancer, anti-HIV [9] and insecticides [10]. In this paper, we report the toxicity of methanol extract from stem of *E.geniculata* Ortega against armyworms (*Spodoptera. litura*) larvae. The extracted compound had been isolated and identified as triterpenoid namely lupeol acetat.

EXPERIMENTAL SECTION

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on NMR Agilent 500 MHz (^{1}H) and 125 MHz (^{13}C) spectrometer consol DD2 system. TMS was used as an internal standard and $CDCl_3$ as the solvent. Melting points were measured in a micro Fisher-John apparatus (uncorrected). Merck silica gel G 60 (230-400 mesh) and Si gel Merck G 60 (70-230 Mesh) were used in column chromatography, thin layer chromatography (TLC) analysis was performed on precoated Si Gel plates (Merck Kiesel gel 60 GF_{254}, 0.25 mm 20 x 20 cm).

Plant sample preparation

The stem of E. *geniculata* Ortega was collected in March 2012 from Inderalaya, Ogan Ilir district South Sumatra province, Indonesia. The plant species and genus classification was checked by Bogorience Herbarium. The voucher specimen (EB182015) is available for inspection in organic chemistry laboratorium Sriwijaya University, Inderalaya, South Sumatra. Toxicity assay was carried out by using spinach (*Amaranthus sp*) leaves (5 cm lenght)

Extraction

The powdered stem (700 g) of *E. geniculata* Ortega (air dried) was macerated three times with methanol (4 L for each extraction) at room temperature. The solvent was removed in vacuo to produce a black residue (20 g).

Toxicity Assay: Preparation of test insect

The larvae of army worms (*S. litura*) (5 cm lenght) were collected from Tanjung Laut village, Banyuasin district South Sumatra. The Armyworms larvae were transported to the laboratory using a plastic bottle with gauze. The bottle has dimension of 20 cm height and 15 cm diameter. The army worm larvae feed with spinach leaves and allow to adapt in the laboratory for 24 hours [11].

The treatment of the test insect to the concentration of plant extracts

Prior to treatment of test insect, *S.litura* larvae were fasted for 24 hours [12]. Test solutions were prepared by taking 8 g extract, 1 mL tween 80 as emulsifier and 10 mL of distilled water were added in order to obtain mother liquor as much as 15 g. Five variation of concentrations (1, 2, 3, 4, 5 (g/v) %) were made from mother liquor. Two leaves of spinach (5 cm lenght) dipped into each solution prepared and then placed into a plastic container (15 cm diameter and 15 cm height). Five army worms was inserted into each plastic container and covered with gauze and left for 24 h. The observations were done after 24 h by count their mortality. Three replications were made for each experiment. The lethal concentrations were determined by probit analysis [11]

$$\% \text{ Mortality} = P = r/n \times 100$$

r= amount of mortality, n= number of initial larvae

Isolation

A portion of methanol extract (1.4 g) was fractionated in a column chromatography (Si gel 50 g, diam 15 cm) using a mixture n-hexane: ethylacetate, (99:1, 95:5 and 90:10) to obtain five fractions (fr A-E). Fr.C (70.4 mg) was subjected into flash column chromatography (FCC, diam 1 cm) eluted successively using solvent gradient of n-hexane: $CHCl_3$ (1%, 5% and 10%) to give 7 fractions (C1-C7). Recrystalization of C2 fraction yield a pure compound (63 mg).

RESULT AND DISCUSSION

The toxicity Assay

The toxicity was evaluated against the armyworms larvae (*S. litura*) using no-choice test. The spinach leaves were immersed to various concentrations of methanol extract and observed after 24 hours. The result of treatments at (Figure 1) 1−4 % concentration exhibited all of the leaves were feed by the armyworms larvae but not for 5% concentration. Although all of leaves were consumed by armyworm larvae at 1-4% concentration but mortality percentage of larvae was shown at 5% with LC_{50} obtained at 3.92%.

The extract at 1−4% concentration showed no deterrent effect indicated by spinach leaves were eaten completely. On the contrary at concentration 5%, armyworm did not consumed leaves entirely but showed the highest % mortality (60%). Methanol extract on low concentration (1−2%) exhibited low % mortality, the armyworm possibly able to neutralize toxins in methanol extract given. The low mortality calculated were 6,7−30% can be caused by the ability of *S. litura larvae* to netralize the toxins of methanol extract which were given [11]. According to Tentirawe (2007) at low concentration the toxin is neutralized by insects through the activation of the microsomal mixed-function oxidase (MFO) genetically present in the insect's body. MFO has important roles in degrading and deactivate pesticides and other synthetic compounds in the insect's body [13]. At higher concentration i.e. 5% the toxins distrupted the digestion of armyworm which leads them to their death.

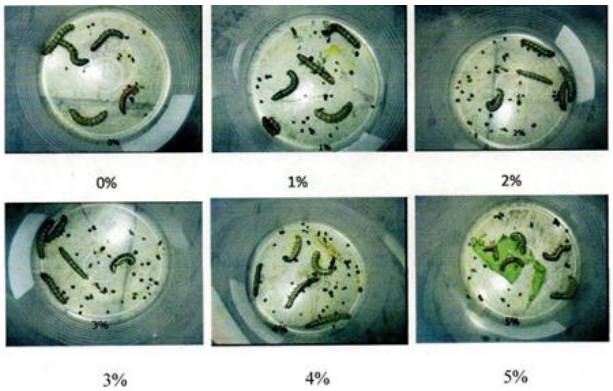

Figure 1. Mortality assay of methanol extract of *E. geniculata* Ortega stem against armyworms (*S. litura*) larvae after 24 hours

Isolation and Identification

A triterpenoid compound had been isolated from methanol extract. Isolation process were conducted through extraction, fractionation and purification using several chromatography techniques. Elucidation structure were determined by ^{1}H-NMR, ^{13}C-NMR, DEPT, HSQC and HMBC. The isolated compound (63 mg) was obtained as white solid m.p 205-207°C. ^{13}C-NMR spectrum showed 32 signals carbon and one of them was identificated as carbonyl carbon of ester at δ_C 171.0 (CH_3-$\underline{C}$OO-) and 21.3 ($\underline{C}H_3$-COO-) for methyl of ester.

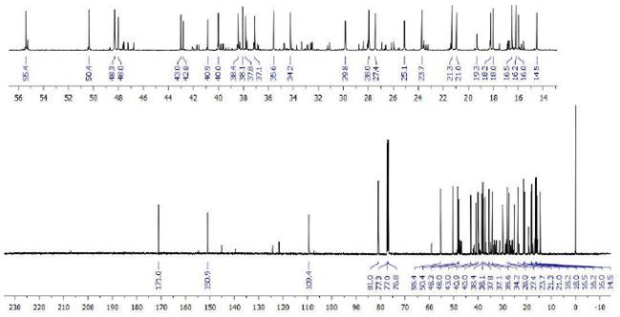

Figure 2. ^{13}C-NMR spectrum of lupeol acetate ($CDCl_3$)

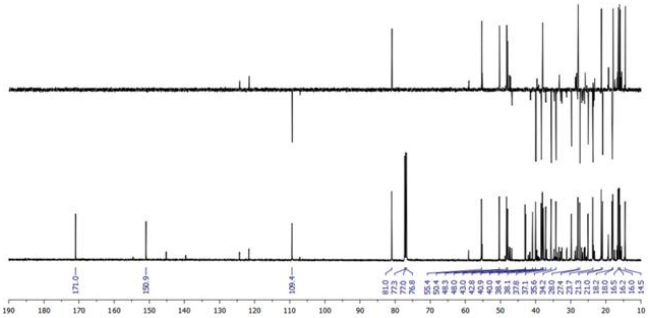

Figure 3. DEPT Spectrum of Isolated Compound

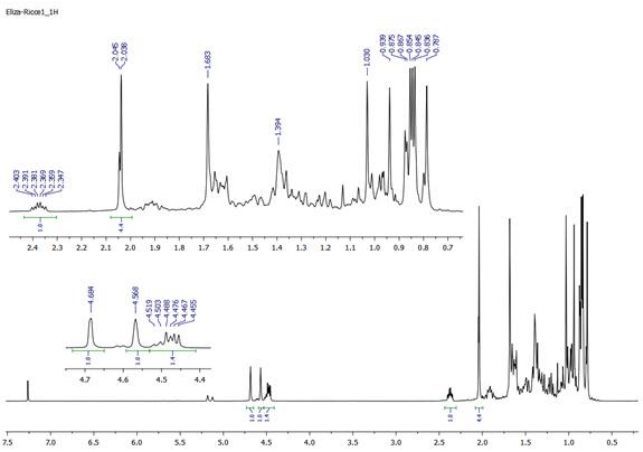

Figure 4. ^{1}HNMR Spectrum of Isolated Compound

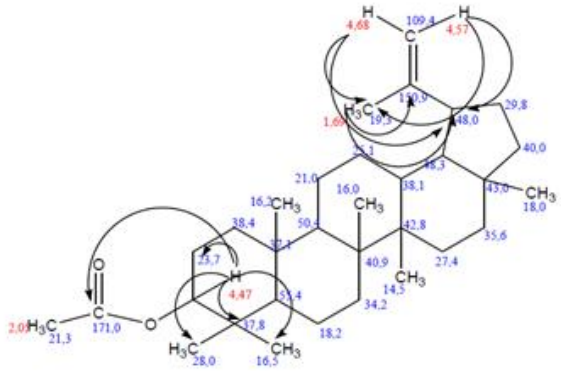

Figure 5. Structure and some HMBC data of isolated compound (lupeol acetate)

Table 1. The Percentage of larvae *S. litura* due to the treatment of methanol extract of *E. geniculata* Ortega stem

Concentration of extract (%)	∑ larvae of *S.litura*	Percentage of mortality
0	15	0
1	15	6.7
2	15	30
3	15	33.3
4	15	46.7
5	15	60

The DEPT spectrum (135 MHz) showed 8 signals of methyl, 11 signals of methylene, 6 signals of methyne and 7 signals of quartener carbon. ^{13}C-NMR and the ^{1}H-NMR spectrum (Table 2) showed two signals vinylic proton at δ_H 4.57 (1H, s, H-29a) and 4.68 (1H, s, H-29b) attributed to terminal methylene proton of lupan-type triterpenes. In addition, the other signal at δ_H 4,47 (1H, dd, J=10 Hz) indicated proton at C-3, while signal for proton methyl of acetyl group revealed at 2.05 (3H, s). Data of isolated compound was deduced by HSQC and HMBC spectrum. The HMBC spectrum was used to indicate position of acetate moety. There was long range correlation between H-3 (δ_H 4,47) with carbonyl carbon of acetyl (δC 171.0) that confirmed acetyl group bonded at C-3. Comparing data analysis of spectrum for the sample with database reference, we concluded that the isolated compound is assigned as lupeol acetate [14].

Tabel.2 ^{13}C-NMR chemical Shift data of isolated compound ($CDCl_3$, 500 Hz and 125Hz)

Position carbon	DEPT	δ_C refference (ppm)	δ_C of isolated compound
1	CH_2	38.4	38.4
2	CH_2	23.7	23.7
3	CH	81.0	81.2
4	C	37.8	37.8
5	CH	55.4	55.4
6	CH_2	18.2	18.2
7	CH_2	34.3	34.2
8	C	40.9	
9	CH	50.4	50.4
10	C	37.1	37.1
11	CH_2	21.0	21.0
12	CH_2	25.1	25.1
13	CH	38.1	38.1
14	C	42.9	42.8
15	CH_2	27.5	27.4
16	CH_2	35.6	35.6
17	C	43.0	43.0
18	CH	48.0	48.5
19	CH	48.3	48.0
20	C	150.9	150.9
21	CH_2	29.9	29.8
22	CH_2	40.0	40.0
23	CH_3	28.0	28.0
24	CH_3	16.5	16.5
25	CH_3	16.2	16.2
26	CH_3	16.0	16.0
27	CH_3	14.5	14.5
28	CH_3	18.0	18.0
29	CH_2	109.4	109.4
30	CH_3	19.1	19.3
2’	CH_3	21.3	21.3
1’	C=O	170.8	171.0

CONCLUSION

The methanol extract of Katema plant demonstrate toxicity against armyworm (*Spodoptera liture*) with the highest mortality at 5% concentration with LC_{50} 3.92%. A pentacyclic triterpenoid compound namely lupeol acetate had been sucessfully isolated and identified from this extract.

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