

## ISOLATION OF 8-HIDROXY-6,7-DIMETHOXY COUMARIN FROM JARAK TINTIR STEM (*Jatropha multifida* L.) AND ITS TOXICITY VALUE USING BRINE SHRIMP LETHALITY TEST (BSLT)

### ISOLASI DARI 8-HIDROKSI-6,7-DIMETHOKSI KUMARIN DAN TOKSISITAS BATANG JARAK TINTIR (*Jatropha multifida* L.) MENGGUNAKAN BRINE SHRIMP LETHALITY TEST (BSLT)

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#### ABSTRACT

*Jatropha multifida* L. (jarak tintir) was a shrub, annual, and had  $\pm 2$  m high. Empirically jarak tintir sap was used as traditional medicine by Indonesian people for a long time. Only limited studies were conducted regarding its chemical compound. It was reported that multifidone (diterpenoid compound from the stem) had an activity against cancer cells in vitro. This study aimed to test the toxicity of various extracts (*n*-hexane, ethyl acetate, and methanol) with Brine Shrimp Lethality Test (BSLT) of *Jatropha multifida* L. stem. A Fraxidin (8-hidroxy-6,7-dimethoxy coumarin) has been isolated from ethyl acetate fraction based on highest cytotoxic with  $LC_{50}$  value 3.69  $\mu\text{g/mL}$ . The isolated compound was elucidated to gain chemical structure base on spectroscopic data (UV-Vis Spectrofotometric, IR Spectrofotometric, and NMR). Toxicity of fraxidin was tested on BSLT and showed no potential activity with  $LC_{50}$  value  $> 500$   $\mu\text{g/mL}$ .

**Key words:** Fraxidin, BSLT, coumarin, *Jatropha multifida* L.

#### ABSTRAK

*Jatropha multifida* L. (jarak tintir) adalah semak yang memiliki tinggi  $\pm 2$  m. Secara empiris getah Jarak tintir digunakan sebagai obat tradisional oleh penduduk Indonesia sejak lama. Penelitian yang dilakukan tentang senyawa kimia Jarak tintir masih terbatas. Menurut penelitian, multifidone (senyawa diterpenoid dari batang Jarak tintir) memiliki aktivitas terhadap sel kanker in vitro. Penelitian ini bertujuan untuk mengetahui toksisitas berbagai macam ekstrak (*n*-hexane, etil asetat, dan metanol) dari batang *Jatropha multifida* L. dengan Brine Shrimp Lethality Test (BSLT). Sebuah Fraksidin (8-hidroksi-6,7-dimetoksi kumarin) telah diisolasi dari fraksi etil asetat berdasarkan sitotoksik tertinggi dengan nilai  $LC_{50}$  3.69  $\mu\text{g/mL}$ . Senyawa yang diisolasi diuraikan untuk mendapatkan basis struktur kimia pada data spektroskopik (UV-Vis Spektrofotometri, IR Spektrofotometri, dan NMR). Toksisitas fraksidin diuji dengan BSLT dan tidak menunjukkan potensi aktivitas dengan nilai  $LC_{50} > 500$   $\mu\text{g/mL}$ .

**Kata kunci:** Fraksidin, BSLT, kumarin, *Jatropha multifida* L.

#### INTRODUCTION

*Jatropha multifida* L. (jarak tintir) is a shrub, annual, and had  $\pm 2$  m high. Empirically Jarak tintir sap has been used as a traditional medicine by Indonesian people for a long time (Hutapea, 2000). It was reported that multifidone (diterpenoid compound from the stem) has an activity against cancer cells in vitro with  $IC_{50}$  value was 45-160  $\mu\text{M}$  (Das *et al.*, 2008b). Another diterpenoid compound from the stem had also been found, such as 15-O-acetyl japodagrone, (4E)-jatrogrossidenta-dione (Das *et al.*, 2008a),

multidione (Das *et al.*, 2009), multifidanol and multifidenol (Kanth *et al.*, 2011). Cytotoxic assay of Jarak tintir leaves has been done and given  $LC_{50}$  value 0,91  $\mu\text{g/mL}$  of *n*-hexane fraction (Honasan, 2012).

Brine shrimp lethality test (BSLT) was one of toxicity assays that used toxic bioactive study from natural products. This metode is known as bioassay-guided fractionation from natural products, because it is easy, fast, cheap, and good enough in reproducible (Meyer *et al.*, 1982). This study reports on the isolation, toxicity test, and structure elucidation of fraxidin (8-hidroxy-6,7-dimethoxy coumarin) from ethyl acetate fraction.

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## MATERIAL AND METHODS

### Sample Preparation

*J. multifida* stem is collected from Manoco garden, Lembang, West Java-Indonesia, and determined in Herbarium Bandungese School of Life Science and Technology, ITB. The stem was dried under morning sunlight. The dried stems were grinded until a crude drug powder was obtained.

### Characterization of Crude Drug

The crude drug was examined microscopically and characterized such as moisture, total ash, water soluble ash, acid insoluble ash, water soluble extract, and ethanol soluble extract content (Depkes, 2000).

### Phytochemical Screening of Crude Drug and Extract

Phytochemical screening was done to detect secondary metabolite, like alkaloids, flavonoids, polyphenols, tannins, quinones, saponins, monoterpenoids-sesquiterpenoids and terpenoids-triterpenoids (Farnsworth, 1966).

### Extraction

Continous extraction was performed with Soxhlet apparatus using three solvents with increasing polarity (n-hexane, ethyl acetate, and methanol).

### Brine Shrimp Lethality Test (BSLT)

All extracts were tested using BSLT toxicity assay with various concentrations. Lethal concentration 50% (LC<sub>50</sub>) was counted and obtained from Probit analysis. The lowest LC<sub>50</sub> of extract was the most toxic and has potential activity in cytotoxic (Meyer *et al.*, 1982).

### Isolation

Ethyl acetate fraction was fractionated using vacuum liquid chromatography (VLC) with gradien elution (dichloromethane-ethyl acetate-methanol). Fraction 6K, 7K and 8K were fractionated with VLC with gradien elution (dichloromethane-ethyl acetate-methanol). Sub fractions 8-10 were then decanted by some organic solvents to obtain isolate 1.

### Chemical Compound Characterization and BSLT Assay Isolate 1

Isolate 1 was characterized using specific spray reagent, melting point assay, UV-Vis, infrared spectrophotometry, and H-NMR, C-NMR and 2D-NMR spectrometry. The toxicity of isolate 1 was tested with BSLT.

## RESULT AND DISCUSSION

### Characterization Crude Drug

Microscopic examination showed fragments of parenchyma, crystal, and tracheid (Figure 1). The characterization of crude simplicia is water content  $3.02 \pm 0.05$  % v/w; total ash content  $5.17 \pm 0.54$  % w/w; water soluble ash content  $2.04 \pm 0.15$  % w/w; non-soluble acidic ash content  $0.36 \pm 0.07$  % w/w; water soluble extractable matter  $11.46 \pm 0.57$  % w/w; ethanol soluble extractable matter  $7.56 \pm 0.33$  % w/w and all parameters fullfil the regulation quality of crude drug.

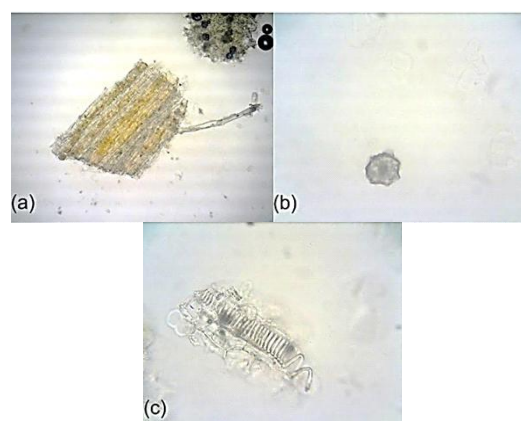


Figure 1. Microscopic examination crude drug of *J. multifida* L. in chloral hydrate, (a) parenchyma (100x), (b) crystal (400x), (c) tracheid (400x).

### Brine Shrimp Lethality Test (BSLT)

Brine shrimp lethality test (Table I) of extract and isolate1 was done between 1-100 µg/mL against *Artemia salina*. Probit analysis showed LC<sub>50</sub> of methanol, ethyl acetate, and n-hexane extracts were 4.85; 3.69 and 18.14 µg/mL respectively. Ethyl acetate was the most active and toxic. A compound called as cytotoxic if the compound has LC<sub>50</sub> ≤ 30 µg/mL (McLaughlin dan Jerry, 1991).

Table I. Brine Shrimp Lethality Test (BSLT) of extracts and Isolate1

Extract	Methanol	Ethyl Acetate	n-hexane	Isolate 1
LC <sub>50</sub> (ppm)	4.85	3.69	18.14	>500

### Phytochemical Screening

The crude drug showed only alkaloids and tannin that gave negative result. Ethyl acetate as most active extract had flavonoid, polyphenol, quinon, monoterpen-sesquiterpen, and steroid-triterpenoid for compound that responsible to its activity.

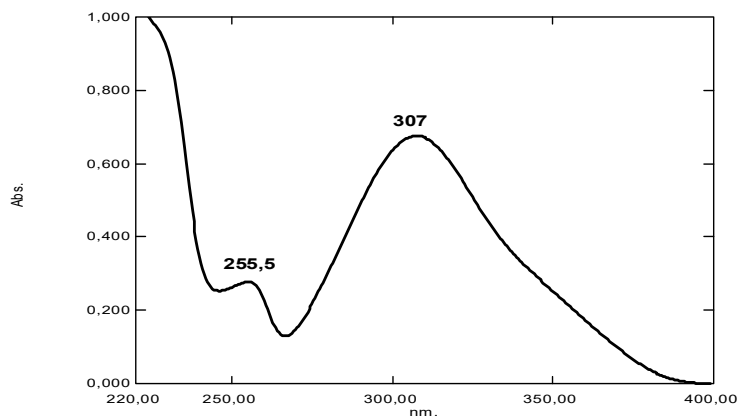


Figure 2. UV spectrum of isolat 1 in methanol

Table II. Phytochemical Screening Crude Drug and Ethyl Acetate Extracts of *J. Multifida* L.

Compound	Reagent	Crude Drug	Extract
Alkaloid	Dragendorff	-	-
Flavonoid	Zn, amyl alcohol	+	+
Polyphenol	FeCl <sub>3</sub>	+	+
Quinon	KOH 1%	+	+
Saponin		+	-
Tannins	Gelatin 1%	-	-
Monoterpen-sesquitepen	Vanilin-SO <sub>4</sub>	+	+
Steroid-Triterpenoid	Liebermann Bourchard	+	+

Table III. Comparison <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data of isolate 1 with Fraxidin

Position	Isolate 1 (Aseton-d <sub>6</sub> )		Fraxidin (CDCl <sub>3</sub> )	
	δ <sub>H</sub> (ppm) (500 MHz)	δ <sub>C</sub> (ppm) (125 MHz)	δ <sub>H</sub> (ppm) (400 MHz)	δ <sub>C</sub> (ppm) (100 MHz)
2	-	160.7039	-	161.4
3	6.2911 <i>d</i>	115.5018	6.26 <i>d</i>	114.4
4	7.8600 <i>d</i>	144.9752	7.60 <i>d</i>	144.4
5	6.7983 <i>s</i>	101.1943	6.44 <i>s</i>	99.7
6	-	150.9939	-	150.2
6-OCH <sub>3</sub>	3.8773 <i>s</i>	56.5453	3.83	56.1
7	-	140.9119	-	140.4
7-OCH <sub>3</sub>	3.8850 <i>s</i>	61.2191	3.91	61.0
8	-	139.3095	-	138.5
9	-	139.3762	-	138.3
10	-	115.7498	-	114.1

### Chemical Compound Characterization

Isolate 1, white solid, 115.1 mg, mp 195.8° - 196.8° C were obtained using two step vacuum liquid chromatography (VLC) with gradien elution (dichloromethane-ethyl acetate) from ethyl acetate extract. UV-Visible Spectrophotometer: Isolate 1 showed 2 peaks on λ<sub>max</sub> 255.5 and 307 nm (Figure 2.)

IR Spectrophotometer (KBr medium): 3228.84 cm<sup>-1</sup>(-OH); 3055.24; 3016.67 cm<sup>-1</sup> (aromatic C-H); 2989.66; 2947.23; 2839.22 cm<sup>-1</sup> (aliphatic C-H); 1697.36 cm<sup>-1</sup> (C=O) and 1612.49; 1570.06; 1496.76 cm<sup>-1</sup> (aromatic C=C).

Analysis of <sup>1</sup>H-NMR (acetone-d<sub>6</sub>, 500 MHz) showed presence of 9 protons. Doublet signal on δ<sub>H</sub> 6.2911 ppm (*d*, 1H, *J*=10), and δ<sub>H</sub> 7.8600 ppm (*d*, 1H, *J*=10) showed correlation due to the signals and certain signal for unsubstitute coumarin in orto position on pyron ring (Murray *et al.*, 1982). Methoxy group (-OCH<sub>3</sub>) showed δ<sub>H</sub> 3.8773 (*s*, 3H) and δ<sub>H</sub> 3.8850 (*s*, 3H) signals.

Analysis of <sup>13</sup>C-NMR (acetone-d<sub>6</sub>, 125 MHz) showed presence of 11 carbons. Signals on δ<sub>C</sub> 56.5453 and 61.2191 ppm came from 2 methoxy groups (-OCH<sub>3</sub>). Signal on δ<sub>C</sub> 160.7039 ppm came from carbonyl group (C=O) (Pretsch *et al.*, 2009).

The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  data of isolate 1 were founded to be identified as 8-hydroxy-6,7-dimethoxy coumarin (fraxidin).

The structure of fraxidin showed on figure 3 was confirmed by comparison of 2D-NMR spectral data with earlier published literature of Fraxidin data (Rumzhum *et al.*, 2012).

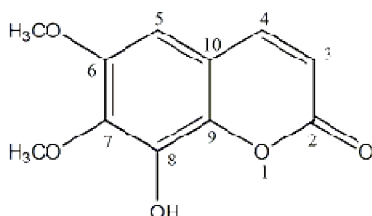


Figure 3. 8-hydroxy-6,7-dimethoxy coumarin

## CONCLUSION

Ethyl acetate extract of *Jatropha multifida* L. had toxicity value with  $\text{LC}_{50}$  3.69  $\mu\text{g/mL}$  with BSLT assay. Isolate 1 was succesfull isolated and identified as 8-hydroxy-6,7-dimethoxy coumarin and not classified as toxic compound after BSLT assay.

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