Trad. Med. J., January - April 2017
Vol. 22(1), p 21-24
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 26-07-2016
Revised : 16-01-2017
Accepted : 22-03-2017

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)

Akhirul Kahfi Syam[1,2*], Muhamad Insanu[2], Komar Ruslan Wirasutisna[2]
[1] Jenderal Achmad Yani University Cimahi-Indonesia
[2] School of Pharmacy Institut Teknologi Bandung-Indonesia

ABSTRACT

Jatropha multifida L. (jarak tintir) was a shrub, annual, and had ± 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 µg/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 µg/mL.
***Key words**: Fraxidin, BSLT, coumarin, Jatropha multifida L.*

ABSTRAK

Jatropha multifida L. (jarak tintir) adalah semak yang memiliki tinggi ± 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 µg/mL. Senyawa yang diisolasi diuraikan untuk mendapatkan basis struktur kimia pada data spektroskopik (UV-Vis Spektrofotometrik, IR Spektrofotometrik, dan NMR). Toksisitas fraksidin diuji dengan BSLT dan tidak menunjukkan potensi aktivitas dengan nilai LC_{50} > 500 µg/mL.
***Kata kunci**: Fraksidin, BSLT, kumarin, Jatropha multifida L.*

INTRODUCTION

Jatropha multifida L. (jarak tintir) is a shrub, annual, and had ± 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 µM (Das *et al*., 2008b). Another ditepenoid 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 µg/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.

Corresponding Author: Akhirul Kahfi Syam
Email: akhirulkahfisyam@yahoo.co.id

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_{50}) was counted and obtained from Probit analysis. The lowest LC_{50} 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 ± 0.05 % v/w; total ash content 5.17 ± 0.54 % w/w; water soluble ash content 2.04 ± 0.15 % w/w; non-soluble acidic ash content 0.36 ± 0.07 % w/w; water soluble extractable matter 11.46 ± 0.57 % w/w; ethanol soluble extractable matter 7.56 ± 0.33 % w/w and all parameters fullfil the regulation quality of crude drug.

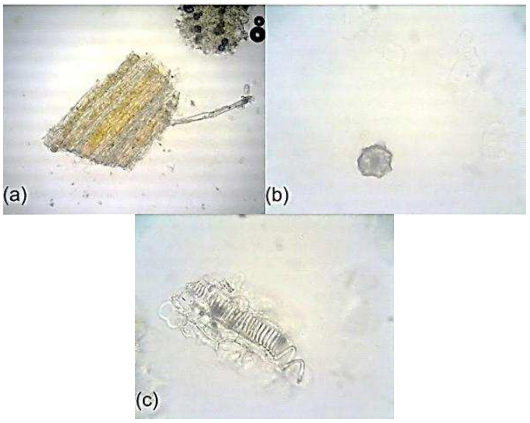

Figure 1. Microscopic examination crude drug of *J. multifida* L. in chloral hidrate, (a) parenchyma (100x), (b) crystal (400x), (c) tracheid (400x).

Brine Shrimp Lethality Test (BSLT)

Brine shrimp lethality test (Table I) of extract and isolate1 was done between 1-100 µg/mL againts *Artemia salina*. Probit analysis showed LC_{50} 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 $LC_{50} \leq 30$ 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_{50} (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.

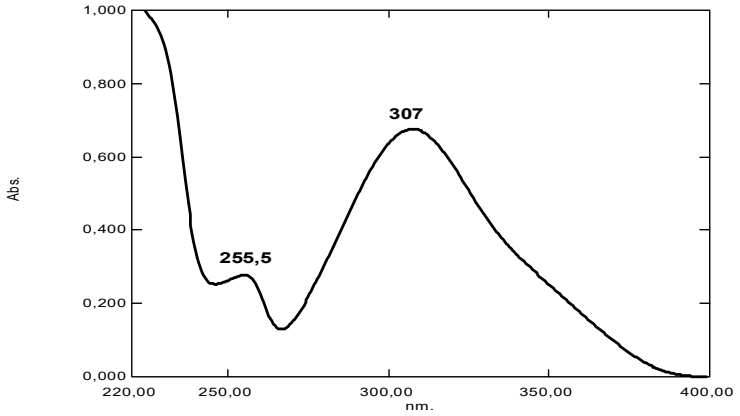

Figure 2. UV spectrum of isolat 1 in methanol

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	$FeCl_3$	+	+
Quinon	KOH 1%	+	+
Saponin		+	-
Tannins	Gelatin 1%	-	-
Monoterpen-sesquitepen	Vanilin-SO_4	+	+
Steroid-Triterpenoid	Liebermann Bourchard	+	+

Table III. Comparison ^{1}H-NMR and ^{13}C-NMR data of isolate 1 with Fraxidin

Position	Isolate 1 (Aseton-d_6)		Fraxidin ($CDCl_3$)	
	δ_H (ppm) (500 MHz)	δ_C (ppm) (125 MHz)	δ_H (ppm) (400 MHz)	δ_C (ppm) (100 MHz)
2	-	160.7039	-	161.4
3	6.2911 *d*	115.5018	6.26 *d*	114.4
4	7.8600 *d*	144.9752	7.60 *d*	144.4
5	6.7983 *s*	101.1943	6.44 *s*	99.7
6	-	150.9939	-	150.2
6-OCH_3	3.8773 *s*	56.5453	3.83	56.1
7	-	140.9119	-	140.4
7-OCH_3	3.8850 *s*	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 λmax 255.5 and 307 nm (Figure 2.)

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

Analysis of ^{1}H-NMR (acetone-d6, 500 MHz) showed presence of 9 protons. Doublet signal on δ_H 6.2911 ppm (*d*, 1H, *J*=10), and δ_H 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_3) showed δ_H 3.8773 (*s*, 3H) and δ_H 3.8850 (*s*, 3H) signals.

Analysis of ^{13}C-NMR (acetone-d6, 125 MHz) showed presence of 11 carbons. Signals on δ_C 56.5453 and 61.2191 ppm came from 2 methoxy groups (-OCH_3). Signal on δ_C 160.7039 ppm came from carbonyl group (C=O) (Pretsch *et al.*, 2009).

The ^{1}H-NMR and ^{13}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).

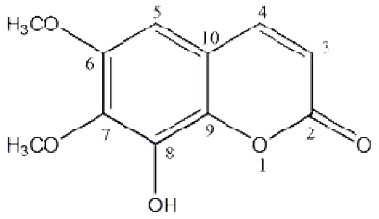

Figure 3. 8-hydroxy-6,7-dimethoxy coumarin

CONCLUSION

Ethyl acetate extract of *Jatropha multifida* L. had toxicity value with LC_{50} 3.69 µg/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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