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Extraction, fractionation and Cytotoxicity Test of *Merremia peltata* (L.) Merr., (Fam. Convolvulaceae) Leaves

Yohannes Alen[1], Puspita Sari[2], Yufri Aldi[1], Yulianis[2], Shuhei Nakajima[3], Niomichi Baba[3] and Akmal Djamaan[1,4*]

[1]*Faculty of Pharmacy, University of Andalas, Padang, Indonesia*
[2]*Department Pharmacy, School of Health, Harapan Ibu, Jambi, Indonesia*
[3]*Graduate School of Natural Products and Bioresources Chemistry, Okayama University, Japan*
[4]*Laboratory of Biota Sumatra, University of Andalas, Padang, Indonesia*

ABSTRACT

The study of cytotoxic effects of methanolic extract and their fractions of leaves Merremia peltata (L.) Merr., (Fam. Convolvulaceae) had been done by Brine Shrimp Lethality Method. Results showed that the methanolic extract have cytotoxic (LC_{50}) 19.68 ppm, n-hexane fraction 22.03 ppm, ethyl acetate fraction 130.92 ppm, and methanolic fraction 532.11 ppm respectively, while the water residue fraction have no activity below 1.000 ppm.

Keywords: extraction, fractionation, cytotoxicity, *Merremia peltata* (L.) Merr.

INTRODUCTION

The plant namely *Merremia peltata* (L.) Merr.) is one kind of species from Convolvulaceae family. This plant is perennial herbaceous vine, which often wrapped around the other plants. The plant's stem is smooth that may up to 20 m length and has spiral end. Leaves are simple, alternate with purple vine beneath the leaves, leaf margin is waxy and its has milky white sap. Synonym of this plant are *Convolvulus peltatus* (L.), *Ipomoea peltata* (L.), Choisy, *Ipomoea nymphaeifolia* Blume, *Merremia nymphaefolia* (Dietr.) Hall. Fil. *Operculina peltata* (L.), Hall. Fil [1].

Some local communities in Indonesia have been using leaves of this plant traditionally as an anti-cancer medicine (especially for breast cancer), diarrhea, abdominal pain, cough, sore eyes, wound, inflammation and for wound compress, it also used for helping the birth process. Research about *Merremia peltata* had been conducted, and it had known that this plant has biology activity as anti-HIV-1 (EC 31.3 µg/ml). Other research showed that this plant acted as anti-bacteria on 500 ppm concentration[2]

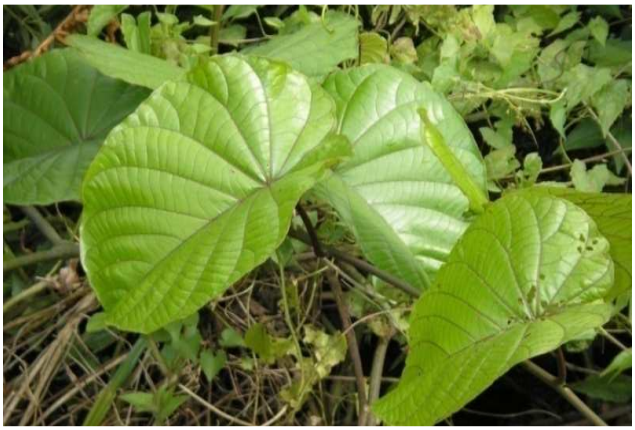

Figure 1. Profile of *Merremia peltata* (L.) Merr leaves

Knowledge about the benefits of *Merremia peltata* (L.) Merr., many researcher interested to fractionating the extract and prove the toxicity of this plant's extract and fraction (LC_{50}) towards cell of shrimp larvae (*Artemia salina* Leach.) using Brine Shrimp Lethality Biossay method. According to the literature, if result of LC_{50} extract test showed value under $\leq$ 1.000 ppm, the extract stated as cytotoxic active in inhibits shrimp larvae (*Artemia salina* Leach.) growth [3,4].

Cytotoxic test with brine shrimp method is initial method to test the cytotoxic compound, before using continuation method such as cancer or bacteria cell test. Cyotoxic is one of characteristic compounds which able to inhibit or kill the organisms cell. From phytochemical screening test on fresh leaves, the plant have chemical compound contents of secondary metabolites such as terpenoid, steroid, saponin, and phenolic [1, 4].

MATERIALS AND METHODS

Plant Materials Collection

Plant materials such as *Merremia peltata* (L.) Merr. fresh leaves collected in June 2012, at Bypass area, Km-22, Padang City, West Sumatra, Indonesia. This species then identified at The Herbarium of Andalas University, with specimen number of Far/03/2006.

Method

This research used extraction method with maceration and fractionation. To proves sample fractionated completely, thin layer chromatography was conducted with *n*-hexane: ethyl acetate (7:3) as eluant.

Extraction and Fractionations

As much as 3.2 kg fresh leaves were finely chopped and macerated with methanol until all samples completely soaked in solvent. Each bottle was used 2 liter solvent, and it was soaked for 5 days. The result from maceration then filtered with glass funnel which coated with filter paper. From this method was obtained maceration filtrate. The filtrate combination from each maceration then evaporated with *in vacuo* method with vacuum distillation, which resulted thick methanol extract as much as 710 gram (22.18% w/w). Then, it was fractionated using solvent with different polarity, i.e. *n*-hexane, ethyl acetate and methanol. Each solution then evaporated *in vacuo* that was resulted thick fraction of *n*-hexane 7.5 gram (1,05% w/w), thick fraction of ethyl acetate 7.5 gram (1.05% w/w), thick fraction of methanol solution 40 gram (5.63% w/w) and residual thick fraction 655 gram (92.25% w/w), each fraction then tested for its cytotoxic activity.

Cytotoxic Activity Test with Brine Shrimp Lethality Bioassay

The test was done with 5 concentrations variation i.e 1.000, 500, 100, 50 and 10 ppm. Each concentration was done for 3 replication. Each of extract, *n*-hexane fraction, ethyl acetate fraction, methanol fraction and water residual fraction was pipette based on predetermined concentration. Sample then put into each vial with varied concentration and each solvent sample evaporated until it was dried. After that, sample added with DMSO 100 µl for each vial and sea water added until ½ part of vial limit which calibrated for 5 ml. Shrimp larvae as much as 10 individual then

added into the vial and it was stirred until homogenous and incubated for 1x24 hours. After 24 hours incubation, total number of life larvae was counted for each vial.

RESULTS AND DISCUSSION

Specifically, this plant has some differences beyond another look alike plants because of leaves with the purple vine. The method used in cytotoxic activity is Brine Shrimp Lethality Bioassay. This method was selected because it is initial test for cytotoxic activity, did not take long in the process and the tools used is simple. Cytotoxic activity was used *Artemia salina* Leach shrimp larvae, this larvae was obtained by incubated the eggs for 48 hours.

To dissolve the methanol extract, *n*-hexane fraction, ethyl acetate fraction, dissolved methanol fraction and residual fraction, DMSO was used. Addition of DMSO into vial test aim to help dissolve process of water hard-soluble compound, so the solution was finely distributed. The maximal addition of DMSO is 100 µL, because with that concentration DMSO will not caused any harm towards shrimp larvae.

Toxicity of one leaves extract towards *Artemia salina* Leach. shrimp larvae can be determined by the LC_{50} value as shown in Table 1, 2 and 3. The plants stated as toxic if LC_{50}value is 30-1.000 ppm, the extract stated as extremely toxic if it has LC_{50} value is under 30 ppm and non-toxic if LC_{50} value is over 1.000 ppm. Pure compound is stated as toxic if it has LC_{50} value < 200 ppm [5,6,7]. The level of toxicity showed the potential activity as anti-tumor.

Tabel 1. Probit value according to percentage of death.

% death

%	0	1	2	3	4	5	6	7	8	9
0		2.674	2.946	3,119	3.249	3.355	3.445	3.524	3.595	3.659
10	3.718	3.773	3.825	3.874	3.920	3.964	4.006	4.046	4.085	4.122
20	4.158	4.194	4.228	4.261	4.294	4.326	4.357	4.387	4.417	4.447
30	4.476	4.504	4.532	4.560	4.587	4.615	4.642	4.668	4.695	4.721
40	4.747	4.773	4.798	4.824	4.849	4.900	4.925	4.950	4.950	4.975
50	5.000	5.025	5.050	5.075	5.100	5.126	5.151	5.202	5.202	5.227
60	5.253	5.297	5.305	5.332	5.358	5.385	5.413	5.468	5.468	5.496
70	5.524	5.553	5.583	5.613	5.643	5.674	5.706	5.772	5.772	5.806
80	5.842	5.878	5.915	5.954	5.994	6.036	6.080	6.175	6.175	6.227
90	6.282	6.341	6.405	6.476	6.555	6.645	6.751	6.881	7.054	7.326

Standard Probit value

Table 2. Result of cytotoxic activity test from extract, *n*-hexane fraction, ethyl acetate fraction, dissolved methanol fraction and residual fraction of *Merremia peltata* (L.) Merr. leaves

Sample	Amount of Shrimp Larvae Deaths with Concentration (ppm)					Lc_{50} (ppm)
	1.000	**500**	**100**	**50**	**10**	
Extract	9	8	6	-	-	19.68
	9	8	6	-	-	
	10	9	10	-	-	
Mean	9.3	8.3	7,3	-	-	
n-hexane fraction	10	10	10	5	3	22.03
	10	10	10	4	4	
	10	10	10	4	2	
Mean	10	10	10	4.3	3	
Ethyl acetate fraction	10	10	3	-	-	130.92
	10	10	3	-	-	
	10	10	3	-	-	
Mean	10	10	3	-	-	
Dissolved methanol fraction	8	6	2	2	4	532.11
	6	3	4	2	2	
	5	3	5	4	4	
Mean	6.3	4	3.6	2.6	3.3	
Residual fraction	0	0	0	-	-	>1.000
	0	0	0	-	-	
	0	0	0	-	-	
Mean	**0**	**0**	**0**	-	-	

Annotation: Amount of death larvae was counted

Table 3. Probit LC_{50}analysis of methanol extract of *Merremia peltata* (L.) Merr. Leaves, Calculation for $LC_{50}$with Regression Equation: Methanol Extract of *Merremia peltata* (L.) Merr. Leaves

Methanol extract	Larvae amount for each series	Amount of death larvae Replication			Mean	Deaths %	Log Concentration	Probit Value
		1	2	3				
1000	10	9	9	10	9.3	93%	3	6.476
500	10	8	8	9	8.3	83%	2.698	5.954
100	10	6	6	10	7.3	73%	2	5.613

y = a + bx
a =3.968; b = 0.797
Accordingly, y =3.968+ 0.797x
For LC_{50}, respons is 50%, so probit (y) value is 5.00

Accordingly,
y = a + bx

5 = 3.968+0.797x
5-3.968 = 0.797x
x = 1.032/0.797
x = 1.294
LC_{50} = anti log x
= anti log 1.294
= 19.68 ppm

The extract activity that was showed is high enough, this condition allegedly because the extract contains the terpenoid and phenolic compounds. Hexane fraction also showed high cytotoxic activity, it is allegedly because hexane fraction contains non-polar terpenoid contents [7]. This condition was in accordance with primary component of secondary metabolite test result showed in Table 4.

Table 4. Initial test result of primary chemical compound of secondary metabolite from *Merremia peltata* (L.) Merr

Number	Chemical Compound	Reagent	Result
1	Alkaloid	Mayer	-
2	Flavonoid	HCl/Mg	-
3	Terpenoid/Steroid	Acetate anhydrate:H_2SO_4 conc	+/+
4	Saponin	Water/Foam	+
5	phenolic	$FeCl_3$	+

Annotation: + : reacted - : unreacted

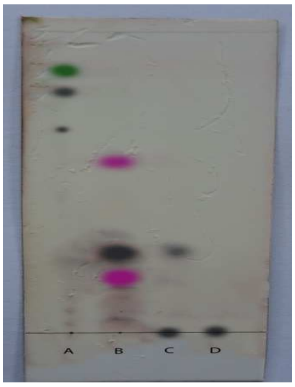

Figure 1. Profile of each fraction of Thin Layer Chromatography after addition of Vanilin Sulphate Acid Reagent

Annotation:
(A) *n*-hexane fraction (B) ethyl acetate fraction (C) dissolved methanol fraction
(B) and (D) residual fraction.

The result of thin layer chromatography analysis of each fraction showed that fractionation process was in accordance with polarity level and it was finely done as shown in Figure 1. The result of the spraying with $FeCl_3$, *n*-

hexane fraction and dissolved methanol fraction showed blackish-blue spot that allegedly contains phenolic compounds. Ethyl acetate fraction was showed two colors change, pink and purple spot that means the fraction was contains triterpenoid compound and positively reacted with vaniline sulphate acid.

Initial test result of primary chemical content from *Merremia peltata* (L.) Merr.. leaves, showed presences of terpenoid, steroid, sponin and fonolic compound. From 3.2 kg fresh samples of *Merremia peltata* (L.) Merr. was obtained methanol thick extract 710 gram (22.18%ww/w). After fractionated, it was obtained 7.5 gram (1.05%ww/w) *n*-hexane fraction, 7.5 gram (1.05% w/w) ethyl acetate fraction, 40 gram (5.63% w/w) dissolved methanol fraction and 655 gram (92.25%) residual fraction from the total weight of thick extract. From cytotoxic activity test with *Brine Shrimp Lethality Bioassay* method was obtained LC_{50} value of *Merremia peltata* (L.) Merr. leaves methanol extract as much as 19.68 ppm, and 22.03 ppm of *n*-hexane fraction, 130.92 ppm of ethyl acetate fraction, 532.11 ppm dissolved methanol fraction and for residual fraction was over 1.000 ppm. Brine shrimp lethality bioassay (BST) is an efficient, rapid and inexpensive assay for testing the bioactivity of plant extracts. It is an excellent choice for elementary toxicity investigations based on the ability to kill laboratory-cultured Artemianaupli [9]. Studies have demonstrated a positive correlation between the brine shrimp lethality and oral lethality test in mice in medicinal plant research [10].

CONCLUCION

From 3.2 kg *Merremia peltata* (L.) Merr. leaves was obtained as much as 710 gram (22.18% w/w) thick methanol extract, 7.5 gram (1.05% w/w) *n*-hexane fraction, 7.5% gram (1.05%w/w) ethyl acetate fraction, 40 gram (5.63% w/w) methanol fraction and 655 gram (92.25% w/w) residual fraction. Cytotoxic test result showed that activity value (LC_{50}) of each methanol extract is 19.68 ppm, *n*-hexane fraction is 22.03 ppm, ethyl acetate fraction is 130.92 ppm, dissolved methanol fraction is 532.11 ppm and residual fraction is over 1000 ppm. The result of phytochemical content test from *Merremia peltata* (L.) Merr. leaves showed the presence of terpenoid, steroid, saponin and fenolic compound.

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