Open Acces

Int. J. Trop. Vet. Biomed. Res.
Vol. 1 (1) : 45-49; May 2016

www.jurnal.unsyiah.ac.id/IJTVBR
E-ISSN : 2503-4715

Cytotoxic Potential of n-Hexane Extract of *Calotropis gigantea* L. Leaves

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Abstract

The present study was done to determine the cytotoxic potential of n-hexane extract of Calotropis gigantea L. leaves and its fractions. Here, dried leaves of C. gigantea L. were macerated using n-hexane to obtain crude extract of 21.16 g (1.03%). The components of n-hexane extract of C. gigantea L. leaves were separated with vacuum-liquid chromatography resulted 4 fractions which are A (0.5 g), B (0.9g), C (5.29 g), and D (6.25 g). Phytochemical screening indicated that the extract contained various secondary metabolic compounds such as steroids, terpenoids, saponins, flavonoids, coumarins, and phenolics. Cytotoxic potenital of the crude extract of C. gigantea L. and its fractions was examined using brine shrimp lethality test (BSLT). The LC50 values of C. gigantea's n-hexane extract and its fractions that determined by Finney method were 272.27 (n-hexane extract), 31.62 (fraction A), 43.65 (fraction B), 33.89 (fraction C) and 20.98 (fraction D) µg/mL, respectively. The most active was D fraction contained terpenoids, flavonoids, and phenolics which were assumed contribute to its cytotoxic potential. These results suggested that n-fraction of C. gigantea might possess antitumor or pesticidal activities..

Key words : Calotropis gigantea, secondary metabolites, cytotoxic potential, Finney method, BSLT.

Background

Indonesia has many lowland and highland plants that may bring benefits to human life as sources of foods and medicines. More interests have been devoted to investigate into chemical ingredients of some medicinal plants (Heyne, 1987). One of herbs that commonly used as traditional medicines by community in Aceh province is *Calotropis gigantean*. Scientific information regarding to active compounds and pharmacological effects of the plant that grows in the dry land with tropical climate is still limited (Khare, 2004).

Traditionally *C. gigantea* was used as analgesic, toothache and ear medicines as well as sprains and epilepsy and wound healers (Kasahara, 1986; Kamuhabwa, *et al*., 2000; Pathak and Argal, 2007; Saratha, *et al.*, 2009; Ravi *et al.*, 2011). A variety of compounds have been isolated from this plant include β-Amyrin, taraxasterol, gigantin, giganteol, β-sitosterol (Elakkiya, *et al*., 2012) and calotropin (Wang, *et al*., 2008). Calotoxin, uskharidin and voruskharin were also isolated from *C. gigantea* leaves (Bhat and Sharma, 2013). Habib and Karim (2011) who tested pharmacological effects of methanol extract or petroleum ether and chloroform fractions from *C. gigantea* in mice identified anticancer activity of methanol extract and chloroform soluble fractions of this plant. Hasballah (2013) recently investigated cytotoxic compounds extracted from *C. gigantea* stem bark. Using Brine Shrimp Lethality Test (BSLT) she found that LC_{50} values of n-hexane extract of *C. gigantea* stem is 61.67 ppm on *Artemia salina*.

According to Juniarti *et al*. (2009), a substance is said to be active or have toxic properties when the LC_{50} values are less than 1000 ppm for the extract and less from 30 ppm for the pure compounds. Therefore, it is important to isolate cytotoxic

compounds from n-hexane extract of leaves of *C. gigantea* guided by BSLT. The BSLT using the larvae of *A. salina* as experimental animals is one of preliminary methods used to test natural materials which are considered toxic (Obuotor and Onajobi, 2000; Pisutthanan, *et al*., 2004).

Materials and Methods

Plant Materials

Calotropis gigantea plants were collected from the wild growing populations in Alue Naga village, Syiah Kuala Subdistrict, Banda Aceh, Indonesia. The plants were then identified in the Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Syiah Kuala University.

Preparation of extract

The leaves of *C. gigantea* were air dried at room temperature, powdered (2,000 g) and macerated with n-hexane for 3x24 hours. Filtrate resulted were concentrated using rotary vacuum evaporator resulted in 21.16 g (1.03%) thick n-hexane extract. The extract was kept in desiccators for about two weeks. Small amounts of extract were evaporated to dry before the cytotoxicity tests were carried out. The dried extracts were weighed, dissolved in Tween 80 and used as stock. The rest of extracts were kept in freezer for further use.

Fractionation of concentrated n-hexane extract

Concentrated n-hexane extract, 10 g, were drawn and its components are separated using a Vacuum Liquid Chromatography (VLC). Stationary phase was Silica gel 60 G (100 g) whereas n-hexane and ethyl acetate with gradient elution of 9:1 were used as a mobile phase. This optimum condition was determined based on TLC analysis. Fractions resulted was collected using 100 mL Erlenmeyer flasks. The fractions were combined according to similarity of stain patterns after being eluted with an eluent system obtained and also sprayed with reagent seric sulfate. This is called the combined fractions. Then, the concentrated extracts and combined fractions were used for chemical identification of bioactive compounds and BSLT test.

Screening of secondary metabolite composition

Composition of bioactive compounds of *C. gigantea* leaves n-hexane extract and its combined fractions was determined using standard proceduresdescribed by Harbone (1998).

Bioassay

The procedure for BSLT was modified from the assay described previously by Meyer *et al*. (1982). A serial dilution was prepared by diluting stock extract in Tween 80 In triplicateseveral concentrations of leaves extract and its fractions were added in equal volume with 100 mL of vehicle suspension containing 10 nauplii. In this test used to determine dose response relationship, a control group was set with vehicle The nauplii were drawn through a glass capillary, placed in test tube containing sample and filled up artificial sea water up to 5 mL. The tubes were incubated at room temperature (25-29 ˚C) for 24 hours. Then, the numbers of dead (non-motile) nauplii in each tube were counted and LC_{50} values were estimated. The percentage of lethality was determined by comparing the mean of died larvae of the test with those treated with positive conrol vincristine sulfate. LC_{50} values were obtained by plotting concentration o extracts versus percentage of lethality using statistical method of Finney's probit analysis.

Results and Discussion

Maceration of the dried leaves of *C. gigantea* in n-hexane yielded a blackish green extract. Fractionation of the extract by VLC on silica gel gave 42 fractions (Figure 1.). According to similarity of stain patterns in the TLC analysis, the fractions were combined into four combinations designed as fraction A (fractions 1-6), fraction B (fractions 7-8), fraction C (fractions 9-11), and fraction D (fractions 12-42).

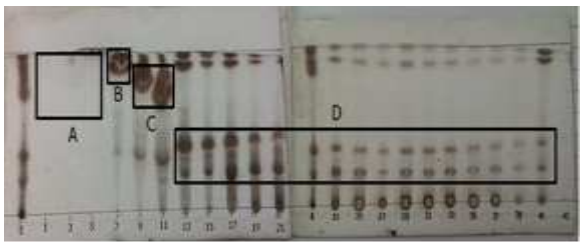

Figure 1. The chromatogram of *C gingantea* fractions

Qualitative phytochemical screening

Phytochemical compounds were screened in the n-hexane leaves extract of *C. gigantea* and fractions through a qualitative method. The results indicated the presence of steroids, terpenoids, flavonoids, coumarins, phenols as presented in Table 1. These compounds have been showed strong antioxidant property and other bioactivity important for medical purposes. The antioxidant may prevent and cure cancer and other diseases by protecting the cells from cellular damage caused by free radicals - the highly reactive oxygen compounds (Caragay, 1992; Steenkamp and Eloff, 2007; Usman *et al*., 2012).

Table 1. Phytochemical screening of leaves extract of *C. gigantea* and its fraction

Chemical constituents	n-hexane extract	Fractions			
		A	B	C	D
Steroids	+	-	-	-	-
Terpenoids	+	-	-	-	+
Saponins	-	-	-	-	-
Flavonoids	+	-	-	-	+
Coumarins	+	-	-	+	-
Phenols	+	-	-	-	+

+: presence; -: absence

Cytotoxicity bioassays

The cytotoxic effects of the extract and its fractions were determined by using brine shrimp lethality test (BSLT) (Ghisalberti, 1993). Brine shrimp nauplii mortality and LC_{50} values after treatment with the n-hexane extract of *C. gigantea* leaves and its fractions are shown in Table 2.

Table 2. Cytotoxic activity of n-hexane extract of leaves of *C. gigantea* and its fractions

Examined Sample	LC_{50} (µg/mL)	Compound Type
n-hexane extract	272.27	Terpenoids, steroids, phenols, flavonoids, and coumarins
A Fraction	31.62	Undetected
B Fraction	43.65	Undetected
C Fraction	33.89	Coumarins
D Fraction	20.98	Terpenoids, flavonoids, and phenols
Positive control	0.6	Vincristine sulfate

The LC_{50} for n-hexane extract of *C. gigantea* andfractions obtained from Finney method were 272.27, 31.62, 43.65,33.89 and 20.98 µg/ml, respectively (Table 2). In toxicity evaluation of crude extracts by BSLT, LC_{50} values less than 1000 µg/mL were considered significantly active (Loomis *et al*., 1978), whereas for a fraction of a test solution is said to be active if it has LC_{50} value <100 µg/ml and the smaller the LC_{50} value, the more active a test solution (Juniarti, *et al*., 2009).

Results of this study suggested that all the test samples were lethal to brine shrimp nauplii. However, D fraction demonstrated the highest toxcity potential. These positive results indicated that these bioactive compounds, especially fraction D, may possess antitumor or pesticidal activity. Therefore, further purification of highly active fraction of the n-hexane extract of *C. gigantea* leaves and elucidation of the structure of the potential active compounds may lead to the discovery of the new cytotoxic compounds.

Conclusion

Results of our study revealed that the n-hexane extract of *C. gigantea leaves* and its fractions exhibited cytotoxic potential.
Further study is required to further isolate and purify the most toxic D fraction in order to obtain the lead compound responsible for

the activity as well as to investigate its therapeutic potential as an anticancer drug.

Acknowledgement

We would like to thank to Project Hibah Bersaing, Syiah Kuala University, the Ministry of Educational and Cultural Affair (No: 498/UN11/S/LK-BUPT/2014) for financial support.

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