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TOXICITY AND ANTIOXIDANT ACTIVITIES OF EXTRACT OF n-HEXANE, H_2O, AND ETHYL ACETATE FROM THE LEAVES OF DURIAN, *Durio zibethinus L.*

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ABSTRACT

Steroids are secondary metabolites abundantly found in plants. Traditionally, the leaves of durian, *Durio zibethinus* L. (Malvaceae) have been used as an antipyretic and analgesic by communities in Indonesia. This research was performed to discover the potency of some extracts from the leaves of *D. zibethinus* L. as antioxidants and investigate their toxicity. The extracts of durian leaves, *D. zibethinus* L. was carried out with 96% ethanol, then partitioned with water, *n*-hexane, and ethyl acetate respectively. The antioxidant and toxicity screening was done by Brine Shrimp Lethality Test (BSLT) and free radical scavenging method using diphenyl-1-picylhydrazyl (DPPH). Brine shrimp lethality test (BSLT) gave a toxicity LC_{50} of 195.13; 85.37; and 453.78 ppm for *n*-hexane, ethyl acetate and water extracts, respectively. Free radical scavenging method using diphenyl-1-picrylhydrazyl gave an antioxidant activity IC_{50} 90.58; 48.04; and 171.78 ppm for water, *n*-hexane, and ethyl acetate extracts, respectively. Result of the studies paves the way for further studies on isolation and characterization of the chemical compound as antioxidant and anticancer.

Keywords: Toxicity, Antioxidant, Steroids, *Durio zibethinus* L., Malvaceae

INTRODUCTION

Steroids are secondary metabolites mostly analyzed in plants, as these are major constituents of plant pigments. Steroids are used as drugs or food supplements due to their strong antioxidant activity[1]. *Durio zibethinus* L. (local name: durian) is species from the Malvaceae family and traditionally has been used as an antipyretic and analgesic. Phytochemical screening revealed that the fruit of this plant contains flavonoids and polyphenol. Pung Manee, Mon Thong, and Chani are a cultivar of Durian have high bioactivity and the main of it were the total polyphenols contributors to the overall antioxidant capacity[2]. Ethanolic extract from durian seed contains complex secondary metabolites, including proanthocyanine oligomer[3]. The aim of this research is to discover the toxicity and antioxidant activity of water, *n*-hexane, and ethyl acetate extracts from the leaves of durian (*D. zibethinus* L.).

EXPERIMENTAL

Materials used in this research were powdered leaves of *D. zibethinus* L. collected in Desa Pangaribuan, Sibolga, , Sumatera Utara Indonesia. Chemicals and solvents such as 96% ethanol, *n*-hexane, ethyl

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acetate (Sigma), diphenyl-1-picrylhydrazyl (DPPH), 1% dimethyl sulfoxide (DMSO), vitamin C (Merck), cysts of *Artemia salina* (leach) (Wudi Fengtai Aquaculture Co Ltd, China).

Extraction and Purification

The leaves of durian (*D. zibethinus* L.) powder (1.8 kg) macerated with ethanol 96% 6 times. Maceration process that had done at room temperature[4]. Then partitioned with *n*-hexane and ethyl acetate 3 times. All extracts are evaporated and weighed (see the scheme of the extraction process in Fig.-1).

Toxicity Test by Brine Shrimp Lethality Test (BSLT)

The toxicity of extract (water, *n*-hexane, and ethyl acetate) was measured by using Brine Shrimp Lethality Test (BSLT) method based on Meyer et al., 1982.[5] The concentration of the sample were 10, 100, 1000 µg/mL and toxicity measurements were performed in three repetitions. Using this method, the toxicity of all extracts with LC_{50} value is the concentration of the extract that gives like 50% mortality rate.

Antioxidant Activity Test by Free Radical Scavenging (FRS) Method

The test activity of antioxidant in extracts with Molyneux[6] using a reagent 4 mM DPPH (diphenyl-1-picrylhydrazyl). Calculated DPPH (inhibition) using the formulation of antioxidant activity test by free radical scavenging (FRS):

$$\text{Inhibition (\%)} = [(A_o - A_1) / A_o] \text{ X } 100$$

The absorbance of blank as A_o and absorbance of the sample as A_1. The concentration of inhibition IC_{50} is the concentration of antioxidant value that has inhibit 50% (mg/mL) of free radicals. Value of IC_{50} was obtained from interline between 50% concentration inhibition axes and the formula Y = a + bx, x showed IC_{50} value and Y = 50. Accessed to the National Cancer Institute, the active compound from the extract by Institute, compound or extracts are studied to have good activity of antioxidant if it has IC_{50} value < 20 mg/mL[6].

RESULTS AND DISCUSSION

Extraction and Partition

Extraction of 1.8 kg leaves of durian gave 185.2 g of ethanol extract. The Extract of ethanol was partitioned with water, *n*-hexane, and ethyl acetate successful to get extracts of water, *n*-hexane, and ethyl acetate. The yield of the extracts and debt to equity ration (DER) obtained from ethanol extract listed in Table-1.

Table-1: The Yield of Extracts Obtained from the Leaves of Durian, *Durio zibethinus* L.

Extract	Weight (g)	Yield (%)*	Debt to equity ratio**
Ethanol	185.2	10.29	9.72
n-hexane	14.3	0.79	125.87
Ethyl acetate	3.56	0.20	505.61
Water	126.7	7.04	14.20

*Calculated from dry material (1.8 kg leaves of durian) $\frac{185.2}{1800}$ x 100 %

**Calculated by $\frac{1800}{185.2}$

Screening of extract ethanol from durian leaf such as steroids/terpenoids, flavonoids, an glycosides[7]. The result of extraction and partition for samples showed that the water extract had the highest yield (7.04%) among other extracts (0.2 - 0.8%). This result showed that water extract possibly contained a polar compound with a high molecular weight such as glycosides, polyphenols or some polysaccharides which had a correlation with a yield of the extraction process.

Toxicity of Extracts

The value of the toxicity with BSLT displayed that the extract of ethyl acetate gave the highest percentage of mortality with the lowest LC_{50} of 85.37 ppm. The toxicity test results can be seen in Table-2.

Antioxidant Activities of Extract

The test results of antioxidant free radical scavenging on all extracts showed that ethyl acetate extract gave the highest percentage inhibition (85.57%) with IC_{50} of 48.05 ppm. These results indicate that the

flavonoids and polyphenols contained in durian act as an antioxidant.[2,3.] The antioxidant activity for all extracts can be seen in Table-3.

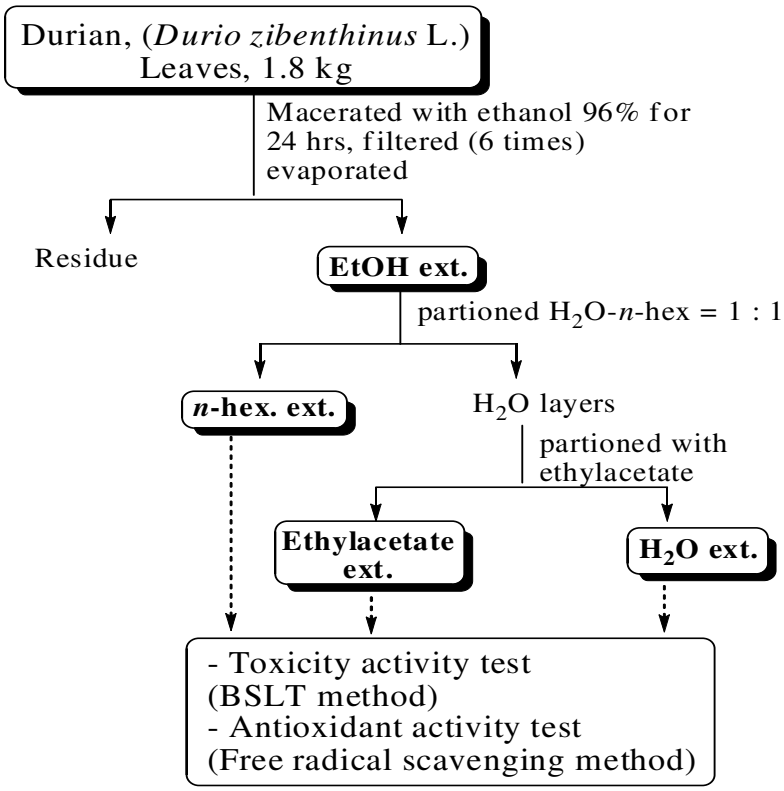

Fig.-1: Scheme of Extraction and Partition Process for Leaves of Durian (*Durio zibethinus* L.).

Table-2: Toxicity of all Extracts Obtained from the Leaves of Durian, *Durio zibethinus* L.

Extracts	Conc. (ppm)	Total amount of larva	Amount of dead larva	LC_{50} (ppm)
Water	10	45	0	453.787
	100	45	5	
	500	45	34	
Ethyl acetate	10	45	6	85.375
	100	45	18	
	500	45	45	
n-hexane	10	45	4	195.132
	100	45	7	
	500	45	42	

Table-3: Activity of Antioxidant in Gained of Extracts from The Leaves of Durian, *Durio zibethinus* L.

Extracts	Concentration (ppm)	IC_{50} (ppm)
Water	5	90.583
	10	
	25	
	50	
	100	
Ethyl acetate	5	48.047
	10	
	25	

	50	
	100	
n-hexane	5	171.953
	10	
	25	
	50	
	100	
Vitamin C	1	4.320
	3	
	5	
	7	
	9	

CONCLUSION

Ethyl acetate extract has a mortality percentage (LC_{50} = 85.37 ppm) and IC_{50} value (48.04 ppm), which is considered as a strong antioxidant (< 50 ppm). This has potential as an antioxidant bioactive compound resource with moderate toxicity.

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