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Characteristics, Efficacy and Safety Testing of Standardized Extract of *Croton tiglium* Seed from Indonesia as Laxative Material

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Abstract: Identification and taxonomy analysis conducted at Herbarium Bogoriense at Research Centre for Biology, Indonesian Institute of Sciences Bogor. The name of the plant was *C. tiglium* L. The result of analysis on *C. tiglium*, ethanol extract as laxative material using the intestinal transit method showed treatment group that received dosage 0.06 mL/30 g b.wt. (72.5%) was significantly different compared to negative control (48.4%) or positive control (50.6%) which showed the weak effect as laxative at the dosage of 0.75 mL/30 g b.wt. It showed that ethanol extract of *C. tiglium* seed at dosage 0.06 mL/30 g is effective as laxative. The test result of the treatment using dosage 0.06, 0.04, 0.026 and 0.07 mL/28 g of body weight showed the mice population response 100, 60, 40 and 40% consecutively. The Thompson and Weil analysis result showed the ED_{50} was at 0.027 mL or equal to 639,5 g kg^{-1} b.wt. The LD_{50} was at 0.0707 equals with 1674,5 mg kg^{-1} b.wt. Safety limit is the range of dosage that cause the lethal effect and the dosage that gives the intended effect. The safety limit is represented by the comparison of LD_{50}/ED_{50}. Calculation result that the extract safety limit was LD_{50}/ED_{50} = 0.0707/0.027 = 2.7.

Key words: *Croton tiglium*, ethanol extract, efficacy, safety test

INTRODUCTION

Indonesia is one of the countries with mega diversity for medical plants in the world with the second highest biodiversity after Brazil. There are 40,000 types of flora in the world and 30,000 of them can be found in Indonesia and 940 of them is known to be beneficial as medicines that have been used traditionally generations by generations by many different ethnics in Indonesia. This biodiversity is a national asset that has high value for the development of agromedicine industry in the world (Dictionary of Natural Products, 1982; Hutapea, 1994).

Recently, the tendency of going back to nature lifestyle, with the believe that consuming natural medicines is relatively safer than that of synthetic ones, has risen the world demand of natural medicines so that the prospect of medical plant for both foreign and domestic markets is significantly larger (Kardono, 1991; Gupta, 1994).

The Kamandrah (*C. tiglium* L.) is one of the medical plants that can easily be found in Indonesia. In Central Kalimantan, the seed is widely used as laxative (Sangat *et al*., 2000). Even so, the people's knowledge of using the plant as laxative is merely information passed down in generations so that we do not know yet the active ingredients contained in the plant.

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Most of the traditional medicines developed by natural selections do not meet the scientific requirements of modern medication. In order to make the usage of traditional medicines accountable, a research must be conducted to find the active ingredients and its efficacy and safety (BPOM, 2005; Fabio *et al*., 2006).

To determine whether a medical plant can be used as medicine or not, it has to be non-toxic. The safety limit of a medicine is set by index/coefficient called therapeutic index. This index is safety measurement of therapeutic effect and toxic effect. The higher the therapeutic index of a medicine, the safer it is (Dipalma, 1971; Mutcshler, 1986).

In the development of pharmaceutical industry, the traditional medicines are categorized into four groups, traditional medical herbs, standardized extract, phytopharma and supplement/nutraceutical (BPOM, 2005). The group used in this research was the standardized extract. It is the result of pre-clinical extracting from the natural source and has been proven to be effective both in its efficacy and its safety (Okokon *et al*., 2004).

This research is intended to provide information about the characteristics of the *C. tiglium* seed and find out its efficacy and the safety of *C. tiglium* standardized extract which can give qualitative contribution as standardized extract material.

MATERIALS AND METHODS

Plant materials: *C. tiglium* seeds, were collected from its natural habitat in Tamiang Layang Forest, in the boundary of Central Kalimantan, Indonesian between June and September. The plant was identified and taxonomists analysis conducted at Herbarium Bogoriense at Research Centre for Biology, Indonesian Institute of Sciences Bogor.

Animals: Forty eight male albino mice of similar age and weight (30-40 g) were selected for this study.

Instrument and general procedures: Magnetic stirrer, buchner funnel, filter paper, analytical balance, candle jar, candle, swap, blender, centrifuge, ice box, autoclave, incubator, laminar flow, microscope, object glass, micro pipet, magnifying glass, petridish, colony counter, reaction tube, sterilized bottle and measurement flask.

Preparation of *C. tiglium* seeds organic-soluble extracts: The seed is refined, sun-dried and grinded into powder. About 200 g powdered seeds were extracted continuously by maceration using hexane solvent (3×1 L) and evaporated at 35°C to yield the ethanol-soluble extract. The residues 108 g were macerated with 747 mL ethanol for 24 h, repeated for 3 times and ethanol-soluble extract was obtained. The extract was dried using vacuum-oven at 35°C. Yield of ethanol extract used in the experiment is 18.6%. Extract is in the oily form (i.e., 100 g seed = 3.15 mL ethanol extract).

Identification and taxonomy evaluation: Identification and plant evaluation was conducted as empirical study to confirm that the plant used for the research is true the same plant that has been widely used by many generations as traditional medicine. This identification and taxonomical evaluation was conducted by Herbarium Bogoriense at the Research Center for Biology, Indonesian Institute of Sciences (LIPI) Bogor.

Efficacy testing of the extract as laxative

Intestinal transit method: According to Anonymous (1982), the experiment was conducted on male mice (ddY) weighing 28-40 g. Previously, the mice were acclimatized for one week. The animals that have normal mice feces during acclimatization were grouped in five different treatments.

Group 1 was control group (-) received water 0.1 mL/30 g body weight (BW), group 2 received Oleum Ricini (OR) dosage 0.75 mL/30 g b.wt. (1 mL OR = 0.0304 g) as control group (+) and the other groups received *C. tiglium* ethanol extract dosage 0.03 (group 3), 0.06 (group 4) and 0.09 mL/30 g b.wt. (group 5) (1 mL extract = 0.0315 g seed). We used Oleum Ricini (OR) for positive control because it a commercial laxative medicine (Ansel, 1989).

Prior to the treatment, mice were not fed for 18 h but still given water *ad libitum*. At min 0 (t0) the object was given intragastric using gastric hose and with the same method it was given norit dosage 0.1 mL/10 g at min 45 (t45). At the end of the experiment, at min 65 (t65) the mice were euthanized using chloroform and the intestines were taken out. The length of intestine from pylorus to rectum and the length of intestine passed by norit marker were measured. The ratio of the length of intestine passed by marker (A) and the total length of the intestine (B) represented intestinal transit.

$$\text{Intestinal transit} = (A/B) \times 100\%$$

Defecation method: The animals tested were male mice that have normal waste characteristic. Average weight at the time of the experiment was 30 g. The animals were grouped into 5 different treatments. Group 1 was given water with solvent as negative control, group 2, 3, 4 were the groups that were given ethanol extracts dosage 0.030,

0.060 and 0.090 mL mice^{-1} (1 mL extract = 0.0315 g seed). Group 5 was given OR dosage 0.75 mL hamster^{-1} (positive control) (1 mL OR = 0.0304 g). The mice were put into individual cage and the characteristics of the waste were observed every 30 min for 4 h.

The characteristics of waste covered the quantity, weight and consistency. The data was analyzed using ANOVA and continued with SNK when it showed significant gap on $p<0.05$. Statistical Analysis of waste was conducted using Mann Whitney Test.

Safety limit test of the extract as laxative: The animals tested were male mice weighing 30-40 g. Observation was conducted for 3 h on waste characteristics. The animals showing soft waste were said to positively responded to treatment. The treatment was the dosage of ethanol extract 0.060, 0.040, 0.026 and 0.017 mL/30 g b.wt. The data were collected to be analyzed to count for the effective dosage (ED_{50}) using the formula (Thompson and Weil, 1952; Loomis, 1994) :

$$\text{Log } ED_{50} = \log D + d(f+1).$$

The animals tested were adult male mice ddY weighing 30-40 g. Observation was conducted for 24 h. The treatment was giving ethanol extracts with the dosage 0.060, 0.040, 0.026 and 0.017 mL/30 g b.wt. The parameters were the number of death, symptoms during experiment and relative toxicity level. Counting the Lethal Dosage (LD_{50}) using the formula (Laurence and Bacharach, 1964):

$$\text{Log } LD_{50} = \log D + d\,(f+1).$$

The data collected from both effective Dosage test and Lethal Dosage were analyzed to determine extract safety limit using the calculation (Laurence and Bacharach, 1964; Loomis, 1994):

$$\text{Safety Limit} = LD_{50}/ED_{50}.$$

RESULTS AND DISCUSSION

Identification and taxonomy analysis conducted at Herbarium Bogoriense at Research Centre for Biology, Indonesian Institute of Sciences Bogor, confirmed that the plant used in the experiment was indeed the same plant used by the people for many generations. The name of the plant was *Croton tiglium* L.

The purgative effect of *C. tiglium* was conducted by studying the effect on the intestinal transit marker and waste characteristics of the test animals. The result on the effect of using ethanol extract of on *C. tiglium* seed to intestinal transit and intestine length on different treatments can be shown in Table 1.

Table 1: Effect of treatment dosage on intestinal transit

Treatments	Dosage (mL g^{-1} b.wt.)	Intestinal transit (%)
Control-(Water)	0.10	48.36^{a}
DI	0.03	61.89^{bc}
DII	0.06	72.52^{c}
DIII	0.09	65.08^{bc}
Control + (OR)	0.75	50.60^{b}

The result of ANOVA analysis showed that the ethanol treatment had significant impact on the Intestinal Transit of the tested animals. To know the differences between groups, further test was conducted using SNK test. Significance test result showed treatment group that received ethanol extract dosage 0.06 mL/30 g b.wt. (72.5%) of body weight was significantly different compared to negative control (48.4%) or positive control Oleum ricini (50.6%) which showed the weak effect as laxative at the dosage of 0.75 mL/30 g b.wt. of body weight. It showed that ethanol extract of *C. tiglium* seed at dosage 0.06 mL/30 g is effective as laxative.

Using defecation method for observation was based on the consideration that experiment object which functions as laxative will change the defecation pattern of the testing animals marked by the increase of the defecation frequency, waste consistency that became softer or liquid and/or the increasing of waste production mass. The method was used to evaluate the laxative effect of ethanol extract, followed by observing the characteristics of the feces produced by mice in 4 h. The result of analysis of variables indicated that the amount of waste and the weight of the waste on the group that was given ethanol extract dosage and both positive and negative control did not have significant impact to the amount and the weight of the waste produced by testing animals.

However, even the result of analysis of variables indicated the amount and weight of the feces was not statistically significant, the amount of feces produced by mice with the treatment of ethanol extract 0.06 mL (DII) was 9.9 higher compared to other treatments. DI (8.2), DIII (6.4), negative control (8.7) and positive control (9.0). While the result of waste weight owed that the treatment produced lighter weight compared with positive and negative control. The cause was the feces produced by the mice had been transformed softer until it melted so the weight was decreased as shown in Table 2.

Observation on the characteristic of the waste that showed mice waste treated by ethanol extract showed that waste characteristic were varied from hard to medium soft to liquid soft (dosage 0.03 and 0.09) and hard to liquid (dosage 0.06). On the other hand, the group treated with OR as positive control produced waste with the

Table 2: Effect of treatment dosage to the amount of feces

	Treatments				
n	Control-(Water = 0.1 mL)	DI (0.03 mL)	DII (0.06 mL)	DIII (0.09 mL)	Control + (OR = 0.75 mL)
1	9.00	19.00	14.00	2.00	4.00
2	15.00	8.00	14.00	7.00	8.00
3	6.00	5.00	12.00	9.00	5.00
4	7.00	10.00	9.00	8.00	0.00
5	14.00	1.00	3.00	0.00	13.00
6	9.00	17.00	8.00	7.00	14.00
7	5.00	9.00	0.00	2.00	13.00
8	13.00	1.00	6.00	5.00	16.00
9	3.00	10.00	13.00	9.00	16.00
10	6.00	2.00	20.00	15.00	1.00
Average	8.70	8.20	9.90	6.40	9.00
SD	4.083	6.268	5.915	4.376	6.1604

Table 3: Effect of treatment dosage on the feces weight (g)

	Treatments				
n	Control-(Water = 0.1 mL)	DI (0.03 mL)	DII (0.06 mL)	DIII (0.09 mL)	Control + (OR)
1	1.60	1.30	1.40	0.50	1.20
2	1.30	1.10	2.00	0.90	0.40
3	1.80	0.50	1.40	1.00	2.20
4	0.80	0.80	0.80	1.10	0.40
5	1.50	0.00	0.60	0.00	1.90
6	2.10	1.80	0.40	1.20	0.70
7	0.50	0.80	0.00	0.40	1.50
8	0.90	0.00	0.70	0.82	1.80
9	1.80	0.60	1.50	0.80	2.70
10	0.90	0.10	1.20	1.00	0.60
Average	1.32	0.87	1.11	0.86	1.34
SD	0.52	0.52	0.52	0.26	0.81

characteristic ranged from hard to liquid and the characteristic of the negative control feces ranged from hard to semi hard as shown in Table 3.

The result of benefit test of ethanol extract as laxative material using the intestinal transit method showed that the treatment with the dosage 0.06 mL/30 g b.wt. was the best treatment with significant impact in both testing method. The result of observation on the feces characteristics using defecation method was consistent with the result of test using the intestinal transit method. Therefore, it was obvious that ethanol extract contained tetradecanoic acid compound that has laxative effect. The effective dosage of the ethanol extract as laxative was 0.06 mL/30 g (DII) of body weight with the observable effect of increasing transit time and modifying characteristics of feces. Based on Mass Spectrum Chromatogram (Mangunwidjaja *et al*., 2006), it was found that this extract content tetradecanoic acid, which predicted has functions as laxative material (Dictionary of Natural Products, 1982). According to Ansel (1989), active ingredient compound can be classified as medicine if it is within the appropriate dosage and called as toxic if given exceeding the dosage or not functioning if given below standard dosage.

This test was conducted to see how far the ethanol extract is safe to consume. The experiment was conducted to determine the effective dosage and lethal dosage on the tested animals. The test result of the tested animals mice conducted on several parameters of the tested animals that showed positive respond as shown in Table 4.

Table 4: Effect of treatment dosage on the positive response of the tested animals

Treatments	Dosage (mL g^{-1} b.wt.)	Animal response (%)	Effective dosage (ED_{50})
AI	0.060	100	0.027 mL g^{-1} b.wt. equals with
AII	0.040	60	639.5 mg kg^{-1} b.wt.
AIII	0.026	40	
AIV	0.017	40	

The test result of the treatment using dosage 0.060 (AI), 0.040 (AII), 0.026 (AIII) and 0.017 mL/30 g b.wt. (AIV) of body weight showed the response 100, 60, 40 and 40% consecutively. Therefore it can be said that the lower the dosage of the ethanol extract the lower the tested animals respond. The Thompson and Weil (1952) analysis result showed the effective dosage (ED_{50}) was at 0.026 mL/30 g b.wt. or equal to 635 mg kg^{-1} body weight.

The lethal dosage (LD_{50}) test showed the higher the dosage, the higher the level of the animal death. The test result shown in Table 5. The Thompson and Weil analysis showed Lethal Dosage (LD_{50}) was at 0.0707 mL equals with 1726.7 mg kg^{-1} b.wt.

Safety limit is the range of dosage that cause the lethal effect and the dosage that gives the intended

Table 5: Effect of treatment dosage to the number of dead mice

Treatments	Dosage (mL g^{-1} b.wt.)	Animal death (%)	Lethal dosage (LD_{50})
BI	0.200	100	0.0707 mL g^{-1} b.wt. equals with
BII	0.100	75	1674.5 mg kg^{-1} b.wt.
BIII	0.050	50	
BIV	0.025	25	

effect. According to Loomis (1994), the safety limit is represented by the comparison of LD_{50}/ED_{50}. Calculation result that the extract safety limit was LD_{50}/ED_{50} = 0.0707/0.026 = 2.7x. This extract can be classified as medium toxicity (0.5-5 mg kg^{-1} b.wt.) (Loomis, 1994). Judging from the result, the extract can be classified as medium toxic with narrow safety limit of 2.7 times the effective dosage (Loomis, 1994; Laurence and Bacharach, 1964). According Mitra *et al.* (2003) the active extract may be administered in different dosage forms like capsules in which 5 mL may contain 50-250 mg of active extract of *Picrorrhiza kurroa* for clinical administration in human beings to produce as laxative action.

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