Activity Test of Laban Bark Infusion (*Vitex Pubescens* Vahl) In Mice (*Mus Musculus*) As Antidiarrheal

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ABSTRACT

Diarrhea is an endemic disease and also a potentially becomes Incident Outside Normal (ION). ION itself explained as emergence or increasing incident pain and or meaningful death in an epidemiology manner of area in a period certain time, and is a possible situation that lead to plague occurrence. An acute diarrhea could result in fatal events up to death if not handled fast. The use of traditional drug culture in Indonesia itself has also increased every year along with the thinking pattern of society that want to return to nature (back to nature). Traditional drug plant alone is still easily found in nature, one example the plant is laban plant (*Vitex pubescens* V.) The purpose of this study was to determine if there is an activity in laban bark as an antidiarrheal. The method used was a quantitative experimental with true experimental design study pretest and posttest control group design. Samples were mice in one negative control group (blank), two positive control groups (Loperamide HCl and New Diatabs), and three Laban Bark Infusion (LBI) groups (LBI 13,2 [g] LBI 26,4 [g], and LBI 52,8 [g]). Analyzed with Kruskal – Wallis test with a 95 [%] confidence level. The results of this study were that the most significant found in the LBI 52,8 [g] group. Based on the results of the conclusion that there is an activity in Laban Bark Infusion as an antidiarrheal.

INTRODUCTION

Diarrhea is an endemic disease and also a potentially becomes Incident Outside Normal (ION). ION itself explained as emergence or increasing incident pain and or meaningful death in an epidemiology manner of area in a period certain time, and is a possible situation that lead to plague occurrence. An acute diarrhea could result in fatal event up to death if not handled fast. Diarrhea interpreted as escalation of frequency and declining consistency of feces disposal compared with a normal pattern of individual intestine. This often is a symptom to systemic disease. Acute diarrhea usually defined as duration not more than 14 days, persistent diarrhea occurred more than 14 days, and chronic diarrhea is longer than 30 day in duration. Most diarrhea cases caused by viral, bacterial, or protozoan infections, and generally healed alone. According to Ministry of Health of Indonesia there were 10 Incident Outside Normal (ION) in 2018 that spread in 8 provinces, and 8 cities. With 756 patients treated and 36 patients died.

The use of traditional drug culture in Indonesia itself has also increased every year along with the pattern of thinking society that want to return to nature (back to nature). Traditional drug plant alone still easy found in nature, one example of the plant is laban plant (*Vitex pubescens* V.). According to Widjaja, in Indonesia Borrneo is one of the bioregions or in other words have great diversity of flora and plant laban still found easily enough in Borneo especially the wood frequently used as board because it has strong durability[1]. As for the laban bark usually used by the Dayak community as a drink brew like a tea. Steeping laban bark in a manner empirically known could lower diarrhea symptom according to public Dayaks in Borneo. Usually public Dayak in Borneo use laban bark with a hand grip to determine as how much to use, where a hand grip here is referring to adult hand grip. Research conducted using the average grip of the researcher as reference to how many laban bark will be taken, and the average was 13,2gram which then will be converted for mice use.

LITERATURE REVIEW

There was a study on Laban plant before that studies about the Laban's leaf ethanol extract with results as the extract has antidiarrheal effect on white male mice indicating that Laban leaf has an effect as antidiarrheal[2]. This journal was used as a reference by the author as guide to do a research on Laban bark. Also, there was another journal used by the author as guide to conduct phytochemicals test to determine the secondary metabolites in the Laban's bark. And there're few other journals used as guide to conduct research on this study[3].

MATERIALS AND METHODS

Materials

The materials used in this study were laban bark, aquadest, lieberman burchard reagent, mayer reagent, dragendorff reagent, HCl, chloroform, magnesium powder, gelatin, concentrated sulfuric acid, FeCl3, mice (mus musculus) loperamide HCl, new diatabs, aquadest, Na CMC 0.5%, castor oil.

The tools needed in this study were an analytical balance, mouse cage, wire ram, mice drinking bottles, oral probe, infusion vessel, cloth, glass bottle container, knife, scissors, mortar and stamper, stopwatch, beaker glass, measuring cup, syringe, test tube, stir bar, dropping pipette, funnel glass, test tube, test tube rack, test tube clamp, denaturated alcohol candle[4].

Methods

This study is quantitative experimental using true experimental with design study pretest and posttest control group design method in quantitative non parametric analysis. Design study of pretest and posttest group design that is conducted on two group, one group given intervention and the other group as a control later will be observed before and after giving the intervention. Analyzed with Kruskal – Wallis test with a 95 [%] confidence level[5].

In this study, the first thing that was done was a phytochemical screening to determine the secondary metabolites contained in laban bark infusion. From this test, the secondary metabolites that will be tested in the infusion were alkaloids with dragendorf reagent, flavonoids with the Shinoda tester, tannin with the reactant FeCl3, there are also saponins, namely the formation of stable foam with a height of 1-3 cm for 30 seconds, lastly steroids and terpenoids.

The test animals used were male white mice (Mus musculus) where the mice were divided into 6 groups consisting of negative controls with 0.5% Na CMC, two positive controls with Loperamide HCl and New Diatabs, and three groups with infusion dose variants. Laban bark (IKKL) namely IKKL 13.2 g, IKKL 26.4 g and 52.8 g where each group consisted of 5 test animals which were then eliminated into 4 test animals. The antidiarrheal activity test can be seen from two aspects, namely based on the frequency of defecation and the consistency of the stool or stool from the sample.

Before the test on mice are conducted, the mice were to be acclimatized for 7 days. This was done so as to avoid bias on the results. After the mice were acclimatized, the test conducted in the Sari Mulia University laboratory, Banjarmasin, South Borneo, Indonesia. And the last thing to be done is the data analysis.

RESULTS AND DISCUSSION

Phytochemical Screening

In this study, the first thing that was done was a phytochemical screening to determine the secondary metabolites contained in Laban bark infusion. From this test, the secondary metabolites obtained were alkaloids marked in orange with a white precipitate or in the form of brick red with dragendorf reagent. Then the flavonoids were obtained which were marked with brownish red with the Shinoda tester. The tannin content was obtained with the reactant $FeCl_3$ where the infusion sample could be directly mixed with $FeCl_3$ because it had gone through boiling and was positive with a greenish brown color. There are also saponins, namely the formation of stable foam with a height of 1-3 cm for 30 seconds by shaking the infusion, then adding concentrated hydrochloric acid (HCl) the foam is still formed and does not break. The secondary metabolites of steroids and terpenoids were not found in the infusion test that was carried out, in contrast to the results of secondary metabolite testing from previous studies. This is thought to be caused by the place where the material was obtained, namely Laban bark which was obtained in the Central Borneo area, different from previous research which obtained the material from the West Borneo area[6].

Stool Consistency

Acute diarrhea usually defined as duration not more than 14 days, persistent diarrhea occurred more than 14 days, and chronic diarrhea is longer than 30 day in duration. In this study the samples were induced with castor oil which the samples later contracted with acute diarrhea.

Table 1 Average Results of Stool Consistency

No.		Na CMC 0.5 [%]	Loperamide HCl	New Diatabs	LBI 13.2 [g]	LBI 26.4 [g]	LBI 52.8 [g]
			CONSISTENCY				
1	*Pre-test*	1	1	1	1	1	1.25
2	*Posttest*	2.38	2.31	2.5	2.55	2.31	2.26
Difference		1.38	1.31	1.5	1.55	1.31	1.02

* Description: ≥0-1 = Solid
>1-2 = Soft
>2-3 = Liquid

Based on the above results, the average stool consistency obtained, only the score difference average of LBI 52.8g which has score difference the average 1.02 where score the showing improvement on consistency feces becomes solid. While others still are not yet have score mean difference close to 1.

Defecation Frequency

Escalation of defecation frequency is one of the indicator for diarrhea which also required in the evaluation

Table 2 Average Results of Defecation Frequency

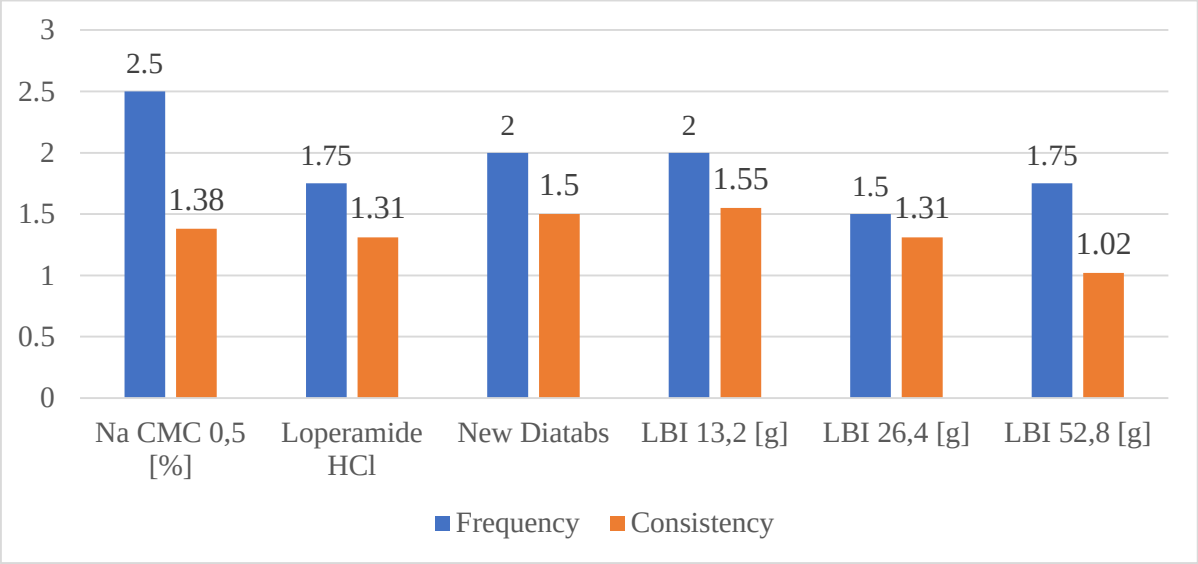
No.	Na CMC 0.5 [%]	Loperamide HCl	New Diatabs	LBI 13.2 [g]	LBI 26.4 [g]	LBI 52.8 [g]

FREQUENCY							
1	*Pre-test*	2.5	2.5	2.5	2.5	2.25	2.25
2	*Posttest*	5	4.25	4.5	4.5	3.75	4
Difference		2.5	1.75	2	2	1.5	1.75

Based on the results above, the defecation frequency obtained at the mean difference value shows the value below the pretest, which means that the frequency value improves after being given intervention.

Value of Changes in Stool Consistency and Defecation Frequency

Figure 1 Value of Changes in Stool Consistency and Defecation Frequency



In the figure above, the consistency value is obtained from the division between the number of stool consistency divided by the number of defecation frequencies. This is intended to determine the consistency of the mice's feces based on the number of mice's defecation frequencies.

Results of Significance Analysis of Stool Consistency Parameters and Defecation Frequency

Table 3 Results of Significance Analysis of Stool Consistency Parameters and Defecation Frequency

No.		Na. CMC 0,5 [%]	Lopermaide HCl	New Diatabs	LBI 13,2 [g]	LBI 26,4 [g]	LBI 52,8 [g]
CONSISTENCY							
1	Na CMC 0,5 [%]	-	.108	.078	.139	.020*	.018*
2	Loperamide HCl	.108	-	.065	.178	.642	.017*
3	New Diatabs	.078	.065	-	1.000	.065	.017*
4	LBI 13,2 [g]	.139	.178	1.000	-	.137	.017*

5	LBI 26,4 [g]	.020*	.642	.065	.137	-	.137
6	LBI 52,8 [g]	.018*	.017*	.017*	.017*	.137	-
			FREQUENCY				
1	Na CMC 0,5 [%]	-	.096	.343	.343	.061	.343
2	Loperamide HCl	.096	-	.617	.617	.495	.874
3	New Diatabs	.343	.617	-	1.0	.343	.647
4	LBI 13,2 [g]	.343	.617	1.0	-	.343	.647
5	LBI 26,4 [g]	.061	.495	.343	.343	-	.752
6	LBI 52,8 [g]	.343	.874	.647	.647	.752	-

Statistically, the results showed that there was no significant difference in the frequency parameter for all test groups, while there was a significant difference in the consistency parameter. It is said to be significant if the p-value <0.05 LBI dose of 26.4 [g] and LBI dose of 52.8 [g] have a difference with Na. CMC 0.5 [%], Loperamide HCl, New Diatabs, and LBI dose of 13.2 [g]. While the LBI dose of 26.4 [g] and LBI dose of 52.8 [g] did not have a significant difference.

Based on Figure 1, the consistency of the LBI 52.8 [g] dose parameter has a lower consistency value than all groups. This means that LBI 52.8 [g] has stool consistency which is on average denser than all groups. Meanwhile, based on the defecation frequency graphical data at the LBI dose of 26.4 [g], it shows a lower decrease in defecation frequency compared to all parameters. A low consistency value indicates an improvement in the condition of the stool, namely returning to solidity or normal, while the frequency shows a decrease graphically but it is still not statistically significant.

According to Wells, diarrhea is an increase in the frequency of defecation and a decrease in stool consistency compared to the normal intestinal state of an individual[7]. This is consistent with the parameters of frequency of defecation and stool consistency which can be seen in Figure 1 where there are differences in the parameters of consistency and frequency when compared to the negative control. From the dose parameters, LBI 26.4 [g] and LBI 52.8 [g] had different antidiarrheal activity against Na CMC 0,5 [%], and LBI 13.2 [g] only showed a slight difference.

CONCLUSIONS

Based on the results of the Activity Test of Laban Bark Infusion (Vitex pubescens Vahl) in Mice (Mus musculus) as Antidiarrheal, it can be concluded that Laban bark infusion contains secondary metabolites in the form of alkaloids, flavonoids, tannins, and saponins, and Laban bark infusion has antidiarrheal activity in doses of 26.4 [g] and 52.8 [g] on white male mice induced by oleum riccini or castor oil which were seen based on the parameters of defecation frequency and stool consistency. The dose of 52.8 [g] is a better dose than the dose of 26.4 [g].

REFERENCES

[1] Widjaja. The Flora of Indonesia 2014. IGARSS 2014 (Issue 1). 2014.

[2] Yasnita, Khori. Harahap, Urip. Antidiarrheal Effect Test of Laban Leaf Ethanol Extract (Vitex quinata Lour. F.N. Williams) on Male Mice. North Sumatera University. *Repository of North Sumatera University*. 2018.

[3] Pribadi, A. J., Abdul, G., Thamrin, R., & Rahmadi, A. Identification of Phytochemical Content of Alaban Bark (Vitex Pubescens Vahl) Based on Elevation in Pelaihari. 02(4), 595–604. 2019.

[4] Amilia, R., Kresnamurti, A., Faizah, A. K., Klinik, B. F., Farmasi, P., & Medical, F. Writhing Test Analgesic Activity of Fish Oil in BALB/c Male Mice (Mus musculus) with Writhing Test Method. *Pharmaceutical Journal of Indonesia*, 17(01), 13–21. 2020.

[5] Surahman. Research Method. *Ministry of Health of Republic Indonesia*. 2016.

[6] Rinaldi F, F., Ibrahim, A., Fadraersada, J., & Rijai, L. Identifikasi Metabolit Sekunder dan Pengujian Toksisitas Ekstrak Metanol Kulit Kayu Laban (Vitex pinnata L.) dengan Metode Brine Shrimp Lethality Test (BSLT). *Proceeding of Mulawarman Pharmaceuticals Conferences*, *4*(1), 133–139. https://doi.org/10.25026/mpc.v4i1.172 . 2016.

[7] Wells, B. G., DiPiro, J. T., Schwinghammer, T. L., & DiPiro, C. V. *Pharmacotherapy Handbook,Tenth Edition*. In McGraw-Hill Companies. 2017.

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