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Immunomodulatory Effectiveness of Aqueous *Obat Pahit* Extract of Lingga Malay Ethnic on White Rats (*Rattus novergicus*)

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Immunomodulatory; Lingga; *Obat pahit*; Riau Archipelago

Abstract

Obat pahit has been generally known and believed by Lingga Malay society as anti-aging agent. However, the study of *Obat pahit* is not scientifically proven. This research was aimed to prove immunomodulatory ability of *Obat pahit* potion from Lingga, Riau Archipelago. This study used white rats as an animal modelling, and *Staphylococcus aureus* as bacteria tester. The rats had been treated with aqueous *Obat pahit* extract from three TMPs on dose scales of 0.09, 0.18 and 0.27 mL/200g of body weight through oral administration for 7 days. Furthermore, on the 8th days, the experiment animals were injected by the preparation of bacteria tester through intraperitoneal administration in the amount of 0.5 mL/200 gram of body weigth and subsequently incubated for 1 hour after the injection. There were 2 observed parameters on this study, i.e efectivity and capacity of phagocytosis by leukocytes. The observation of leukocytes-phagocytocis activity was carried out by making a smear preparat samples of peritoneum fluid from rats. After the observation under microscope on a magnification of 100 times. The result was obtained the *Obat pahit* from Kalan PMT swere more effective on dose 2, while from SP4 and Linau TMPs were much more effective on dose 1. It is therefore, using these data of the results, the advanced doses scale of this *Obat pahit* would not be necessary. *Obat pahit* potion from Malay Lingga Malay Ethnic could become raw materials of immunomodulatory herbal medicine based on traditional knowledge. It also potentially as a standardized herbal.

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INTRODUCTION

The immune system is a mechanisms of body defense which palys role to respond any antigents coming outside from the body (Prakash *et al.,* 2013; Chakraborthy, 2009). When the body attacked, the common antigents of our body will automatically stimulate the immune system (Subowo, 2014). Furthermore, this mechanism will protect the body from any microorganism infections, such as bacteria, virus, fungi, and other disesease pathogents (Levinson, 2006; Sunnitha, 2015). When the immune system working unwell, our body will be easily susceptible of disease infections. There are several possible factors of affecting the immune system, such as environment factors, food, life style, stress, age, and hormones (Abbas *et al.,* 2014; Bratawidjaja, 2002).

Immonumodulator is still a debated compounds or materials to be explored. Its basically function is to develop materials which are able to increase the responses of immune system or to reverse the imbalance of immune systems (Djauzi, 2003; Patil *et al.,* 2012). Improving the immune systems can be done with many practices, like consuming supplements which are as immunomodulator (Saroj *et al.,* 2012; Silalahi, 2005). The usage of immunomodulatory drugs can become a prevention treatment of infecting diseases.

Obat pahit is one of the traditional herbals which is generally consumed by society of Malay Ethnic from Lingga in Riau Archipelago. In Lingga District, the knowledge is an intrinsic typical among the indigenous groups and is inherited from their great ancestry by oral communication (Fitmawati et al, 2017a). This potionof *Obat pahit* is consumed by local people as a body energy keeper (immunomodulator and antioxidants) (Fitmawati *et al.,* 2017a; Fitmawati *et al.,* 2017b). Additionally, it isalso believed as a youthful agent. Every village in Lingga has a Traditional Medicine Practitioner (TMP) who is still mixing various of traditional herbals according to their inherited knowledge (Fitmawati *et al.,* 2017). Most of the famous TMPs come from Kalan Village, SP4 Village and Linau Village.

By having the variety of TMPs and the composition of current *Obat pahit* ingriedients, it is therefore required to conduct a study regarding the activity and the capacity of macrofagecells phagocytosis on white rats of winstar strain which are treated by the aqueous *Obat pahit* extract from Lingga, Riau Archipelago. This is required as an attemptdue tothe empirical evidence of developing *Obat pahit* on commercial purposes and improvisation of product value in Lingga Malay society. The purposes of the research is to prove immunomodulatory ability of *Obat pahit* potion from Lingga, Riau Archipelago. And the benefit from this research is *Obat pahit* potion from Malay Lingga Malay Ethnic could become raw materials of immunomodulatory herbal medicine based on traditional knowledege.

METHODS

Materials and Tools

Materials used for this research were the analyzed samples of *Obat pahit* collected from Lingga Malay society, Riau Archipelago. Other materials were white rats and chemical materials for analysis purposes like gel nutrient, alcohol 70%, NaCl solution 0.8%, Giemsadye, methanol, and distillated water.

Preparation of Animal Modelling

The used animal modelling on this study was white rats of winstar strain aged 6 months with average body weight of 20-300 grams. The total number of rats used for immunomodulatory activity test of AOPE was 36, which was divided into 12 groups of treatments. Before giving the aqueous extraction, every group of treated rats was firstly maintained during more less one week for environmental adaptation, then their health and body weight were daily controlled as well as its food intake. White rats were placed in modified cages made by netting wires and wooden rafters with a square shape. Every cage was scaled of 33 cm x 43 cm x 18 cm, and given on the base of rice husk. The cages cleaning was carried out for two times a week. During the maintenance and treatement of rats, the animal modellingfed a standard food and distillated water via *ad libitum*.

Preparation of Aqueous *Obat pahit* Extract (AOPE) with Boiling Method

The potions were consisted of three available packgings, which of 100 grams of each was boiled in 100 mL of distillated water as a solvent. Then it was cooled on room temperature for several minutes, and about 20 mL of it was poured into measuring cup. This stage was repeated for 6 times on every treated rats.

Doses Scale Determination for Animal Modelling

Determination of dose scale was done by converting a commonlly human-consumed doses of 200 mL, with the convertion factor of rats was 0.018. The created samples were100 grams of doses in 1000 mL, which resulting a convertion dose

as given as doses scale to treated rats. This convertion dose is relevant of weight samples multiplied to maximal volume or 3.6 / 100 x 5 = 0.18 mL x 5 becoming 0.9 mL x kg of body weight. It is established as doses of 2 routes of orally ABH-ME administration. These doses were next made in stages of 0.4 mL/ kg BW, 0.9 mL / kg BW, and 1.35 mL / kg BW.

Immunomodulatory Effectiveness Test of *Obat pahit*

The total of treated animals was randomly grouped and divided into 12 groups and each of them consists of 3 white rats. All extract administrations were orally conducted every 2 times a day for 1 week using *spuit disposible* sized 1 mL without needle. All treatments were displayed on Table 1.

Table 1. Treatements of Immunomodulatory Effectiveness of Long-life Herbals

No Group	Treatments	Description
I	Treated white rats with immunomodulatory drugs	Positive Control
II	Treated white rats with-CMC Na	Negative Control
III	Treated white rats with-distillated water	Normal Control
IV	Treated white rats with AOPE TMP 1 by dose 1	
V	Treated white rats with AOPE TMP 1 by dose 2	
VI	Treated white rats with AOPE TMP 1 by dose 3	
VII	Treated white rats with AOPE TMP 2 by dose 1	
VIII	Treated white rats with AOPE TMP 2 by dose 2	
IX	Treated white rats with AOPE TMP 2 by dose 3	
X	Treated white rats with AOPE TMP 3 by dose 1	
XI	Treated white rats with AOPE TMP 3 by dose 2	
XII	Treated white rats with AOPE TMP 3 by dose 3	

Bacteria Test

The tested bacteria were *Staphylococcus aureus* (SA) No. ATCC 12600. Embedded SA on Nutrient Mueller Hinton Broth (MHB) gel was then diluted on sterile pepton broth suspension using sterile syringe and susbequently incubated inside incubator on 35-37^{0}C.

Phagocytosis Test

On the 8th days, every treated rats was intrapertoneally injected by SAusing formula dose as following as : BW/SW x MD; BW: Body Weight (grams), SW: Standard Weight (200 grams), MD: Maximum Dose (0.5 mL). After infection procedure, all treated rats were rested for 1 hour. The treated rats were subsequently anesthetized by chloroform and then abdominally dissected by using surgical instruments. The peritoneum fluid of rats was taken out by pippet, and dropped on object glass to make smear preparat samples. Before stainning of Giemsa, they were fixed on object glass by using methanol for minutes. After that, they were windy dried for 20 minutes and rainsed by flowing water. When completely dried, the smear samples were observed under microscope using immersion oil on 10x-100x of magnification, and then analysed the activity and capacity of the macrofage-cells phagocytosis. The phagocytosis activity was determined according to the number of active cells on the phagocytosis process of 100 of phagocyte cells. The phagocyte capacity was established from the number of bacteria that has been ingested by 50 active-phagocyte-cells.

Data Analysis

The determination of siginificantly differences of AOPE effect on several used doses of the activity and capacity of macrofage-cell phagocytosis was analysed by ANOVA *one way* test and further test of LSD.

RESULT AND DISCUSSION

The Immunomodulatory Effectiveness Test of Aqueous *Obat pahit* Extract (AOPE)

This immunomodulatory effectiveness study has explored two aspects: activity and capacity of phagocytosis. Phagocytosis cells are predominantly important in the removal of bacteria and parasites from the body. They engulf these foreign bodies and degrade them using their powerful enzymes (Ranjith *et al.,* 2008; Saroj *et al.,* 2012). Phagocytosis activity is the number of phagocyte cells that is ingesting antigents like bacteria, whilst phagocytosis capacity is the number of bacteria cells that have been ingested by phagocytic cells. As following is figure of peritoneum fluid smear samples of treated rats.

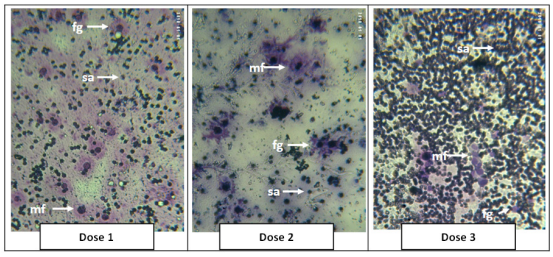

Figure 1. Peritoneum fluid smear display of treated rats from boiling medicines of TMP 1 Kalan; Fg. Phagocytosis, mf: Macrofage, sa: *S. Aureus*

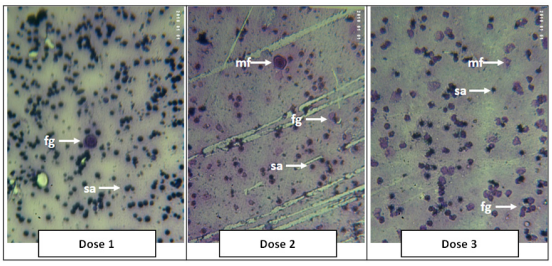

Figure 2. Peritoneum fluid smear display of treated rats from boiling medicines of TMP 2 SP4; Fg. Phagocytosis, mf: Macrofage, sa: *S. aureus*

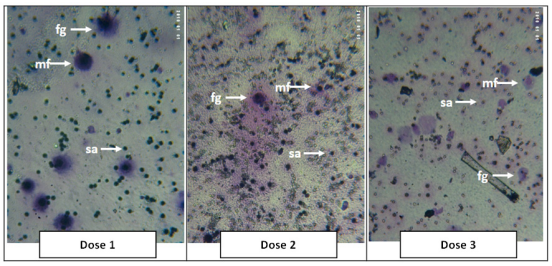

Figure 3. Peritoneum fluid smear display of treated rats from boiling medicines of TMP 3 Linau; Fg. Phagocytosis, mf: Macrofage, sa: *S. Aureus*

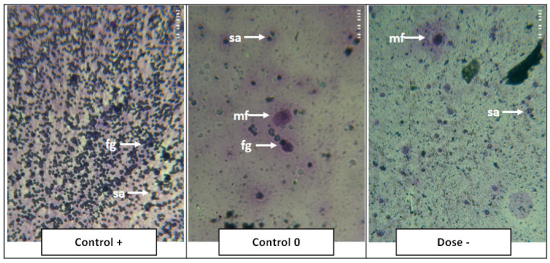

Figure 4. Peritoneum fluid smear displays of treated rats for control:Stimuno, control 0: distillated water, control – :CMC Na 1%, ; Fg. Phagocytosis, mf: Macrofage, sa: *S. aureus*

On Peritoneal fluid smears on the Figure 1-4, it can be seen the difference of each treatment both in the activity and capacity of phagocytic cells. In general, all treatments of AOPE showed the activity nor capacity of phagocytosis. On the control group, it was very significantly difference in contrast amongst control (+), control neutral and control (-). To determine the quantity of activity and capacity Lingga AOPE, it was analyzed by counting the active-phagocytic cells and phagocyted bacterial cells. Here is the data of calculation phagocytic activity and capacity value in all groups.

Table 2. Average Activity of AOPE Phagocytosis

TMP	Dose 1	Dose 2	Dose 3	C+	C0	C-
Kalan	80	97	97			
SP4	94	92	95			
Linau	96	91	93			
Stimuno				90		
Distillated water					66	
CMC Na 1%						62

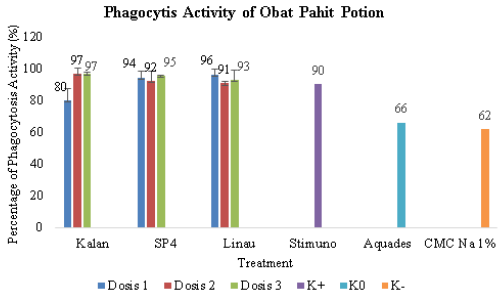

Figure 5. Activity Percentage of AOPE Phagocytosis

Generally, the score of all AOPE of phagocytosis activity on the treated rats showed that the highest score was positive contro, also it was even from 2 tested AOPEs, i.e SP4 and Linau that have showed the highest number of positive control by using low doses scale.

From capacity aspect, positive control has a bigger score than Lingga AOPE which was tested on treated white rats. However, all of AOPE ingriedients that have been tested having scores above average of control. To observe the differences amongst treated gorups, it has been anaylzes by ANOVA *one way* with further test of LSD.

Table 3. Capacity Average of AOPE Phagocytosis

TMP	Dose 1	Dose 2	Dose 3	C+	C0	C-
Kalan	95	187	136			
SP4	168	157	107			
Linau	169	141	112			
Stimuno				404		
Distillated water					122	
CMC Na 1%						82

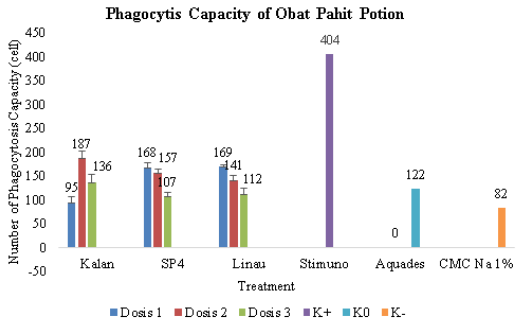

Figure 6. Capacity Score of Phagocytosis of Lingga *Obat pahit*

Table 4. Further LSD Test on Phagocytosis Activity of All Groups ofAOPE

Potion		Phagocytosis Activity (%)
	Aquades (K0)	65 ± 3.21a
Control	Stimuno (K+)	90 ± 2.08c
	CMC Na 1% (K-)	62 ± 1.52a
	Dose 1	80 ± 7.54b
Kalan	Dose 2	97 ± 3.51c
	Dose 3	97 ± 11.84c
	Dose 1	94 ± 4.04c
SP4	Dose 2	92 ± 6.08c
	Dose 3	95 ± 0.57c
	Dose 1	96 ± 11.23c
Linau	Dose 2	91 ± 1.52c
	Dose 3	93 ± 11.018c

In a further test LSD phagocytic activity with treatmen of Kalan AOPE showed activity in the control (-) and control (0) was not significantly different, this group had lower activity than the other group, dose group 1 had a higher activity than the control group (-) and control (0), but lower than the dose above as well as the positive control. In terms of positive control activity was not significantly different Kalan AOPE treatment at a dose 2 and dose 3. In a further test of LSD, phagocytosis activity with treatment AOPE of SP4 showed that activity in the control (-) and control (0) was not significantly different, this group had lower activity than the other group, all doses tested in rats with AOPE of SP4 did not have a significant difference compared to control (+). In a further test of LSD, phagocytosis activity with AOPE treatments of Linau showed that activity in the control (-) and control (0) were not significantly different, this group had lower activity than the other groups, all doses tested in mice. But, the first dose of Linau AOPE showed the better activity than the other groups.

Table 5 . Further LSD Test on Phagocytosis Capacity of All Groups of AOPE

Potion		Phagocytosis Capacity
	Aquades (K0)	122 ± 4.04c
Control	Stimuno (K+)	404 ± 3.21f
	CMC Na 1% (K-)	82 ± 2.88a
	Dose 1	95 ± 11.54a
Kalan	Dose 2	187 ± 15.04e
	Dose 3	136 ± 15.50c
	Dose 1	168 ± 7.63e
SP4	Dose 2	157 ± 16.07d
	Dose 3	107 ± 33.29b
	Dose 1	169 ± 21.73e
Linau	Dose 2	141 ± 17.50c
	Dose 3	112 ± 12.58b

In a further test of LSD, phagocytic capacity with AOPE treatment of Kalan showed dose 1 has a capacity that is not high and equal with another control. However, at a dose of 2 there was an increase, even higher than above doses 3. AOPE of Kalan showed good capacity at doses commonly consumed by people, i.e a dose of 2, although it was not equal with a positive control in phagocytosis of bacteria test. In a further test of LSD, phagocytosis capacity of AOPE treatment of SP4 showed that all treatments were significantly different from one another, dose 1 was the best dose of AOPE SP4 in terms of capacity and tends to decline with rising doses used.

In a further test of LSD, phagocytosis capacity of AOPE treatments from Linau showed that all treatments were significantly different from one another, the dose 1 was the best dose of AOPE SP4 in terms of capacity and tends to decline with rising the used doses, as relevant as the phagocytosis capacity owned by Linau and SP4. Immunomodulatory effectiveness was influenced by activity and phagocytosis capacity in fluids of living organisms (Nastiti, 2014; Shibata & Glass, 2009; Shalhoub *et al.,* 2011). In study (Ranjith *et al.,* 2008), we found that the aqueous extract of *Tinospora cordifolia* was effective in boosting phagocyte mediated immune response in vitro. The extract at a concentration of 5 µg/ml showed 200% increase in phagocytic ability of macrophages as compared to control. *T. cordifolia* is reported to benefit the immune system in a variety of ways.

In AOPE Kalan, it can be seen that doses of *Obat pahit* which were tested to mice are effective at a dose of 2 (the usual doses taken by Lingga community), means that the use of AOPE Kalan does not require treatment dose increase in its use, as well as the AOPE SP4 and Linau that had higher antioxidant activity than Kalan, both of these *Obat pahit* showed that at the first dose it has had better effects than other doses, so that the use of these herbal medicines does not need to be increased in dose scale. Highest immunomodulatory effects regarding to macrophage phagocytosis. Its high immunomodulatory effect is related to high content of phenolic compounds. The phenolic compounds are potential antioxidant (Nastiti *et al.,* 2014). Antioxidative activity is closely related to the immunomodulatory activity (Fitmawati *et al.,* 2017b). Changes in cellular oxidant status provides a stress to immune system cells (Krifa *et al.,* 2012). The antioxidative action promotes redox-sensitive pathways responsible to control immune cell function. From this research, there is an inevitable need to judiciously exploit and utilize the immunomodulatory properties of the unique medicinal plants like *Obat pahit* in modern medicine.

The result was obtained the *Obat pahit* from Kalan PMT swere more effective on dose 2, while from SP4 and Linau TMPs were much more effective on dose 1. It is therefore, using these data of the results, the advanced doses scale of this *Obat pahit* would not be necessary. *Obat pahit* potion from Malay Lingga Malay Ethnic could become raw materials of immunomodulatory herbal medicine based on traditional knowledege. It also potentially as a standardized herbal.

CONCLUSION

Aqueous herbal medicines extract showed an increase of phagocytosis against SA, but declining at the highest dose. The best immunomodulatory activity contained in TMP 3 (TMP from the Linau village). *Obat pahit* being tested on white mice had the effect which is able to increase the activity and capacity of phagocytosis while still under the control (+). Activity and capacity of SP4 and Linau *Obat pahit* effectively modulate the immune system.

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