

Formulation and Evaluation of Langsat (*Lansium domesticum* Corr.) Peel Ethanol extracts Lotion as Anti-Mosquito Repellent

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ABSTRACT

The repellent is a material that works in protecting humans from mosquito bites. Langsat peel (*Lansium domesticum* Corr.) is one of the plants having function as repellent, anti-mosquito containing triterpenoid compound. The purpose of this research was to formulate the langsat peel ethanol extract into anti-mosquito lotion and to determine the effectiveness of repellent anti-mosquito from the best formulation of the langsat peel ethanol extract. The Optimization of the lotion basis was performed on three variations of cetyl alcohol concentrations i.e. 3%, 5%, and 7% respectively and obtained F2 containing 5% Cetyl alcohol as the optimum base. The F2 lotion basis formula was formulated into lotion with three concentrations of 10%, 20%, and 35%. The three lotions formula of the langsat peel ethanol extract was tested by organoleptic test, preparation, spreading test, adhesion test and stability test, including pH and viscosity exposed at 15-30°C, 4°C and 40°C. All formulas were tested for irritation using rabbits for 3 days and did not show any erythema and edema. Tests of the effectiveness of anti-mosquito were done on rabbit using mosquitoes. The results obtained showed that langsat peel extract 20% and 35% had effectiveness as anti-mosquito repellent and can be formulated into lotion dosage form.

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Introduction

Indonesia is an archipelago country which is rich in natural materials from flora and fauna. One of them is Gorontalo Province having a lot of herbal medicine which used some people to prevent diseases. Besides that, Gorontalo has a high level to gain risk of dengue hemorrhagic fever (DHF) which caused by mosquito [1].

A mosquito is one of the insects that have a role as a vector of disease agents. Diseases transmitted by mosquitoes are still a health problem for people, both in urban and rural areas, such as Dengue Hemorrhagic Fever (DHF), Malaria, Filariasis (Elephant Foot), Chikungunya and Encephalitis. Extraordinary events (KLB) which in recent years tend to increase the number of cases and death [2]. Repellent is an ingredient that has the ability to protect humans from mosquito bites when applied to the skin surface [3] but the repellent available on the market today contains lots of synthetic chemicals in large quantities can irritate the wearer. Related to the adverse effects of repellent synthesis encourage scientists to look for alternative anti-mosquitoes that do not harm human beings such as materials that come from nature. Some plants have insect repellent properties, can be a natural mosquito repellent or as a natural insecticide mosquito repellent.

One of the plants having the potential as a repellent is langsat peel (*Lansium domesticum*). In ancient times, people used the traditional way to repel mosquitoes by burning of langsat peel and reinforced with a fairly sticky sap [4]. The repellent advantage of the skin of this leaf is natural (enviro oriented) so as not to interfere with breathing [5,6]. Langsat peel is often used in cosmetics such as scrubs, lotions and creams. Lotion is a semi-solid preparation of emulsions intended for external use. Lotion is an easy to apply preparation with even distribution [7].

Based on the research by Haryanto (2014) showed langsat peel got positive results containing triterpenoid, saponin, quinone and tannin. According to Nishizawa et al (2012) stated that triterpenoids are secondary metabolite compounds having functioned as repellent [10]. In this study, the extract ethanol of langsat peel was formulated into lotion including its evaluations.

Materials and methods

Materials

Balance, rotary evaporator, beaker, magnetic stirrer, centrifuge, pH meter, langsat peel, Ethanol 96%, *Sweet almond oil*, filter paper, distilled water, Span 80®, Tween 80® Crodamol SS®, cetostearyl alcohol, cetyl Alcohol, Glycerin, Citric acid, sodium citrate, α -tocopherol, DMDM Hydantoin, propylene glycol, Liebermann Burchard. All chemical materials were purchased from Brataco Chemical Pharmaceuticals.

Methods

Sample 400 g of the langsat peel was extracted by maceration and soaking the sample in a 96% ethanol solvent for 3 times 24 h while occasionally stirring. The langsat peel extract was filtered to be separated between the residue and the filtrate. The filtrate was partitioned by using n-Hexan solvent at a ratio 1: 1. Separated partition results and taken top layer. The upper layer was obtained from the preceding treatment which was put into the evaporator to separate the langsat peel extract and the solvent. Furthermore, it accommodated the viscous extract obtained on the Erlenmeyer flask.

Phytochemical Screening

Triterpenoid examination was done by Liebermann Burchard reaction. The 2 ml test solution was evaporated in the test tube. The extract was added 0.5 ml of anhydrous acetic acid and 2 ml of concentrated sulfuric acid through the tube wall. The color change of solution was brownish red indicating the presence of a triterpenoid [8].

The Optimization of Lotion Basis

The optimization of this lotion basis using the emulsion basis, the first step in making the lotion preparation was to mix the oil phase (almond oil and Span 80) and water phase (Tween 80 and water). Both phases were heated at 60-70°C. After

dissolution between tween 80 and Span 80 in each phase, additional additives were added according to their solubility. The water phase and oil phase were mixed by using an electric stirrer ultraturax at a speed 3200 RPM for 2 min. Stirring was done up to both homogeneous phases. On the optimal basis used 3 variations of cetyl alcohol concentration i.e. 3%, 5%, 7% respectively. The best concentration of cetyl alcohol will be used to make lotion formulation based on the viscosity and separation [9].

Formulation of ethanol extracts of langsat peel

The first step in the preparation of a lotion of langsat peel ethanol extract was to mix the soluble ingredients in the water phase and the soluble ingredients in the oil phase. Both phases were heated at a temperature of 60 - 70°C which then the second phase was mixed using Ultra-turax with a speed of 3200 RPM for 2 min. In the preparation of langsat peel extract utilized 3 different concentrations of 10%, 20%, and 35%.

Evaluation of langsat peel ethanol extract lotion

The organoleptic Evaluation

The organoleptic test included visually observed colors, odors and homogeneity. All formulas were marked by panelist (10 people) giving scoring. Quantitative descriptive analysis profiling techniques were used for organoleptic assessment of lotions with open line scales for scores ranging from 0 to 10 for the following flavor, colors and homogeneity attributes [19].

The pH Evaluation

The evaluation of pH of the lotion preparation used the pH meter tool. 1 g lotion was checked and diluted with distilled water up to 10 ml. The pH meter electrode was immersed in the treated solution, the pH meter needle was allowed to move until showing a fixed position, the pH

indicated by the pH meter needle was recorded [11].

The Viscosity Evaluation

25 g of langsat peel lotion were put into the container, then mounted rotor no. 1 and make sure that the rotor was immersed in the test preparation. The Brookfield Viscometer tool switched on and the rotor can rotate. Observed was pointing needles of the Viscometer leading to a number on the viscosity scale for the available rotor no 1, when the needle was pointing in a stable direction, that number represents its viscosity [11].

The Freeze Thaw Evaluation

The lotion preparation was kept at 4°C for 24 h, then discharged and placed at 40°C for 24 h, the process was calculated 1 cycle. This experiment was conducted for 7 cycles. The results of the cycling test were compared with the previous preparation [9,11].

The Centrifugation Evaluation

The preparation of the lotion was fed into a centrifugation tube then centrifuged at 3750 RPM for 30 min. This centrifugation test described the stability of the lotion due to the effect of equal gravity for 1 year [10,16].

The Spreading Power Evaluation

0.5 g lotion was carefully placed on transparent plastic coated graph paper, left for a moment (15 sec) and the area given by the preparation and calculating, then covered again which was given plastic load of 1 g, 2 g, and 5 g and left for 60 sec, the increased area given by the preparation and can be calculated [12].

The Sticky Power Evaluation

0.25 g samples were placed over 2 glass objects that have been determined, and then pressed with a load of 1 kg for 5 min. After that the load was lifted from the glass object, and then was mounted

on the test equipment. The test equipment was loaded 80 g and then recorded the time of removal of lotion from the glass object [13].

Skin Irritation Study

Skin irritation test using healthy rabbits, there were no injuries or skin disorders. Three groups (n=3) of albino male rabbits (1.5-2 kg) were used in this study. Negative control (no application) were applied (n=3) on the dorsal side (2 cm²) of properly shaven skin of rabbits. The formulation was removed after 72 h and examined for any signs of erythema and edema. Undesirable skin changes, i.e. change in color and change in skin morphology, were visually checked in for periods of 1h, 24 h, 48 h, and 72 h. The resulting reactions were compared and scored against a control group (n=3).

Test of Repellent Effectiveness

The test was carried out into 3 mosquito cages measuring 29.4 x 28.5 x 32.8 cm, into each contained 20 mosquitoes. The test was done by applying lotion from langsung peel ethanol extract on rabbit skin as much as 1.5 g using a brush. The rabbits that had been smeared with lotion then put into the experimental box for 5 min for the first observation, then the rabbit was removed for 30 min, and put back into the test box for 5 min for second (second) observation, and the rabbit re-issued in 30 min then re-inserted for 5 min for the third observation. During the experiment, rabbit skin was not washed and treatment (lotion) group was not added lotion. This was to see the durability of repellent protection. The Observed and counted were done to gain the number of mosquitoes that perched during this experiment [14].

Statistical Analysis

Data on the relationship between each formulation and the obtained results of physical evaluation were analyzed by one way ANOVA (Analysis of Variance) method using SPSS 16.

Results and Discussion

Maceration Method

400 g langsung peel were extracted by maceration method for 3 times 24 h using 96% ethanol solvent and partitioned using N-Hexan which yielded 13 g extract.

According to Handa that maceration is a technique use in wine making and has been adopted and widely used in medicinal plants research. Maceration involved soaking plant materials (coarse or powdered) in a stoppered container with a solvent and allowed to stand at room temperature for a period of minimum 3 days with frequent agitation [19]. The processed intended to soften and break the plant's cell wall to release the soluble phytochemicals. After 3 days, the mixture is pressed or strained by filtration. In this conventional method, heat is transferred through convection and conduction and the choice of solvents will determine the type of compound extracted from the samples [20].

Phytochemical Screening

The phytochemical scheme was a qualitative analysis of secondary metabolite compounds. In this study, phytochemical screening test used Lieberman Burchard reagent. The phytochemical screening results gained a change in the color of brownish red indicating positive triterpenoids [15].

Lotion Basis Optimization

Optimization of the lotion basis that can be seen in **table 1**. The purpose of this optimization basis was to obtain a stable and a good lotion basis. The material used in this formulation was prepared in an emulsion lotion o/w. This study used the combination two surfactants i.e. tween 80 and span 80 considering these preparations were formulated into emulsion o/w. Tween 80 was a water-soluble emulsifying agent which was capable to form an emulsion type o/w. Otherwise, span 80 was a nonionic emulsifying agent, in which the lipophilic group was more dominant. According to Tungadi (2018) stated that tween 80 and span 80 acted as emulsifier to stabilize the interface of the water-in-oil internal emulsion and a hydrophilic emulsifier for the external interface of the oil-in-water emulsion [1]. In addition, this basis optimization used 3 variations of concentration of cetyl alcohol as a viscosity enhancer. The result of basis optimization can be seen in **table 1**, based on the

results obtained in F2 and F3 showed no separation. However, F3 gave the appearance of the dosage form resembles cream preparation because it had a high viscosity so it cannot be poured. According to Ansel (1989) stated that the lotion is a topical preparation without drugs for topical use, which permeate the water evenly, so the viscosity of the lotion is lower than cream [14]. Therefore, F2 can be selected as a stable basis. This is consistent with Handa et al. stated that before making formulation, pre-formulation study has important role to get good and stable formulation. It involves the characterization of a drug's physical, chemical, and mechanical properties relating to other ingredients which used in the preparation. In dealing with lotion pre-formulation, the important aspect is to understand the emulsion behavior of a given lotion under a variety of stress conditions such as freeze-thaw, temperature, shear stress among others to identify mechanisms of degradation and its mitigation [21].

Table 1. The optimization of lotion basis.

Materials	The amount of materials (%)		
	F1	F2	F3
Oil phase			
Sweet Almond oil	15	15	15
Span 80	1,4	1,4	1,4
Crodamol SS	0,5	0,5	0,5
Alpha tocopherol	0,02	0,02	0,02
Cetyl Alcohol			
Cetostearyl Alcohol	3	5	7
Water phase			
Tween 80	3,598	3,598	3,598
Propylene glycol	5	5	5
Glycerin	5	5	5
Citric acid	0,294	0,294	0,294
Sodium citrate	0,137	0,137	0,137
DMDM Hydantoin	0,5	0,5	0,5
Aquadest	100	100	100
Results	S	NS	NS

S: separate; NS: not separate

Lotion Formulation

Preparation of *Langsat peel* extracts

This stage of the active substance or *langsats* peel ethanol extract was made in several concentration variations (**table 2**) which then was added to the basis of F2. After the active ingredient was added on the basis, three different concentrations of *langsats* peel ethanol extracts were obtained, including F2A, F2B, F2C. The three formulas of

langsats peel ethanol extract were centrifuged using centrifugation. The purpose of centrifugation was to see the separation of the dispersed phase due to the formation of cream or agglomeration when the emulsion was exposed to centrifugation [16]. In the centrifugation evaluation results showed no separation in the three lotions formula of *langsats* peel ethanol extract.

Table 2. The formulas of *langsats* peel extract lotion.

Materials	The amount of materials (%)		
	F2a	F2b	F2c
Langsat peel extract	10	20	35
Oil Phase			
Sweet Almond oil	15	15	15
Span 80	1,4	1,4	1,4
Crodamol SS	0,5	0,5	0,5
Alpha Tocoferol	0,02	0,02	0,02
Cetyl Alcohol	5	5	5
Cetostearyl Alcohol	5	5	5
Water Phase			
Tween 80	3,598	3,598	3,598
Propylene glycol	5	5	5
Glycerin	5	5	5
Citric acid	0,294	0,294	0,294
Sodium citrate	0,137	0,137	0,137
DMDM Hydantoin	0,5	0,5	0,5
Aquadest	100	100	100
Results	NS	NS	NS

NS: not separate

Evaluation of the dosage form

The organoleptic evaluation

Based on the scoring from panelists, the three formulas were observed organoleptically including color, odor and homogeneity. The observations described F2A had a white color ivory, while F2B and F2C had a yellowish white color. The color change of each formula was caused by the yellow color from the extract of *langsats* peel resulting in the change of color in the dosage. The greater concentration of *langsats* peel extract used was the more yellow the resulting

preparation. However, these three formulas had the same distinctive odor of skin odor and showed homogeneous preparations.

The pH evaluation

This evaluation at room temperature with temperature 15-30°C was measured by using pH meter.

Based on **table 3** showed that the pH measurement results for 7 cycles or T₀-T₃₀ was about ± 5, it corresponded to a skin pH of 4.5-7.0 [11].

Table 3. The pH Measurement of stability test.

Cycle	pH		
	F1	F2	F3
0	5,64±0,005	5,66±0,011	5,65±0,005
1	5,59±0,005	5,49±0,011	5,63±0,01
2	5,70±0,015	5,60±0,02	5,68±0,005
3	5,69±0,017	5,58±0,011	5,57±0,005
4	5,78±0,011	5,61±0,01	5,64±0,01
5	5,70±0,01	5,65±0,011	5,67±0,005
6	5,73±0,011	5,64±0,005	5,68±0,005

All values are reported as mean ± SD (n=3)

The viscosity evaluation

The three formulas were followed by the stability of *langsat peel ethanol extract*. This evaluation at room temperature 15-30°C was measured by using a pH meter and Brookfield Viscometer (table 4). According to Martin (1993), viscosity is

a pressure statement of a liquid to flow, the lower viscosity has the higher the resistance. The lotion has a lower viscosity of cream and gel preparations which make it easier to use (spread more evenly than cream). Lotion is suitable for hairy areas such as scalp and spreads in the form of thin films making it more economical [11].

Table 4. The lotion viscosity after stability test.

Cycle	Viscosity (Cp)		
	Formula I	Formula II	Formula III
0	1703±11,01	1697±4,58	1672±4,35
1	1685±5,13	1680±7,09	1681±3,05
2	1688±4,58	1584±4,58	1593±3
3	1460±5	1581±2,51	1542±4,35
4	1562±4,35	1697±6,08	1596±5,29
5	1514±4,5	1670±4,16	1651±4,16
6	1625±4,72	1647±2,51	1648±7,5

All values are reported as mean ± SD (n=3)

The Freeze Thaw evaluation

The Freeze thaw evaluation was performed for 7 cycles, including viscosity and pH. For each cycle exposed at 40°C for 48 h which placed at 4°C for 48 h. The purpose of this Freeze Thaw evaluation was to evaluate the shelf-life of all formulas. In addition, the objective of the Freeze thaw test was to obtain the desired results at the shortest possible time by storing samples under conditions

designed to accelerate the occurrence of changes that normally occur under normal conditions [11].

The results showed that pH change during storage at 40°C and 4°C for 7 cycles can be seen in table 5. Based on the results of pH measurement, the pH of the dosage was about ± 5, it corresponded to human skin pH 4.5-7,0 [17]. As for the evaluation of viscosity measured using a Brookfield Viscometer for 7 cycles, the results showed the viscosity change during storage at 40°C and 4°C for 7 cycles. The resulting of viscosity was about ± 1700 cp.

Table 5. The results of Freeze Thaw evaluation.

Cycle	pH		
	Formula I	Formula II	Formula III
0	5,69±0,005	5,72±0,005	5,75±0,005
1	5,70±0,005	5,72±0	5,68±0,01
2	5,74±0,01	5,73±0,03	5,73±0,005
3	5,80±0,01	5,79±0,01	5,76±0,01
4	5,81±0,01	5,80±0,01	5,75±0,01
5	5,75±0,01	5,75±0,01	5,79±0,005
6	5,78±0,01	5,78±0,01	5,50±0,005

All values are reported as mean ± SD (n=3)

The spreading power evaluation

Evaluation of the scattering power of the lotion of longsat peel ethanol extract used loads of 1, 2 and 5 g of load. The spreading power test was done to see how far the lotion can spread evenly when applied to the skin.

The results showed the difference of power dissipation of each formula (table 6). This was caused by the difference of the viscosity of each of

the formulas because spreading was inversely proportional to viscosity. The more dilute a lotion, the greater the spreading power [17, 18]. From the results obtained by spreading lotion of a longsat peel ethanol extract was ranging from 3.5 to 5 cm. According to Laverius (2011) stated that the semisolid spreading capacity was about > 5 cm, so the spreading power of longsat peel ethanol extract qualified from semi solid dosage form.

Table 6. The results of spreading power evaluation

Load (g)	Formula	Results (cm)
1	F _{2A}	L = 4,5 W = 4
	F _{2B}	L = 4 W = 4
	F _{2C}	L = 4 W = 3,5
2	F _{2A}	L = 3,6 W = 3,6
	F _{2B}	L = 4 W = 4
	F _{2C}	L = 3,5 W = 3,5
5	F _{2A}	L = 5 W = 5
	F _{2B}	L = 4,5 W = 4,5
	F _{2C}	L = 4,5 W = 4,5

L:length; W: width

The sticky power evaluation

Chemical composition of *Crupina crupinastrum*

The power test was attached to all formulas using a load of 1000 g and 80 g for 5 min. From the adhesive test results showed the results of the three formulas having different adhesiveness due to differences in the viscosity of each formula. This

viscosity difference occurred due to the difference of the concentration of the active substance which has a thick viscosity and can affect the viscosity of the formulas (**table 7**).

Table 7. The results of the sticky power evaluation.

Formula	Time (second)
F _{2A}	6
F _{2B}	9
F _{2C}	9

Table 8. The results of effectiveness test on mosquito

The treatment	The number of mosquitoes		
	F _{2A}	F _{2B}	F _{2C}
The first five minues	1	0	0
The second five minutes	0	0	1
The third five minutes	2	1	0

Table 9. The results of irritation test on rabbits

Rabbit	Formula	Observation tme						Irritation index
		24 hours		48 hours		72 hours		
		Erythema	edema	Erythema	edema	Erythema	edema	
1	F _{2A}	0	0	0	0	0	0	0
2	F _{2B}	0	0	0	0	0	0	0
3	F _{2C}	0	0	0	0	0	0	0

Test of Repellent Effectiveness

From the test results of each formula showed that F_{2A}, F_{2B}, and F_{2C} had effectiveness as anti-mosquito. The concentration of 10% of langsat peel ethanol extract (F_{2A}) can be seen that 1 mosquito that perched on the first 5 min and 2 mosquitoes on the third 5 min. The concentration of 20% (F_{2B}) and F_{2C} with the concentration of 35% showed that mosquitoes which perched in the same amount that was 1 mosquito at the different time (**table 8**). This was caused by the different concentrations of each formula, the

greater the concentration of langsat peel ethanol extract and better effectiveness of anti-mosquito. While on the positive control showed the absence of mosquitoes perched.

The last stage was an irritation test conducted for 3 days or for 72 h using a rabbit as animal model. This irritation test was performed to see the presence of erythema and edema that occurred after smearing the lotion preparation of langsat peel ethanol extracts, thus preventing hypersensitivity reactions before use in humans [17, 18]. Observation of this irritation test used the Draize method by looking at the appearance of

erythema and wound shape, and the magnitude of edema [17]. The observation and irritation index calculations was obtained the three formulas did not show any irritation (erythema and edema). It can be seen in **table 9** that all the formulas were not irritating.

Conclusion

Langsat peel ethanol extract (*Lansium domesticum* Corr.) can be formulated into lotion dosage form at the concentrations of 10%, 20%, 35% and was stable based on stability evaluation. Besides that, the preparation of langsat peel lotion at the concentrations of 20% and 35% showed good effectiveness as anti-mosquito repellent.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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