JNPDD
Journal of Natural Product for Degenerative Diseases
https://journal.univpancasila.ac.id/index.php/Natural

Development of Gel Preparations from a Combination of Iler Leaf Extract and Patchouli Oil as a Repellent Against *Aedes aegypti* Mosquitoes

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Received: 23 August 2023 / Accepted: 7 September 2023

ABSTRACT: Dengue Hemorrhagic Fever (DHF) is an abnormal health condition caused by the dengue virus through the bite of the Aedes aegypti mosquito. The use of repellent to prevent mosquito bites can provide protection against DHF. DEET, a synthetic repellent with usage that has spread throughout the world, is reported to have toxic effects and cause irritation. Leaves of iler and patchouli oil are plants of the Lamiaceae family with repellent activity. This study aimed to obtain a repellant gel combination of iler leaf extract and patchouli oil. Gel preparations are made with a combination of Iler Leaf Extract and Patchouli Oil at concentrations of 12.5%, and 2%. Physical and chemical quality parameter test, stability test, acute dermal irritation test, and repel effectiveness test were tested on Aedes aegypti mosquitoes. Gel preparations showed a homogeneous physical appearance, greenish-brown color with an aromatic distinctive odor, a viscosity value of 16000 cPs, pH 6.32, and thixotropic flow properties. Gel preparations are effective as a mosquito repellent for 3 hours with a protection power of 56.82%. The gel preparation storage temperature, besides the resulting gel, does not irritate, and it is physically stable at 4 ± 2°C and 25 ± 2°C and is slightly less stable at 40 ± 2°C.

KEYWORDS: Aedes aegepty; formulation; repellent; iler leaf; patchouli oil.

1. INTRODUCTION

Dengue Fever (DHF) is an abnormal body health condition caused by dengue virus infection in humans due to female mosquitoes, which is the main cause, namely *Aedes aegepty*. WHO estimates that there are 390 million cases of dengue infection annually (between 284–528 million), and there are likely to be as many as 96 million cases (67–136 million) with a variety of clinical symptoms. According to a different study, 3.9 billion people in 128 countries are considered at risk of contracting the dengue virus based on prevalence rates, and 70% occur in Asia[1]. In Indonesia, a significant problem with dengue disease is often an outbreak disease (KLB).

If mosquitoes feed on the blood of people suffering from dengue fever, they can become infectious agents of the *Dengue* virus mosquitoes suck the blood of dengue fever sufferers. The virus that mosquitoes carry into their bodies will eventually be produced in the hemocoelum and enter the saliva to be ready to be transmitted. *Aedes aegypti* is one of the mosquitoes that acts as an infectious agent which is the most important disease-spreading organism of the *Dengue* virus[1]. *The female aegypti aedes* have multiple feeding properties, which allows mosquitoes to suck blood repeatedly to meet the need for blood in one gonotrophic phase[2]. Therefore, control of the vector is needed.

Based on the Regulation of the Minister of Health No. 374 of 2010 concerning Vector Control, vector control can be done by managing the physical or mechanical environment, the use of biotic, and chemical agents, both on vectors and their breeding sites and/or community change behavior and can maintain and develop local wisdom as an alternative[3]. Other efforts were also made to minimize vector contact with humans with the use of synthetic compounds. In this case, using mosquito repellent accompanied by personal protection such as repelling is one of the integrated vector control efforts with the greatest success rate because it can provide individual protection and is more practical.

How to cite this article: Wahyuni AN, Purba AV, Djamil R, Development of gel preparation from a combination of iler leaf extract and patchouli oil as a repellent against mosquitoes Aedes aegypty. JNPDD. 2023; 1(1): 23-30.

https://doi.org/10.58511/jnpdd.v1i1.4676
ISSN: 0000-0000

Repellents are preparations that can be applied directly to the skin, clothing and other surfaces to avoid contact with insects and mosquitoes. Synthetic repellents derived from *diethyltoluamide* (DEET) are known as repellent products that are widely used in the community, where the active substance is widely present in topical dosage forms and generally contains a concentration of 10-15%. Several studies have shown that the application of DEET-based repellents causes toxic effects that vary in degree in humans such as irritation of the mucous membrane to the occurrence of DEET insensition in *Aedes aegepti*[4] mosquitoes.

The toxic effect on synthetic repellents leads to a hypothesis of a new alternative in the manufacture of repellents with ingredients that are safe to use. The use of natural ingredients as a substitute for synthetic compounds in mosquito repellent repellents is considered to provide more profit because in addition to containing the main active compounds that have bioactivity as repellents, natural materials also contain various additional compounds that have the potential to boost the repelling activity of these plants synergistically[5].

Previous studies have shown several plants with repellent activity in mosquitoes, including the families Meliaceae, Rutaceae, Annonaceae, Lamiaceae and Zingiberaceae[5]. The Lamiaceae family, such as Rosemary and Patchouli essential oils, were shown to have a >96% protection against *Aedes aegypti* mosquitoes for 3 hours[6]. Patchouli oil contains repellent bioactivity and is a fixative that can bind to other compounds to maintain its aroma longer.

In Indonesia, one of the Lamiaceae families that are commonly found is iler plants, which also have repellent and insecticidal activity with the presence of saponin compounds, flavonoids, eugenol, tannins, carvacol, ethylsalicylate, alkaloids, thymol, and camphor found in the leaves of the iler plant[7,8,9]. Even so, there have been no further studies on the application of the use of iler leaf extract as a repellent and the effects it causes on humans.

This study aims to create a dosage formulation of a combination of iler leaf extract with patchouli oil as a repellent against *A. aegypti* mosquitoes. The preparation is made in the form of a gel because it has an excellent spreading ability on the skin, provides a cooling effect, is easy to wash, and physiologically does not inhibit hair function. In addition, the dosage form of the gel was chosen because it adapts to the volatile properties of natural ingredients.

2. MATERIALS AND METHODS

2.1. Materials

The ingredients used are dried of iler leaves, patchouli oil, *Aedes aegypti* eggs, 70% ethanol (Ika Pharmindo), water, sugar solution, pellets (*fish food*), carbopol (Lubrizol), TEA (Merck), methyl paraben, Sorbitol (Brataco). While the tools used include Brookfield RV (Haake Visco Tester 6®), pH Meter (Mettler Delta 320 pH Meter), Mortar and stamper, Becker glass, water bath, glass object, oven, refrigerator, vacuum rotary evaporator, blender, oven, glassware, pet confinement, shaker, analytical balance, counter, desiccator, water catcher, test cage (made of gauze wire) measuring ± 27 cm x 27 cm x 27 cm with a hole diameter of ± 14 cm, latex gloves, filter paper, funnel, aluminum foil, stopwatch, pipette, aspirator, analytical scale, stirrer, evaporating cup, stirrer rod, and spatula.

2.2. Methods

This study used an experimental method with a post-test design in the control group. The gel preparation is made with a combination of Iler Leaf extract and Patchouli Oil at a specific concentration, where optimization of the gel preparation had been carried out first. Then the preparation's physical and chemical quality parameter tests are carried out, such as stability tests, safety tests, and repellent effectiveness tests against *Aedes aegypti* mosquitoes. The safety test of the practice is carried out by the acute dermal irritation test method. Meanwhile, the repellent effectiveness test is carried out based on the procedure recommended by the *World Health Organization Pesticides Evaluation Scheme* (WHOPES).

The research was conducted from December 2016 to August 2017 at the Research Laboratory of the Faculty of Pharmacy, Pancasila University, with ethical testing by the Health Research Ethics Committee of the Faculty of Medicine, University of Indonesia. Data analysis used the *anova one-way* test and the *multiple comparison* test to determine the differences between groups.

2.3. Procedure

The examination of dried iler leaves was determined at the Herbarium Bogoriense LIPI Bogor and patchouli oil based on the SNI quality standard 06-2385-1991[10.11.] In addition, the examination of other additives is based on the monograph of each material. The manufacture of iler leaf extract is carried out starting from the selection of ingredients and drying then dissolved in *70% ethanol* in a ratio of 1: 10 and filtered. The results of the first filter are re-dissolved in a ratio of 1: 5 and filtered then steamed using a rotary evaporator at a temperature of ± 60 0 C until all ethanol compounds evaporate.

Preliminary tests of the extract were carried out on volunteers against *Ae mosquitoes. Aegypti* already bred. The volunteer's left arm acts as a control with a smear of 1 ml of 70% alcohol for 30 seconds, and the number of mosquitoes perched there is calculated by the criteria that testing can be started when there are >10 mosquitoes perched. Upon completion, the arm is removed and neutralized with a rinse of water and soap. The procedure is repeated at the lowest extraction dose in the presence of a dose increase at each repetition. At the end of the test, 1 ml of alcohol is applied to the right arm, allowed to dry for about a minute, then in place of the right in the same cage to ensure that no more than 10 mosquitoes perch on the arm in the same time period[12]. The ratio of mosquitoes perched on the treated arm to mosquitoes perched on the control arm was used to calculate % protection power[13].

The gel formula is made into 3 formulas and 1 placebo. The placebo/positive control did not contain iler leaf extract and patchouli oil. Formula 1 contains only iler leaf extract (12.5%) while formula 2 contains only patchouli oil (2%). Formula 3 contains a combination of iler leaf extract (12.5%) and patchouli oil (2%). All preparations are made with the same supporting materials with the same portion, namely Carbopol 940 (0.6g), sorbitol (6g), methylparaben (0.1), TEA (1.5), and water ad 100%. Evaluation of formula gel preparations is carried out physically and chemically[14.15.] Physical evaluation is carried out by organoleptic examination including the color, odor, and homogeneity of the gel and determination of viscosity and flow properties[16]. The determination of viscosity is carried out at various speeds while the determination of flow properties uses a force curve (dyne/cm^2) with a shear speed (rpm). Evaluation of chemical is carried out by measuring pH.

The effectiveness test of the preparation was carried out with the same procedure as the preliminary test of the extract by replacing the extract into a formula gel preparation with an observation period of 3 minutes every 1 hour for 6 hours[12]. The effectiveness value is calculated by the percentage of the protective power of the preparation. The stability test carried out is an accelerated stability test, namely at a temperature of 4 ± 2 ^{o} C, 25 ± 2 ^{o} C and at a temperature of 40 ± 2 ^{o} C for 12 weeks by observing organoleptic, homogeneity, pH, viscosity and flow properties of the preparation[16]. Freeze *thaw* test using temperature variations of 4 ± 2 ^{o}C for 24 hours and temperatures of 40 ± 2 ^{o}C for 24 hours for 3 repeated cycles[17].

Safety test of the preparation (irritation test) was carried out on 3 male albino rabbits with healthy skin. The test was carried out at a dose of 0.5 g on test preparations on or semi-solid. Initially, the test preparation was carried out on 1 rabbit with 3 patches with different locations and durations (3 minutes; 1 hour;4 hours). After that, if it is suspected that there are no corrosive properties, then the preparation is used on 3 rabbits with 1 patch each with a duration of 4 hours. Assessment of the irritant response is based on a score of 0-4 on the formation of erythema and the formation of edema. Observations of erythema and edema are carried out at the 1st, 24th, 48th, and 72nd hours after the patch is opened. Observation of reversibility is carried out until the 14th day if at the 72nd hour no irritating properties are found on the skin. After the test treatment is completed, the experimental animal will be sacrificed by means of euthanation[18]. The value of the irritation index is assessed at a certain time interval after exposure to the test preparation[18].

3. RESULTS

The extraction process of 1000 g of iler leaves (*Plectranthus scutellarioides* (L.) R. Br) on 70% ethanol solvent yielded a yield of 8.214%. The results of the iler leaf extract examination show that the extract is in accordance with MMI both in terms of organoleptic, pH, water content (<11), ash content (<3), and specific gravity. Phytochemical screening also showed negative content results in steroids and positive results in alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides. Patchouli oil meets the SNI 06-2385-2006 standard except for patchouli content where patchouli oil has a patchouli content of 28.24% while the minimum SNI standard is 30%.

The results of the preliminary effectiveness test based on WHOPES recommendations showed that the concentration selected for Iler Leaf extract was 12.5% where it had a protective power of 69.68%. And the concentration of patchouli oil chosen is 2% where it has a protective power of 83.12%. The preparation is made with a stirring rate of 200 rpm with a time period of 30 minutes.

Table 1 shows the characteristics of preparations based on the resulting organoleptic. F1 preparations produce a slightly clear brown color while F2 preparations produce *a* non-clear/transparent opaque white color. As for the preparation F3, it produces a greenish-brown and non-transparent color. In terms of smell, the whole preparation produces a characteristic aromatic aroma. At physical character review, the entire preparation is homogeneous. All preparations have a pH that matches the physiological pH of the skin (4.5-6.5) with thixotropic flow properties.

Table 1. Organoleptic characteristics of the preparation

Parameter	Positive control	F1	F2	F3
Color	Clear white	Slightly clear brown	White *Opaque*	Greenish Brown
Construction	Distinctive	Distinctive	distinctive	distinctive
Homogeneity	Homogeny	Homogeny	homogeneous	homogeneous
Viscosity	16000	15000	16500	16000
Flow properties	thixotropic	Thixotropic	thixotropic	thixotropic
pH	6,12	6,40	6,15	6,32

3.1. Stability test

The results of the preparation stability test showed that there was a change in the color of the preparation at a temperature of 40 ± 2 ˚C where the dosage color tended to become more cloudy and darker. However, there were no significant changes in the aroma, homogeneity, and flow properties of the preparation (data were not shown). The pH changes in each formula, both at storage temperatures of 4 ± 2°C, 25 ± 2°C and 40 ± 2˚C, are generally still in the skin's pH *balance* range and the pH changes that occur are not too large. The statement suggests that the gel has a relatively consistent pH. As for the viscosity of gel preparations in all formulas, it undergoes changes. The viscosity of the preparation in each test formula at a temperature of 4 ± 2 °C and 40 ± 2 °C tends to decrease while the viscosity of the preparation in each test formula at a temperature of 25 ± 2 ˚C tends to rise although not too significantly. From the results of this viscosity measurement, it is also known that the flow properties of each test formula stored within 12 weeks under different temperature conditions do not show changes in flow properties still have a thixotropic flow property of [16.17.]

Table 2 shows the results of the stability test by cycle test *(freeze thaw* test) where organoleptically, during the test cycle there is no change in terms of shape, smell and color as well as homogeneity in each test preparation. The pH of the preparation during the test cycle, also did not show significant changes in pH. The pH produced in the normal pH range of the skin (4.5-6.5) is 5.31-6.36. The whole preparation has an insignificant decrease in viscosity. This shows that the gel base selected in the Mamu formula produces a stable gel. Where this test aims is to ensure the physical fitness of the gel under the influence of *stress* temperature[16].

3.2. Safety Test of the Preparation

The preparation safety test is carried out by the method of acute dermal irritation test. The results of the dermal acute irritation test on placebo and gel preparations showed that at 1-72 hours observation at a temperature of 4 ± 2°C, 25 ± 2°C, and a temperature of 40 ± 2°C, there was no erythema or edema. In addition, the results of the irritation index calculation showed a result of 0.0, meaning that the mosquito repellent gel preparation combined with iler leaf extract and patchouli oil is very light irritant (*negligible*).

3.3. Repellent Effectiveness Test

Figure 1 shows that the longer the time of application of the gel on the surface of the skin, the less protective power as a mosquito repellent. This shows that the gel preparation is only effective as a repellent for 3 hours (for F2 and F3) starting from the time the preparation is applied to the skin surface, where the protective power is still above 50%, which is 50-77.36%. F3 preparations have better protection compared to

single formulas such as F1 and F2. In addition, the three formulas have a relatively lower protection power compared to the positive control of 58.14-90.57%.

The results of statistical analysis showed a p value of <0.05, meaning that at the α value of 5% there was a significant difference in protection between the positive control groups, F1, F2, and F3. The results of the *multiple comparison* test showed a difference between the positive control group and F1 except at the 2nd hour. The positive control group has a significant difference in protection power with F2 except in the 2nd and 3rd hours. The positive control group had a significant difference in protection power with F3 in the 5th and 6th hours. The F1 group has significant differences with F3 at the 4th hour.

Table 2 Preparation Stability Test Results with *Freeze Thaw* Test

Formula	Cycle To-	Organoleptic			PH	Viscosity
		Color	Construction	Homogeneity		
Positive control	1	Clear white	distinctive	homogeneous	6,15	18500
	2	Clear white	distinctive	homogeneous	6,08	18000
	3	Clear white	distinctive	homogeneous	6,00	17500
F1	1	Slightly clear brown	distinctive	homogeneous	6,36	17500
	2	Slightly clear brown	distinctive	homogeneous	6,28	16000
	3	Slightly clear brown	distinctive	homogeneous	6,15	16000
F2	1	White opaque	distinctive	homogeneous	5,98	18750
	2	White opaque	distinctive	homogeneous	5,84	18000
	3	White opaque	distinctive	homogeneous	5,31	17500
F3	1	Greenish Brown	distinctive	homogeneous	6,28	18000
	2	Greenish Brown	distinctive	homogeneous	6,12	18000
	3	Greenish Brown	distinctive	homogeneous	6,07	17500

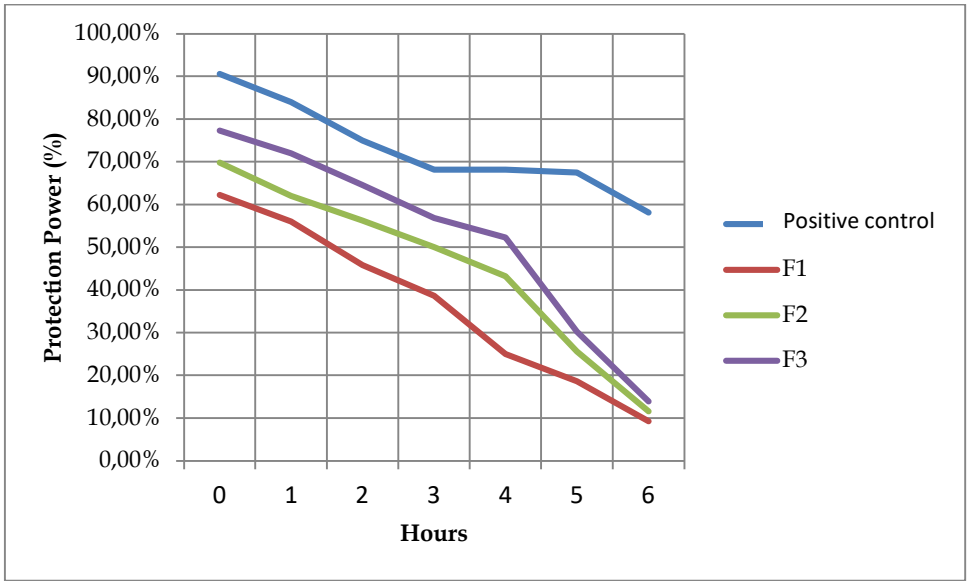

Figure 1: The protective power of the repellent gel preparation

4. DISCUSSION

The preparation uses Patchouli oil which has an alcohol patchouli content of 28.24%. This does not meet the SNI standard for patchouli oil, which is 30%. Differences in patchouli levels can be caused by many factors including the quality of the patchouli raw materials used, differences in the distillation process, to differences in treatment and storage places. In this study, the preparation was stirred with a time of 30 minutes at a speed of 200 rpm. The speed and duration were selected based on the results of observations of the homogeneity of mosquito repellent preparations which were considered optimal, namely homogeneous and slightly bubbles. The resulting bubbles should be as few as possible in order to facilitate the filling process into the container and maintain the uniformity of the dosage weight at the time of filling.

The choice of gel dosage form is due to the preferred properties of the gel, namely comfortable and soothing skin, easy to apply, not greasy, easy to wash, high adhesion and good drug release[24]. In addition, the composition of the active ingredients used in each formula is different, but still uses the same base concentration.

Carbopol was chosen as a *gelling agent* because it is relatively non-toxic, non-irritating, does not cause hypersensitivity reactions on human skin. In the presence of Carbopol the resulting gel mass will appear clear, not cloudy, and have a good shape. Due to its hydrophilic properties, Carbopol can easily dissolve in water at low concentrations (between 0.5 and 2%). The Carbopol used in this study is a type of Carbopol 940 because it has a good viscosity so that it is effective in the formation of gels with high viscosity and the ability to make clear gels[19]. Sorbitol in this formula functions as a humectant because it is able to keep the water content of the cosmetics preparation maintained[19]

The difference in dosage characteristics, especially between F1 and F2 is due to the active ingredient used in the form of oil so that when mixed into the gel base, an emulgel is formed, because the active ingredient in the form of oil reacts with the *gelling agent*. All preparations (formulations) of the gel are physically homogeneous. The statement shows that the components of the gel can dissolve and mix thoroughly. In addition, the characteristics of the preparation have a different pH. The pH of the preparation can be influenced by variations in the content of the active components contained in the gel, but in the resulting preparation it still meets the pH requirements of the skin physiologist. If it is below the pH, it can irritate the skin, and it can dry the skin if it is above the pHcalled[20]. Therefore, the resulting preparation can increase *the acceptability* of the wearer.

The viscosity of the preparation is relatively homogeneous. The viscosity of the preparation should not be too high or too low, because if the gel that is too viscous will be removed from the container, and if it is too aqueous it will not last long in the skin when applied. In addition, the preparation has the quality of thixotropic flow, which is typical for semisolid preparations due to its high consistency in containers but easy to pour and easy to disperse.

The results of the stability test showed that the aroma was getting less and less. Hal this is due to the aromatic compounds of the active ingredients that begin to disappear due to evaporation. In addition, discoloration also occurs during the test process caused because the formula is not resistant to heating temperatures. Given that gel preparations are stored in sealed containers, the viscosity of the preparation is also relatively decreased allegedly because water from the environment is likely absorbed by the gel preparation during testing. From these observations, it was found that the gel available from each formula did not undergo synthesis. The results of the *cycle* test also showed that there was no change in shape, smell, and color as well as homogeneity of the test preparation. This can be an indicator of the stability of the preparation organoleptically.

Gel preparations do not have irritant properties after testing at three varying temperature conditions. The overall results showed a very mild irritation index value of 0.0. This shows that the mosquito repellent gel preparation resulting from the combination of iler leaf extract and patchouli oil is non-irritant and does not cause irritation to the user's skin after use. This answers another alternative need for the use of synthetic repellents in the industrial market where there are indications of mild-severe irritation in users[18].

The results of the protection test show that the F2 and F3 gel preparations have a protective power of 50-77.36% for 3 hours with a tendency to decrease every hour. This is because the longer the gel stays in the open air, the essential oils contained in the iler leaf extract and patchouli oil will evaporate and their concentration decreases slowly in the gel preparation. For F1 has the lowest protection power because of the three formulas, F1 has the lowest viscosity, so that the active substances from the natural ingredients used are more volatile.

Variations in the protective power of each formula are possible due to the difference in synergistic effects between iler leaf extract and patchouli oil; besides that, it can also be caused by the fixative effect of patchouli oil in the preparation so that the active substance is longer retained in the preparation. If you compare the test preparations (F1, F2, and F3) with the positive control, the protective power of the three test preparations is smaller than the positive control protection power of 58.14-90.57%. The defensive power of positive controls also decreases over time but is still better than the test formula.

5. CONCLUSION

Combining iler leaf extract and patchouli oil in the gel preparation formulation meets the physical and chemical quality requirements. The resulting gel preparation shows a homogeneous physical appearance, greenish-brown color with a characteristic aromatic odor, with a viscosity value of 16000 cPs, pH 6.32 and has thixotropic flow properties. In addition, the preparation is also effective as a mosquito repellent for 3 hours with a protective power of 50-69.81% and no irritant effect based on the results of the dermal acute irritation test.

Acknowledgements: None

Author contributions: Concept – A.N., A.P., R.D.; Supervision – S.A.; Resources – A.N., A.P., R.D. ; Materials – A.N. ; Data Collection and/or Processing – A.N., A.P., R.D.; Analysis and/or Interpretation – A.N., A.P., R.D.; Literature Search – A.N., A.P., R.D. ; Writing – A.N.

Conflict of interest statement: The authors declared no conflict of interest.

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