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Test The Effectiveness of Kesum Leaf N-Hexana Extract Gel (Polygonum Minus Huds) as A Repellant Against Mosquitoes

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ABSTRACT

In some research shows that kesum contains several chemical compound that have a repellent activity such as alkaloid and essential oil which consist of terpenoids (β-Caryophyllen, α-curcumene, geraniol and geranial). It is important to support the effect by made a formulation that can give better repellent effect. The purpose of this research is to know whether n-heksane extract of kesum leaf have a effect as mosquito repellent and how about its effectivity when it was formulated in gel product with different concentration. Effectivity test was done by using a number of mosquitous that have been grow up from its pupae. The test was done in a mosquito box with 30 female mosquitos. Test group being used are positive control (Autan®), negative control (a hand that smeared with nothing), gel base, extract 3%, F1 (gel that contain 0,5% extract), F2 (gel that contain 1,5% extract) and L3 (gel that contain 3% extract). The test is done on all of a test groups for 15 minutes every single hour from 08.00-15.45 in West Indonesia Times. It is done in three time repetition on three different people. The results of the effectivity test is analyzed statistically by use One Way ANOVA with confidence level 95% and then it analyzed with Post Hoc Tukey. The results shows that F3 give the best repellent effect between F1 and F2. F3 group is better than extract 3% (without formulation). In repellent potention shows that the best effect is given by positive control (89,45%) and it is followed by F3 (85,78%), F2 (79,12%) and F1 (73,62%). Evaluation of gel is done on F3 for a mounth (31 days) every three days. The results shows that gel has a few change in all variable of evaluation, but it is good enough esthetically for a month and can be used on the skin.

1. Introduction

Diseases that are transmitted by mosquito vectors (intermediaries) are still a heavy burden for most tropical countries, including Indonesia. Diseases that are transmitted through mosquito bites are still endemic in many areas in Indonesia and claim thousands of lives every year, including West Kalimantan.The easiest and simplest prevention to do is to try to prevent the skin from being infested by mosquitoes that cause the dangerous diseases mentioned above, by using repellents (insect repellents) that are applied to the exposed skin.

Repellents currently available and still widely used contain DEET (N,N-Diethyl-3-Methylbenzamide) which is the main insect repellent. DEET has proven to be very effective and has been widely used for more than 50 years and is still the most effective repellent. Repellents containing DEET can provide full protection from 2-8 hours, depending on the concentration of DEET. However,

on daily use, DEET can cause several kinds of dangers, including a decrease in the permeability of the blood-brain barrier in certain brain areas, unsafe in children because it can cause encephalopathy, and can cause neurodegeneration in the form of nerve cell death (Tjahjani, 2008). .

Research to find plants that are efficacious as repellants needs to be done. This effort is an effective way because plant chemical compounds are generally plant secondary metabolites that are safe for use in humans. The examination of the effectiveness of the repellent is based on the fact that the chemical compounds in these plants can work as mosquito repellents. According to Othman (2009) one of the plants that can provide repellent effectiveness is Polygonum minus Huds or known as the kesum plant. The results showed that the kesum plant (Polygonum minus Huds) contained sesquiterpenoid compounds. The sesquiterpenoid compounds that have been identified from the essential oil using the GC-MS method are -curcumene, where in previous studies showed that this -curcumene compound was effective as a repellent against insects (Baharum, 2010). Another study also showed that kesum leaves (Polygonum minus Huds) contain 2 main essential oils, namely dodecanal (17.7%) and beta-caryophyllene (17.8%), which is thought to play a role in repellant against mosquitoes is beta-caryophyllene which is thought to play a role in mosquito repellent. has been tested by exposing a number of mosquitoes to the white guinea pig probandus (Othman, 2009). Furthermore, from the identification of kesum plant metabolites (Polygonum minus Huds), long chain aldehydes and terpenoids were found, including geraniol and geranial (Maroof, 2010). These two active compounds from the results of other studies are known to have activity as mosquito repellants (Gunawan, 2009). In addition, other studies have also shown that the kesum plant contains alkaloid compounds which are also active as insect repellants (Mustanir, 2008).

The use of repellent compounds derived from plants directly is less effective because of the nature of some compounds that are volatile and quickly disappear from the skin, so it is necessary to make them in an appropriate dosage form so that they are easy to use and more durable. In this research, kesum leaf extract will be formulated in the form of a gel. Gels, which are usually also called jellies, are semisolid systems consisting of suspensions made of small inorganic particles or large organic molecules, penetrated by a liquid (Depkes RI, 1995).

Gel is a clear, transparent preparation so that it does not leave stains on the wearer. In making the gel, additional ingredients are needed, one of the additional ingredients used in this study is carbopol. Carbopol functions as an agent that can increase the viscosity of the gel (Rowe, 2006), so that with increasing viscosity, the adhesive power of the gel on the skin will also be longer and the repellant effect will also be greater.

2. Method

Tool

The tools used in this research are erlemenyer (pyrex®), beaker glass (pyrex®), funnel glass (pyrex®), measuring cup (pyrex®), digital scale (precisa®), evaporator (heidolph®), box insect, porcelain dish, watch glass, plastic pot, mortar and stamper, glass jar, hot plate (memmert®), oven (memmert®), spatula, spatula, vial, crucible glass, object glass, viscometer stormer (Biuged®) , pH meter (pHepp®)

Ingredient

The ingredients used are n-hexane extract of kesum leaves, glycerin (pharmaceutical), carbopol 940 (pharmaceutical), aquadest, triethanolamine (pharmaceutical), sodium metabisulfite (pharmaceutical), and female mosquitoes.

Test preparation formulation

Kesum leaves are separated from the stalks, washed with running water and aerated. Next, the coriander leaves are mashed with a chopper and in a blender. After that, maceration was carried out with n-hexane for 3x24 hours and repeated 3 times. The viscous extract was obtained through the evaporator process.

The gel is made by expanding carbopol in a mortar with hot water, then stirring. Glycerin and sodium metabisulfite were mixed until homogeneous, then put into the developed carbopol, grinded until homogeneous. Add water to the mixture to the desired volume, grind it slowly until it gets a gel-like

mass. Then the extract was added and crushed again. After that, add TEA drop by drop while stirring slowly until a clear gel is formed.

Repellent Effectiveness Test

a. Negative Control Test

The arm was put in a cage containing 30 female mosquitoes for 15 minutes. The number of mosquitoes that landed on the arm was counted from 08.00-15.45 with an interval of 1 hour. The experiment was carried out 3 times.

b. Positive Control Test

2 grams of Autan® is applied to the arm and then placed in a cage containing 30 female mosquitoes for 15 minutes. The number of mosquitoes that landed on the arm was counted from 08.00-15.45 with an interval of 1 hour. The experiment was carried out 3 times.

c. Extract Effectiveness Test

A total of 0.06 grams of the extract was bathed until it melted and then rubbed on the arm, then the arm was put in a cage containing 30 female mosquitoes for 15 minutes. The number of mosquitoes that landed on the arm was counted from 08.00-15.45 with an interval of 1 hour. The experiment was carried out 3 times.

d. Gel Base Effectiveness Test

The arm was smeared with a gel base without extract as much as 2 grams and then put in a cage containing 30 female mosquitoes for 15 minutes. The number of mosquitoes that landed on the arm was counted from 08.00-15.45 with an interval of 1 hour. The experiment was carried out 3 times.

e. Test the Effectiveness of Gel Formulas

A total of 2 g of Formula 1 gel (0.5% extract) was applied to the arm, then the arm was put in a cage containing 30 female mosquitoes for 15 minutes. The number of mosquitoes that landed on the arm was counted starting at 08.00 – 15.45 with an interval of 1 hour. The experiment was carried out 3 times. The same treatment was also carried out using a gel with formula 2 and formula 3.

Evaluation of Physical and Chemical Properties of Gel Formula

The formula used in the evaluation is the formula that provides the best repellency. The evaluation was carried out by observing and measuring the physical and chemical properties of the gel. Physical evaluation is meant by organoleptic observation, measurement of viscosity, spreadability, and adhesion, while chemical evaluation is measurement of pH. Observations and measurements were made on day 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, and day 31.

Data analysis

The data obtained in the form of the number of mosquitoes that perch on the probandus arm. The data were analyzed using the SPSS program to determine the significant differences between the experimental groups. Then the analysis is continued by calculating the percentage of repelence with the formula:

$$\%R = \frac{a-b}{a} \times 100\%$$

Description :

R = Repelence

a = Number of mosquitoes perched on negative control.

b = The number of mosquitoes that landed on the treatment.

3. Results and Discussion

Table 1. Average data of mosquitoes perched per hour of treatment

Treatment Hours	Negative Control	Positive Control	Extract 3%	Gel Base	F1 (0.5%)	F2 (1.5%)	F3 (3%)
J1 (08.00-08.15)	24	0	0	0	0	0	0
J2 (09.15-09.30)	22.67	0	0	1.67	0.67	0.67	0
J3	22.33	0.67	1.67	4	2	1.67	1.67

(10.30-10.45)							
J4 (11.45-12.00)	20	2	5	5	6.33	4	2.33
J5 (13.00-13.15)	19.67	3	7.33	6.67	7.67	9.33	3.67
J6 (14.15-14.30)	18.67	4.33	12.67	12	10.67	10	4.33
J7 (15.30-15.45)	18	5.33	12	13	11	11	8.67
Total	145.34	15.33	38.67	42.34	38.34	36.67	20.67

The results of the one way ANOVA test for all treatment groups showed a significant difference in values in all hours of treatment ($p < 0.05$), so it was feasible to continue with post hoc in each group being compared. One way ANOVA test in the treatment group formulas 1, 2 and 3 showed a significant difference in all treatment groups at 4 hours, 5 hours and 6 hours by producing a significant number (p) <0.05, namely at -4 0.022 ; hours-5 0.010 and hours-6 0.001 so it is necessary to do a post hoc Tukey test with the aim of knowing specifically the significant differences between the groups.

Table 2. Tukey Post Hoc Test Results in Treatment Groups F1, F2 and F3

Treatment Hours	Group name	Significant Value	Conclusion
	F1 – F2	1,000	Not significantly different
J2	F1 – F3	0.269	Not significantly different
	F2 – F3	0.269	Not significantly different
	F1 – F2	0.679	Not significantly different
J3	F1 – F3	0.679	Not significantly different
	F2 – F3	1,000	Not significantly different
	F1 – F2	0.133	Not significantly different
J4	F1 – F3	0.018	Significantly different
	F2 – F3	0.302	Not significantly different
	F1- F2	0.428	Not significantly different
J5	F1 – F3	0.042	Significantly different
	F2 – F3	0.009	Significantly different
	F1 – F2	0.797	Not significantly different
J6	F1 – F3	0.002	Significantly different
	F2 – F3	0.003	Significantly different
	F1 – F2	1,000	Not significantly different
J7	F1 – F3	0.161	Not significantly different
	F2 – F3	0.161	Not significantly different

From the table it can be explained that at hour-1, hour-2 and hour-3 there was no significant difference between the treatment groups, this was because at that hour the number of mosquitoes that landed was still in close range, meaning that the three formulas could still work. effectively up to 3 o'clock. However, at 4 hours there was a significant difference in value between formula 1 and formula 3, namely 0.018. These results indicate that at 4 hours the formula 1 group experienced a surge in the number of mosquitoes that landed, so it can be said that the formula 1 group did not provide an effective repellant effect anymore, while formula 2 and formula 3 were still not significantly different. At the 5th hour, it turned out that the treatment groups of formula 2 and formula 3 were significantly different, namely 0.009, while formula 1 and formula 2 there was a significant increase in value from 0.133 to 0.428. These results indicate that at 5 hours, the formula 2 treatment group has begun to reduce its effectiveness while formula 3 is still holding on. The significant increase in the values of formula 1 and formula 2 from 4 to 5 hours showed that the number of mosquitoes that landed in the formula 2 group at 5 hours was almost the same as the formula 1 group. At 6 hours, it turned out that formula 3 could still be said to be effective because still significantly different from formula 1 and formula 2. At 7 o'clock

the formula 3 group did not give a significant difference with formula 1 and formula 2. almost equal to the formula 1 and formula 2 groups.

Table 3. Tukey's Post Hoc Test Results in the Positive Control Treatment Group, Formula 3 and 3% Extract

Treatment Hours	Group name	Significant Value	Conclusion
J3	Post – F3	0.165	Not significantly different
	Post – Extract	0.165	Not significantly different
	Extract – F3	1,000	Not significantly different
J4	Post – F3	0.939	Not significantly different
	Post – Extract	0.051	Not significantly different
	Extract – F3	0.077	Not significantly different
J5	Post – F3	0.869	Not significantly different
	Post – Extract	0.037	Significantly different
	Extract – F3	0.069	Not significantly different
J6	Post – F3	1,000	Not significantly different
	Post – Extract	0.002	Significantly different
	Extract – F3	0.002	Significantly different
J7	Post – F3	0.136	Not significantly different
	Post – Extract	0.009	Significantly different
	Extract – F3	0.136	Not Significantly Different

Based on the table, at the 6th hour there was a significant difference between formula 3 and the 3% extract, meaning that until the 6th hour formula 3 was still actively providing a repellant effect while the 3% extract was no longer effective. This proves that the extract in the form of a gel is more effective than the unformulated extract.

The next analysis carried out was between the positive control and formula 3. The aim was to see the limits of the ability of the gel formula formula 3, which is the formula with the best effect, being able to compete with the positive control. The results of the SPSS data processing showed that up to the 7th hour there was no significant difference in value with a significance value (p) > 0.05, meaning that formula 3 was still able to compete with the positive control in maintaining its repellency effect until the 7th hour.

Table 4. Percentage of Repellent Potential of All Groups per Hour of Treatment

	Negative control	positive control	Extract 3%	Gel Base	F1 05%	F2 1.5%	F3 3%
J-1 (08.00-08.15)	0	100	100	100	100	100	100
J-2 (09.15-09.30)	0	100	100	94.13	97.04	98.54	100
J-3 (10.30-10.45)	0	98.52	92.52	82.09	94.04	92.52	92.52
J-4 (11.45-12.00)	0	88.35	75	75	71.65	83.35	88.35
J-5 (13.00-13.15)	0	84.75	62.74	66.09	62.74	52.57	81.34
J-6 (14.15-14.30)	0	74.99	32.14	35.72	42.85	46.44	76.81
J-7 (15.30-15.45)	0	70.39	33.33	27.78	38.89	40.72	51.83

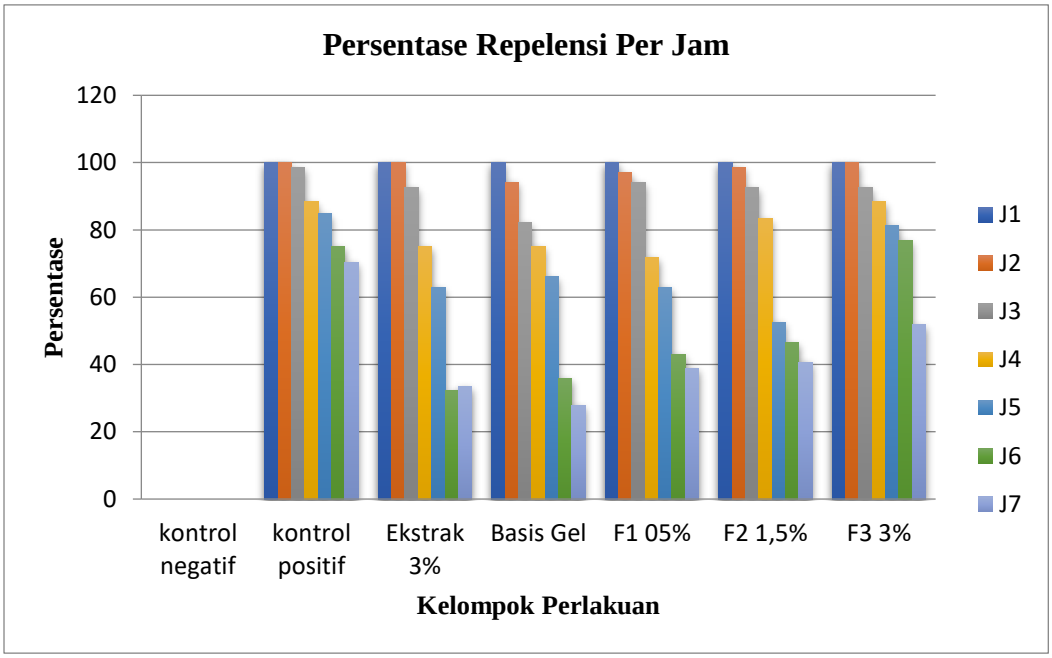

Fig 1. Bar Chart Repellent Potential of All Groups Per Hourly Treatment

Based on the table and diagram above, it can be seen that at every hour in the treatment group formulas 1, 2 and 3 there is a difference in the ability or potential for repellency, where the increase in treatment hours decreases the potential for repellency. The diagram also shows a significant decrease in formula 1 seen from the 3rd hour to the 4th hour, namely from 94.04% to 71.65%. In formula 2, there is a significant decrease in the potential for repellency at 4 to 5 hours, namely from 83.35% to 52.57% and in formula 3 a significant decrease in potency occurs from 6 to 7 hours, namely from 76.81 to 51.83.

The Pesticide Commission of the Ministry of Agriculture (1995) stated that a repellent preparation is said to be effective if up to the 6th hour its ability or repellency potential is at least 90%. However, in the test results that were carried out at 6 hours, none of the experimental groups were said to be effective in meeting the standards, including positive control and formula 3 which gave potency above 90% only up to 3 hours. However, in general the n-hexane leaf extract Kesum can be used as a repellent against mosquitoes, although it cannot be said to be effective according to the Pesticide Commission of the Ministry of Agriculture.

The results of the evaluation showed that there were changes both physically and chemically from the gel preparation after 31 days of storage. Organoleptic test showed a change in the color of the preparation that occurred on the 25th day of storage where the color became lighter. The viscosity test showed a change from 125.72 poise on the 1st day to 117.89 poise on the 31st day. Likewise with the dispersion test, there was a change from 4.1 cm to 4.47 cm, the adhesion test from 18.70 to 15.73 seconds and a shift in the pH value from 6.7 to 6.4.From the evaluation results that have been carried out for 1 month of storage, it can be concluded that there is a change in the preparation, both physically and chemically, but the change is not too significant so that it does not reduce the aesthetic value of the preparation and is still acceptable by the user for use on the skin.

4. Conclusion

Kesum leaf n-hexane extract gel (Polygonum minus Huds) can be used as a mosquito repellent although it cannot be said to be effective according to the standards of the Pesticide Commission of the Ministry of Agriculture, namely with a minimum repellency potential of 90% until the 6th hour. The gel that gave the best effect as a repellent in this study was a gel with a concentration of n-hexane extract from kesum leaves (Polygonum minus Huds) as much as 3%. Gel formula 3 (containing 3% extract) had the same effectiveness as the positive control group until the end of the treatment. During storage for 1 month (31 days), the gel underwent physical and chemical changes, but did not show a significant change in the test value, so it did not reduce the aesthetic value of the preparation and was still feasible in terms of use.

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