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Formulation and Evaluation of Gel Contains the Combination of Ethanol Extract Basil leaves (*Ocimum sanctum* L.) and Soursop Leaves (*Annona muricata* L.) as a Mosquito Repellent

			
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ABSTRACT

The purpose of the present combination is to develop a mosquito repellent with a combination of active ingredient combination of ethanol extract of basil leaves (*Ocimum sanctum* L) and the soursop leaves (*Annona muricata* L). Mosquito repellent gel made as many as three formulas containing combinations of ethanol extract of basil leaves and soursop leaves (10% : 2%), Carbopol 940 with varying concentrations of 0.5%, 0.75%, and 1.0% as a gelling agent. Gel are evaluated physically and chemically via organoleptic examination, homogeneity, viscosity, flow properties, the scatter and pH. The formula of qualified physical and chemical stability testing conducted at room temperature, 4°C and 40°C. The results showed that the preparation is stable at room temperature and 4°C. Basil leaves extract provides optimum power protection at a concentration of 6% amounting to 93.17% and soursop leaves extract at a concentration of 8% amounting to 87.12%. The combination gel formulation mosquito repellent ethanol extract of basil leaves and the soursop leaves (10% : 2%) have the capability of protection from 59.60 to 92.62% with 120 minutes long protection. Based on observations and test the stability of power protection, it can be concluded that the best formula is with a concentration of carbopol 940 is 1.0% as a gelling agent.

INTRODUCTION

Mosquitoes are species that are very disturbing and harmful to humans. One of the effects clearly felt is Dengue Hemorrhagic Fever. Anti-mosquito topical products that are circulating today contain harmful ingredients that besides harmful to mosquitoes too dangerous for humans.

A very dangerous substance contained in a topical anti-mosquito is Diethyltoluamide or DEET. Almost all anti-mosquito topical products circulating in Indonesia with active ingredient DEET (Diethyltoluamide) in concentrations of 10-15%. DEET is very corrosive, anti-mosquito topical products can not be stored in plastic containers PVC or iron because in a week will erode the lining.

Of the types of mosquito repellent products on the market, mosquitoes topical preparations such as mosquito repellent gel classified product is favored because of its practical utility can be used anytime and anywhere. Additionally, gel formulation has many advantages when compared to other semisolid preparations such as creams or ointments. Preparations in the form of a gel have the ability to absorption of the active either on the skin, easily washable with water, used in hair and skin lesions with high adhesive power that does not clog pores.

The plant material such as extract of basil leaves (*Ocimum sanctum L.*) contains eugenol which can function as a repellent. Eugenol is a class of compounds essential oils which have a very distinctive aroma and flavor disliked by mosquitoes. While the leaves of the soursop (*Annona muricata L.*) has acetogenin compound that becomes toxic to mosquitoes and it's not like mosquitoes. These substances can be used to make anti-mosquito gel preparation, because of the smell and the taste is not like mosquitoes. The combination of the two plants is expected to work synergistically as a mosquito repellent.

MATERIALS AND METHODS

Material

The eggs of *Aedes aegypti* L. Strain Liverpool derivative/F101, basil leaves (*Ocimum sanctum L.*) and soursop leaves (*Annona muricata L.*), carbopol 940 (Lubrizol), methylparaben (BRATACO), propylparaben (BRATACO), Propylene Glycol-400 (BRATACO), triethanolamine (BRATACO), disodium EDTA (BRATACO) and aquadest.

Method

Introduction test of the extracts : ⁽⁶⁾

Basil leaves and extract concentration of soursop leaves are used in a formula determined based on these introduction test results. This study used 5 series of the single concentrations (2%, 4%, 6%, 8% and 10%) and 5 series of the combination concentrations (2%:10%, 4%:8%, 6%:6%, 8%:4%, and 10%:2%) in 1 ml ethanol 70% with 3 times replication then sought doses to obtain the percentage of thrust against the mosquito *Aedes aegypti* L. >50%, to assess the effective dose of ethanol extract of basil leaves and the soursop leaves, repellent activity test conducted following the procedures recommended by WHOPEs. Repellent activity test using human subjects, methods have been selected in accordance with the actual conditions of use (WHOPEs, 2009).

The calculation of concentration extracts for introduction test

M1	V2	M2	$V1 = \frac{V2 \cdot M2}{M1}$	Pengulangan (V1 x 3)
100%	1 ml	2%	0.02 ml	0,06 ml
100%	1 ml	4%	0,04 ml	0,12 ml
100%	1 ml	6%	0,06 ml	0,18 ml
100%	1 ml	8%	0.08 ml	0,24 ml
100%	1 ml	10%	0,10 ml	0,30 ml

Mosquitoes Hatching Eggs

The eggs of *Aedes aegypti* L. inserted into a glass or plastic bowl containing water media for 1-2 days to hatch into the larval instar stage I-IV which lasts for 7-8 days. During the development period, the larvae were fed in the form of pellets up to the phase did not eat that pupa. Pupa transferred into a bowl of water and left in the media in the cage for 1-2 days to develop into adult mosquitoes. The female mosquitoes will be split with the male mosquitos and fed in the form of sugar water.

Preparation of gel formulation

Carbopol 940 dispersed in aquadest with a stirrer speed of 500 rpm to get a clear solution. Then let stand for 24 hours before use. Methylparaben and propylparaben then dissolved in

propylene glycol and stirred with a stirring rod until a homogeneous mixture was generated. Dissolve the extract into the mixture, stirring until completely dissolved. Dissolve Disodium EDTA in Aquadest. Stir until homogeneous. Carbopol 940 which has been expanding neutralized with Triethanolamine, stirring with a stirrer until homogeneous. Enter into the basis of all the mixture. Stir using a stirrer speed of 200 rpm for 30 minutes. Evaluation of anti-mosquito gel formulation.

Evaluation of Physical and Chemical Preparations Gel

Physical and chemical properties like organoleptic color and smell, homogeneity, dispersive power, and pH of gel were determined. Determination of the viscosity and flow properties. Viscosity measurement is done by Brookfield viscometer with speeds from 1; 2; 2.5; and 4 rpm, and turned 4; 2.5; 2; and 1 rpm. The obtained result was plotted against shear stress (dyne/cm^2) and shear rate (/sec). The viscosity examination conducted for 3 months on storage at 4°C and room temperature, 4 weeks at a temperature of 40°C.

The power protection activity of gel

Before and after the experiment each test area (forearm of volunteers) are washed with soap and rinsed with water, then dried. The left arm as a control incorporated into the mosquito cage. Observe and record the number of mosquitoes that landed within a period of 30 seconds, if the mosquitoes landed more than 10 the testing can begin. After 30 seconds the arm is removed carefully from the cages of mosquitoes. Then the right arm smeared with gel performed mosquito repellent. Enter your hand back into the cage to be observed for 30 seconds. The arm should not move during the test. Observe the number of mosquitoes that land on the arms of volunteers. Continue observations at 30, 60, 120, 240, and 480 minutes, and observe the number of mosquitoes that land.

Stability Test

Performed gel stability test includes organoleptic (color and smell), homogeneity, dispersive power, pH, viscosity and flow properties. Evaluated at low temperature ($4\pm 2^\circ\text{C}$) and room temperature ($25\pm 2^\circ\text{C}$) within 3 months with observation every 1 month, and high temperature ($40\pm 2^\circ\text{C}$) for 4 weeks with observation 1 week once.

Irritation Test

Toxicity tests carried out by the method acute dermal irritation test in rabbits. Rabbits were used bodied, with a body weight of about 2 kg (rabbits used were New Zealand white rabbits, male aged 4-5 months, weighing rabbits between 1.8 to 2 kg). (BPOM Chief Regulation No. 7 of 2014 About Non-Clinical Toxicity Test By In Vivo).

RESULTS AND DISCUSSION

Power Protection Activity Test Results Ethanol Extract Basil and Soursop Leaf.

The power protection test of mosquitoes for ethanol extract of basil leaves are most effective at a concentration of 6% that is 93.17%. The power protection test of mosquitoes for ethanol extract of soursop leaves are most effective at a concentration of 8% that is 87.12%.

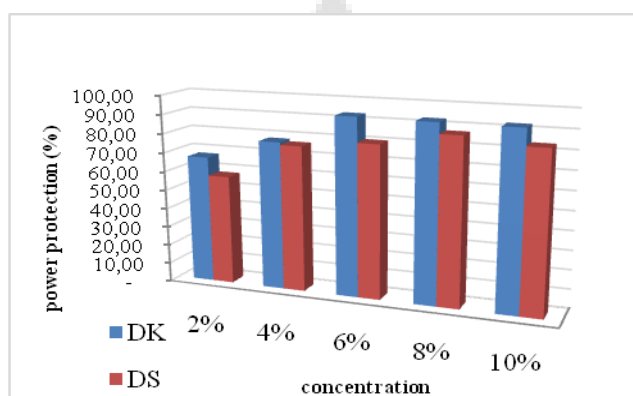


Figure 1. The result of the power protection tests from the single extract

The power protection test of mosquitoes for a combination of ethanol extract of basil leaves and soursop leaves are most effective at a concentration of 10%:2% is 93.94%.

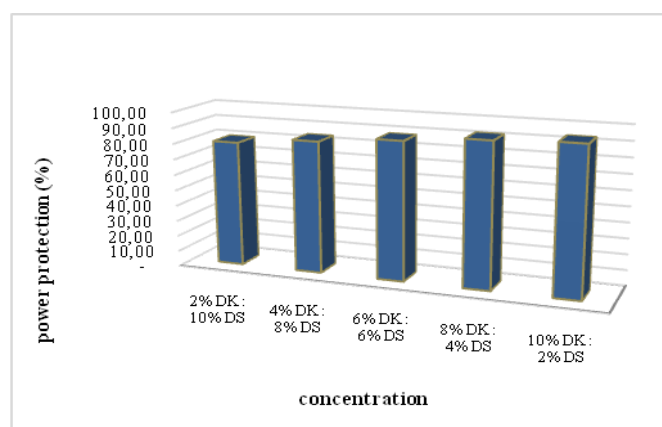


Figure 2. The result of the power protection tests from the combination extract

Formulation Design

Formula Materials	Weight (% b/b)		
	F1	F2	F3
Combination of ethanol extract of basil leaves and soursop leaves	X	X	X
Carbopol 940	0,5	0,75	1
Methylparaben	0,2	0,2	0,2
Propylparaben	0,02	0,02	0,02
Propylene glycol	5	5	5
Triethanolamine (TEA)	qs	qs	qs
Dinatrium EDTA	0,05	0,05	0,05
Aquadest	Add 100	Add 100	Add 100

Information :

X: indicates the concentration of the results at introduction test are 10%:2%.

Evaluation of gel preparation

Organoleptic

Mosquito repellent gel is made shows the physical appearance of a homogeneous, dark green color and distinctive smell aromatic.

The test results in Viscosity and Flow Properties

The viscosity grades 53125-220000 cps, thixotropic flow properties.

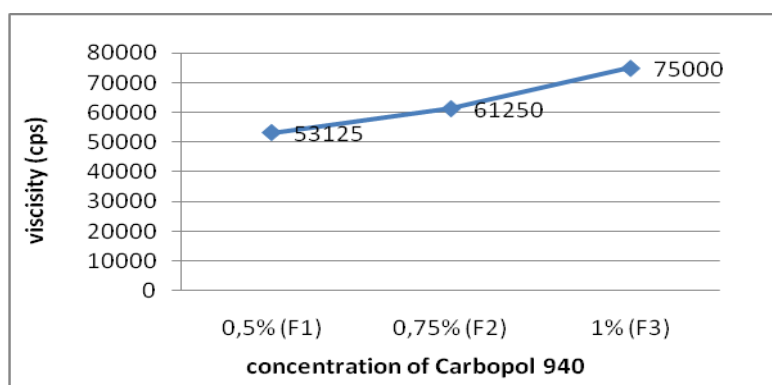


Figure 3. Graph of the ratio between the concentration of Carbopol 940 with mosquito repellent gel viscosity combination of ethanol extract of basil leaves and the soursop leaves

So it can be concluded that the higher the concentration of Carbopol 940, it can increase the viscosity of the gel, the viscosity increased because Carbopol can inflate when dispersed in water to form a colloidal dam.

Thixotropy flow properties have properties, ie viscosity can be reduced with increasing shear rate. Downhill curve shape on the left ascending curve, which indicates that the emulsion has a lower viscosity at any shear rate curve price lower than an ascending curve. It shows the breakdown of the structure that is not re-established immediately if stress is reduced or eliminated.

Coverage Test Results

Dispersive power gel preparations obtained from 2236.80 to 2758.58 mm². The higher the concentration of Carbopol 940, the wide-spread decreases (smaller) in each formula. This suggests that the effect on the concentration of Carbopol 940 gel deployment area. Rising concentrations of Carbopol will increase the viscosity and reduce the size of the spread gel formulation so that the gel becomes more difficult to spread.

pH

pH 5.75 to 6.42 (terms of skin pH: 4.5 - 6.5). The addition of a combination of ethanol extract of basil leaves and leaves of the soursop can lead to a decrease in mosquito repellent gel pH becomes more acidic.

Test Results Power Protection mosquito repellent gel

In the effectiveness test of mosquito repellent gel, in each formula (F1, F2, and F3) used a combination of ethanol extract of basil leaves and leaves of the soursop with a concentration ratio of 10%:2%.

Treatment	Repellency (%)			
	0	1	2	3
Stability				
Positive control	100,00	98,41	98,55	98,25
F1	85,29	83,28	80,55	81,43
F2	86,39	80,79	81,10	82,93
F3	87,37	84,13	85,94	85,26

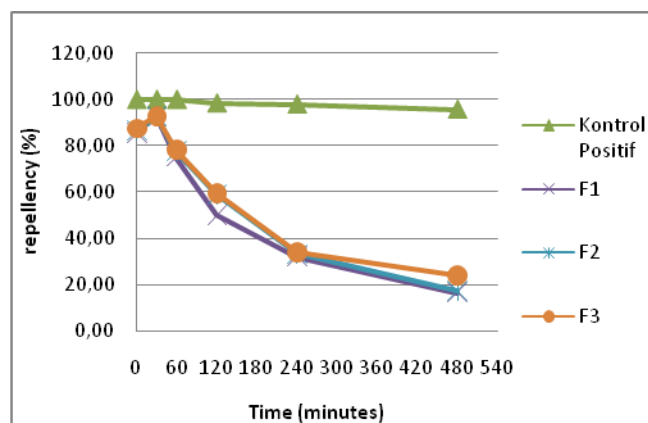


Figure 5. Comparison Chart Power Protection of Mosquito Repellant Gel Combination Ethanol Extract and Basil Leaf Soursop against Time

Mosquito repellent gel combination of ethanol extract of basil leaves and leaves of the soursop has a maximum power protection that is 92.59 to 92.66% at 60 minutes and effective as a mosquito repellent for \pm 120 minutes (2 hours).

Stability Test Results of Mosquito repellent Gel

Gel formulation of mosquito repellent generated stable at room temperature ($25\pm 2^\circ\text{C}$) and cold temperature ($4\pm 2^\circ\text{C}$), with the physical appearance of a homogeneous, dark green color and distinctive smell aromatic viscosity grades 42500-207500 cps, properties thixotropic flow, dispersive power from 2082.02 to 3061.50 mm^2 , pH 5.60 to 6.54 (terms of skin pH: 4.5 - 6.5).

Irritation Test Results

Mosquito repellent gel combination of ethanol extract of basil leaves and the soursop leaves does not cause skin irritation in experimental animals after application for 3 consecutive days (72 hours) with irritation index of 0.

CONCLUSION

The ethanol extract of basil leaves and leaves of the soursop proven to give effect to the mosquito repellent. The combination of extracts of basil leaves and the soursop leaves can be formulated into gel formulation has met the quality requirements of physics, and chemistry. Gel mosquito repellent combination of ethanol extract of basil leaves and the soursop leaves does not cause irritation.

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