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## DEVELOPMENT OF AN ANTI-MOSQUITO SPRAY GEL FORMULATION FOR A COMBINATION OF MARIGOLD FLOWERS (TAGETES ERECTA L.) AND CHRYSANTHEMUM FLOWERS (CHRYSANTHEMUM INDICIUM L.)

### Abstrak

Nyamuk merupakan vektor penyakit menular yang harus dikendalikan. Upaya pengendalian vektor nyamuk salah satunya dengan menggunakan produk anti nyamuk diantaranya gel spray anti nyamuk. Produk anti nyamuk kebanyakan tersusun dari bahan kimia yang membahayakan kesehatan dan lingkungan, sehingga diperlukan alternatif anti nyamuk dari bahan alami. Tujuan dari penelitian ini adalah untuk mengetahui formula gel spray anti nyamuk yang efektif dari kombinasi bunga marigold dan bunga krisan. Penelitian ini menggunakan metode eksperimental untuk menemukan formulasi gel spray yang paling efektif dari persentase bunga marigold dan bunga krisan yang berbeda-beda. Variasi yang digunakan adalah 0%, 2,5%, 5% dan 10%. Pengujian dilakukan dengan menggunakan 30 nyamuk betina dewasa. Hasil yang didapatkan melalui ekstraksi bunga marigold menunjukkan rendemen sebesar 8,922% dan rendemen bunga krisan sebesar 4,560%, Pada uji organoleptik yang dilakukan pada 10 panelis menunjukkan bahwa rata-rata para panelis berpendapat suka (pada range 4-5) terhadap kesukaan terhadap warna, aroma, tekstur dan reaksi setelah 10 menit pemakaian gel spray. Uji pH menunjukkan bahwa keempat formula gel spray memiliki pH 6 atau netral. Hasil uji homogenitas yang dilakukan dengan kaca objek menunjukkan keempat formula gel spray adalah homogen. Daya proteksi gel spray formula 2,5% adalah 18,01%; gel spray formula 5% adalah 66,29% dan gel spray formula 10% adalah 88,61%. Dari hasil tersebut maka dapat diketahui formula dengan konsentrasi ekstrak bunga marigold dan bunga krisan sebanyak 10% dapat menjadi gel spray anti nyamuk paling efektif. Diharapkan hasil penelitian ini dapat bermanfaat bagi masyarakat dalam hal penggunaan anti nyamuk yang ramah lingkungan dan aman bagi kesehatan.

**Kata kunci:** Gel Spray, Bunga Marigold, Bunga Krisan, Anti Nyamuk.

### Abstract

Mosquitoes are vectors of infectious diseases that must be controlled. One of the efforts to control mosquito vectors is by using anti-mosquito products, including anti-mosquito gel spray. Most anti-mosquito products are composed of chemicals that are harmful to health and the environment, so alternative anti-mosquito products made from natural ingredients are needed. The aim of this research is to determine an effective anti-mosquito gel spray formula from a combination of marigold flowers and chrysanthemum flowers. This research uses experimental methods to find the most effective gel spray formulation from different percentages of marigold and chrysanthemum flowers. The variations used are 0%, 2.5%, 5% and 10%. Tests were carried out using 30 adult female mosquitoes. The results obtained through the extraction of marigold flowers showed a yield of 8.922% and a yield of chrysanthemum flowers of 4.560%. The organoleptic test carried out on 10 panelists showed that on average the panelists thought they liked (in the range 4-5) their preference for color and aroma, texture and reaction after 10 minutes of using the gel spray. The pH test showed that the four gel spray formulas had a pH of 6 or neutral. The results of the homogeneity test carried out with a glass object showed that the four

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gel spray formulas were homogeneous. The protective power of the 2.5% gel spray formula is 18.01%; gel spray formula 5% is 66.29% and gel spray formula 10% is 88.61%. From these results, it can be seen that a formula with a concentration of marigold and chrysanthemum flower extracts of 10% can be the most effective anti-mosquito gel spray. It is hoped that the results of this research will be useful for society in terms of using mosquito repellent that is environmentally friendly and safe for health.

**Keywords:** Gel Spray, Marigold Flowers, Chrysanthemum Flowers, Mosquito Repellent

## INTRODUCTION

Mosquitoes are the main insect vectors that cause various important tropical diseases in Indonesia such as malaria, dengue fever, chikungunya, lymphatic filariasis and Japanese encephalitis. Mosquitoes are a type of insect belonging to the order Diptera and the Family Culicidae (Sianipar et al, 2018). Mosquitoes constitute one of the most significant threats to human and animal health throughout the world (Norris and Coat, 2017).

Dengue fever (DHF) is a disease that is transmitted by mosquitoes. The disease transmitted by mosquitoes apart from dengue fever (DHF) is malaria which usually attacks Indonesian people. North Sulawesi has long been known as an endemic area for malaria and high cases of dengue fever infection (Worang et al., 2013). Until now, in the 26th week of 2023, cases of dengue fever in Indonesia are still being recorded in Indonesia. In 2022 there were 143,266 cases recorded with 1,237 deaths, while in 2023 until the 26th week there were 42,690 cases recorded with 317 deaths (Anonymous, 2023).

A total of 1,120 cases of dengue hemorrhagic fever were found in North Sulawesi during January-June 2023. Manado was the area with the highest spread with 230 cases. So the government is wary of an outbreak in 2024. In Manado City every month an average of 44 cases are found. The peak of spread occurred in January with 53 cases, at the peak of the rainy season climate (Kristian, 2023).

Malaria is also a disease caused by mosquitoes. The highest fluctuating number of malaria cases in Indonesia was in 2022, namely 3.1 million, a 56% increase from the previous year. Indonesia is in 2nd position in malaria cases after India in first position based on WHO 2022 data (Anonymous, 2023).

Understanding vector ecology, use of control tools and instruments, and use of insecticides are important factors that must be considered in controlling vector-borne diseases. However, some synthetic pesticides, for example from the organophosphate, carbamate and pyrethroid groups, have negative effects on humans when used to control mosquitoes. After repeated use, this vector can become resistant to synthetic pesticides (Marini & Sitorus, 2019).

Currently, the dosage forms that are widely used as mosquito repellents or repellents are in the form of anti-mosquito spray (spray), lotion and electric mosquito repellent which contain synthetic chemicals such as DEET or N,N-diethyle-m-toluamide (Suhud et al., 2018). Therefore, choosing an alternative mosquito repellent that does not have a negative impact on humans and is safe for repeated use, especially using natural ingredients with ingredients controlled by biological control, needs to be carried out and developed for the community.

The use of synthetic mosquito repellents at high doses is not proven to directly repel mosquitoes, but instead the negative impact of their use on the body will be greater. Gel spray is a modified mosquito repellent preparation made from a combined gel and spray preparation.

One of the plants that can be used as a natural anti-mosquito gel spray is marigold flowers (*Tagetes erecta* L.) and chrysanthemum flowers (*Chrysanthemum indicum* L.). Based on the results of research conducted by Marini & Sitorus (2019), there are 13 types that are easy to find in Indonesia and have the ability to act as repellents with an average repellency of above 60%. One of them is marigold flowers. In other research by Marini, et al (2019). Testing the mosquito repellent power against the *Aedes aegypti* vector provided an average protection power of over 90% in lotion preparations although the anti-mosquito protection power decreased to 30-60% after 2 or 4 hours of testing. From the results of research conducted, marigold flowers contain secondary metabolite compounds, namely flavonoids, saponins, tannins and steroids/triterpenoids which function as insecticides (Kusmiati, 2011). Meanwhile, research by Werdhitasari (2007) showed that the results of administering chrysanthemum flower filtrate (*Chrysanthemum cinerariaefolium*) in K3 treatment with a concentration of 0.07% had a

significant effect on the death rate of *Aedes aegypti* mosquitoes where the number of deaths of *Aedes aegypti* mosquito larvae reached 50% or LC50. In Putra's research (2020), the results of testing chrysanthemum extract at a 100% dose showed the ability to fight mosquito bites at 89.6% for 1 hour, 76.3% for 2 hours, 63.0% for 3 hours, 59.1% for 4 hours, 47.5% for 5 hours and 43.6% for 6 hours. So it can be stated that within 2 hours to 6 hours chrysanthemum flowers are optimal in repelling mosquito bites.

## METHOD

This research was conducted at the Jueusan Biology Biofamaca and Biomolecular Laboratory, Faculty of Mathematics, Natural and Earth Sciences, Manado State University and the Forensic Chemical Biology Laboratory, North Sulawesi Regional Police. This research was carried out from October to November 2023.

The tools used in this research are analytical balance, measuring cup, beaker glass, watch glass, mortar and stamper, knife, porcelain cup, rotary evaporator, funnel, stirrer rod, dropper pipette, spatula, autoclave, stopwatch, filter paper, object glass, pH stick, and preparation container, namely a spray bottle. The materials used in this research were marigold flowers, chrysanthemum flowers, distilled water, carbopol, glycerin, NaOH, disodium edetate, NaCl, 96% alcohol.

The population involved in this research is adult mosquitoes in the area which have been sorted as female mosquitoes. The samples used were 30 adult adult mosquitoes.

The initial stage is to breed mosquito larvae by making a water storage medium in a container (bucket and baking sheet) containing dirty stagnant water mixed with a little clean water, then placing it in a place where lots of mosquitoes gather, thus allowing mosquitoes to land on the media that has been made. Check after 48 hours, if there are mosquito eggs floating on the media. If it is there, provide nutrition (fish food) every day for the larvae which will turn into mosquitoes. If there are the desired number of larvae, move the container to the mosquito cage that has been made in advance in a place that has been set to be dark until they become adult mosquitoes.

This research will carry out experiments, according to the title, to obtain an effective formulation for anti-mosquito gel spray from marigold and chrysanthemum flower extracts. The research design is RAL (Completely Randomized Design) non-factorial pattern with treatment (t) = 5 and replications (r) = 3. Where the treatment consists of:

F0 = Provision of anti-mosquito product formulas with commercial brands

F1 = Providing mosquito gel spray formula with 0% extract

F2 = Providing mosquito gel spray formula with 2.5% extract

F3 = Providing mosquito gel spray formula with 5% extract

F4 = Providing mosquito gel spray formula with 10% extract

The research will be carried out with the following procedures:

### 1. Making Simplicia

Fresh marigold flowers are picked for the flowers, then sorted, washed and cleaned until only the flower crown remains, then dried by airing, then after drying, they are ground using a blender until they form a powder and weighed. The same thing is done with standard chrysanthemums. Grind the samples and weigh 500g each.

### 2. Making Extracts

Making the extract begins by mixing simplicia with 96% ethanol in a ratio of 1:2 in a glass jar and then closing it. . Macerate for 3 days, stirring occasionally for 1 hour. After 3 days the sample was filtered using filter paper to obtain a filtrate, and to obtain the extract of marigold and chrysanthemum flowers using a rotary evaporator via evaporation (Anonymous, 2000).

### 3. Making Anti-Mosquito Gel Spray

Anti-mosquito gel spray is formulated according to the gel spray formula with a total preparation amount of 60 ml, namely as follows: Prepare tools and materials. Carbopol is developed in distilled water in a mortar, stirred slowly until it dissolves using a stamper until a homogeneous mixture is formed. Dissolve NaOH, disodium edetate, and NaCl in distilled water in a glass beaker, stir until it becomes a homogeneous mixture. Add glycerin to the beaker glass, stir until homogeneous. Add little by little the carbopol that has been developed into the beaker glass, stir until homogeneous. Marigold and chrysanthemum flower extracts in a 1:1 ratio were

added to each preparation to produce 4 spray gels with concentrations of 0%, 2.5%, 5% and 10%. The finished preparation is put into a spray bottle (Rahmat, 2021).

According to Rahmat (2021), the ingredients for making gel spray, which are the results of previous research which have been modified, are used using the measurements shown in Table 1.

Table 1. Ingredients for Making Gel Spray

Material	Formulation			
	F0	F1	F2	F3
Extract	0 %	2,5 %	5 %	10 %
Karbopol	0,24 gr	0,24 gr	0,24 gr	0,24 gr
NaOH	0,12 gr	0,12 gr	0,12 gr	0,12 gr
Gliserin	9 gr	9 gr	9 gr	9 gr
Dinatrium edetat	0,06 gr	0,06 gr	0,06 gr	0,06 gr
NaCl	0,06 gr	0,06 gr	0,06 gr	0,06 gr
Aquades	<i>Disesuaikan hingga volume mencapai 60mL</i>			

Information :

F0: Anti-mosquito spray gel preparation formula with 0% extract

F1: Anti-mosquito spray gel preparation formula with 2.5% extract

F3: Anti-mosquito spray gel preparation formula with 5% extract

F4: Anti-mosquito spray gel preparation formula with 10% extract

#### 4. Organoleptic Test

Organoleptic/sensory testing is a method of testing using human senses as the main tool for assessing product quality. Assessment using this sensory tool includes specifications for the quality of appearance, smell, taste and consistency/texture as well as several other necessary indicators (Anonymous, 2014). The test was carried out on 10 panelists who observed and assessed objectively and wrote down the assessment results on the form prepared.

#### 5. Test pH

According to SNI 16-4946.1-1998, the pH of mosquito repellent preparations is between 4.5 – 7. The more acidic an ingredient comes into contact with the skin, the more difficult it is to neutralize it and the skin will become tired of it. Skin can become dry, cracked, and easily infected. So measuring the pH of a preparation is necessary (Tranggono and Latifah, 2007). pH measurements are carried out with a pH stick. The preparation is sprayed on a pH stick and the pH value is observed.

#### 6. Homogeneity Test

The homogeneity test results on all preparations are considered stable in terms of homogeneity parameters. This is based on the results obtained that there are no solid particles in the spray gel, as well as the absence of spray gel forming which is still lumpy or uneven in the preparation (Mangalik et al, 2023). Homogeneity testing is carried out by taking a small amount of the preparation and placing it on an object glass, then covering it again with another object glass so that the preparation is in the middle of the two object glasses. Observe for inhomogeneities and coarse particles.

#### 7. Test Spray Conditions

In this test, what is observed is the spray condition of the spray gel preparation, whether the preparation comes out of the spray bottle badly or well. (Suyudi, 2014).

#### 8. Test Protection Power

This test is determined based on the percentage of mosquito rejection of the test arm using the anti-mosquito gel spray formula, and compared with the negative control arm or without the addition of extracts in the formula. The protective power is obtained from the number of perches on the negative control minus the number of mosquitoes that perched on the test arm (formula) then divided by the number of perches on the negative control (Rasydy et al, 2020).

Determination of mosquito protection power was carried out based on the standard pesticide efficacy testing method of the Department of Agriculture, Jakarta. 1-HL 4/9-95 which has been modified. The test is carried out by inserting the arm for 10 (ten) seconds alternately into the test cage containing the female mosquito. Then the number of mosquitoes that landed was counted, after that the arm was moved to repel the mosquitoes that landed and then exposed again for the next 10 seconds. This activity was carried out three times (three repetitions) on each arm. All treatments were tested simultaneously. The protective power against mosquito disturbances can be determined using the formula:

$$Dp = \frac{(K - P)}{K} \times 100$$

Information:

DP : Protection Power

K : Perch numbers with gel spray control arm do not contain marigold and chrysanthemum flower extracts.

P : Figures are perched on the arms smeared with gel spray containing marigold and chrysanthemum flower extracts

## RESULTS AND DISCUSSIONS

### 1. Plant Extraction

From the extraction results of marigold and chrysanthemum flowers (Figure 7) using the maceration method, it shows that the extraction results of marigold flowers are dark brownish yellow, while the results of extracting chrysanthemum flowers are yellow.



Figure 1. Extract Results

Extraction was carried out using a 1:2 ratio between simplicia and solvent. The calculation of the total extract yield from the two samples is shown in Table 2. 500 grams of dried marigold flowers were used and then macerated using 96% ethanol solvent with a volume of 1,000 mL which then obtained 44.61 grams of extract with a total extract yield of 8.922%. The dried chrysanthemum flowers used were 500 grams then macerated using 96% ethanol solvent with a volume of 1,000 mL which then obtained 22.8 grams of extract with a total extract yield of 4.56%.

### 2. Organoleptic Test

Organoleptic tests were carried out on dosage formulations that were produced from a combination of marigold flowers and chrysanthemum flowers. The results of the physical test of the gel spray that has been made are shown in Table 3. The gel spray preparation from the flower extract of a combination of marigold and chrysanthemum flowers that is formed is brownish yellow in color. The higher the concentration of the extract used, the more intense the color produced.

Table 2. Organoleptic Observation Results

Organoleptis	Formulation			
	F0	F1	F2	F3
Aroma	No smell	Special Extract	Concentrated	Concentrated

<b>Color</b>	Clear	Yellow	Dark yellow	Dark yellow
<b>Texture</b>	Gentle	Gentle	Gentle	Gentle
<b>Reaction to use</b>	Light	Light	Light	Light

The panelists were assessed on a scale of 1-5 (1 really doesn't like it, 2 don't like it, 3 neutral, 4 like it, 5 really like it) regarding the aroma, color, texture and reaction during 10 minutes of use. With 10 panelists who carried out organoleptic tests. The data obtained from the 10 panelists (Table 4) was then added up according to assessment categories and an average was taken. The results show that the average of the panelists is like it (in the range 4-5). Regarding the aroma, the panelists thought that the gel spray had a distinctive and unique scent, so most of the panelists chose to like it. The color of the gel spray is very thick and bright, making the average panelist choose the answer they like. The panelists also generally chose to like their assessment of the texture because it has a light texture, gives up easily and is the same as the texture of gels that are sold and even used daily, especially moisturizing gels or hand sanitizers during the pandemic. Because it uses ingredients that are commonly used in making gel spray. An assessment of the reaction after 10 minutes of use is also carried out in this test with the aim of testing whether there is irritation. The panelists generally voted that they liked it because the gel spray did not cause any reaction and even had a cooling reaction when used and absorbed into the surface of the skin.

Table 3. Organoleptic Test Results

<i>Panelist code</i>	<i>Aroma</i>	<i>Color</i>	<i>Texture</i>	<i>Reaction after 10 minutes</i>
<b>1</b>	4	4	3	3
<b>2</b>	4	4	5	4
<b>3</b>	3	4	4	4
<b>4</b>	4	5	4	5
<b>5</b>	3	4	5	5
<b>6</b>	2	4	5	5
<b>7</b>	4	5	5	5
<b>8</b>	3	4	3	3
<b>9</b>	4	4	5	4
<b>10</b>	3	4	4	4
<b>Total</b>	34	42	43	42
<b>Average</b>	<b>3,4</b>	<b>4,2</b>	<b>4,3</b>	<b>4,2</b>



Figure 2. Marigold and Chrysanthemum Flower Spray Gel Formula

### 3. pH Test

Test the pH of the gel spray for each concentrate using a universal pH stick (Figure 9). The results shown in Table 5 pH gel spray meet the requirements because it shows pH = 6 on the universal pH test litmus paper. Ideally topical preparations have a pH value that is the same as the skin's pH, namely 5-7 so that irritation does not occur on the skin surface (Sujono et al., 2014).

Table 4. Gel Spray pH Test Results

Losion dengan konsentrasi ekstrak	Nilai pH
0 %	6
2,5 %	6
5 %	6
10 %	6



Figure 3. pH Gel Spray

#### 4. Homogeneity Test

The homogeneity test that has been carried out on all gel spray preparations is shown in Figure 10. The results of the gel spray homogeneity test with each different concentration observed between the object glasses (Table 7) can be seen that the ingredients that make up the gel spray and the extract have been mixed very well, No coarse particles are visible so it can be said to be homogeneous.

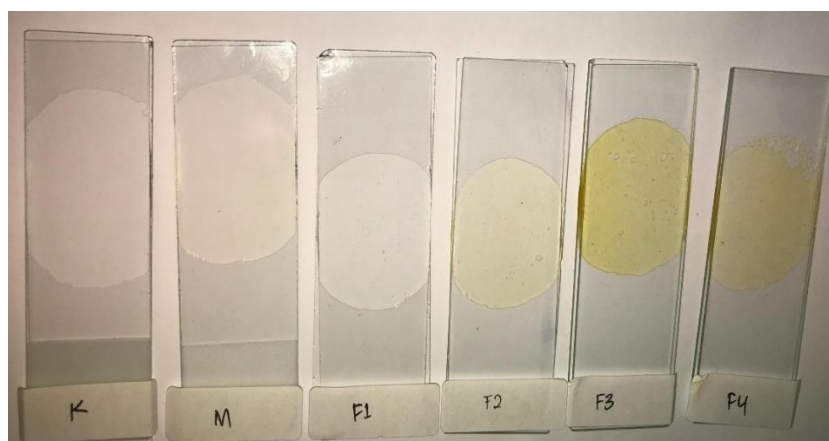


Figure 4. Homogeneity analysis with a glass object

Table 5. Gel Spray Homogeneity Test Results

Gel Spray Formulation Concentration	Result
0 %	Homogeneous
2,5 %	Homogeneous
5 %	Homogeneous



<b>10 %</b>	Homogeneous
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This test was carried out to determine the condition of the gel spray spray, whether it could spray out of the applicator well, badly or not at all. Spray each preparation on mica plastic at a distance of 3 cm, then observe the spray conditions and the shape of the spray results. With the following standards:

Bad 1: doesn't spray out

Bad 2: sprays out, but not in the form of particles but in the form of droplets/clumps.

Bad 3: sprays out, but particles are too big.

Good: sprays out uniformly and in the form of small particles (Kamishita et al, 1992)

Tabel 6. Kondisi Semprotan Gel Spray

Concentration	Spray Conditions
<b>0%</b>	Good
<b>2,5%</b>	Good
<b>5%</b>	Good
<b>10%</b>	Good

#### 5. Protection Power Test

Testing of the protective power against mosquito bites with the anti-mosquito gel spray formula combined with marigold and chrysanthemum flowers was carried out by spraying the formula with a predetermined concentration onto human arms and then placing them in a testing drum containing 30 female mosquitoes for 10 seconds per arm. If there are mosquitoes that land, count them, then move your arm to repel the mosquitoes and expose them again 10 seconds later for up to 3 treatments.

Table 7. Protection Power Test Results

Concentration	T1	T2	T3	Average
Merk X	100%	100%	100%	100%
0%	0,08%	0,10%	0,10%	0,09%
2,5%	12,50%	20%	21,54%	18,01%
5%	63,33%	69,23%	66,33%	66,29%
10%	87,77%	86,65%	91,42%	88,61%

The results obtained from testing the protective power of gel spray (Table 8) show that there are differences in the protective power of the four gel sprays with extract concentrations of 0%, 2.5%, 5% and 10%. Soffell brand commercial gel spray was used as a positive control to see and calculate the difference in protective power of the four gel spray formulations. It can be seen from the results obtained that the negative control for this test is gel spray with a concentration of 0% because it does not contain marigold and chrysanthemum flower extracts. The gel spray formula with an extract concentration of 2.5% has an average protective power of 18.01%. The gel spray formula with an extract concentration of 5% has an average protective power of 66.29%. Meanwhile, the gel spray formula with an extract concentration of 10% has the highest protective power, namely 88.61%. From these results it can be seen that the higher the concentration of the extract used, the higher the protective power provided against mosquito bites. It can be noted that the results in the sig section of table 8 anova  $P < 0.05$  means  $H_0$  is rejected and  $H_1$  is accepted. So statistically there is a real difference between lotion formulas with 0%, 2.5%, 5% and 10% extracts on the protective power of mosquito stings. Because there are differences, a further Duncan Multiple Range Test will be carried out.

#### 6. Discussion

The research results show that the most effective concentration as an anti-mosquito spray gel is a concentration of 10% because it has almost no mosquito repellent power.

Marigold flowers contain the compound eugenol. Eugenol emits a distinctive odor that is detected through the mosquito's antennae. This odor is later interpreted by the mosquito as



something that must be avoided which will then change the mosquito's behavior not to land (Irfayanti and Jasmiadi, 2022).

Eugenol has neurotoxic compounds that can cause insects to become inactive. Neurotoxicity works in the process of suppressing the insect's nervous system which can be characterized by the insect's body feeling soft and limp when touched (Siti et al, 2013 in Nindatu & Noya, 2018). So marigold flowers can be used as an anti-mosquito because marigold extract contains the compound eugenol.

Chrysanthemum flowers contain pyrethrins or pyrethroids. Pyrethroid insecticides are one of the most widely used vector control tools because of their efficacy against the toxicity of beneficial insects. Pyrethroids are used for indoor and outdoor spraying as well as mosquito nets. In addition, it is popularly used in clothing. (Anonymous, 2006; Kittayapong, 2017). Pyrethroids exert insecticidal effects on gated sodium channels (VGSC) located in the neuronal membrane. When pyrethroids bind open channels, they prevent their closure, thus prolonging the action potential and causing rapid paralysis of the insect, which is known as knockdown or kdr and ultimately results in death of the mosquito.

The smell of marigold flowers can repel mosquitoes, combined with chrysanthemum flowers which contain pyrethroid insecticide compounds which are combined in a gel spray preparation to produce the best formula for repelling mosquitoes. So the combination of marigold flowers and chrysanthemum flowers can be said to be a synergistic formula because it produces a more effective mosquito repellent effect when combined.

## CONCLUSIONS

Marigold and chrysanthemum flower extracts, when formulated, can be used as anti-mosquito products in gel spray preparation. Gel spray with an extract concentration of 10% is the most effective formula in repelling mosquitoes. The combination of marigold flowers and chrysanthemum flowers prepared as a gel spray can be said to be the best formula because it is synergistic as a mosquito repellent.

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