

# Methanol Extract Lotion of *Tagetes erecta* L. Flower as A Repellent Against *Culex quinquefasciatus*

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## ABSTRACT

Most Lymphatic Filariasis is transmitted by *Culex quinquefasciatus*. DEET is effective to repel mosquitoes, but it has side effects. Marigold (*Tagetes erecta* L.) flower contains flavonoids and terpenoids which are potential repellents. This study aims to determine the effectiveness of the methanol extract lotion of *T. erecta* as a repellent against *Cx quinquefasciatus*. This is an experiment with a posttest-only control group design. There were seven concentration groups, including control and treatment groups of 2%, 4%, 8%, 16%, and 32% extract. The lotion was applied to the skin of the guinea pig. There were 25 female mosquitoes per cage for each experiment. One Way Anova and Probit used to analyze the data. The result showed that there was a significant difference among the research groups ( $p < 0.01$ ), of which 16% and 32% have significant values ( $p$  0.106 and 0.350 compared with DEET respectively).  $RC_{50}$  and  $RC_{90}$  are in 11.078% and 32.131% respectively, with  $RT_{50}$  and  $RT_{90}$  at the hour of 2.398 and 1.496 respectively. It concluded that the methanol extract lotion of *T. erecta* was effective to repel *Cx quinquefasciatus* at 16% concentration, and potency against 50% and 90% are in 11.078% and 32.131%, at the hour of 2.398 and 1.496 respectively.

**Keywords:** Lymphatic Filariasis, Repellent Potency, *Culex quinquefasciatus*, *Tagetes erecta* L.

## 1. BACKGROUND

Lymphatic filariasis or elephantiasis is a chronic infectious disease caused by filarial worms such as *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* which are transmitted through mosquito bites. One of the vectors is the *Culex quinquefasciatus* mosquito which is a vector of *Wuchereria bancrofti*. In Indonesia, there are still 236 regencies/cities that are endemic for filariasis in 2017. The province with the highest number of filariasis-endemic districts/cities in Papua, with 23 endemic districts/cities. In 2017 there were 12,677 chronic cases of filariasis that occurred in Indonesia, a decrease from the previous year, which was 13,009 cases in 2016. [1]

Transmission of filariasis can be prevented by using repellents. Repellents can contain a variety of active chemicals, one of which is N,N-diethyl-meta-toluamide (DEET) which is very good to use because it has high mosquito repellency, but unfortunately can cause type 4 hypersensitivity reactions and irritation. [2]

Several studies have been conducted to determine alternative repellents against *Aedes sp.* by using plants such as Kecombrang flower (*Etilingera elatior*), [3] Cinnamon (*Cinnamomum burmannii* Bl), Papaya (*Carica papaya*) leaves, Tembelekan (*Lantana camara*) leaves, Betel (*Piper betle* Linn) leaves, Zodia (*Euvodia graveolens*) leaves, and Marigold (*Tagetes erecta* L) leaves. [4] Marigold leaves are thought to be used as a repellent because they contain saponins, flavonoids, tagetiin, alpha-terthienyl, helenium, and flavoxanthin. [5] Ingredients such as terthienyl [6] and

pyrethrin can provide biocidal and mosquito repellent effects. [7] Marigold plants are often found in Indonesia and have a pungent odor and are not liked by insects. This study aimed to analyze the effectiveness of Marigold flower (*T. erecta* L.) methanol extract lotion against *Cx quinquefasciatus* mosquitoes. The results of this study are expected to add to the knowledge base regarding the potential of Indonesian plants, especially the *T. erecta* flower as a repellent to support the control of lymphatic filariasis in Indonesia.

## 2. MATERIAL AND METHODS

This research is a laboratory experimental study with a post-test-only control group design. This research was conducted to test the effectiveness of the lotion of methanol extract of *T. erecta* L. flower as a repellent against *Cx quinquefasciatus*. This study used guinea pigs as subjects which were divided into 7 treatment groups, namely the negative control (treated by lotion without active ingredients), a positive control (treated by 13% DEET lotion), and 5 treatment groups which treated by flower methanol extract with concentrations of 2%, 4%, 8%, 16%, and 32%.

The independent variable in this study was the concentration of Marigold flower (*T. erecta* L.) methanol extract lotion with a concentration of 2%, 4%, 8%, 16%, and 32%. The dependent variables of this study were the percentage of mosquitoes that did not perch, Repellent Time ( $RT_{50}$  and  $RT_{90}$ ), and Repellent Concentration ( $RC_{50}$   $RC_{90}$ ). Confounding variables include temperature, biting time, and

the condition of hunger or whether the mosquito is controlled during the study by homogenizing the conditions.

The materials used were 10 kg of wet *T. erecta* L. flower petals, 5 L of 70% methanol, 10% sugar solution, cetyl alcohol, stearic acid, lanolin, glycerin, metal parabens, triethanolamine, and DEET-based repellent.

The tools used are drying cabinet, blender, scales, filter paper, stirrer, water heater, porcelain cup, potio for lotion container, 7 mosquito cages measuring 35 x 35 x 35 cm<sup>3</sup> as a test chamber, hair shaver, sugar solution tube, mosquito aspirator, pipette, stopwatch, and *handschoen*.

The research was conducted at the Pharmacy and Parasitology Laboratory, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta. The manufacture of Marigold flower methanol extract lotion was carried out at the Pharmacy Laboratory. *Culex quinquefasciatus* mosquitoes were obtained from nature and then reared in the Parasitology Laboratory. A total of 125 female *Cx quinquefasciatus* were transferred to 7 mosquito cages using a manual aspirator, where each mosquito cage contained 25 mosquitoes. Furthermore, in the 7 mosquito cages, one guinea pig that has been selected at random is added.

The guinea pigs were first adapted for 7 days in the Experimental Animal Laboratory. Furthermore, the guinea pig is shaved on the back area of 5 x 5 cm<sup>2</sup>. Treatment is done by applying lotion with various concentrations according to the group. The guinea pig was put into a holder and then tested on mosquitoes that had previously been starved for 24 hours. Each group observed the number of mosquitoes that landed on the surface of the guinea pigs' back skin every 5 minutes at the beginning of every hour for 6 hours. After 5 minutes in the mosquito cage, the guinea pig was removed from the cage and the holder, put in the guinea pig cage to wait for the next treatment.

Observations on the subjects were carried out starting at 5 pm until 10 pm, according to the periodicity of the mosquito biting time. The data observed included the number of mosquitoes that perched on the surface of the guinea pigs' back skin. Observations were repeated 3 times on different days.

The data were analyzed using Probit to obtain RT and RC values. The test of normality and homogeneity of the data used the Shapiro-Wilk Test and Levene Test. Test the significance of differences between groups using One Way Anova followed by the Post Hoc test using LSD to determine the significant differences between each research group. This research has received ethical approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Muhammadiyah Yogyakarta.

#### 4. RESULT AND DISCUSSION

This study was an experimental study to determine the effectiveness of Marigold flower (*T. erecta* L.) methanol extract lotion as a repellent against *Cx quinquefasciatus* with a post-test only control group design. The results of the study

in the form of the average percentage of repelling power of Marigold flower methanol extract lotion at each observation time are presented in Table 1.

**Table 1. The average percentage of repelling power of Marigold flower methanol extract lotion based on the time of observation in each group**

	Average Repellency at Every Hour Observation (%)						Average
	H-1	H-2	H-3	H-4	H-5	H-6	
2%	84±0	80±0	76±1	72±1	72±0	68±1	75±6
4%	84±1	84±0	84±1	76±1	76±1	72±1	79±5
8%	88±1	88±1	84±0	84±1	80±0	76±1	83±5
16%	96±1	96±0	96±0	96±0	96±1	92±0	95±2
32%	100±0	100±0	96±1	96±0	96±0	96±0	97±2
C +	100±0	100±0	100±0	100±0	100±0	100±0	100±0
C -	88±1	84±2	80±2	76±1	72±2	64±2	77±0

Table 1 shows that the percentage of Marigold flower methanol extract lotion started to be effective from concentrations of 16%, which appears to have repellence > 90% for up to 6 hours. The repellence of Marigold flower methanol extract lotion seemed to decrease the lower the concentration.

To find out which group was the most effective in repelling mosquitoes, a one-way ANOVA analysis was carried out followed by a post hoc analysis. The results of the one-way ANOVA analysis showed that there was a significant difference between the study groups (p < 0.01). The results of the post hoc analysis are presented in Table 2.

Based on Table 2, there appears to be no significant difference between the positive control group and the treatment group with concentrations of 16% and 32% (p 0.106 and 0.350). This means that the repelling power of the *T. erecta* flower methanol extract lotion of 16% and 32% is as effective as the lotion of DEET. The effectiveness of Marigold flower (*T. erecta* L.) methanol extract lotion as a repellent can be proven by using probit analysis to determine RT<sub>50</sub>, RT<sub>90</sub>, RC<sub>50</sub>, and RC<sub>90</sub>.

**Table 2. Post Hoc Test of Repellency of Marigold Flower Methanol Extract Lotion against *Cx. quinquefasciatus***

Research Groups	P-Value	
C -	2%	0.482
	4%	0.482
	8%	0.040*
	16%	0.000*
	32%	0.000*
C+		0.000*
(DEET)		

2%	4%	0.164
	8%	0.007*
	16%	0.000*
	32%	0.000*
	C+	0.000*
	(DEET)	
4%	8%	0.000*
	16%	0.000*
	32%	0.000*
	C+	0.000*
	(DEET)	
8%	16%	0.000*
	32%	0.000*
	C+	0.000*
	(DEET)	
16%	32%	0.482
	C+	0.106
	(DEET)	
32%	C+	0.350
	(DEET)	

Based on the Probit Analysis of the data on the number of mosquitoes that landed, the RT<sub>50</sub> and RT<sub>90</sub> were 2.398 and 1.496, respectively. This means that Marigold flower methanol extract lotion can repel 50% of the test mosquitoes up to 2.398 hours and can repel 90% of test mosquitoes up to 1.496 hours.

**Table 3. Repellent Time (RT) of *T. erecta* L. Flower Methanol Extract against *Cx quinquefasciatus***

Repellent Time	Hour
RT <sub>50</sub>	2.398
RT <sub>55</sub>	2.226
RT <sub>60</sub>	2.088
RT <sub>65</sub>	1.971
RT <sub>70</sub>	1.865
RT <sub>75</sub>	1.767
RT <sub>80</sub>	1.674
RT <sub>85</sub>	1.584
RT <sub>90</sub>	1.496

**Table 4. RC Value of Marigold Flower Methanol Extract Lotion against *Cx quinquefasciatus***

RC Value	Concentration
RC <sub>50</sub>	11.078
RC <sub>55</sub>	13.143
RC <sub>60</sub>	15.240
RC <sub>65</sub>	17.408
RC <sub>70</sub>	19.693
RC <sub>75</sub>	22.158

RC <sub>80</sub>	24.904
RC <sub>85</sub>	28.104
RC <sub>90</sub>	32.131

Based on Probit Analysis, it was found that RC<sub>50</sub> at a concentration of 11.078% and RC<sub>90</sub> at a concentration of 32.131%. This indicates that the concentration of lotion for *T. erecta* L. flower methanol extract required to repel 50% - 90% of *Cx quinquefasciatus* mosquitoes was 11.078% and 32.131%, respectively.

The results showed that there were differences in the effectiveness of each treatment group with lotion repelling power against test mosquitoes. Table 4 shows that the treatment group with a higher concentration of lotion had a higher percentage of repulsion compared to the treatment group with a lower concentration of lotion. This statement is by Mirawati *et al.*, (2018) which states that the more active substances in a lotion, the higher the protective power, thus causing fewer mosquitoes to land.[8]

WHO states that to be used as a repellent, the standard repellency of protection that must be achieved is 90% for 6 hours after the application of the repellent. [9] In this study, the concentration group that met the WHO standard was the concentration group of 16% with an average percent repellency of 95% and the concentration group of 32% with an average percent repellency of 97% for 6 hours of observation.

The results of this study are different from the results of Shinta's research, (2020) [10] which tested the flower extract of *T. erecta* on *Aedes sp.* Research conducted by Shinta (2020) shows that at a concentration of 10% it already has a 90% repulsion. Our research showed that *T. erecta* flower extract had 90% repellency at 16% concentration (lower repellency). This is likely due to species differences. *Cx quinquefasciatus* has a larger body size and a longer flight range than *Aedes*. [11] This is probably the reason for the repulsion of the *T. erecta* against *Cx quinquefasciatus* appearing weaker than against *Aedes sp.* This needs to be further proven.

Several other studies related to the effectiveness of *T. erecta* flower extract, namely by Zen & Asih, (2017) [12] and Rusmartinni *et. al* (2018) [2] which proved that the flower repellent of *T. erecta* L. against *Aedes aegypti*. at a concentration of 10% only had a repulsion of 86.8% and at a concentration of 10%, 15%, and 20% had a repulsion to the *Aedes aegypti* mosquito, respectively, of 63.6%, 76.2%, and 79.2% so that it did not meet the WHO standard criteria.

Differences may occur due to differences in the type of mosquito tested and the solvent used. The type of *Cx quinquefasciatus* mosquito has better repellent protection than *Ae aegypti* and *Anopheles aconitus*. [6] *Culex quinquefasciatus* is more sensitive to repellent than other mosquito species such as *Aedes aegypti* because this mosquito is more tolerant of some natural oils that are sensitive to *Cx quinquefasciatus*. [13] Further research is needed on the differences in the level of

repellent resistance against various types of mosquitoes because previous studies used the same type of mosquito, namely the *Ae aegypti*, but had different repellency.

The effect of using ethanol and methanol solvents can also affect the results of the repelling power of Marigold flower extract lotion. Solvents have the principle of dissolving. This principle explains that polar solvents will dissolve polar compounds and preferably non-polar solvents will dissolve nonpolar compounds. [14] The flavonoids contained in Marigold flowers (*T. erecta* L.) are polar compounds, so based on the principle of like dissolves like, polar compounds will dissolve in polar solvents such as ethanol and methanol. [14]. Methanol solvent has low polarity [15] so methanol is not good at dissolving extracts of flavonoids contained in Marigold flowers (*T. erecta* L.). Ethanol solvent has a level of polarity that resembles the level of the polarity of flavonoids, so ethanol is more effective in dissolving extracts of flavonoid compounds. [15] Terpenoid substances contained in Marigold flowers have non-polar polarity so they are easier to extract in non-polar solvents. [16]

The active substances in Marigold flowers that can have a mosquito-repelling effect are Terpenoids and Flavonoids. Flavonoids are substances that provide a repellent effect because they interfere with the behavior, growth, and development of mosquitoes. [17]. Terpenoids have a biocidal effect and can be used as a repellent by inhibiting nerve signals, metabolism, and mosquito movement. [7] The smell of Marigold flowers detected by mosquito antennae through chemical receptors will cause nerve impulses which will be continued to the mosquito antenna lobes so that mosquitoes do not land and bite. [2]

The number of test mosquitoes that landed on the guinea pigs' back's skin increased with the length of observation time. This can be indicated by a decrease in the average percentage of lotion repellency. In Table 4, the decreased repellency of the lotion started from the 4th to 5th hour of observation. This is because the *Cx quinquefasciatus* mosquito is more active at biting the 4th and 5th hours of observation, starting at 8 pm – 10 pm, which corresponds to the time of the circadian biting pattern of the *Cx quinquefasciatus* mosquito, namely at 9 pm – 1 am Indonesian time. [18] The decrease in lotion repellency is also influenced by the length of time the lotion is used, which is caused by increased evaporation of chemical compounds which can result in loss of odor from the lotion contained in the Marigold flower methanol extract lotion, resulting in a decrease in lotion repellency. [8]

## CONCLUSION

Marigold flower (*Tagetes erecta* L.) methanol extract lotion was effective as a repellent against *Culex quinquefasciatus* with the lowest concentration of 16%, having RC<sub>50</sub> at 11.078%, RC<sub>90</sub> at 32.131% and RT<sub>50</sub> at 2,398 hours, and RT<sub>90</sub> at 1,496 hours.

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## CONFLICT OF INTEREST

There is no conflict of interest between the authors.

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