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Etlingera elatior FLOWER OIL ANALYSIS AND ITS PROTECTION LEVEL TEST IN MOSQUITO REPELLENT LOTION

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ABSTRACT

Etlingera elatior flowers contains flavonoids, terpenoids, saponins, and tannins. This study aims to determine the content of oil compounds from Etlingera elatior flowers in n-hexan extract using the GC-MS method and to find out their protective level towards Anopheles mosquitoes compared to the mosquito repellent lotions on the markets. Extraction was carried out by maceration method using n-hexane solvent and it was purified using 96% ethanol. The analysis oil was carried out by compound derivatization method from Etlingera elatior flower's oil through transesterification using sodium methanolic and analyzed using GC-MS. The analysis showed that Etlingera elatior flower's oil contained 22 types of compounds and five of them had % AUC values between 9 - 22.16% consisting of 9,12-octadecadienoyl chloride, 9-hexadecanoic methyl ester, octadecanoic methyl ester, 9-octadecenoic tetradecil ester, and dodecanoic methyl ester. The results of the effectiveness test in the form of anti-mosquito lotion showed that the mosquito repellent lotion of Etlingera elatior flower F3 (7.5%) had a significant difference with the mosquito repellent lotion that had been sold on the market.

Keywords: Mosquito repellent, Etlingera elatior flower, GC-MS, lotion

Introduction

Mosquitoes are animals belonging to the Insect class, Diptera, and Culicidae Family Mosquitoes live on almost around the world except in Antarctica. Mosquitoes can live in between 1,250 meters below sea level up to 5,500 meters above sea level. Genus that is often associated with human life are Anopheles, Culex, Aedes, and Mansonia[11].

These insects often interfere with humans and animals with their bites, basically mosquitoes are harmless insects but these mosquitoes can act as

vectors of disease in humans and animals that can cause various diseases in humans such as vector chikungunya disease, malaria, dengue (Dengue Hemorrhagic Fever), and filariasis[11].

In general, the ways to avoid mosquito vectors and non-vector diseases can be done by avoiding mosquito bites and preventing transmission of diseases originating from mosquitoes, one of them is by using mosquito repellent or repellent[13].

Repellent is a chemical or non-chemical that has the ability to prevent mosquitoes from perching and biting on the skin. Repellent can block sensory function towards mosquitoes target so that it can provide protection from mosquito bites in individuals who use repellent for a certain period of time[22].

Chemical repellents that are often used to prevent mosquito bites, namely diethylmetatoluamide (DEET). The use of DEET can cause hypersensitivity and irritation reactions. This compound can be absorbed more quickly percutaneously which is around 6 hours with a concentration of 50% while excretion within 12 hours is 10%. This shows that the absorption rate of DEET is much greater than the speed of its extraction[22].

The existence of side effects caused by the use of diethylmetatoluamide, the researcher encourages this study to look for alternatives that have low side effects that can be used as a repellent, one of them is from the use of natural ingredients. Natural materials that can be function as repellents are terpenoids, alkaloids, quinones and flavonoids[22].

Etlingera elatior flowers have been known for a long time by Indonesian people as ornamental plants, vegetables, and traditional medicines. *Etlingera elatior* flowers contains flavonoids, terpenoids, saponins, and tannins[12]. *Etlingera elatior* flower has the main essential oil components, such as dexal, dodecalal, 1-dodecanol, dodecyl ester, dodecanoic acid, 1-dodecanol, 3-methyl-1-oxo-2-buten1- (2,4,5-trihydroxy phenyl) and 1 -tetradecena[19,20].

According to a previous study conducted by Renaninggalih *et al* in 2014, the essential oil of *Etlingera elatior* flower had a potential repellent activity with a repelency index of 94.38%[15]. The next study was conducted by Zulfikar et al in 2017. It was found that the ethanol extract of the kecombrang flower can be used as a repellent with an average rejection time of mosquito bites for 5.4 minutes with an extract concentration of 25%[22].

Every plant including *Etlingera elatior* flower contains vegetable oil to support its growth. Oil is one type of lipid, namely neutral lipids. Oil is also an ester of glycerol molecules and three fatty acid molecules. Oil is an organic compound that is available in nature and is not soluble in water but soluble in nonpolar organic solvents such as n-hexane[8].

At this era, the analysis of fatty acid compounds generally uses gas-liquid chromatography equipment so the researchers were interested in developing an analysis using the fatty acid derivatization method of *Etlingera elatior* flowers using GC-MS.

This oil can also be formulated into pharmaceutical preparations, specifically lotions because lotion can form a system of dispersion or emulsion with an oil[10].

Therefore this study aims to identify the compounds contained and their levels in *Etlingera elatior* flower's oil and to determine whether the *Etlingera elatior* flower oil's lotion can function as a repellent compared to lotions that have been sold in the market.

Material and Methods

Equipments

The equipments used in this study are GC-MS (QP2010S SHIMADZU), analytic balance (Mettler Toledo), rotary evaporator (EYELA OSB-2100), (Memmert oven), viscometer (DV-1 BROOKFIELD), and tools glass.

Ingredients

The ingredients used in this study are Etlingera elatior flower, n-hexane, reagent of vanillin-sulfate, ethanol, NaOH, methanol, H_2SO_4, carbomer, stearic acid, cremophore, TEA, propylene glycol, DMDM hidantoin and Aquades**.**

The Processing of ingredients into Crude Material

The processing of ingredients into crude material includes wet sorting, peddling, drying, dry sorting, and grinding into powder.

Isolation of *Etlingera elatior* Flower's Oil

Isolation of *Etlingera elatior* flower oil was carried out by using the maceration method with n-hexane until all components were dissolved. Evaporation of solvents was carried out using a rotary evaporator. Concentrated extract was dissolved using 96% ethanol and then distilled and oil phase was taken.

The Quality Test for *Etlingera elatior* Flower's Oil

Testing the quality of *Etlingera elatior* flower oil includes organoleptic test, specific gravity analysis, solubility in ethanol and terpenoid test.

Analysis of The Compounds of *Etlingera elatior* Flower's Oil

The analysis of *Etlingera elatior* flower's oil was carried out using GC-MS. The sample preparation was carried out by the transesterification method using sodium methoxide. The samples are mixed with sodium methanolic then heat for 10 minutes. After the samples are already cold enough, add concentrated sulfuric acid and n-hexane. The n-hexane phase was taken to be analyze

Formulation of Lotion

Lotion was made by melting the oil phase of cetyl alcohol and stearic acid. Then mixed with the water phase, namely the cremophore RH 40 in a hot state until it forms a base. After that, add additives include carbomer which has been dissolved in water and added TEA, then propylene glycol and DMDM are added to hydrolyoin and water as solvents[16].

The Evaluation of Lotion

The evaluation of lotion included organoleptic observation, pH test, homogeneity test, viscosity test, scattering power test, cycling test, hedonic test, and preliminary irritation test.

The Effectiveness Test of Mosquito

The effectiveness of mosquitoes repellent was carried out in a box using 25 mosquitoes. The right hand was smeared with lotion while the left hand was smeared with lotion without active substance. Then the hand is inserted into a box and how many mosquitoes are perched in 10 seconds. The repetition is done for about 10 times and repeated every hour 5 times. Then calculated as protective level.

Table 1. Lotion Formulation

Ingredients	Formula 0 (w/v)	Formula 1 (w/v)	Formula 2 (w/v)	Formula 3 (w/v)	Functions
Oil	0	2,5	5	7,5	Active substance
Cetyl Alcohol	0,5	0,5	0,5	0,5	Oil phase
Carbomer	0,5	0,5	0,5	0,5	Thickener
Stearic Acid	2	2	2	2	Oil phase
Cremophor	0,039	0,039	0,039	0,039	Water phase
TEA	0,5	0,5	0,5	0,5	Basifiers
Propylene glycol	2	2	2	2	Humectant
DMDM hydantoin	0,5	0,5	0,5	0,5	Preservative
Aquadest	Add 100	Add 100	Add 100	Add 100	Solvent

RESULTS AND DISCUSSIONS

Isolation of *Etlingera elatior* Flower's Oil

The results of isolation were concentrated extract for about 7.55% and the yield of *Etlingera elatior* flower oil for about 2.55%. Maceration using n-hexan because of the nature of non-polar oil which can dissolve in organic solvents, one of which is n-hexane. The concentrated extract which was obtained then it dissolved using 96% ethanol to dissolve compounds that are more ethanol part

Quality Test for *Etlingera elatior* Flower's Oil

The results of purification tests of *Etlingera elatior* flower's oil showed that the organoleptic test of *Etlingera elatior* flower's oil had a liquid form like oil, then the distinctive smell of *Etlingera elatior* flowers and blackish brown color. Moreover, *Etlingera elatior* flower's oil has a specific gravity of 0.9405.

Furthermore, to dissolve in ethanol, *Etlingera elatior* flower's oil cannot dissolve in a ratio of 1: 9.

Analysis of *Etlingera elatior* Flower's Oil

GC-MS results showed that *Etlingera elatior* flower's oil produced a chromatogram with 22 peaks. Which was shown that there were 22 identified compounds. The following were the results of the chromatogram and a list of identified compounds.

The 5 compounds that have the highest %AUC are 9,12-octadecadienoyl chloride, 9-hexadecenoate methyl ester, octadecanoic methyl ester, 9-octadecenoic tetradecil ester, and dodecanoate methyl

ester. Fragmentation Patterns of 5 compounds that created the *Etlingera elatior* flower's oil can be seen in the picture below.

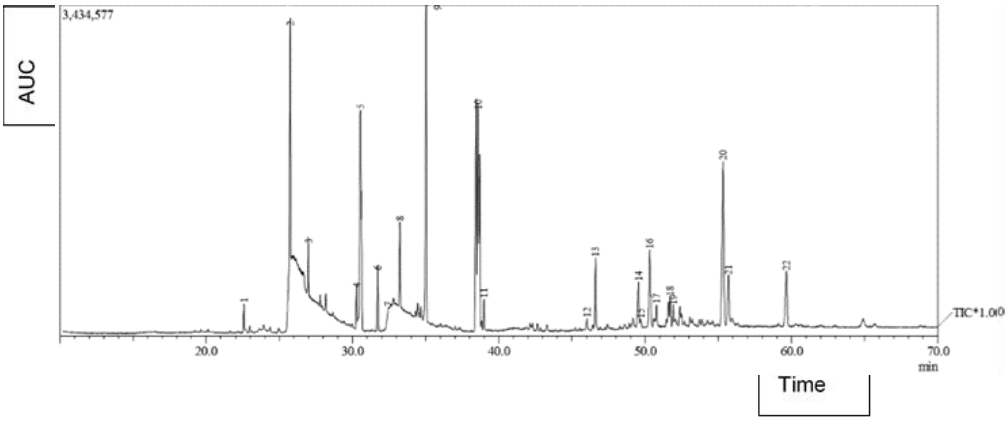

Figure 1. GC-MS chromatogram

Table 2. Compounds from Each Peak in the Chromatogram of *Etlingera elatior* Flower's Oil

Peaks	R.Time	% AUC	SI	BM	Compounds
1	22.592	1	95	184	Dodecanal
2	25.75	9	94	214	Dodecanoic Metil Ester
3	26.992	1.51	92	230	Dodecanal Dimetilasetat
4	30.292	1.37	82	338	Palmitatdehide Dialil Acetat
5	30.55	11.95	89	268	9-hexadecenoic Metil Ester
6	31.742	1.9	89	184	2-dekenoic Metil Ester
7	32.467	5.64	90	170	10-undeken-1-ol
8	33.25	3.16	92	272	Metil 3-metoksitetradekanoic
9	35.042	11.49	94	298	Oktadecanoic Metil Ester
10	38.583	22.16	90	298	9,12-Oktadecadienoyl klorid
11	38.992	0.89	96	298	Oktadecanoic Metil Ester
12	46.033	0.49	92	382	Tetrakosanoic Metil Ester
13	46.617	2.58	87	268	9-octadeken-1-ol
14	49.558	2.15	90	242	2-hexyl-1-decanol
15	49.717	0.37	86	298	1-eicosanol
16	50.317	3.87	79	436	9,12,15-octadecatrionoic Metil Ester
17	50.792	1.35	86	268	9-octadeken-1-ol
18	51.725	2.14	90	230	Dodecanal dimetilacetat
19	51.942	1.17	85	478	9-hexadecenoic Hexadecyl Ester
20	55.342	9.78	84	478	9-octadecenoic Tetradecil Ester
21	55.708	2.6	73	153	7-azabicyclo 4.1.0 heptana
22	59.667	3.46	84	478	9-octadecenoic Tetradecil Ester

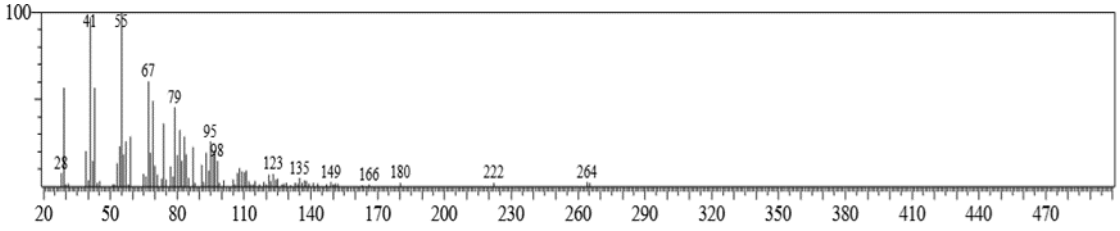

Figure 2. The Mass Spectrum of Peak 10 is 9,1 2-Octadecadienoyl chloride

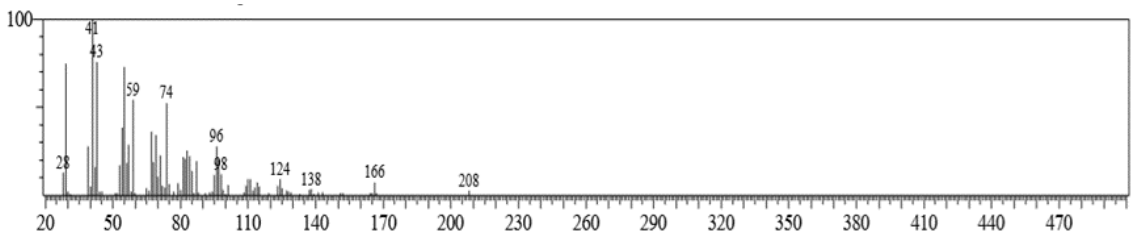

Figure 3. The Mass Spectrum of Peak 5 is 9-Hexadecenoic Methyl Esters

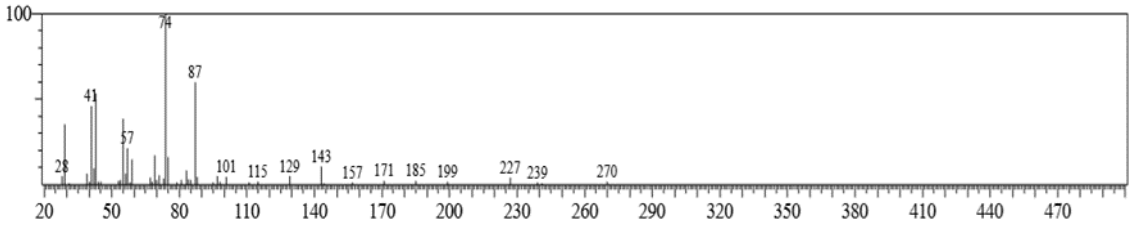

Figure 4. The Mass Spectrum of Peak 9 is Oktadekanoic Methyl Ester

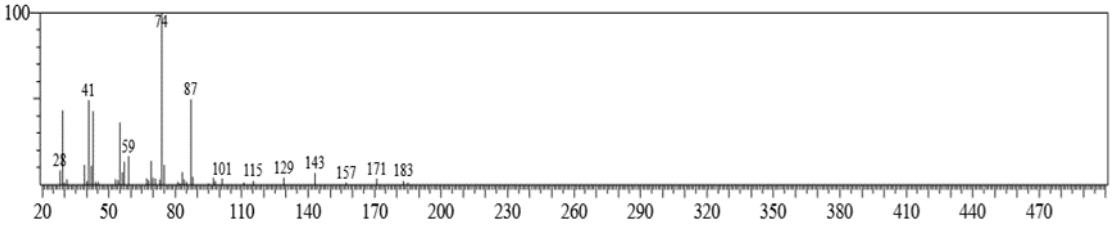

Figure 5. The Mass Spectrum of Peak 2 is Dodecanoic Methyl Esther

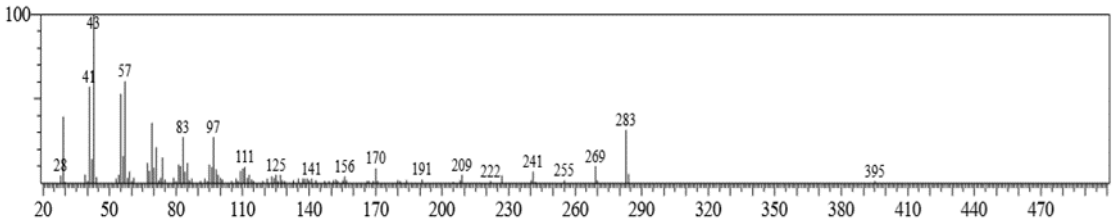

Figure 6 The Mass Spectrum of Peak 20 is 9-Octadecenoic Tetradecyl Ester

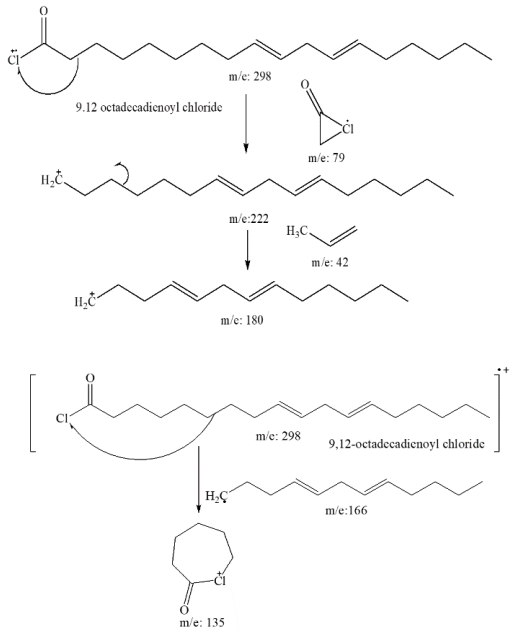

Figure 7 Fragmentation patterns 9,12-octadecadienoyl chloride

Estimated fragmentation patterns of 9,12-octadecadienoyl chloride compounds can be seen in the figure below. The presence of these compounds is characterized by the appearance of peak m/z = 135 which is a characteristic of the aliphatic chloride fragmentation pattern and appears at the peak m/z = 79

Estimated fragmentation pattern of the 9-hexadecenoic methyl ester compound can be seen in the figure below. The presence of these compounds was indicated by the emergence of the peak m / z = 74 which is the result of fragmentation through the McLaferrty mechanism by releasing $C_{14}H_{28}$ and it also reappear at the peak m / z = 59.

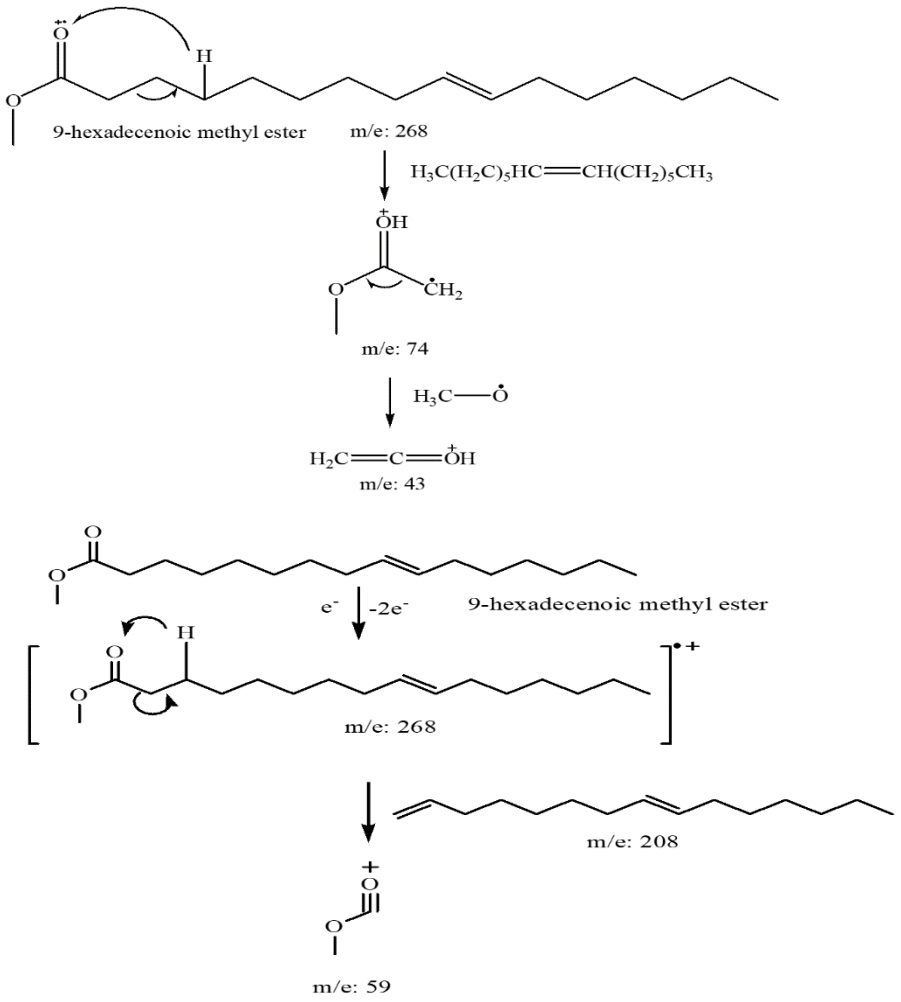

Figure 8. Fragmentation patterns 9-hexadecenoic methyl ester

Estimated fragmentation pattern of octadecanoic methyl ester compounds can be seen in the figure below. The presence of these compounds was characterized by the emergence of the peak m / z = 74 which is the result of fragmentation through the McLaferrty mechanism by releasing C16H31 and it also reappear at the peak m/z = 59 releasing $C_{17}H_{34}$.

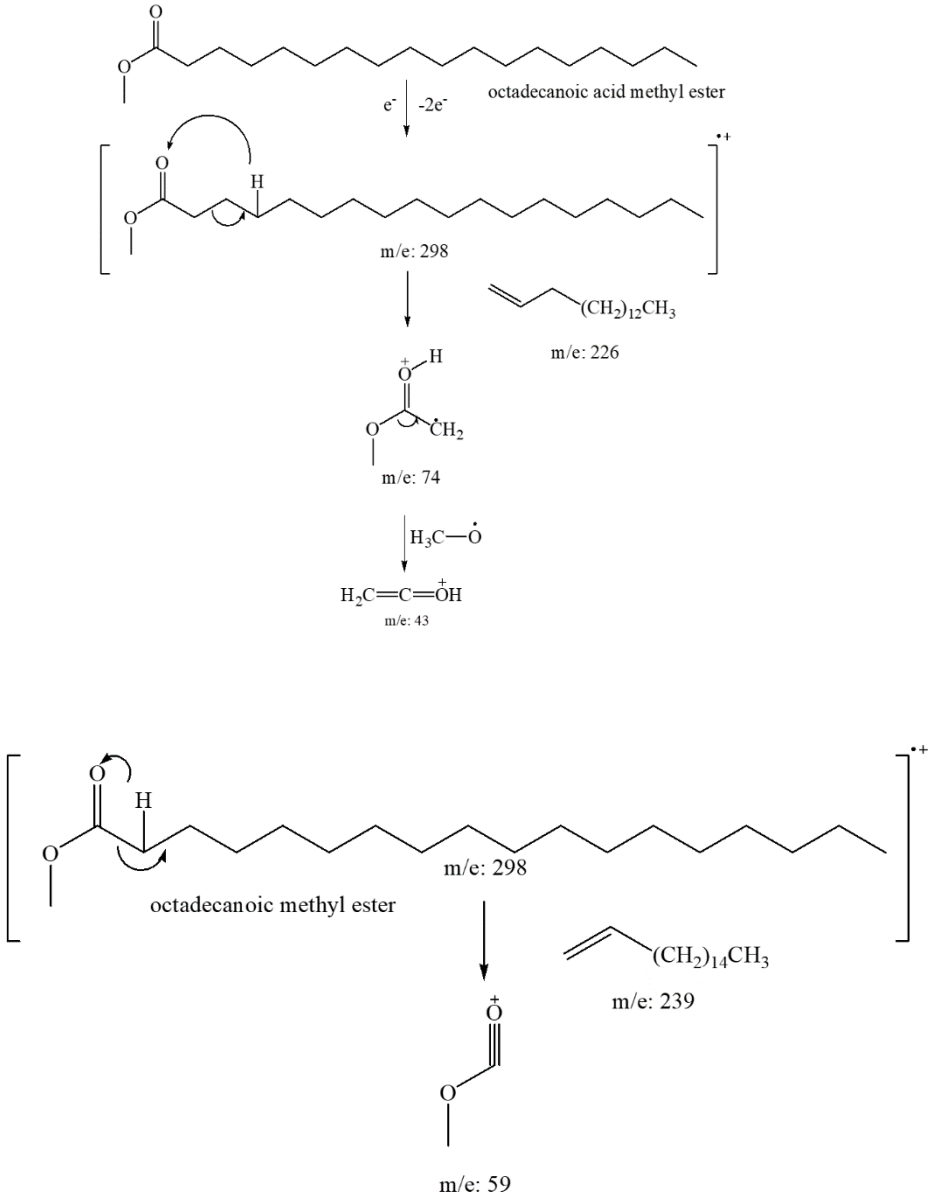

Figure 9. Fragmentation patterns octadecanoic methyl ester

Estimated fragmentation pattern of Dodecanoic Methyl Esters compound can be seen in the figure below. The presence of these compounds was characterized by the emergence of peak m/z = 74 which is the result of fragmentation through the McLaferrty mechanism by releasing $C_{10}H_{20}$ and it also reappear at peak m /z = 183 by releasing H_3CO.

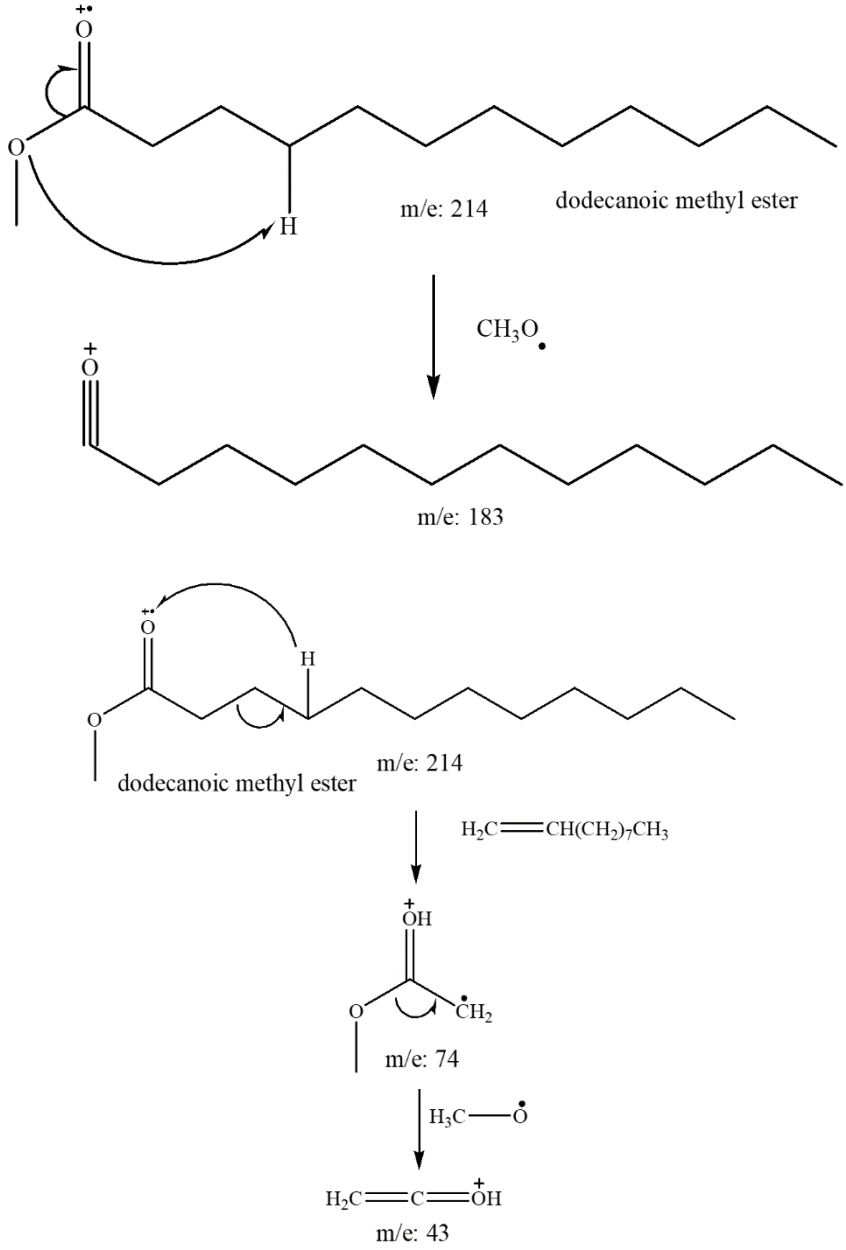

Figure 10. Fragmentation patterns Dodecanoic Methyl Esters

Estimated fragmentation pattern of the 9-Octadecenoic Tetradecyl Ester compound can be seen in the figure below. The presence of these compounds is indicated by the appearance of peak m/z = 255 which is the result of fragmentation through the McLaferrty mechanism by releasing $C_{16}H_{32.}$

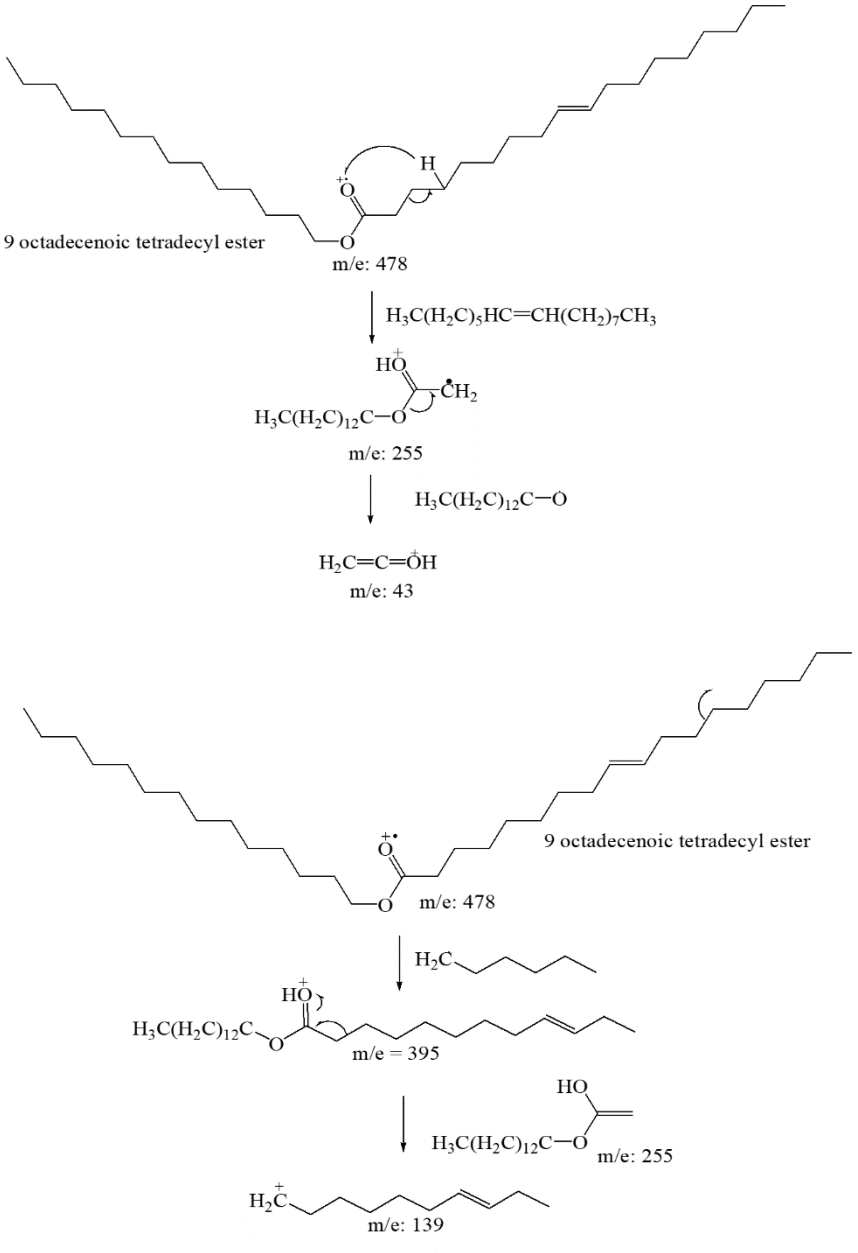

Figure 11. Fragmentation patterns 9-Octadecenoic Tetradecyl Ester

Lotion Formulation

In this lotion formulation, concentration variations were conducted from the *Etlingera elatior* flower's oil. The concentration used were the concentration of 0%, 2.5%, 5% and 7.5%. Based on the results of the preformulation study, the lotion was made using oil ingredients as active substances, cetyl alcohol and stearic acid as oil phase, carbomer as thickener, Cremophore as water phase, triethanolamine as basifier, propylene glycol as humectant, DMDM hydantoin as preservative and distilled water as a solvent. The oil phase is melted in order to facilitate dissolution and mixing with the water phase. Mixing between the oil phase and the water phase must be in a hot condition in order to avoid phase separation and the stirring must be intense enough. The addition of triethanolamine into carbomer aims to develop carbomers because the presence of alkalis will make carbomers expand.

The Effectiveness Test of Mosquito

From the results of statistical analysis, it was found that the data of protective level was not normal because it has a significance of <0.05. Because of that, the analysis continued by using the Kruskall-Wallis method to find out the differences in all the formulas tested. According to the results of testing with the Kruskall-Wallis, it was obtained a significance value of <0.05 which means that there are significant differences from each formula to the protective level.

To find out the difference between one formula and another, this study was continued by using the Mann-Whitney method. Based on the results of data analysis, it was found that there are significant differences between 3rd formula and positive control formula. On the other hand, 1st and 2nd formula do not have significant differences to positive control formula. The result showed that the 3rd formula has a significant difference in protective level to the positive control

Evaluation of Lotion

Organoleptic observation was done by looking at the physical condition of the lotion using the five senses with the parameters of the shape, color and smell of its form. Observations were carried out once every 7 days for 28 days and the results of organoleptic observations showed that the form of lotion from day 1 until day 28 had a semi-solid form, brownish white color and distinctive odor like *Etlingera elatior* flower.

The pH test was carried out using universal indicators. pH testing was conducted in order the pH is in accordance with the pH of the skin to avoid irritation during the utilization process. Observations were carried out once every 7 days for 28 days and the test results from day 1 until day 28 stated that the pH of the lotion was 5. Based on the results of testing the pH , it can be concluded that the lotion was good because

the pH range of the lotion supply required was

pH 4.5 – 7[2].

Homogeneity test was carried out by observing at the lotion by using a microscope with a magnification of 100x and 400x. The tests were carried out once every 7 days for 28 days. The test results from day 1 until day 28 showed that the lotion is homogeneous with the spread of oil evenly on its lotion.

Table 3. The Observation Results of Viscosity Test

Formula	Day	Rpm	Viscosity (cp)
F3	1	100	2640
	7	100	2400
	14	100	2120
	21	100	1830
	28	100	1730

Description: F3= The lotion formula of *Etlingera elatior* lower's oil with 7.5% concentration

The viscosity test was carried out by uses a Brook Field viscometer with a rotation speed of 100 rpm and it also uses spindle number 6. The test was carried out once every 7 days. The viscosity test results showed that the longer the dosage is stored, the lower the viscosity of the lotion. This might occur due to the influence of pH. The carbomer used in the lotion have functions as a thickener. Carbomer itself has characteristics that it can expand when it is in an alkaline environment. On the other hand, in medicinal ingredients that made to have acidic pH, the number of ionised carboxylic groups in carbomers decreases so that they reject the carboxyl group which causes a development in the decreased carbomer structure[7].

Table 4. The Observation Result of The Spreadability Test

Formula	Day	Observation
F3	1	5 cm
	7	5.2 cm
	14	5.2 cm
	21	5.3 cm
	28	5.4 cm

Description: F3= The lotion formula of *Etlingera elatior* flower's oil with 7,5 % concentration

Spreadability test was carried out by giving the load to the medicinal ingredients then it will be measured by the diameter of the spread of the medicinal ingredients. The load used in this study was 100 grams. Tests are carried out in once every 7 days. The test results stated that the average spreadability of the medicinal ingredients was 5.2 cm.

Conclusions

The a nalysis of *Etlingera elatior* oil using
GC-MS showed that compounds contained in isolates of *Etlingera elatior* flower's oil are vegetable oil compounds. The compounds identified included 9,12-Octadecadienoyl chloride, 9-Hexadecenoic Methyl Esters, Octadecanoic Methyl Esters, 9-Octadecenoic Tetradecil Esters, and Dodecanoic Methyl Esters.

There is a significant difference between the protective level on all the formulas which was used, but not so with 1st dan 2nd formula the significant difference between them was appear with positive controls. Meanwhile in 3rd formula, there is a significant difference between the level of positive control protection level and as the best formula

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