UTILIZATION OF THE FLOWER OF *Tagetes Erecta* LINN AS A REPELLENT AGAINST *Aedes Aegypti* L (DIPTERA: CULIDAE) MOSQUITO

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ABSTRACT

The use of *Tagetes Erecta* Linn flowers as a repellent against the *Aedes Aegypti* L mosquito (Diptera: culidae) has been carried out. Extraction of 570 grams of *Tagetes* flower samples was carried out by maceration using methanol to produce 44 grams of thick green extract. Phytochemical test results showed the methanol extract of *Tagetes* flower samples were positive for flavonoids. Compound fractionation was carried out and obtained 3 fractions, namely water fraction, acetone fraction and n-hexane. The results of the phytochemical test showed that the n-hexane fraction was negative for flavonoids, while the water and acetone fractions were positive for flavonoids. The total yield of flavonoids in the water and acetone fractions was 8293.2692 mg/100 grams and 4964.1148 mg/100 grams, respectively. The water fraction of *Tagetes* flower extract was tested for active compounds using the GC-MS instrument. Test the effectiveness of the repellent effectiveness of *Tagetes* flower compounds, the lowest was 5% at 5 hours at 9.35% and the highest was 20% at 1 hour at 60.58%.

Key words: *flavonoid*, GC-MS, repellent

INTRODUCTION

Dengue Hemorrhagic Fever (DBD) is a disease caused by dengue virus which travels quickly and can cause death in a short time. Mosquitoes are vectors of dengue hemorrhagic fever (DBD) which is transmitted through the *Aedes Aegypti* L mosquito. yourself from mosquito bites is to use repellent. Repellents can be made from chemicals or natural. The ingredient found in the repellents on the market is DEET (Diethyltoluamide), which is an aromatic amide that is effective for use in repellent products, also known as N,N-diethyl-meta-toluamide or m-DET. The use of repellents containing DEET is indeed very effective in repelling mosquitoes, but there are many negative effects that can be felt if used continuously, ranging from insect resistance, polluting the environment and poisoning humans and other insects that are not targeted. Therefore, to reduce the negative effects caused, efforts are needed to overcome this, namely by using vegetable insecticides.

Several studies have been conducted using plants as repellents, namely by Darwis (2010), from the results of the study it is known that rosemary leaf extract (Rosmarinus officianalis) is effectively used as a repellent against *Aedes Aegypti* mosquitoes by 5%. *Tagetes* flower (*Tagetes Erecta* Linn) contains secondary metabolites of flavonoids, saponins, tannins and steroids/triterpenoids that function as

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biological insecticides (Kusmiati, 2011). Based on the results of research by Zen et al., 2017, T.erecta flower extract has the potential to be used as a repellent against *Aedes Aegypti* mosquitoes with the highest protective power at a concentration of 10% at 88.86%, while the lowest protection power at 6% concentration was 47.76%. Liquid preparations of electric mosquito repellent made from *Tagetes* flower extract with a concentration of 60% have been proven to be effective in killing *Aedes Aegypti* mosquitoes (Wardani et al, 2019). From the description above, the author wants to know the potential repulsion of *Tagetes* flowers against the *Aedes Aegypti* mosquito.

MATERIAL AND METHODS

Materials

The materials used were *Tagetes* flowers, virus-free *Aedes Aegypti* mosquito, methanol, n-hexane, acetone, H2SO4, Mg powder, HCl, 10% NaOH.

Tools

Blender, analytical balance, water bath, oven, drip plate, beaker, micro pipette, filter paper, aluminum foil, stirring rod, measuring flask, measuring pipette, glass funnel, rotary evaporator, dropper, separating funnel, and GC-MS.

Methods

Sample preparation

A total of 7000 grams of *Tagetes* flowers were washed and then air-dried and blended to obtain a sample of 570 grams of *Tagetes* flowers in powder form then macerated using methanol solvent at room temperature for 24 hours then the filtrate obtained was collected and evaporated with a rotary evaporator (at 50^0C) so that concentrated methanol extract was obtained.

Fractionation of flavonoid compounds

The concentrated methanol extract was dissolved in 100 mL of water-methanol mixture. After that, the methanol was evaporated and partitioned with acetone solvent, to obtain acetone solution fraction and water fraction. back with n-hexane as solvent. The n-hexane extract was collected and evaporated to obtain a thick n-hexane extract. The partition results were evaporated at 45^0C to obtain a thick extract of n-hexane, after that the yield of each fraction was calculated and the flavonoid test was carried out again.

Flavonoid phytochemical test

Phytochemical tests of flavonoid compounds were carried out on the Macerated *Tagetes* flower extract and the partitioned fraction

a. Test with 10% NaOH: A small sample is given a few drops of 10% NaOH. The reaction is positive if there is a specific color change.

b. Wilstatter test: A small sample is added with concentrated Mg and HCl powder. The reaction is positive if there is a specific color change.

c. Smith-Matcalfe Bate Test: A few drops of concentrated H_2SO_4 were added to the sample, heated for 5 minutes. The reaction is positive if there is a specific color change.

Total flavonoid test

a. Preparation of quercetin standard solution

Weighed 0.01 gram of standard quercetin and dissolved in 100 mL of 50% ethanol concentration. The standard solution was then made with variations in concentration, namely 4 ppm, 8 ppm, 12 ppm, 16 ppm, and 20 ppm each 5 mL, prepared successively for tube 1, pipette 0.02 mL standard and added ethanol 4.98 mL, tube 2 pipette 0.04 mL standard and add ethanol 4.96 mL, tube 3 pipette 0.06 mL standard and add ethanol 4.94 mL, tube 4 add 0.08 mL standard and add ethanol 4.92 mL, tube 5 is added 0.10 mL standard and 4.9 mL ethanol. For a 5 mL ethanol pipette blank, 1 mL of $AlCl_3$ was added to each concentration variation, incubated for 30 minutes and the absorbance was measured at a wavelength of 415nm. After getting the absorbance value, a linear regression equation is made which is

$y = ax + b$

b. Extract analysis

A total of 0.1 gram of *Tagetes* flower sample was dissolved in 5 mL of 50% ethanol. Samples were homogenized and centrifuged at 3000 rpm for 15 minutes, the supernatant was filtered. The resulting filtrate is diluted to a volume of 5 mL, 0.5 mL is pipetted again and 0.5 mL of 50% ethanol is added. The extract was put in a test tube and then 1 mL of $AlCl_3$ was added and incubated for 30 minutes and its absorbance was measured at a wavelength of 415nm. After obtaining the absorbance value of the sample, the concentration could be calculated.

Repellent activity test

The repellent activity test was carried out using female *Aedes Aegypti* mosquito test animals. *Aedes Aegypti* mosquito eggs came from the Parasite Laboratory of the Faculty of Medicine, Udayana University. given boiled chicken liver as a food source. After the pupa turns into an adult mosquito, the mosquito will be given a sugar water solution. The mosquitoes used as test animals were female *Aedes Aegypti* mosquitoes aged 2-5 days.

The concentration variations of the *Tagetes* flower fraction used were 5%, 10%, 15% and 20%. The positive control

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used was a lotion containing 15% DEET and the negative control was ethanol. The repellant activity was tested by applying the extract on the forearm of 6 volunteers. who are 20-30 years old for each concentration group. The test object used is the arm to the joints of the hand to the elbow and the palm of the hand to the wrist is covered with rubber gloves. The arm is first washed with non-perfume soap and then rinsed with water until clean then smeared with extracts of *Tagetes* flower samples whose concentration variations have been determined. The test was carried out with the arm inserted into a mosquito cage containing 30 mosquito populations for 15 minutes of exposure time, the test was carried out for 5 hours divided into 5 periods with a time interval of 1 hour per period and repeated 4 times. The percentage effectiveness of the *Tagetes* flower sample is calculated by the equation (1)

Protective Power:

$$\frac{number\ of\ mosquitoes\ perched\ on\ control - number\ of\ mosquitoes\ perched\ on\ treatment}{number\ of\ mosquitoes\ perched\ on\ control} \text{ x } 100\% \ldots(1)$$

Analysis of active compounds

GC-MS analysis was performed using the GC 7890B method with Agilent MSD 5977B. Helium (99%) was used as the carrier gas at a flow rate of 1.0 mL/min. The column temperature used is a programmed temperature with an initial temperature of 70^0C held for 5 minutes, increased by 10^0C/minute to 290^0C and held for 3 minutes. The database used is WILLEY09TH.

Data analysis

The statistical tests used in this study were normality test, homogeneity test, One Way ANOVA test and Tukey HSD test using IBM SPSS Statistics 24 software.

RESULTS AND DISCUSSION

The thick extract of *Tagetes* flower obtained in this study was tested for phytochemical screening. The screening results are presented in Table 1. In the *Tagetes* flower fractionation, three solvents with different polarities were used and three fractions were produced, namely the water fraction, acetone fraction and n-hexane fraction. The results of the phytochemical screening of the *Tagetes* flower fraction are presented in Table 2.

Table 1. Phytochemical screening the tick extract of Tagetes

Phytochemical Screening	**Reagents**	**Color Changes**	**Description**
Alkaloid	2N HCl + Mayer Dragendorff reagent	Chocolate has orange brown precipitate	+
	2N HCl + Mayer Dragendorff reagent	Chocolate has red precipitate	+
Flavonoid	Mg + HCl	Red	+
	NaOH 10%	Brown	+
	H_2SO_4 + heated	Red	+
Tannin	FeCl3 1%	Blackish green	+
Saponin	H_2O + HCl 1N	Orange + Stable foam	+
Steroid	Liberman Baucard	Green	+

Description :
(+) : positive for the phytochemical screening carried out
(-): negative for the phytochemical screening carried out

Tabel 2. Phytochemical screening of the *Tagetes* flower fraction

Phytochemical Screening	**Reagent**	**Colour change**	**Description**		
			FA	**FS**	**FN**
Alkaloid	2N HCl + Mayer Dragendorff reagent	Chocolate has orange brown precipitate	+	+	+
	2N HCl + Mayer Dragendorff reagent	Chocolate has red precipitate	+	+	+
Flavonoid	Mg + HCl	Red	+	+	-
	NaOH 10%	Brown	+	+	-
	H_2SO_4 + heated	Read	+	+	-
Tannin	FeCl3 1%	Blackish green	-	+	-
Saponin	Heated H_2O + HCl 1N	Orange + stable foam	+	-	-
Steroid	Liberman Baucard	Green	-	-	-

Description:
FA : Water fraction
FS : Acetone fraction
FN : Fraction n-hexane
(+) : positive for the phytochemical screening carried out
(-): negative for the phytochemical screening carried out

Determining total flavonoid content aims to determine the total flavonoid content in *Tagetes* flower samples. In the total flavonoid test using quercetin solution

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as a comparison. According to Markham, 1988 flavonoids will be more soluble in water and polar solvents because of the bonds with sugar groups, so that flavonoid compounds are more bound to the water fraction so that it can affect the total flavonoid test value. The total flavonoid test was only carried out on the water fraction and the acetone fraction because these two fractions in the phytochemical screening test were positive for flavonoid compounds. From the results of the measurement of the absorbance of the quercetin solution, it is known that the regression equation and R value are obtained. Based on the calculations, the linear regression equation is obtained y = 0.0022 x - 0.0001 with R^2 = 0.9992.

The results of the total flavonoid test are presented in Table 3. Based on the calculation of the flavonoid content of the water and acetone fractions, it is known that the water fraction has a greater total flavonoid value so that the water fraction of the *Tagetes* flower extract was continued by analysis of active compounds using the GC-MS instrument.

Table 3. Total flavonoid test results on *Tagetes*

Sample	Flavonoid levels	
	%	mg/100 g
Tagetes extract water fraction (FA)	8.2933	8293.2692
Tagetes extract acetone fraction (FS)	4.9641	4964.1148

Analysis of active compounds using GC-MS was carried out on the aqueous fraction of the *Tagetes Erecta* flower extract. This was done because the water fraction of *Tagetes* flower had a higher total flavonoid value than the acetone fraction of *Tagetes* flower extract.

Based on the results of the analysis in Table 5.4, it can be seen that the active compounds contained in the *Tagetes* flower samples include *Isololiolide, 4-hydroxy-3,5 dimethoxybenzoic acid, Ethyl 9H-fluorene-9-carboxylate, ethyl ester.* Based on WILLEY09TH library with retention times of 16,719 minutes, 17,478 minutes, and 21.436 minutes, respectively, which were analyzed with Full Scan Mode. Full Scan mode is a mode for analyzing all compounds in the sample, while SIM mode is an operating mode without recording the

entire spectra, but only certain ions (Moffat, et al, 2004).

Polyphenols have 4 major groups, namely phenolic acids, flavonoids, stiben, and lignans. The compound *4-hydroxy-3,5 dimethoxybenzoic acid* or what can be called syringic acid is a derivative of phenolic compounds from the phenolic acid group of the hydroxybenzoic acid group which is useful as an antimicrobial and deterrent (Bitzer et al, 2004). Deterrent is found in plants that can be used as a deterrent or inhibitor of certain insect behaviors, such as research by Prajapati et al, 2005. Essential oils from 10 medicinal plants were extracted and used to determine insecticidal activity, prevention of egg laying (oviposition-deterrent) and repellent effects.) against Anopheles stephensi, *Aedes Aegypti*d and Culex quinquefasciatu.

Fragmentation ions of the compound *4-hydroxy-3,5 dimethoxybenzoic* acid were 198, 155, 127, 93, 53, 29. According to Pubchem, the highest peak fragmentation ion of *4-hydroxy-3,5 dimethoxybenzoic acid* was 198 with the highest peak the second was 183 and the third highest peak was 127. This finding confirms that the compound in question based on ion fragmentation is 4-hydroxy-3,5 dimethoxybenzoic acid and is confirmed by the WILLEY09TH database on the GC-MS instrument used.

Based on the analysis using the GC-MS instrument, the flavonoid group has not been found specifically, but it can be ascertained that the water fraction of *Tagetes* flower extract contains flavonoids of 8.2933% based on the total flavonoid test. In testing using GC-MS, 4-hydroxy-3,5 dimethoxybenzoic acid was found which belongs to the polyphenol group which has the potential to be used as a deterrent.

The flavonoid group compounds have not been detected in the water fraction of *Tagetes* flower extract using GC-MS because the concentration of flavonoids is not sufficient to detect, but the compound *4-hydroxy-3,5 dimethoxybenzoic acid* in addition to having the ability as a deterrent, based on the research of Vimaladevi et al, In 2012 it was found that phenolic acid group compounds have the potential to be used as repellents. This causes the water fraction of *Tagetes* flower extract at a concentration of 20% to provide an effectiveness as a repellent of 60.58%. The results of the repellent effectiveness test are presented in Table 5 showing the results of the highest repellent effectiveness test for *Tagetes* flower samples at a concentration of 20% in the first 1 hour, which is 60.58% and the lowest effectiveness test results are shown at a concentration of 5% 5 hours, namely 9.35%.

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Table 4. Analysis of active compounds using the GC-MS instrument

Retention Time	**Compound name**	**% Area Width**	**Biological Activity**
16,719	*Isololiolide*	17,27	Anti-inflammatory
17,478	*4-hydroxy-3, 5-dimeth*	69,99	Deterrent
21,436	*Ethyl 9H-fluorene-9-carboxylate, ethyl ester*	12,74	Indicator of presence of alcohol

Table 5. Repellent effectiveness test results

Concentration / Hour	% Effectivity			
	5%	10%	15%	20%
1	21.17	26.59	41.86	60.58
2	19.20	22.65	38.42	57.14
3	16.25	17.23	33.98	50.34
4	13.29	13.79	29.05	42.35
5	9.35	14.28	23.14	36.45

The data from this study were analyzed using the Kolmogorov-Smirnov normality test, homogeneity, One Way ANOVA, and Tukey HSD test. The normality test showed that all data had a significance value > 0.05 so that all data were normally distributed. The results of the homogeneity test showed a significant value >0.05, which means the research variables are homogeneous or the same. Based on the normality and homogeneity tests that have been obtained, the statistical test can be continued using One Way ANOVA. The results of the One Way ANOVA test obtained a significance value of 0.000> 0.05 so that H0 was rejected and H1 was accepted, so that there was a significant difference in the protective power of the *Tagetes* flower sample between the test groups. For the Tukey HSD test, the significance value was > 0.05 so that all treatment groups showed significant differences in effectiveness.

At each concentration there was a difference in the repellent effectiveness value of the Tagetes flower sample, this was due to the greater the concentration, the higher the active compound content in the Tagetes flower sample. So each concentration difference will have a

different effect. Besides being influenced by concentration variations, the time interval also affects the effectiveness of the Tagetes flower samples. Changes in the effectiveness of the repellent effectiveness of Tagetes flower samples from hour to hour can be caused by evaporation of the active compounds in the sample and absorption by sweat (Debboun et al, 2006).

CONCLUSSION AND RECCOMENDATION

Conclusion

Tagetes flower samples have the potential as a repellent for *Aedes Aegypti* mosquitoes. The active compounds suspected to be contained in the *Tagetes* flower samples are Isololiolide, 4-hydroxy-3,5 dimethoxybenzoic acid, Ethyl 9H-fluorene-9-carboxylate. The most effective concentration of *Tagetes* flower samples was 20% at 1 hour testing time.

Recommendation

Further research is needed on *Tagetes* flower as a repellent against *Aedes Aegypti* mosquitoes by increasing the repellent concentration, modifying extraction and separation techniques and conducting tests using other instruments so that more active compounds are detected.

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