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Vitex Trifolia Plant Control of Mice Environmentally Friendly

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Abstract: Research on, *vitex trifolia* plant control of mice environmentally friendly, aims to determine the chemical compounds in the leaves *Vitex trifolia* and its repellent activity to male mice. The study began by isolating the hexane extract, ethyl acetate extract, and methanol extracts of the leaves of *Vitex trifolia*. Furthermore, all of the extract were tested their repellent activity against male mice. The results showed that the most active is hexane extract and repellent activity percentage for each concentration, are (100 %/2 %, 88%/1%, 88%/0.5%,), compared to the ethyl acetate extract and methanol extract. Furthermore hexane extract was characterized by chromatography gas-mass spectrometry (GC-MS), and was obtained 25 compounds, among these compounds are generally act as a repellents are: 1,8-cineole, caryophyllene, α-pinene, β-pinene, β-phellandrene. Then the hexane extract was fractionated by gravity column, with silica gel stationary phase and a mobil phase elution (hexane-ethyl acetate), and was gained 6 groups of fractions. Each fraction group was tested their repellent activity againts male mice. The results show all fraction groups were active to male mice at concentration of 0.5 % and 0.25 %, and repellent percentage of ranging from 57-100 %. The smallest percentage of the repellent is the fraction of group III (57%), and the greatest in the group of fraction IV (100%). Analysis of these variance were performed using ANOVA *one way Post hoc analysis* procedures, significant differences ($p<0.05$) and ($p<0.01$) conducted using Tukey.
Key word: *Vitex trifolia*, control of mice, repellent, environmentally friendly.

Introduction

Mice are very environment distracting, including humans and plants. From the news in Aceh more than 10 hectares of plants rice with 2.5 months of age was attacked by rat in Labui, Pidie district, and also rat attacking 25 day old rice (http:// www.surya.co.id/2011/12/14)[1]. Pest rat is very difficult handled due to their adaptation to the environment, have a very high power breeding. Female rats (*Rattus argentiventer*), capable of producing 10-12 mice, and can give birth to four times a year, pregnancy for 19-21 days, they will ring again after 48 hours after birthed. In good condition, from three pairs of mice for 13 months will product of 2046 young rats. Rats ready to pregnant again while still breast-fed her first child , thus, each female can produce 2-3 generations of children by the age difference between generations for 1 month. Nursed the last 3-4 weeks, and then weaned, and mice into adulthood[2].

Rodents is omnivorous animals, and eat a varied diet including rice, tubers, nuts, different types of grass, puzzles, insects, snails and small fish. As omnivorous, then rats can utilize a variety of food available, so the mice can more easily and quickly adapt to the environment, as well as selective in choosing food when food is

plentiful. The ability of rats to spend rice and sweet potato respectively around 10 to 23.6 g/day[3]. While cassava, maize pipil, peanuts and fish sauce can be spent each were 20.6 , 8.2 , 7.2 and 4.2 grams/day. When the number of meals prepared at same time, the rice is preferred because most consumed[4].

Chemical pest control is often done, but because rats are intelligent animals that eat the way first taste some food, cause this way is less effective, other chemical substances that pose a residue that can cause problems even new to the environment such as mites resurgensi (resistance) , and could also harm humans if channel water flow to the community.

Many insectisida organophosphate such as parathion, mevinfos, monokrotofos , TEPP and dichlorvos is the most dangerous substances are often associated with humans. While organoklorida like DDT remain in the environment for long periods of time, causing environmental damage because its accumulation in the food chain, and in many cases are carcinogenic, teratogenic, or mutagenic[5].

The environment and agriculture's experts continue to search for natural ingredients that do not harm the environment, which degradable in soil, one of the plants that have been traditionally used by the people of Aceh to repel mice is the of plant *Vitex trifolia*, by putting the the plant twigs in the rice storage, however, research has not been conducted on repellent activity at mice on plant of *Vitex trifolia*

Reseach on the activity of repellent and larvacidal of *Vitex trifolia* relative has been done against the mosquito (*Aedes egypti* and *Culex*)[6,7,8,9,]

Chemical compounds found in *Vitex trifolia* plants are from the class of terpenoids that is labdane diterpene: vitexilactone, rel 5S,6R,8R,9R,10S)-6-acetoxy-9-hidroxy-13(14)-labden-16,15-olide, rotundifuran, vitetrifolin D,vitetrifolin E[10]. Other than that obtaine heptatriacontane, α-pinene, and (4R,5R)-caryophyllene-oxide[11].

Based on the above, the objective of this research is to get information about the active extract of leaves *Vitex trifolia* to repell mice

Material and Method

Plant Material

Leaves of *Vitex trifolia* that grows in the province of Aceh, Darussalam, was collected in March 2013, The plant was identified at Department of Biology, University of North Sumatera, Medanense, Medan.

Animal

All experiment were carried out using breeding 4-6 week old male Swiss Webster mice chosen from animal colony of central animal research facility, University of North Sumatera, Medan. The colony was maintained under controlled condition soft temperature. The experimental protocol has been approved by North of Sumatera University, Mathematic and science Faculty Ethic Committee (Regd. No 53/KEHP-FMIPA/2014).

Testing Phytochemicals

The method used for testing of phytochemical can be found in : Phytochemical methods, Simplified Determina -tion Method to Analyze plant[12].

Isolation The Leaves Of *Vitex Trifolia*

The air dried of leaves of *Vitex trifolia*, 1 kg, was macerated with n-hexane solvent for 3x24 hours, then filtered. the filtrate was evaporated in vacuo were obtained 20 g of hexane extract. Then the residue of the sample was macerated again with ethyl acetate, and methanol each for 3x24 hours and filtered, then the filtrate were evaporated and ethyl acetate extract obtained 25 g and 15 g of extracts methanol respectively. Each of this extract was used as the test material to repell on male Swiss Webster mice.

Having tested repellent activity, that the most active is an extract of hexane, then fractionation performed on the hexane extract and characterized the composition of chemical compounds with GC-MS

Hexane extracts as much as 15 grams were separated using gravity column, the column length 50 cm, stationary phase silica gel GF 60, ≤ 230 mesh, the mobile phase is hexane : ethylacetate (9.5:0.5), produced 70 fractions, in each fraction was 20 mL. These fractions were grouped based stain pattern on their thin layer chromatography (TLC) plate. Based on this, the grouping of hexane extract fractions were divided into 6 groups, namely: Group Fraction I(1-3), the fraction of group II (fractions 4-6), the fraction of group III (7-15) , the fraction of group IV (16-43), the fraction of group V (44-57), and the fraction of group VI (58-70). All group of the fractions were tested their repellent activity against male Swiss Webster mice.

Repellency Test

Before use, the mice were acclimatized for 7 days in laboratory conditions as well as getting enough food and drinks. After 7 days, selected mice were healthy, characterized by weight stable or increased and did not show any abnormal behavior.

There are 3 cages (A, B, C) interconnected on the front with tunnel, 2 parallel cages (A and B), while another one cage (C) is in front of the 2 previous cage (A and B) with a distance of 3 meters is filled with mice (30 mice). Cages of A and B filled with balls containing rice bran, fish meal, and hazelnut, and for B cage beside the same ingredients with A also supplemented with extract *Vitex trifolia*. Each ball weighs 10 g and the extract concentration is from 0.125 to 2%.

Percent repellency is calculated by formula[13],

$$\text{ER (\%)} = \frac{\text{NC} - \text{NT}}{\text{NC}} \text{x100(\%)}$$

ER = percent effective repellency;

NC = number of rats in the control; and NT = number of rats in treatment

Having fasted for 20-24 hours, 30 mice put into the cage C, let the rats 1 hour, and then count the number of rats in cages A and B. Then repellent percentage is calculated based on the above formula [13]

Statistical Analysis

Statistical analysis was performed using *Statistical Product And Service Solution* (SPSS) Program. Analysis of variance were performed using ANOVA *one way Post hoc analysis* procedures, significant differences ($p<0.05$) and ($p<0.01$) using Tukey

Result and Discussion

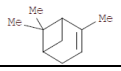
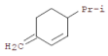
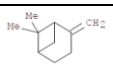
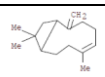
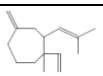
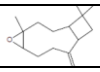
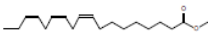
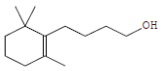
Phytochemical Test Results

Phytochemical test of *Vitex trifolia* leaves contain of secondary metabolites: terpenoids, steroids, alkaloids, and flavonoids.

Characterization results with GC-MS hexane extract performed at the Department of Chemical Engineering Faculty, Unsyiah, of their chemical compounds contained in Table 1.

Table 1. Phytocomponents identified in the ethyl acetate extract of the leaves of *Vitex trifolia* by GC-MS

No.	RT (minute)	Name of compound	Molecular formula	MW	Peak area (%)
1	1.035	Pentane	$Me(CH_2)_3Me$	72	0.36
2	1.069	Butane, 2,2-dimethyl- (CAS) 2,2-Dimethylbutane	Me_3CEt	86	0.66
3	1.105	Pentane, 2-methyl-(CAS)2-methylper	Me_2CHPr	86	23.27
4	1.151	Hexane (CAS) n-Hexane	$Me(CH_2)_4Me$	86	51.29
5	1.218	Cyclopentane, methyl-		84	15.04
6	1.306	Cyclohexane		84	1.29

7	1.722	Benzene, methyl- (CAS) Toluene		92	0.17
8	3.145	alpha.-pinene, (-)-		136	0.43
9	3.630	.beta.-Phellandrene		136	0.04
10	3.693	2-.beta.-pinene		136	0.06
11	3.909	Benzene, 1,3,5-trimethyl- (CAS) 1,3,5-Trimethylbenzene		120	0.48
12	.4.446	1,8-Cineole		154	0.08
13	9.627	Ocimenyl acetate			0.29
14	10.810	trans-Caryophyllene		204	0.09
15	17.357	Cycloheptan, 4-methylen-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-(humulen-"v		204	0.01
16	18.215	Cycloheptan, 4-methylen-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-(humulen-"v		204	0.17
17	18.376	Caryophyllene oxide		220	0.10
18	19.868	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-		292	0.12
19	21.700			332	
20	22.378	1-Cyclohexene-1-butanol, 2,6,6-trimethyl-		196	0.16
21	22.438	(1Ar-(1aalpha,5abeta,9ar(*)))-5a,9,9-trimethyloctahydrobenzo(d)cycloprop(c)oxepin-2,4-dione		236	2.95
22	25.054	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol		306	1.14
23	27.067	Tricyclo[4.3.0.0(7,9)]nonane, 2,2,5,5,8,8-hexamethyl-, (1.alpha.,6.beta.,7.alpha.,9.alpha.)-		206	0.34
24	27.063	Dotriacontane		450	0.17
25	29.620	Tetrapentacontane		758	1.24

Repellency Test Result

Hexane Extract, Ethyl acetate and Methanol

Results of test the repellent activity of hexane extract, ethyl acetate extract of and extracts methanol leaf extract of *Vitex trifolia* on mice with test concentration of 2 % to 0.125 %, can be seen in Figure 1.

From this figure it can be stated that the hexane extract was the most active extract as repellent (can repell the mice: 100 %, 88%, and 88% on concentration *Vitex trifolia* : 2% b/b; 1%b/b; and 0,5%b/b respectively), the hexane extract was then separated by column chromatography to obtain active fraction, the percentage of mice that can be rejected by using a group of fraction contained in figure 2 below.

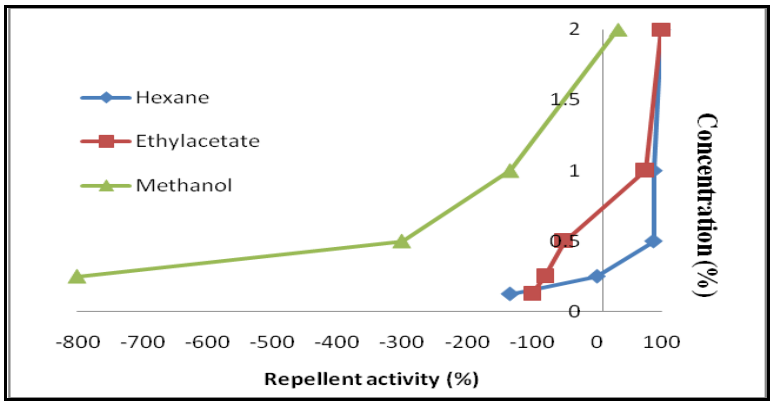


Figure 1. Percent of repellent of mice on the repellent test extract hexane, ethyl acetate, and methanol at concentration of 2% to 0,125%

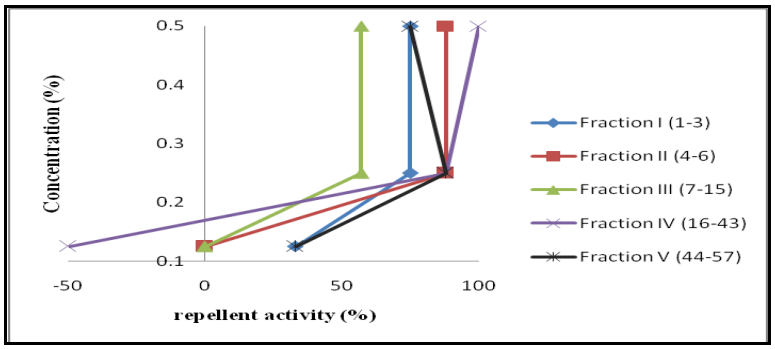


Figure 2. Percent of repellent of mice on repellency test of group fraction hexane leaf extract *Vitex trifolia* at concentration of 0,5% uµ ∗ı 0,125%

In Figure 2 above, it can be seen that the percentage of mice that can be rejected hexane fraction (group fraction) relatively the same that is at a concentration of 0.25 % and 0.5 %. At concentration of 0.125 % hexane fraction (group fraction) less active to repel the mice. Groups fraction (fraction 6 groups), are generally active, but the most active is the fraction IV (100% / 0.5 % and 88%/0.25 % each), while the less active group III fraction (57% / 0.5 % and 0.25%).

Chemical compound in the hexane extract and its fractions are generally composed of compounds monoterpenes (1,8 - cineole, caryophyllene oxide, α - pinene, 2-β- pinene, β - phellandrene) with low molecular weight, that is easily detectable from a far distance. Monoterpenes compounds can affect the nervous system of mice, which can causes mice do not go to near the sample. Other than that, this monoterpene compounds have unpleasant taste that cause mice do not like eat it.

Conclusions

Hexane and ethyl acetate leaf extract *Vitex trifolia* can reject male Swiss Webster mice at concentration: 2%; 1%; and 0,5%, but the most active of these extract is hexane extract. Groups fraction (fraction 6 groups), are generally active, but the most active is the fraction IV (100 % / 0.5 % and 88%/0.25 %), while the less active group III fraction (57 % / 0.5 % and 0.25 %).

Among the active compounds are rejecting mice: Pentane; butane, 1.8 - cineole, caryophyllene oxide, α - pinene, 2 - β - pinene, β – phellandrene.

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